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AGRICULTURE

004-001

ASSESSING THE GROWTH AND PRODUCTIVITY OF CAPSICUM FRUTESCENS AS AFFECTED BY DIFFERENT AMOUNT OF EM BOKASHI

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ABSTRACT

The culinary veggie Capsicum frutescens has been used for a very long period. Capsaicin has been identified as the active ingredient responsible for the flavour that is hot and spicy. The study aimed to evaluate the effects of the different amount of EM-Bokashi in the performance of chili (Capsicum frutescens). This study was carried out at the UCAM campus in Melaka. Between December 2022 and March 2023, the experiment was conducted. It specifically evaluated the effects of EM Bokashi on the growth and yield parameters of chili. It consisted of four treatments and ten replications in each treatment. The Completely Randomized Design (CRD) is applied as an experimental designed. The treatments were the following: Treatment 1 (100ml), Treatment 2 (200ml), Treatment (300ml), and Treatment 4 (no application) per pot. Based on the result of the study, Treatment 3 showed a significant difference among the treatments in terms of height plant. Also, it showed a highly significant difference in the length of canopy and weight of fruits. It is further supported by LSD as it showed significant differences in control and among other treatments. Therefore, the application of 300ml of EM bokashi is recommended for chili production.

Keywords: Capsicum Frutescens, EM Bokashi, Growth Performance.

1.0 INTRODUCTION

Chili (*Capsicum frutescens linn*) is a wild chili pepper native to Central and South America that is genetically related to the cultivated pepper Capsicum Chinese. C. frutescens pepper varieties can be either annuals or short-lived perennials. Flowers are either insect- or self pollinated and are white with a greenish white or greenish yellow corolla. Berries on the bushes are normally upright and ellipsoid-conical to lanceoloid in shape. They are typically small and pungent, measuring 10-20 millimeters (0.39-0.79 in) in length and 3-7 millimeters (0.12-0.28 in) in diameter.

Bokashi is a system of odorless composting by selected "effective microorganism" (EM). This system relies on fermentation rather than putrefaction. Bokashi is made by using an organic material which is inoculated with EM. The concept of EM-Bokashi was discovered and developed by Professor Teruo Higa in 1980s at the University of the Ryukyus, Japan (Higa T, Parr JF, 1994). EM-Bokashi is an effective organic fertilizer in nature farming crop production (Kyan et. al., 1999).

The problem in this study is that most farmers think that organic matter has less effect than chemicals. In addition, farmers are also less knowledgeable about EM-Bokashi and the benefits of organic matter to crops. To meet the growing consumer demand for these vegetables, it became necessary to enhance the productivity of vegetable crops with cost effective.

The objectives of this research are to determine the growth and yield performance of chili with different amount of EM-Bokashi application and to recommend the suitable amount of different rate of EM-Bokashi to be used in chili production.

Due to uncertain weather changes, such as rain. Since fruit production is also influenced by temperature, there will be changes in the yield data. The unpredictable weather and incorrect techniques will also interfere with the development of EM Bokashi. This research was conducted to help farmers in using EM-Bokashi as an addition to improve the growth and yield performance of the chili plants. This study can also help them to identify the suitable amount of EM-

Bokashi in chili plants that give maximum yield to farmers and also can help them to know the benefits of organic matter to crops.

2.0 CHILI (*Capsicum frutescens linn*)

Bird chili (*Capsicum frutescens*) is classified as one of the most pungent chilies in the world, next to the habanero (*Capsicum chinensis*). The pods of bird chilli are small and elongated. They are normally referred to as 'cili padi' or 'cili api' or 'cili burung' interchangeably in Malaysia. For convenience, bird chili will be referred to as cili padi. Most of the bird chili belongs to C. frutescens but Thai cili padi on the other hand, showed typical characteristics of Capsicum annuum. Bird chili that belongs to C. frutescens has a unique flavor and is more aromatic compared to those that belong to C. annuum (R. Melor, 2003).

Chili (*Capsicum frutescens*) is a vegetable commodity with great economic value. This chili is so popular in fresh form that it is widely available in the market. Every year, as the population and food business grow, so does the demand for chili. However, the availability of chili has decreased due to the presence of pests such as Thrips (Thrips parvispinus Karny), fruit flies (Bactrocera sp.), mites (Polyphagotarsonemus latus), leaf lice (Aphididae), and outbreaks of diseases such as ralstonia withers bacteria (Ralstonia) and viruses such as yellow virus disease (Gemini virus). Pest management using natural insecticides is being used to reduce pest assaults (Sari et al, 2020).

Chili padi remains less in importance and is not grown on a large scale in this country. Locally, cili padi is normally grown as backyard crop until lately when the erstwhile steady negligible area of cili padi suddenly increased to 205 ha (Anon. 1999) and the import values showed a sharp rise to about RM39 million (Mohamad Noh Samek, 1999) mainly from Thailand. The sudden increase in demand of cili padi appeared to coincide with the rise in demand for this commodity as raw material in sauce industry.

Capsicum frutescens plants have smooth, medium-sized, elliptical leaves, and slender branches which are 30–120 cm long. The plants attain their full size in a hot climate. A chili plant can produce about 120 pods of intermediate pungency in a season, with Scoville ratings of between 30,000 and 60,000 units. Pods are erect, and exhibit less variation in color, shape, and size than do Capsicum annuum, C. Chinese, and C. baccatum. The phenomenon is probably due to lack of enthusiasm for breeding Capsicum frutescens varieties (Evandro et al, 2011).

2.1 EM BOKASHI

The EM consists of mixed cultures of beneficial and naturally occurring microorganisms applying as inoculants to increase the microbial diversity in soils and plants. Some findings have shown that the inoculation of EM-Bokashi cultures to the soil plant ecosystems improves soil fertility, growth, and yield of crops (Sahta, 2019).

The EM contains up to 80 different species belonging to five primary groups of microorganisms, such as predominant populations of lactic acid bacteria (Lactobacillus plantarum, L. casei, L. fermentum, L. salivarius, L. delbrueckii) and yeasts (Saccharomyces cerevisiae), smaller numbers of photosynthetic bacteria (Rhodobacter sphaeroides, R. capsulatus and Rhodopseudomonas palustris), actinomycetes and mold fungi (Olle and Williams, 2013).

Bokashi have been shown to increase plant nutrient uptake, growth, and yield via different basic mechanisms such as changes in soil structure, nutrient solubility, root growth and morphology, plant physiology, and symbiotic relationships (Iriti et al., 2019). Although the exact mechanism of Bokashi in relation to soil-plant systems is remain controversial, many farmers in Asian countries including Indonesia have adopted the Bokashi technology (Karimuna et al., 2016).

According to Ishimura (2004), the bokashi possess several favorable characteristics on the agriculture, once afford rapid nutrients release, assists on soil physics aspects, as its structure maintenance, besides also support in some diseases and pest's prevention and control, once it has a microorganism's broad range, introduced with the EM utilization, prepared from organism's virgin forest collected. Bokashi is a soil fertility technology amendment in farming systems. This technology can be applied to ameliorate the soil properties to a better condition for plant growth and production (Ginting et al., 2019).

The microbial communities' characteristic of bokashi and compost are similar in that they both include various bacteria, actinomycetes, yeast, molds, and other fungi (Ndona et al. 2012). To promote this diverse group of microorganisms and their coexistence, in the early 1980s, Dr. Teruo Higa, professor at Ryukyus University, Okinawa, developed a mix that he called “effective microorganisms” (EM), which included lactic acid bacteria, yeasts, photosynthetic bacteria, actinomycetes, and fungi (Ndona et al. 2012).

EM has become an important component of bokashi compost. Bokashi compost provides a way to compost food stuff that is typically excluded from aerobic composting, such as meat and dairy products. This system of anaerobic buckets is different, though similar in the use of fermentation, is different from the bokashi fertilizer previously (Nisreen, 2018).

2.3 FERTIGATION SYSTEM ON CHILI

Fertilization through drip irrigation (called fertigation) is expected to increase the effectiveness and efficiency use of fertilizers. Nesthad et al. (2013) stated that growth and yield of chili using fertigation methods was affected by different irrigation levels and different drip system layout. Mods et al. (2015) suggested that the cost to produce 1 kg chili using fertigation technique was lower compared to conventional cultivation.

There is good potential for adoption of drip irrigation and use of water-soluble fertilizers with drip system, i.e., fertigation technique for achieving better productivity and quality in different crops. Micro-irrigation also enables use of fertilizers, pesticides, and other soluble chemicals along with the irrigation water more economically and thus enhancing quality of produce and yield (Singh, 2000). In fertigation the dissolved fertilizer reaches the plant root zone where it is available to the plants directly in solubilized form. The fertilizer in solution form is easy to uptake. Fertigation events can be scheduled several times during the season with fertigation frequencies ranging from daily, twice daily, weekly, or fortnightly. Generally, fertilizer is applied in uniform quantity per application (Singh, 2015).

3.0 DESCRIPTION AREA OF STUDY

This study was carried out at the University College of Agroscience Malaysia (UCAM), situated in Ayer Pa’abas, Alor Gajah, Melaka. To be more specific, this research conducted in close proximity to the hatchery site, with coordinates at Latitude 2.394493 and Longitude 102.167445.

Figure 1: Study Area, University College of Agroscience Malaysia (UCAM)

4.0 METHODOLOGY AND DATA COLLECTION

This research recommends the suitable amount of different volume of EM-Bokashi to be used in chili production. Three treatments were conducted using 100ml, 200ml, and 300ml per polybag/pot and one treatment was conducted without using additional EM-Bokashi. This research also determines the growth and yield performance of chili with different amounts of EM-Bokashi application. Growth and yield performance include parameters of plant height, length of canopy, and weight of fruit.

In the initial preparation of the EM-bokashi, the EM 1 was diluted with molasses in water (40 ml of molasses + 40 ml EM 1 + 4 liters of water). Then, solid ingredients (20 kg rice bran, 1 kg rice hull) were prepared and mixed with water and 4 liters of diluted EM 1 solution at 30-40% moisture content. After mixing the ingredients, cover it and ferment it for three (3) months in a covered area without rain or direct sunlight. During fermentation, gradually remix the mixture if its temperature exceeds 50 C and cover. If the temperature is stable and has a sweet-sour fermented smell, the EMbokashi is ready for use. This method was based on the EM Research Organization (2012).

This research will be carried out with three different volumes of EM-Bokashi using Complete Randomized Design (CRD). In treatment one until treatment three, there is a different volume of EM-Bokashi. Forty (40) sample plants were used. The treatment are as follows: i. Treatment 1 – (T1 = 100ml) ii. Treatment 2 – (T2 = 200ml) iii. Treatment 3 – (T3 = 300ml)
iv. Treatment 4 – (T4 = control)

The Completely Randomized Design (CRD) is method to compare between treatments when environment is adequately uniform. Randomization will ensure there are not subjectively in the distribution of treatment and the effect shall not go out of factor when the treatment will be compared to avoid bias. The polybags for 40 plots will be placed randomly and this technique will ensure there is no intolerance between the treatments. Every polybag will be labelling start from one to 40 polybags with the tape. The letter T1 until T4 will be written on 40 different tapes. From this method, it will be eased to show the location of plot and other papers represent both replication and treatments.

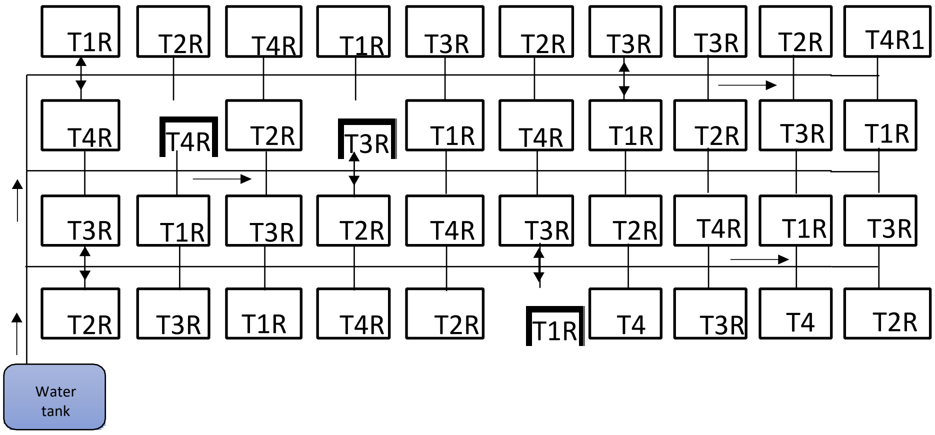

Figure 2: Experimental layout

This research will be carried out through fertigation system. Fertigation system where both water and fertilizers are applied together. It allows for greater efficiency and control compared to traditional fertilizer application methods. There are 4 treatments, and each treatment will be given different amounts of EM-Bokashi. Also, there are 40 polybags where each row will be placed 4 polybags vertically. The benefits of fertigation systems include improved nutrient uptake and increased yields. In addition, fertigation systems can help reduce the environmental impact of fertilizer by limiting the number of nutrients lost to leaching.

5.0 RESULT AND DISCUSSION

5.1 PLANT HEIGHT

Plant height gives an overview of the plant's size and rate of growth. The productive potential of a plant in terms of leaves, fruits, and weight is strongly correlated with its height, an important growth characteristic. A one-way ANOVA was conducted that examined the effect different rate of EM-Bokashi application on plant height chilies. There was a statistically significant difference between different rates of EM-Bokashi on the plant height as demonstrated by one-way ANOVA based on appendix A, (p-value = 0.000).

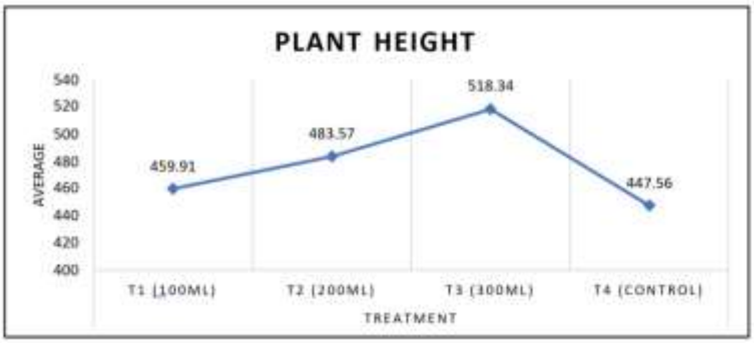

Figure 3: Height of chili with difference rates of EM Bokashi application

According to figure 3, there is statistically significant effect between different rates of EM Bokashi on crop height (100ml), (200ml), and (300ml). From the result, height shows significant difference. Based on the above findings it can be concluded that T3 treatment (300ml) is listed as the best among all treatments. Therefore, the lowest crop height was T4 (control) compared to other treatments. Hence, the plant highest which T3 treatment shows (518.34), and the lowest plant height was T4 treatment with (447.56).

Plant heights varied greatly between treatments as a result. It demonstrates how the amount of nutrients that each plant receives from the fertilizer used varies depending on the treatment. This indicates that the rates of EM Bokashi on application alter the height of the chili plant because there was a substantial variation between all the treatments. According to Quiroz & Céspedes, (2019); Olle, (2021) the increase in plant development using bokashi is associated with an improvement in the quality of the substrate, considering that the bokashi compound improves fertility and biological activity parameters, enhancing plant production. Furthermore, studies showed that organic waste Bokashi improved the plant growth performance in early and later growth, yield, and nutrient. (Xu et al., 2000; Gómez-Velasco et al., 2014; Olle, 2021).

5.2 LENGTH OF CANOPY

The most vital component of the plant's body that provides life might be regarded as its leaves. One of the key jobs of leaves was to use the photosynthesis process to turn sunlight's energy into sugar. The plant used the energy in glucose molecules for cellular growth, flower, and fruit development. A one-way ANOVA was conducted that examined the effect different rate of EM- Bokashi application on length canopy of chilies. There was statistically significant interaction between the effects of different rate of EM Bokashi on length of canopy, based on appendix B, (p-value = 0.002).

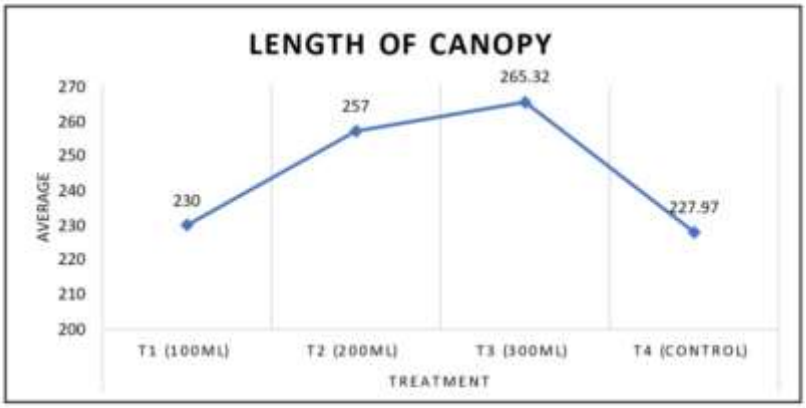

Figure 4: Length of canopy of chili with difference rates of EM Bokashi application

The length of canopy is an important parameter considering the highest performance of chilies. Different rates of EM Bokashi (100ml, 200ml and 300ml) as treatments give an idea about highest number of length canopy at all growth stages of chili cultivar. The length of canopy was determined by different rate of EM Bokashi application. T1 use EM

Bokashi fertilizer were consist about 100ml, T2 treatment (200ml), T3 treatment (300ml), and T4 (control/without EM Bokashi). It was measured that the highest number of length canopy was obtained with 300ml EM Bokashi which is T3 treatment at all growth stages of chili, while the lower number of leaves was determined by T4 treatment (control).

The highest number of length canopy shows that (265.32) followed by T2 treatment (257), and the lowest was T4 treatment (227.97). ANOVA tests showed that 300ml EM Bokashi levels had significant influence on leaf volume (P=0.002). From figure 4 LSD at a rate of 5% shows the treatment of T3 (300ml) a significant difference in the length of canopy on the treatment of T4 (control). This shows that the use of EM Bokashi fertilizer can help increase the growth of chili.

5.3 WEIGHT OF FRUIT

A one-way ANOVA was conducted that examined the effect different rate of EM-Bokashi application on weight of fruits. There was a statistically significant difference between different rates of EM-Bokashi on weight of fruits as demonstrated by one-way ANOVA based on appendix C, (p-value = 0.000).

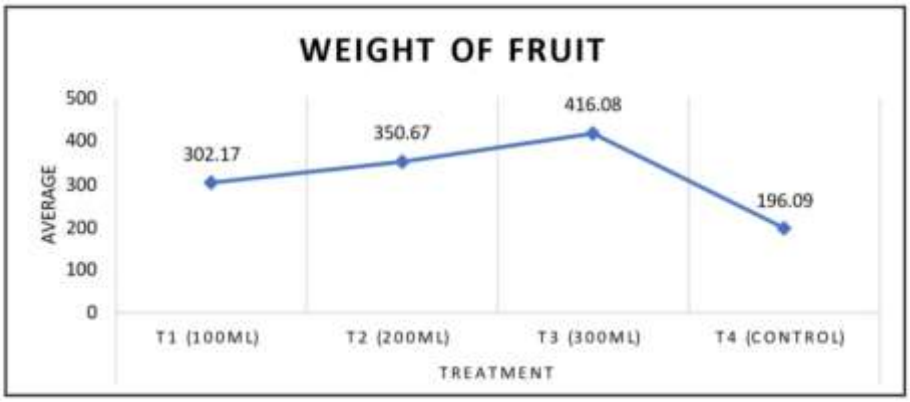

Figure 5: Fruit weight of chili with different rates of EM Bokashi application

The fruit weights of chilies produced among the treatments varied significantly. The significant effect of EM Bokashi fertilizer on fruit weight are because of Bokashi have been shown to increase plant nutrient uptake, growth, and yield via different basic mechanism such as changes in nutrient solubility, plant physiology and symbiotic relationships. Some findings have shown that the inoculation of EM Bokashi cultures to the soil plant ecosystems improves soil fertility, growth, and yield of crops. Figure 5 showed a result that T3 treatment (300ml) had the highest number of weights, followed by T2 (200ml) and T1 (100ml) treatment.

5.4 OVERALL SCORE OF TREATMENT PARAMETER

	OVERALL SCORE TREATMENT			
PARAMETER	**T1**	**T2**	**T3**	**T4**
Plant Height	2	1.5	1	2
Length of Canopy	2	1	1	2
Weight of Fruits	3	2	1	4
TOTAL	7	4.5	3	8
SCORE	**3**	**2**	**1**	**4**

Figure 6: Overall ranking of all treatment parameter different amount of EM Bokashi effect on growth performance of chili.

All the variables that affect the ideal treatment parameter must be considered in this study. Based on the impacts of various amounts of EM Bokashi application on the growing performance of the chili, the ideal treatment should be determined. In this treatment, there are three parameters. Figure 6 compares the performance of each treatment in terms of growth characteristics such plant height, canopy length, and fruit weight. By scoring, the best treatment of different amount of EM Bokashi application was selected based on merit.

The total score is determined to find the best treatment by ranking the treatment. Therefore, to identify the best treatment, comparison of growth performance between all the treatments is the best and most accurate indicator. The EM Bokashi application treatments with the lowest total score are the best; those with the highest total score are the worst.

6.0 CONCLUSION

This study was an evaluation of EM Bokashi in the growth and yield performance of chili. It is concluded that growth and yield attributed characters of chili were significantly affected by EM Bokashi. With the results, it was concluded that Treatment 3 (300ml of EM Bokashi) performed highly significant than the other treatments. The result of study in the plant height showed significant differences among the treatments. Also, the length of canopy and weight of fruits showed highly significant differences among the treatments.

Therefore, the application of treatment 3 (300ml of EM Bokashi) is the highly recommended amount in chilies. Hence, for achieving economically higher yield of chili, the crop may be fertilized with high amount of EM Bokashi. Moreover, further investigation needs to be carried out under field conditions and different locations for getting an optimum yield of chili under low amount of EM Bokashi.

On the other hand, if the study uses a manual system, such as watering twice daily and controlling white fly and other pests to achieve a greater output of fruit and flower, it is advised to use the same rate of 300ml EM Bokashi with a good care system.

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005-005

EFFECTS OF DRYING METHODS ON THE PHYTONUTRITIONAL CONTENT, LIPID PEROXIDATION AND ENZYME INHIBITION PROPERTIES OF TUHAU, *ETLINGERA COCCINEA*

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Etlingera coccinea, commonly known as Tuhau, is a wild ginger species consumed by Borneo natives. Despite being widely used for traditional culinary and medicinal purposes among Kadazan-Dusun community, little attention has been paid to investigating its health-beneficial properties. In this study, the effects of three different drying methods (cabinet drying, sun drying, and freeze-drying) on Tuhau's phytonutritional composition and its inhibition of lipid peroxidation and enzymes (pancreatic lipase and alpha-amylase) associated with human diseases were investigated. While crude fat, protein, and ash content show no significant differences between drying methods, moisture content differs significantly. Freeze-drying significantly increases total carotenoid and total ascorbic acid content compared to other methods. Freeze-drying also yields the highest extraction yield, total phenolic, and flavonoid contents and the lowest malondialdehyde levels following thiobarbituric acid reactive substances (TBARS) assay. It also shows the highest alpha-amylase inhibition and dose-dependent pancreatic lipase inhibition ($p<0.05$). Significant correlations were found between phytochemical content (TPC, TFC) and lipid peroxidation as well as enzymes inhibition. These findings highlight the potential of Tuhau rhizome extract following the freeze-drying method as a natural source of antioxidants and indicate a promising direction for the development of Tuhau rhizome for therapeutic use.

Keywords: Drying Methods, Enzyme Inhibition, *Etlingera Coccinea*, Tuhau, Wild Ginger, Phytonutrients.

009-006

EFFICIENCY IN WATER USE OF OKRA (*ABELMOSCHUS ESCULENTUS*) UNDER DIFFERENT LEVELS OF DRIP IRRIGATION

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ABSTRACT

The okra *(Abelmoschus esculentus)* is an annual herb and vegetable crop grown throughout the tropical regions and subtropical parts of the world. Drip irrigation is commonly used in okra cultivation as it can either apply water directly to the soil's surface or subsurface using buried drip-tape. This study will evaluate the efficiency in water use of okra by applying different levels of water in the drip irrigation system. 4 different levels of water are used by using different valve rotation angles which are 90°(T1), 80°(T2), 70°(T3) and 60°(T4). Each treatment has 7 replications with a completely randomized experimental design (CRD) is applied. The crops were harvested 90 days after planting. Data for growth performance; plant height and yield; fresh weight, fruit length, and the number of fruits are collected and analyzed using one-way ANOVA at 5% significant level using SPSS software. The result of plant height shows that T4: 49.67±1.33 cm had the highest height followed by T3: 44.89±1.25 cm, T2: 40.51±1.29 cm, and T1: 38.24±1.34 cm. The result of okra fresh weight treated with T1 showed the lowest value which is 116.43±33.50 g while T2:129.14±27.23 g, T3:149.84±28.48 g, and the highest weight is T4: 174.29±52.87 g. However, the result of the fruit length of okra treated with different valve rotation angles did not vary significantly. The results of the number of fruits shows significantly different levels as T4: 8.86±1.86 is the highest followed by T3: 7.71±1.11, T2: 7.00±1.00 and T1: 6.29±1.38. The result demonstrated a substantial difference at T4 which is 60° valve rotation that had improved the growth performance and yield of the okra plant. Hence, we can suggest that 60° rotation of the dripping valve can be utilized as the best water level of drip irrigation for commercial okra planting.

Keywords: Okra, Drip Irrigation, Growth Performance, Water Efficiency.

INTRODUCTION

Okra *(Abelmoschus esculentus)* is an annual herb and vegetable crop grown throughout the tropical and subtropical parts of the world (Al-Harbi et al, 2008). Okra is a popular vegetable of high nutritional value, with great acceptance in the market, belongs to the family Malvácea. Small producers are mainly responsible and always take part in the production of this vegetable due to the fact that okra has short growth cycle, is resistant to some pests and also has good production yield (Abubaker et al, 2014). Due to the growing consumer demand, there has been an extensive expansion of okra farming. Malaysia has contributed to the production of okra as a commercial domestic production in 14th position across the world. Roughly, Malaysia produce about 20,000 tons of okra per year and this value has been increasing since 1990.

Irrigation can be characterized as the replacement or substitution of rainwater with another water source (Fereres et al., 2007). Though Malaysia has abundant water resources, the country is still experiencing increased demand for water every year. This can lead to water scarcity if the management of water usage is still on low. Water scarcity prevention should be priority as water efficiency are the key components of good water managements. Thus, by knowing a suitable level of water for crop and good management of water usage are the great examples for water efficiency and control.

Efficient water use now is extremely important and becoming alternative methods of water applications such as drip irrigation. It will make a major contribution to making the best possible use of the scarce available water for crop production. Drip irrigation is a technique that supplies crops with water through a network of pipelines with suitable frequency (Danso et al., 2014). Drip irrigation not only saves water, but also improves the product efficiency and quality, even with poor water quality. A study reported that drip irrigation has great advantages over other irrigation system in terms of water usage efficiency. This has attracted farmers' interest due to the less water requirement and also indirectly doubled the production and yielded better quality produce (Deshmukh et al., 2014).

Okra is a high-water crop despite its considerable resistance to drought (Bhutia et al., 2017). During the year 2009 and 2010, 13.6 and 14.8% higher yield of okra was observed under drip irrigation compared to another irrigation method as reported by Birbal et al. (2013). This is because drip irrigation allows a better use of water through the application of water in the root zone, reduces deep percolation losses, surface evaporation, surface run off and allow crop cultivation even in water scarce areas (Simsek et al., 2004). Even though drip irrigation is more efficient than the other irrigation methods, proper system management is crucial for every user. People need to know for how long plants should be watered by determining the amount of time to water based on the rate of flow of drip emitters (Zwart et al., 2004).

METHODOLOGY AND DATA COLLECTION

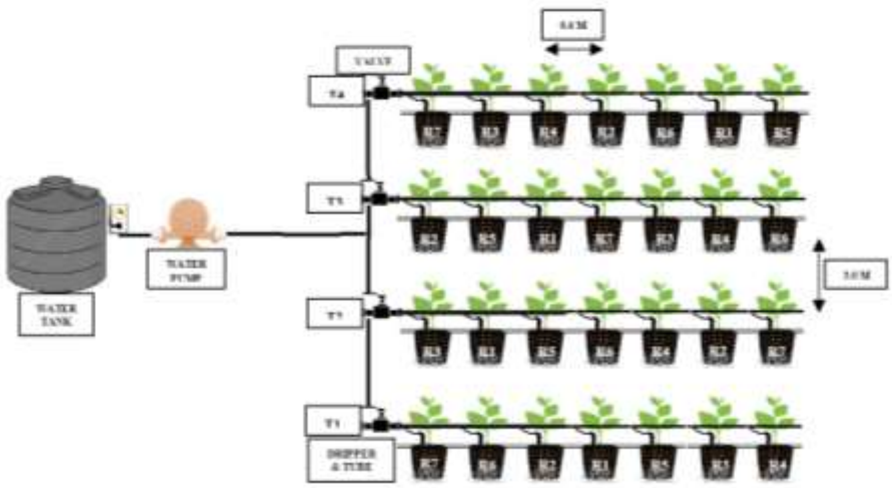
The okra seeds were planted in seed trays using peat moss. One seed was planted in each hole to ensure the uniformity of seed germination and were watered once a day. Then, the seedlings were left to germinate for several days before being transferred into poly bags. Four different levels of water were used to treat the okra using different rotation of valve. Each treatment had 7 replications as outlined in the Figure 1. The following were the treatments used for the okra cultivation:

Table 1: Different rotation of valve used as treatments

T1- 90° valve rotation	T3- 70° valve rotation
T2- 80° valve rotation	T4- 60° valve rotation

Seedlings were transferred after two weeks (14 days) germination in seedling tray. The seedlings were transferred once it grew to 5 cm to 10 cm tall. The process was done carefully to prevent damaging the roots. Seedling transferring was done early in the morning or late in the evening. Two types of fertilizer, NPK green and NPK blue were applied. The water source was ensured to be free from dirt or contaminants to prevent the plants from disease infection. The plants were sprayed with organic pesticide by adding the organic pesticide into water without chlorine, let the mixture sit overnight and add 1/2 cup of cooking oil, 1 tablespoon of mild liquid soap and enough water to fill the jar. The crops were harvested manually after 90 days of cultivation.

Figure 1: Experiment layout



Height of plant was measured in centimetres using measuring tape and ruler. The plants are measured once a week to observe the growth rate. The okra fruit will be harvested after 55 days of planting. Only matured fruits will be chosen for calculation of the number of fruits. The length of the fruit must be determined distance from pedicel attachment to its apex. Fruit weight being collected from 28 plants and represented it in gram (g). Data for growth performance, and yield were analysed using One-way Analysis of Variance (ANOVA) in version of 16.0 and significant differences (P < 0.05) of means among treatment were compared using Duncan's multiple range test using SPSS. The parameters in this study are shown in Table 2 below.

Table 2: Experimental parameters

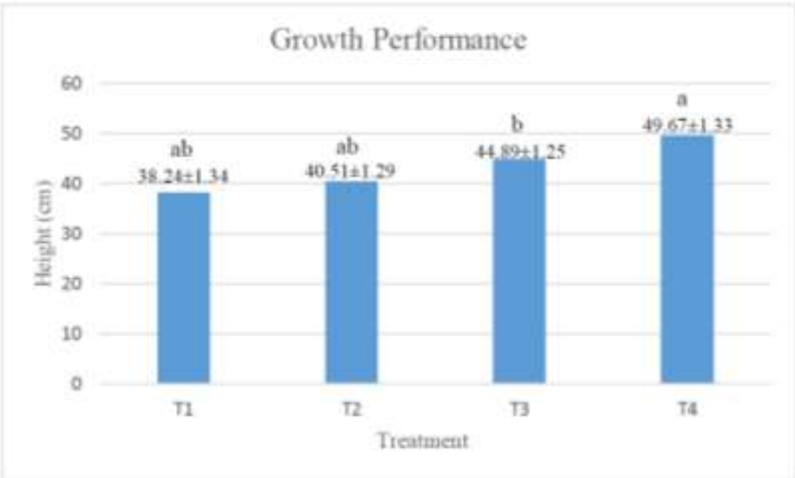
Activities	Parameter	Unit
Growth Performance	Plant Height	Centimetre (cm)
Yield	Fresh Weight	Gram (g)
	Fruit Length	Centimetre (cm)
	Number of Fruit	

RESULT AND DISCUSSION

Plant Height

Generally, plant height provides an overview of the size and growth performance of the plant. The height of plant is an important growth character directly linked with the productive potential of plant in terms of grains, fodder and yield. A one-way ANOVA was conducted that examined the effect of different level of water on the fresh weight of okra *(Abelmoschus esculentus*). There was statistically significant effect between different levels of water on plant height, p = 0.00.

Figure 2: The effect of different level of water on fresh weight of okra. Table indicates (MEAN± STD) are shows that there are significant in result between 4 treatment (P < 0.05).

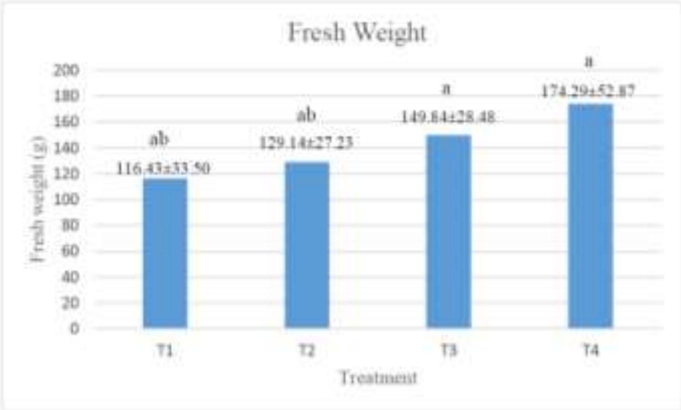


Plant height among four treatments with the different levels of water were varied significantly (P < 0.05). It shows that all plants in four treatments get enough water. Treatment 4 (60° valve rotation) had the highest height which was 49.67±1.33 cm followed by Treatment 3 (70° valve rotation) 44.89±1.25 cm, Treatment 2 (80° valve rotation) 40.51±1.29 cm and the lowest height was found in Treatment 1 (control) 38.24±1.34 cm. Since there is significant result between all treatments, it shows that the difference levels of water did affect the height of the okra plants. According to Amoo et al., (2019), high level of water ensured favourable condition for the growth of okra plant with optimum vegetative growth.

Fresh Weight

Fresh weight of okra *(Abelmoschus esculentus)* treated with different level of water using different valve rotation showed that there was significant difference p = 0.038. The result of okra fresh weight treated with Treatment 1 (control) showed the lowest which 116.43±33.50 g than okra that being treated in Treatment 2 (80° valve rotation) 129.14±27.23 g. Therefore, the fresh weight of okra that received Treatment 4 (60° valve rotation) was the highest which is 174.29±52.87 g than okra in Treatment 3 (70° valve rotation) which fresh weight was lowest 149.84±28.48 g (figure 4.2a) but less than those treated with Treatment 1(90° valve rotation) and Treatment 2 (80° valve rotation).

Figure 3: The effect of different level of water on fresh weight of okra. Table indicates (MEAN± STD) are shows that there are significant in result between 4 treatment ($P < 0.05$).

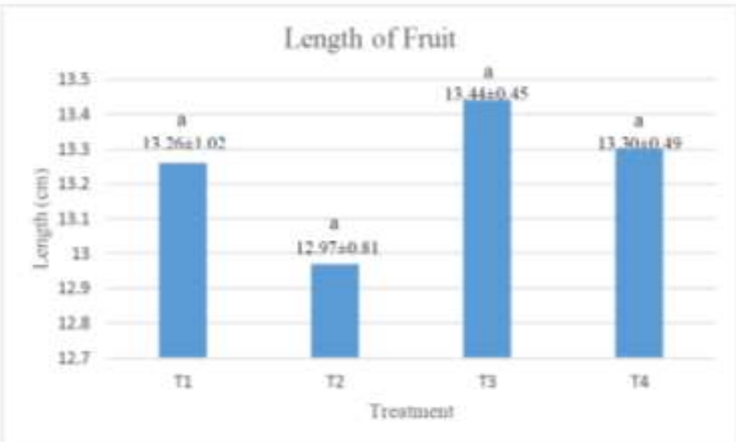


Result showed that there was significant result in number of fruits between all treatments due to the difference level of water applied. According to E.O. Danso et al., 2014, testing irrigation with different levels of water in okra realized that the highest yield of drip irrigated okra is obtained in treatments that receive the highest water levels.

Fruit Length

The provision of the different level of water were not affecting fruit length. A one-way ANOVA was conducted that examined the effect of different level of water on the fruit length of okra. There was no statistically significant effect in different levels of water on the fruit length (p = 0.678). From the table above there was no statistically significant difference in all four treatment.

Figure 4: The effect of different level of water on fresh weight of okra. Table indicates (MEAN± STD) are shows that there are no significant in result between 4 treatment ($P > 0.05$)



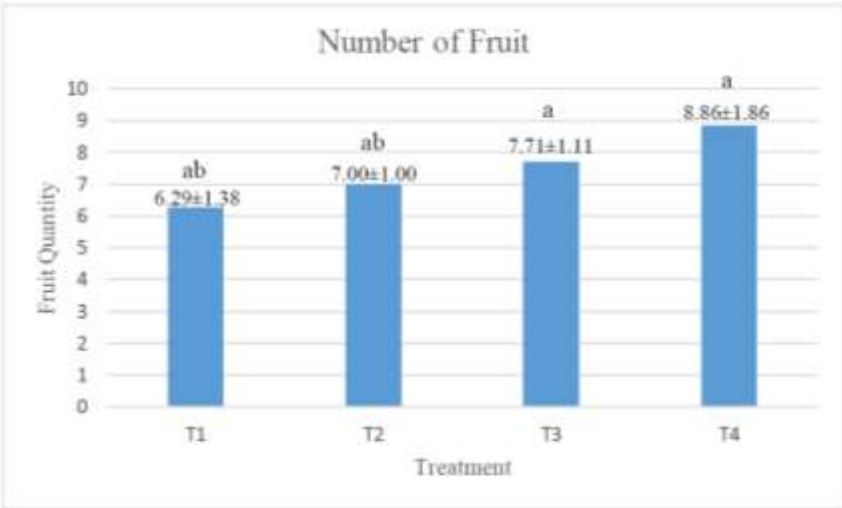
From the multiple comparisons, CRD test were used to determine which treatment have a significant result. Figure 4 shows the mean of fruit length for every four treatment. Since there was no significant difference in different level of water, therefore there are no such different in fruit length between all treatments.

Fruit length of okra treated with different level of water using different valve rotation were not varied significantly. The result of okra fruit length treated with Treatment 3 (70° valve rotation) showed the highest which 13.44±0.45 cm followed by Treatment 1 (control), 13.26±1.02 cm. Therefore, fruit length in Treatment 2 (80° valve rotation) had the lowest 12.97±0.81 cm than okra that being treat in Treatment 4 (60° valve rotation) 13.30±0.49 cm. The non-significant different level of water on fruit length may be due to growth rate of plants. The increasing vegetative growth rate of plants will also increase the number of hormones produced, causing fruit length to increase (S. Choudhary et al., 2012).

Number of Fruit

Generally, number of fruits provides an overview of the yield production of plant to produce fruits. A high number of fruits is claimed to be positively correlated with productivity of a plant. A one-way ANOVA was conducted that examined the effect of different level of water on the fresh weight of okra (Abelmoschus esculentus). There was statistically significant effect in different levels of water on the number of fruits, $p = 0.013$. From the table above there was statistically significant difference in mean of all four treatment.

Figure 5: The effect of different level of water on fresh weight of okra. Table indicates (MEAN± STD) are shows that there are significant in result between 4 treatment ($P < 0.05$).



Number of fruits among four treatments were varied significantly. It shows that plants in four treatments get different amount number of fruits from the water applied. The result of number of fruits showed that the different level of water significantly affected ($P < 0.05$) of each treatment. Treatment 4 (60° valve rotation) had the highest number of fruits which 8.86±1.86 followed by Treatment 3 (70° valve rotation) 7.71±1.11, Treatment 2 (80° valve rotation) 7.00±1.00 and the lowest number of fruits was found in Treatment 1 (control) 6.29±1.38.

Result showed that there is significant result in number of fruits between all treatments due to the difference level of water applied. The higher production occurred in the treatments that submitted to the high-level applications of water. This fact was also confirmed by Bhutia et al., 2017 who noticed an increasing in okra production when plant has a high consumption of water.

OVERALL SCORING

In this research, there was a significant difference in different level of water in parameter of plant height, fresh weight and number of fruits. However, parameter of fruit length did not have a significant difference. This study helps to find only best level of water in all treatment that consists of Treatment 1 (control), Treatment 2 (80° valve rotation), Treatment 3 (70° valve rotation) and Treatment 4 (60° valve rotation).

Scoring purpose is to find the best level of water in all treatments. Table 3 shows the scoring of each treatment based on parameters that had been set in methodology which are plant height, fresh weight, number of fruit and fruit length. By performing scoring method, the best treatment was selected based on merit.

Based on the table 3, the total scores for 60° gave the lowest score of 4, followed by 70°, gave the lower score of 5 after treatment 4. Meanwhile for 90° and 80° showed the same total score with 5.5. In simpler terms, Treatment 4, 60° valve rotation in drip irrigation was the best treatment compared to others treatment which were Treatment 1 (control), Treatment 2 (80°) and Treatment 3 (70°).

Table 3: Total score and ranking for each treatment

Treatment	90°	80°	70°	60°
Plant Height	1.5	1.5	2	1
Fresh Weight	1.5	1.5	1	1
Number of Fruit	1.5	1.5	1	1
Fruit Length	1	1	1	1
TOTAL	**5.5**	**5.5**	**5**	**4**
SCORE	**3**	**3**	**2**	**1**

CONCLUSION

Based on the comparison of four treatment, it was found that Treatment 4 (60° valve rotation) had more drastic result on the growth parameter which is in plant height compared to the other 3 treatment. Treatment 4 recorded the highest mean 49.67 and this can be concluded that Treatment 4 gives the best record on growth performance followed by Treatment 3, Treatment 2 and the last one is Treatment 1. As for yield performance, different level of water gives a different result for each parameter. Yield parameter consists of fruit weight, fruit length and number of fruits. These are greatly affected by different levels of water for each treatment. Maximum number of fruit and fruit weight are recorded under Treatment 4 using 60° valve rotation.

However, the efficiency of water can be seen from the growth performance and yield for okra. It can be seen through the data every week taken to show the growth performance and yield production starting from Treatment 1 (control) until the last treatment. Based on the findings, this can be concluded that Treatment 4 (60° valve rotation) has a highest result on growth performance and yield. Treatment 4 also obtained the best score in the overall scoring. In future, experiment could be conducted in larger scales to further validate the findings.

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010-007

SYNERGISTIC STUDY OF SUPER WATER ABSORBENT AND PLANT GROWTH PROMOTER ON VEGETABLE PLANTS

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This study investigates the synergistic effect of super water absorbent (SWA) and plant growth promoter (PGP) on the vegetable plants. SWA was prepared from sago waste and PGP from chitosan and carrageenan were all prepared by using gamma radiation. Commercial PGP was also used in this study. Synergistic study of SWA and PGP were carried out by using 0.1% of SWA mixed in the soil while, PGPs with concentration of 100 ppm were applied by sprayed onto the plants twice a week. Generally plants with co-application of SWA and PGP shows greater results on plants heights, plants weight and also in leaf area when compared to control.

Keywords: Super Water Absorbent, Plant Growth Propmoter, Gamma Irradiation.

013-008

EXPLORING WATER VAPOR MOVEMENT: ANALYZING DYNAMIC THROUGH OBSTACLE FOR ENHANCED UNDERSTANDING

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ABSTRACT

Water vapor movement become crucial important in industry, agricultural, engineering and also environment studies. Understanding this movement involves considering the fluid dynamics of either liquid water and water vapor, often described through diffusion theory. However, due to the liquid island effect and larger errors in predicting vapor movement in soil, the water vapor enhancement factor was introduced in governing equation of mass and heat, which is commonly known as PdV theory. The theory has undergone several decades of research that aimed at refining and updating the governing equation. Despite these efforts, researchers have been unable to bring out a comprehensive understanding of the new mechanisms consume in vapor movement. One potential advancement suggested in some studies is the role of buoyancy as the new mechanism but without experimental evident. This study seeks to address this gap in knowledge by setting two primary objectives: 1) fabrication four measurement units, designated as A1, A2, A3 and A4. 2) analysis water vapor movement to atmospheric by mass loss of water against time. The result for measurement unit A4 is higher mass loss rate compare to A3. This against to first hypothesis that mass loss A1>A2>A3>A4, the actual result A1>A2>A4>A3. Due to this result, it supports second hypothesis which effect of buoyancy cause the water vapor movement are higher at A4, though the design has obstacle (curve). This could understand of dynamic fluid behavior, potentially solving in mass that will solve problem in mass and heat porous media.

Keywords: Water Vapor; Evaporation; Buoyancy Effect; Climate Change; Governing Equation.

011-009

EFFECT OF CHITOSAN WITH DIFFERENT MOLECULAR WEIGHTS ON KULAI CHILI (*CAPSICUM ANNUM L.*) YIELD

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ABSTRACT

Chitosan has been claimed to be an effective ingredient in promoting plant growth for various crops. However, several studies reported that low molecular weight of chitosan (LMC) induces significant effects on plant growth and yield. Hence, in this study chitosan was degraded into three different molecular weights which were 38780 g/mol (CH), 17560 g/mol (CM) and 6500 g/mol (CL) by gamma radiation to observe their effects on chili. The application concentration of the LMC in this study was fixed at 100 ppm for both seed treatment and foliar application. A set of seeds without LMC treatment was put as Control (C). The observation on day 7 indicated that the highest germination rate was 85.2% while the lowest was 56.3% after treated with CL and CH, respectively. During the nursery stage, the seedlings were sprayed with LMC solutions on day 3, 7, 14, 21 and 28. Results showed that the plant height and number of leaves were insignificantly different between the CM, CL and C treatments. Yet, CH treatment showed the lowest result on plant height and number of leaves during the nursery stage. On day 30, the seedlings were transferred to fertigation pots. The CL, CM and CH were sprayed on day 1 and day 4 every week. The yield was harvested three times during this study period. The total duration of this study was 5 months. The CM treatment contributed the highest yield (45160g) with total of chili number harvested was 3327 fruits, while C indicated lowest yield which was 24290g with total of chili number harvested was 1685 fruits. At the end of the study, it concluded that yield of chili was higher after treated with LMC (CL, CM and CL) than without LMC treatments, C. The LMC treatments increased yield by enhancing flowering or fruiting phase of the chili plants. This study stated that the molecular weight of LMC still plays a crucial role in producing great results, especially on yield.

Keyword: Plant Booster, Polysaccharides, Low Molecular Weight Chitosan, Deacetylated Chitin.

001-016

EXTRACTION AND CHARACTERIZATION OF CELLULOSE FROM SAGO PITH WASTE *(Metroxylon Sago Spp.)*

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ABSTRACT

Cellulose from agricultural waste has gained more attention owing to its high aspect ratio, reinforcing effect and abundance. In this study, cellulose was extracted from sago pith waste (SW) recovered after sago starch extraction. The SW was treated through sodium hydroxide (NaOH) treatment, followed by bleaching treatment. Results revealed that cellulose content increased from 14% to 64% after bleaching treatment. The thermal stability and crystallinity of the CS increased after treatment. The results were supported by Fourier Transform Infrared spectroscopy (FTIR) analyses, which confirmed the removal of non-cellulosic materials. Based on the results, some recommendations can be made on the potential use of extracted cellulose from sago pith waste.

Keyword: Sago Pith Waste, Agricultural Waste, Defibrillation, Cellulose, Extraction.

INTRODUCTION

Sago (*Metroxylon Sago spp.)* palm is one of the crops widely planted in Malaysia that belongs to the *Palmae* family. The residue left behind in the sago starch extraction is called sago pith waste. In Malaysia, according to the Malaysia Statistic Department, over 200,000 metric tonnes of sago starch were produced in 2017. It was reported that one tonne of sago pith waste was formed from every tonne of sago starch produced (dry weight basis). Therefore, it can be concluded that the same quantity of sago pith waste was produced (Bujang and Ahmad, 1999).

Increased agricultural production generates a bulk quantity of agricultural residue after crop harvesting. A significant amount of these wastes remains underutilized. This residue is non-toxic, renewable, and biodegradable. Producing lignocellulosic materials such as cellulose into value-added products has attracted attention among academics and industry. Cellulose can be isolated from various sources, including leaves (Sheltami et al. 2012; Cherian et al. 2010), seeds (Henrique et al. 2013), vegetable by-products such as cassava bagasse (Yue et al. 2015), and many more.

Cellulose from different sources may have different characteristics. To date, not much research has been done on cellulose derived from sago pith waste. It is important to have a set of data on this cellulose in order to explore its potential applications. In this study, cellulose (CS) was extracted from sago pith waste (SW) using NaOH, before bleaching treatment using $CH_3COOH/NaClO_2$. The objective was to study the effect of the bleaching treatment on compositional analysis, crystallinity, structural and thermal properties of the cellulose.

EXPERIMENTAL

MATERIALS

Sago pith waste was obtained from Ng Kia Heng Sago Industry Sdn. Bhd. (Batu Pahat, Johor). Sodium chlorite, $NaClO_2$ and acetic acid, CH_3COOH were obtained from Sigma Aldrich Inc. (St. Louis, MO, USA). Sodium hydroxide, NaOH was purchased from Fisher Scientific Inc. All reagents and solvents were used as received.

METHODOLOGY

EXTRACTION OF CELLULOSE FROM SAGO WASTE

Cellulose was extracted from sago pith waste (SW) as proposed by Yacob et al., (2018) with some modifications. SW was first treated with 2 % NaOH aqueous solution at 60 °C for 1 h, filtered and washed repeatedly with distilled water until pH 7. At this stage, alkaline-treated fibre (AS) was achieved. The AS was then exposed to bleaching treatment with a solution prepared with equivalent parts of 1.7% (w/v) aqueous $NaClO_2$ in water, acetate buffer and distilled water. The mixture was stirred at 80 °C for 4 h. At this stage, cellulose (CS) was successfully extracted from SW.

COMPOSITIONAL ANALYSIS

The chemical composition of all samples was determined according to the standard method of the Technical Association of Pulp and Paper Industry standard (TAPPI). The lignin contents were measured using the T222 om-98 method. The holocellulose contents were estimated using the acid chlorite method. The α-cellulose content was investigated using T203 om-88, while hemicellulose content was calculated by subtracting the α-cellulose content from the holocellulose content.

DETERMINATION OF CRYSTALLINITY INDEX USING X-RAY DIFFRACTION (XRD)

The X-ray diffractograms of untreated and treated SW were obtained using a RÖntgen diffractometer system (PANalytical, Netherlands). The diffracted intensity of Cu-Kα radiation was measured at 40 kV, 30 mA at 25 °C. The diffraction patterns were determined over a range of diffraction angle 2θ from 5° to 40° at a scanning rate of 2 °C/min. The crystallinity index (CI) was calculated using the Segal method given by Eq. (1) (Segal, Creely, Martin, & Conrad, 1958):

$$CI = \frac{I_{002-} I_{am}}{I_{002}}\ x\ 100\% \qquad \text{(Eq. 1)}$$

where I_{002} and I_{am} are the peak intensities of crystalline at 2θ=22° and amorphous at 2θ=18°, respectively.

DETERMINATION OF CHANGES IN FUNCTIONAL GROUPS USING FOURIER TRANSFORM INFRARED SPECTROSCOPY (FTIR)

The Fourier Transform Infrared spectroscopy spectra were recorded on a spectrometer model Bruker Tensor II, Germany in the range of 4000–500 cm^{-1} with a scanning resolution of 2 cm^{-1} to investigate possible changes in functional groups of SW, AS and CS.

DETERMINATION OF THERMAL STABILITY USING THERMOGRAVIMETRY ANALYZER (TGA)

The thermal properties of the samples for each treatment were analysed using a thermogravimetry analyzer. A sample between 5-10 mg, was subjected to N_2 gas and heated from 25 °C to 600 °C at a heating rate of 20 °C/min and a gas flow rate of 60 mL/min.

RESULTS AND DISCUSSION

1. CHEMICAL COMPOSITIONAL ANALYSIS

Table 1 summarised the chemical compositional analysis of SW, AS and CS. The SW contained 38 wt% starch, 14 wt% cellulose, 17 wt% hemicellulose and 4 wt% lignin. Upon alkaline treatment, the cellulose content increased to 63 wt% and further increased to 91 wt% after bleaching. Bleaching caused the breaking down of glycosidic β-ether and hydrolyzed the hemicellulose, hence reducing the hemicellulose content from 17 wt% to 7 wt%. All of the lignin and silica content was removed after bleaching.

Table 1. Compositional analysis data of SW, AS and CS

	Starch (%)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
SW	38.41 ± 1.36	14.31 ± 1.48	17.45 ± 0.90	4.83 ± 1.04
AS	7.05 ± 0.23	63.26 ± 1.50	21.02 ± 3.21	3.03 ± 0.89
CS	-	91.26 ± 2.84	7.84 ± 1.32	-

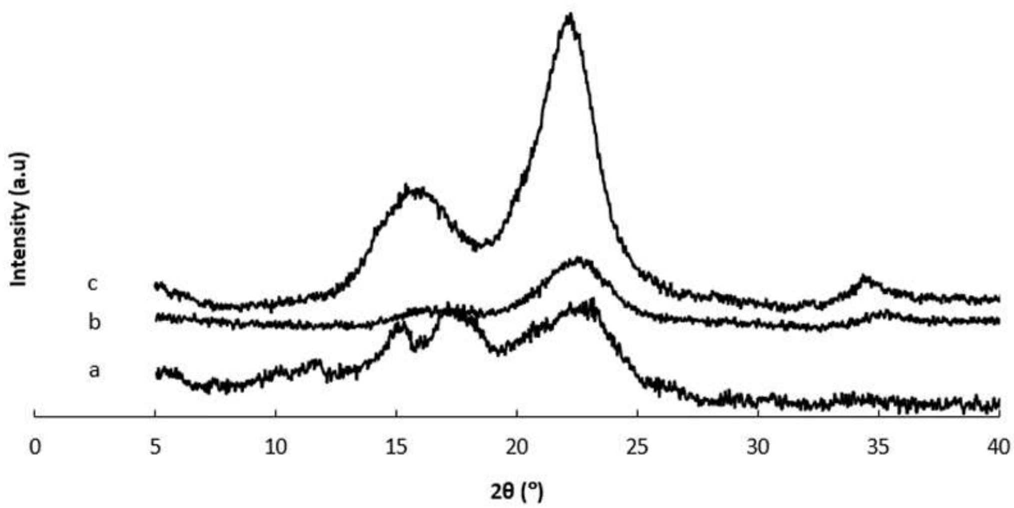
2. CRSYSTALLINITY MEASUREMENTS

Figure 1 shows the XRD diffractogram for SW at different stages of treatment. SW displayed strong and broad peaks at 2θ = 17° and 23°, medium peaks at around 2θ = 5.3° and 15.0° and a couple of weak peaks scattered around 10.2° and 11.7°. These peaks referred to fibres and sago starch granules in parenchyma cells.

After alkaline treatment, AS exhibited three main reflection peaks at 2θ = 15.6°, 22.7° and 34.6°. The 15.6° reflections correspond to the (110) crystallographic planes. The other peaks at 22.7° and 34.6° correspond to the (002) and (004) planes respectively, which are representative crystalline peaks of cellulose I (Gopi et al., 2019).

The CS showed sharper diffraction peaks as compared to SW and CS. The crystallinity indices of SW, AS and CS were found to be 40, 48 and 84% respectively. It is clearly explained that the crystallinity of SW increased during the treatments. This was attributed to the elimination of amorphous compounds (hemicellulose and lignin) of amorphous hemicellulose and lignin.

Figure 1. The XRD pattern fot (a) SW, (b) AS and (c) CS



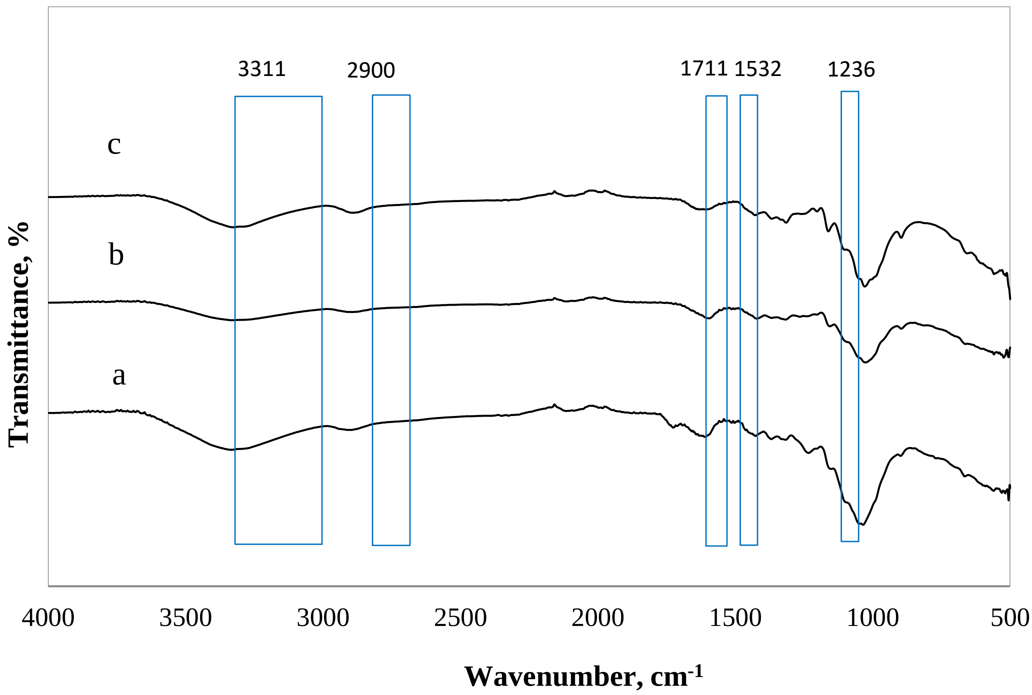
SPECTROSCOPY ANALYSIS

The effect of the treatment on the chemical structure was investigated using FTIR. Figure 2 compares the FTIR spectra of SW, AS and CS. The absorption band in the region of 3650-3650 cm^{-1} was observed in all spectra, corresponding to the OH stretching. The spectra also showed C-H stretching vibration at around 2894 cm^{-1}. As illustrated in the FTIR spectrum of SW, the band at 1711 cm^{-1} is attributed to the carbonyl group, C=O due to the presence of acetyl and uronic ester groups of hemicelluloses or ester linkage of the carboxylic group of the ferulic and *p*-coumaric acids of lignin and hemicelluloses. Peak at 1532 cm^{-1} is attributed to C=C aromatic ring in lignin. The band at 1236 cm^{-1} corresponds to C-O-C (aryl-alkyl ether) in lignin. The presence of these bands was supported by results reported by other researchers (Melikoğlu, Bilek & Cesur, 2019; Fareez et al., 2018).

Upon alkaline treatment, the intensity of peaks corresponded to C=O, C=C and C-O decreased because of the removal of hemicellulose and lignin. However, the complete removal of these components does not take place. Thus, further delignification is needed. The absence of a prominent peak at 1711, 1532 and 1236 cm^{-1} in the CS as shown in Figure 2(c) indicated that the bleaching treatments effectively removed lignin and hemicelluloses from the SW.

Peaks at 800–1500 cm^{-1} region are representing cellulose fingerprint and presented in all spectra. These peaks remained unchanged after treatments indicating that the alkaline and bleaching treatments did not affect the cellulose structure.

Figure 2. FTIR spectra of (a) SW, (b) AS and (c) CS

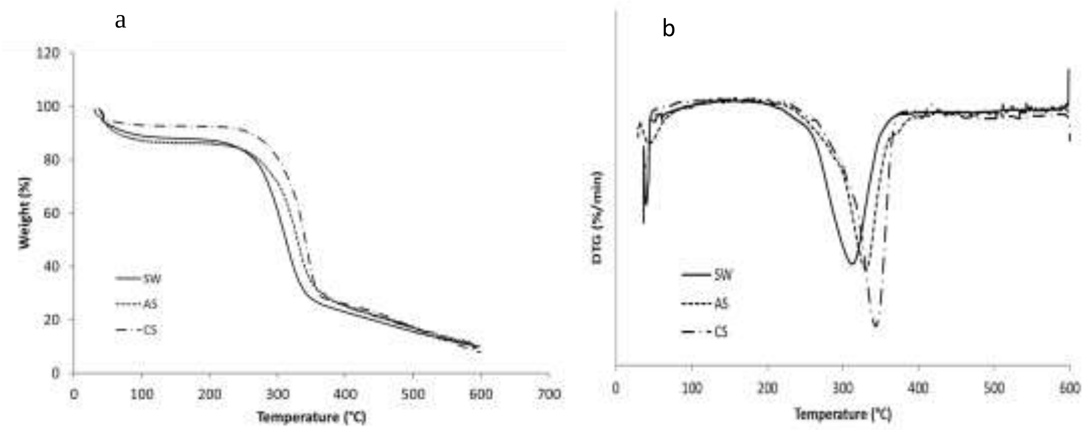


THERMOGRAVIMETRIC ANALYSIS

The thermogravimetry (TG) and derivative thermogravimetry (DTG) results for SW, AS and CS are shown in Figure 3. A small weight loss was found below 100 °C due to the evaporation of moisture or a low molecular weight compound. The weight loss between 200 °C and 370 °C was mainly due to degradation of hemicellulose. The thermal decomposition that occured in the range of 250 °C and 375 °C was associated with the degradation of cellulose and lignin (Hoi and Martincigh, 2013).

From the derivative thermogram curve, the decomposition peak of CS shows narrower with maximum degradation at 343 °C. The maximum degradation was higher than SW and AS due to the removal of hemicellulose and lignin as supported by the results explained in FTIR and compositional analysis.

Figure 3: Thermogram of (a) TGA and (b) DTG for SW, AS and CS



CONCLUSION

Cellulose was successfully extracted from sago pith waste using NaOH followed by $CH_3COOH/NaClO_2$ treatment. The chemical analysis showed extracted cellulose had higher cellulose content and lower hemicellulose content than the original source. This was due to the removal of hemicellulose and lignin during the cellulose extraction process as revealed by FTIR. The thermal stability and crystallinity of the extracted cellulose were found to be higher than the original and alkaline treated fibers. In accordance with these results, the characteristics of the extracted cellulose from SW were well studied and its potential applications could be expanded.

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020-017

GAMMA IRRADIATION-AIDED TREATED SHRIMP SHELL FOR AGRICULTURAL APPLICATION

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ABSTRACT

This current article studies the properties of shrimp shells treated with acidic and alkaline solutions in demineralization and deproteination processes to form chitin. Gamma irradiation was used as a pretreatment to facilitate the deproteination and demineralization processes. Shrimp shells were irradiated by gamma rays at 25 kGy and demineralized under acidic conditions to remove calcium carbonate and other minerals. The removal of proteins was carried out using an alkaline solution. The Field Emission Scanning Electron Microscopy with Energy Dispersive X-Ray Spectroscopy (FESEM-EDX) revealed the composition of macro- and micro nutrients in shrimp shells. The acidic solution process for demineralization was able to remove several elements, including K, Mg, P, Na, Al and $CaCO_3$. Deproteination, in conjunction with the demineralization process, facilitate the improvised removal reactions. Thermogravimetric Analysis (TGA) elaborates on the composition of chitin, protein, and calcium carbonate in shrimp shells treated with acidic and alkaline condition. A large number of beneficial microbes in soil with added treated shrimp shell enhanced soil fertility, as presumed in N, P, K, EC and pH analyses. The absence of pathogenic bacteria in the soil is a good indicator that the soil with treated shrimp shells is safe as soil conditioner with plant disease protection.

Keywords: Shrimp Shell, Gamma Irradiation, Fertilizer.

021-018

PRODUCTION OF CABBAGE (*BRASSICA OLERACEA*) IN CAMERON HIGHLANDS, MALAYSIA: EVALUATING THE CARBON FOOTPRINT (CFP) IN COMPLIANCE WITH MYGAP

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ABSTRACT

The Cameron Highlands, a prominent highland plateau, has been a hub for farming since the colonial era. Over the past five decades, it has rapidly evolved into Malaysia's primary highland farming region. The Malaysia Good Agricultural Practice (MyGAP) is a sustainable approach to resource management in agricultural production, aimed at enhancing farm output while ensuring the production of safe, high-quality food. This method also emphasizes environmental protection and the well-being of employees and workers. Several farms in the Cameron Highlands have adopted the MyGAP system. To understand the ecological impact of highland vegetable production, this study utilized Carbon Footprint (CFP) analysis as a foundation, employing the life cycle assessment (LCA) method to evaluate the total environmental effect across all stages of production, use, and end-of-life phases. A specific focus was on greenhouse gas emissions and their contribution to global warming. The study assessed cabbage (Brassica oleracea) production using the cradle-to-gate approach, which included seed preparation, land preparation, irrigation installation, transplanting, crop management, harvesting, sorting, and packing. Additionally, raw material procurement and transportation to the farms were considered. The findings revealed that the total CFP for cabbage and cabbage shoots per cycle was 2,401.40 kg-CO2e, with land preparation contributing the highest emissions at 1,975.23 kg-CO2e, accounting for 82.25% of the total emissions.

Keywords: Cabbage, Carbon Footprint, Cradle-To-Gate, LCA, MyGAP.

023-020

MICROBIOLOGICAL QUALITY EVALUATION OF DURIAN PASTE AND PULP UPON THERMAL AND NON-THERMAL PROCESSING

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ABSTRACT

The rising trend of durian export globally has encouraged industry players and government agencies to expand the market size of agri-food products. Technological innovations are essential to diversify durian-based downstream food products and to avoid market surplus especially during peak season. For this reason, this study was aimed to evaluate the effect of thermal and non-thermal processing using retort pasteurization and high pressure processing (HPP), respectively on the microbiological quality of durian D24 clone paste and pulp during shelf life study. Specifically, retort treatments of 102 °C for 5 minutes on AL/PE packaging for durian paste and HPP treatments of 6000 Bar for 6 minutes on CPP/PP/PET partially vacuumed packaging for durian pulp were applied based on the optimization of retort and HPP parameter previously done. Based on the microbiological analysis, the durian paste developed using retort pasteurization was found to be safe for consumption within six months of storage at chilled temperature (2 °C ± 7 °C) while its last within two months of storage at room temperature (27 °C ± 2 °C). Interestingly, durian pulp treated with HPP showed a minimum storage of 70 days at chilled temperature whereas durian pulp without pressure treatment (control) showed a minimum storage of 14 days at chilled temperature. In summary, this study emphasized on the importance of microbiological quality in assessing the shelf life of developed durian paste and pulp respectively through effective thermal or non-thermal processing to support market access and comply regulatory requirements for better food sustainability and security.

Keywords: Microbiology, Durian, Retort Pasteurization, High Pressure Processing, Storage Study.

1. INTRODUCTION

Durian (*Durio zibethinus* Murr.) is one of the well-known tropical fruits in Southeast Asia countries, including Malaysia, Indonesia and Thailand. Based on the statistics of Malaysian tropical fruits export, durian has significant import values in international market especially China, United States, Australia and South East Asian countries. For instance, Malaysia's export market shares of durian to Singapore (56%), Hong Kong (29%) and Brunei (4%), respectively with a total of 24,684 metric tonnes in 2021 reflected the growing consumer demand. However, despite the spiking demand for this fruit, durian surplus issue among Malaysia's durian producers still occurs every year that caused the reduction of durian prices especially during peak season. Conventionally, fresh durian only lasted for 3 to 4 days due to natural quality degradation. To overcome this, industry practice involves storage of raw or processed durian at frozen temperature (-18 °C ± 2 °C) however requires high capital investment on freezing chains during storage and marketing. Producers also need to absorb the transportation cost of shell weight if they venture into export market of frozen whole fruit of durian. For this reason, more innovations need to be explored by the food industry especially on minimally-processed durian products with longer shelf life and guaranteed safety at lesser cost. Particularly, durian paste and durian pulp have demonstrated big potentials due to the easiness of consumption of de-shelled durian without having the difficulty of opening the whole fruit of durian. In this study, ripe durian clone D24 paste (without seed) and pulp (with seed) were used to investigate the effect of thermal and non-thermal processing on microbiological safety parameter including its potential to prolong shelf life. Furthermore, durian clone D24 was chosen in this experiment due to its stability in taste and colour, including higher usage among local entrepreneurs due to lower retail prices comparable to other durian clones such as Musang King and Black Thorn.

Moreover, there is a market need for minimally-processed organic foods in line with the increasing consumer awareness on healthier foods with good nutritional properties and optimum attributes of fresh products. The continued demand has strived the industry players to transform traditional foods such as durian to better quality downstream products with the application of novel processing technologies. Hence, retort processing is a method of preserving food by heating it in hermetically sealed containers, meanwhile high pressure processing (HPP) is a non-thermal alternative to inhibit harmful pathogens and spoilage microorganisms on desired foods with minimal effects on nutritional and sensorial properties (Simonin *et al*., 2012). Previous studies on the shelf life extension of pressurized and heat-treated tropical foods such as longan juices, jackfruit, durian (nylon- and skin-film packed) and goat milk have shown encouraging results (Chaikam *et al*., 2014; Tan *et al*., 2019; Chin *et al*., 2020; Mustapa Kamal *et al*., 2021). To the best of our knowledge, studies on the application of retort pasteurization on durian paste and HPP to durian pulp are still fragmented. Malaysia's export market is only limited to frozen durian paste and pulp, frozen whole fruit of durian and dried durian products, thus, the aim for the present study was to investigate the effect of optimized retort and HPP parameters on the microbiological quality of durian paste and pulp, respectively over the storage period under chilled temperature.

2. MATERIALS AND METHODS

2.1 Preparation of durian paste and pulp, including pasteurization and pressurization study

Durian clone D24 were provided by local entrepreneur from Shah Alam, Selangor, Malaysia and further delivered to Food Science & Technology Research Centre (FT), Malaysian Agricultural Research and Development Institute (MARDI) at Serdang, Selangor. After checked for ripeness, durian was cleaned and de-shelled by practising proper hygiene and handling including good manufacturing practices (GMP), subsequently packed into a 15 cm (width) and 22 cm (length) food grade aluminium packaging (AL/PE) for durian paste, whilst vacuum packed into a 17 cm (width) and 25 cm (length) food grade cast polypropylene packaging (CPP/PP/PET). Both types of packaging carried 250 g ± 10 g of durian paste and pulp, respectively. Then, packed durian samples were kept in chilled temperature (2 °C ± 7 °C) for further processing and analysis. For pasteurization study, a Retort unit (Model H60, Type C50, Toyo Seikan Kaisha LTD) in a horizontal water immersion clutch retort located at FT, MARDI was used to process the durian paste. Retort parameter of 102 °C for 5 minutes for durian paste was used in this study based on the optimization study between sterilization and pasteurization effects on durian paste previously conducted. Meanwhile, a HPP unit (Hiperbaric 55, Burgos, Spain) located at Faculty of Food Science and Technology, Universiti Putra Malaysia (UPM) was used to process the durian pulp for pressurization study. The high pressure was generated by a cylindrical pressure chamber, a pressure pump and a hydraulic unit with water as the pressure medium in the HPP chamber. HPP parameter of 6000 Bar for 6 minutes for durian pulp with pressurization maintained at 20 °C processing temperature was used in this study based from the optimization study using Response Surface Methodology (RSM) previously done. In this experiment, microbiological test was carried out 1 hour after retort pasteurization and HPP treatment with all samples analysed by triplicate. Retort-treated samples were kept at chilled temperature (2 °C ± 7 °C) and room temperature (27 °C ± 2 °C) for comparison. HPP-treated samples were kept at chilled temperature (2 °C ± 7 °C) for further analysis. Durian samples without retort pasteurization and HPP were subjected as control samples. The microbiological quality of durian paste and pulp was evaluated on 30 days and 14 days interval, respectively throughout a 180-day and 70-day storage period, respectively.

2.2 Microbiological analysis

Microbial analysis of Total Plate Count (TPC), Yeast & Mould (Y&M), Coliform, *Escherichia coli*, *Staphylococcus aureus* and Psychrotrophic bacterial counts was performed on durian paste and durian pulp according to United States-Food and Drug Administration (US-FDA) Bacteriological Analytical Manual (BAM) standard methods (Feng *et al*., 2002). All microbial data were expressed as number of colony forming units (CFU/g) with plates enumeration based on 25 to 250 CFU/g, respectively except 15 to 150 CFU/g for Y&M. Additional analysis of *Salmonella* was performed on durian paste and durian pulp during the storage period by a method modified from the US-FDA BAM (Feng *et al*., 2002). Isolated colonies that showed typical reactions (Xylose Lysine Deoxycholate and Xylose Lysine Tergitol-4; dark red colonies with black centre, Rambach; bright red colonies) according to manufacturer's instructions were considered as presumptive *Salmonella*. Well isolated colonies were subjected to biochemical tests for confirmation (Merck, Germany).

3. RESULTS AND DISCUSSION

3.1 Microbiological properties during storage study

In this experiment, the Total Plate Count (TPC) in retort-treated durian paste stored at chilled temperature remained below the unsatisfactory limit till the end of the storage study of 6 months (Table 1). Conversely, TPC was observed incremental for retort-treated durian paste stored at room temperature as it continued to reach 6.0 x 10^6 CFU/g (unsatisfactory) on day 60 of storage. As TPC readings being used as quality indicator in most studies, it is notable that lactic acid bacteria (LAB) could also grow in fruit-based products. This might reflect the retort-treated durian paste samples stored at room temperature which was observed still safe for consumption but not limited to other potential quality effects. Meanwhile, TPC growth in HPP-treated durian pulp demonstrated satisfactory results until day 70 of storage proved that aforementioned HPP treatment successfully reduced microbial load of spoilage microorganisms. Relatively, TPC readings in durian pulp without HPP treatment stored at chilled was increased from <25 x 10 CFU/g (satisfactory) on day 0 to 6.0 x 10^5 CFU/g (marginal) on day 56 (Table 3).

Noteworthy, the microbial safety limit for durian paste and durian pulp was referred to China Microbiological Standard for Durian Paste and Durian Pulp including Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand), respectively (Table 5). This was due to the limited reference of microbial safety limit in Malaysia's Food Act 1983 (Act 281) for durian-based products. In term of Yeast and Mould (Y&M), durian samples for both thermal and non-thermal processing showed satisfactory results throughout the storage period (Table 1 & 3). However, untreated HPP samples of durian pulp (control) showed spiking rate of 2.1Y x 10^9 CFU/g at day 28 of storage at chilled, thus resulting in a higher risk of contamination. Although not mentioned in both standards as per Table 5, Y&M including Psychrotrophic could be used as food decay indicators in refrigerated foods. In this study, psychrotrophs were observed non-detectable for durian paste and remained low for durian pulp throughout the storage period (Table 1 & 3). These results are in accordance with the results of Queiros *et al*. (2014) and Tan *et al*. (2019) who reported the lethality of HPP on aerobic mesophilic microorganisms.

This study also demonstrated retort pasteurization successfully inhibited Coliform, *E. coli*, *S. aureus* and *Salmonella* as none of this microorganism were detected in durian paste samples along the storage period of 180 days at chilled temperature (Table 1 & 2). It was worth mentioning that microbiological analysis was not resumed for retort-treated durian paste stored at room temperature after day 60 as the packaging were observed bloated. This might be due to the overgrowth of microaerophilic LAB at room temperature that survived during pasteurization of durian paste. As comparison, retort-treated durian paste stored at chilled temperature was observed stable in term of packaging, hence, microbiology analysis was only continued under the influence of chilled temperature storage. In the pressurization study, HPP-treated samples of durian pulp were found satisfactory as Coliform, *E. coli*, *S. aureus* and *Salmonella* were not detected until the end of storage period at day 70, as opposed to untreated samples of durian pulp that showed a very low level of Coliform, none *E. coli* and *Salmonella* however a surge of *S. aureus* at 3.0 x 10^6 CFU/g, respectively (Table 3 & 4). This higher rate of *S. aureus* was considered potentially hazardous under the Food Standards Australia – New Zealand, hence in this study, durian pulp (control) was considered safe for consumption only under the minimum storage of 14 days. Reduction of microbial activity by thermal processing of food products have also been reported earlier by Watts (2019) whilst Bu *et al* (2022) specifically investigated on the effect of CO_2-assisted high pressure on durian fruit puree for shelf life extension purposes.

Overall, based from the data obtained, a minimum level of pasteurization temperature and holding time of 102 °C for 5 minutes including pressurization level and holding time of 6000 Bar for 6 minutes were sufficient for microbial spoilage prevention and pathogen deactivation in durian paste and durian pulp, respectively. The microbial optimization data obtained proved that both products were safe for consumption, as the merit for food safety goes a long way in term of save from revenue loss, offer quality advantages for market penetration and provide concrete safety assessment for product development.

4. CONCLUSIONS

In summary, our results suggested retort pasteurization parameter of 102 °C for 5 minutes and HPP parameter of 6000 Bar for 6 minutes were effective in retaining the microbiological quality of durian paste and durian pulp at chilled temperature for a minimum of 180 days and 70 days, respectively.

5. ACKNOWLEDGEMENTS

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Analysis / Storage	Total Plate Count (CFU/g)		Yeast & Mould (CFU/g)		Coliform (CFU/g)		Psychrotrophic (CFU/g)	
Retort	DP-C	DP-R	DP-C	DP-R	DP-C	DP-R	DP-C	DP-R
Day 0	<25 x 10 est (1.1 x 10)	1.18×10^4	$<1 \times 10^2$	$<1 \times 10^2$	<1 x 10	<1 x 10	<1 x 10	<1 x 10
Day 30	<25 x 10 est (9.0 x 10)	6.0×10^6	$<1 \times 10^2$	$<1 \times 10^2$	<1 x 10	<1 x 10	<1 x 10	<1 x 10
Day 60	2.52×10^2	NA	$<1 \times 10^2$	NA	<1 x 10	NA	<1 x 10	NA
Day 90	2.02×10^3	NA	$<1 \times 10^2$	NA	<1 x 10	NA	<1 x 10	NA

Day 120	5.05 x 10^2	NA	<1 x 10^2	NA	<1 x 10	NA	<1 x 10	NA
Day 150	<1 x 10	NA	<1 x 10^2	NA	<1 x 10	NA	<1 x 10	NA
Day 180	<25 x 10 est (2.5 x 10)	NA	<1 x 10^2	NA	<1 x 10	NA	<1 x 10	NA

Table 1. Spoilage microorganism counts of durian paste throughout 180-day storage period

Note: <1 x 10 and <1 x 10^2 indicate the microorganisms tested were not detected
<25 x 10 indicate microorganisms tested were very low counts
DP-C = Durian paste stored at chilled temperature (retort-treated)
DP-R = Durian paste stored at room temperature (retort-treated)
NA = Not Available (analysis was not resumed due to bloated packaging)

Table 2. Foodborne pathogen counts of durian paste throughout 180-day storage period

Analysis / Storage	*Escherichia coli* (CFU/g)		*Staphylococcus aureus* (CFU/g)		Presumptive *Salmonella* in 25 g	
Retort	DP-C	DP-R	DP-C	DP-R	DP-C	DP-R
Day 0	<1 x 10	<1 x 10	<1 x 10^2	<1 x 10^2	Absent	Absent
Day 30	<1 x 10	<1 x 10	<1 x 10^2	<1 x 10^2	Absent	Absent
Day 60	<1 x 10	NA	<1 x 10^2	NA	Absent	NA
Day 90	<1 x 10	NA	<1 x 10^2	NA	Absent	NA
Day 120	<1 x 10	NA	<1 x 10^2	NA	Absent	NA
Day 150	<1 x 10	NA	<1 x 10^2	NA	Absent	NA
Day 180	<1 x 10	NA	<1 x 10^2	NA	Absent	NA

Note: <1 x 10 and <1 x 10^2 indicate the microorganisms tested were not detected
DP-C = Durian paste stored at chilled temperature (retort-treated)
DP-R = Durian paste stored at room temperature (retort-treated)
NA = Not Available (analysis was not resumed due to bloated packaging)

Table 3. Spoilage microorganism counts of durian pulp throughout 70-day storage period

Analysis / Storage	Total Plate Count (CFU/g)		Yeast & Mould (CFU/g)		Coliform (CFU/g)		Psychrotrophic (CFU/g)	
Retort	D-H	D-C	D-H	D-C	D-H	D-C	D-H	D-C
Day 0	<1 x 10	<25 x 10 est (1.0 x 10)	<1 x 10^2	<1 x 10^2	<1 x 10	<25 x 10 est (4.0 x 10)	<1 x 10	<1 x 10
Day 14	<1 x 10	1.5 x 10	<1 x 10^2	1.6Y x 10^3	<1 x 10	<25 x 10 est (5.0 x 10)	<1 x 10	<1 x 10
Day 28	<1 x 10	1.3 x 10^3	<1 x 10^2	2.1Y x 10^9	<1 x 10	<25 x 10 est (6.0 x 10)	<1 x 10	1.1 x 10
Day 42	<1 x 10	6.0 x 10^5	<1 x 10^2	1.0Y x 10^8	<1 x 10	<25 x 10 est (8.0 x 10)	<1 x 10	1.8 x 10
Day 56	<1 x 10	NA	<1 x 10^2	NA	<1 x 10	NA	<1 x 10	NA
Day 70	<1 x 10	NA	<1 x 10^2	NA	<1 x 10	NA	<1 x 10	NA

Note: <1 x 10 and <1 x 10^2 indicate the microorganisms tested were not detected <25 x 10 indicate microorganisms tested were very low counts; Y = Yeast Count
D-H = Durian pulp stored at chilled temperature (HPP-treated at 6000 Bar for 6 minutes)
D-C = Durian pulp stored at chilled temperature (without HPP treatment)
NA = Not Available (analysis was not resumed due to high microbial counts)

Table 4. Foodborne pathogen counts of durian pulp throughout 70-day storage period

Analysis / Storage	*Escherichia coli* (CFU/g)		*Staphylococcus aureus* (CFU/g)		Presumptive *Salmonella* in 25 g	
Retort	D-H	D-C	D-H	D-C	D-H	D-C
Day 0	<1 x 10	<1 x 10	<1 x 10^2	<1 x 10^2	Absent	Absent
Day 14	<1 x 10	<1 x 10	<1 x 10^2	4.4 x 10^2	Absent	Absent
Day 28	<1 x 10	<1 x 10	<1 x 10^2	3.0 x 10^6	Absent	Absent
Day 42	<1 x 10	<1 x 10	<1 x 10^2	4.0 x 10^6	Absent	Absent
Day 56	<1 x 10	NA	<1 x 10^2	NA	Absent	NA
Day 70	<1 x 10	NA	<1 x 10^2	NA	Absent	NA

Note: <1 x 10 and <1 x 10^2 indicate the microorganisms tested were not detected
D-H = Durian pulp stored at chilled temperature (HPP-treated at 6000 Bar for 6 minutes)
D-C = Durian pulp stored at chilled temperature (without HPP treatment)
NA = Not Available (analysis was not resumed due to high microbial counts)

Table 5. Microbiological safety limit (unsatisfactory level) based on international standards

Analysis / Reference	Total Plate Count (CFU/g)	Yeast & Mould (CFU/g)	Coliform (CFU/g)	*Escherichia coli* (CFU/g)	*Staph. aureus* (CFU/g)	Psychrotrophic (CFU/g)	Presumptive *Salmonella* in 25 g
CHN	10^5 per g	Not stated	10^2 per g	Not stated	10^3 per g	Not stated	Detected in 25 g
ANZ	≥ 10^6	Not stated	> 10^4	>10^2	10^3 - ≤ 10^4	Not stated	Not stated

Note: CHN = China Microbiological Standard for Durian Paste and Durian Pulp (As per GB/T21270-2007, GB19295-2011, GB29921-2013)
ANZ = Compendium of Microbiological Criteria for Food (Food Standards Australia – New Zealand) for Ready-To-Eat (RTE) foods in which all components of the foods have been cooked but there is some handling before sale or consumption (As at October 2016)

025-023

GENETIC DIVERSITY STUDY OF MPOB-EXOTIC PALM GERMPLASM COLLECTION USING MICROSATELLITE MARKERS

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ABSTRACT

The current plantation crops, such as oil palm, rubber, and cocoa, were introduced during the colonial era as ornamental plants, and these turned out to be billion-dollar industries. MPOB has embarked on the collection of exotic palms with economic potential from Central and South America. In this study, the genetic diversity of MPOB-exotic palms was identified for a comparative study with *E.guineensis* using twelve microsatellite markers. High level of polymorphism (P=98.6%), number of effective alleles (N_e= 2.63), observed heterozygosity (H_o= 0.40), expected heterozygosity (H_e= 0.53) were observed which indicates that the exotic palms has a broad genetic variation. Based on Nei's unbiased measures of genetic identity and genetic distance study, the genetic distance between *Jessenia* and *E.guineensis* was the largest (2.405), indicating low genetic relatedness. The genetic distance between *Oenocarpus* and *Euterpe* was the closest (0.239), indicating high genetic relatedness for both palms. The dendrogram grouped the exotic palms into two major clusters. Cluster I consists of five palm species involving *E.guineensis*, *Babasu*, followed by *Bactris* palm, and the third subcluster consists of *Euterpe* and *Oenocarpus*. *Jessenia* has formed a distinct cluster and is grouped in isolation. Generally, the MPOB-exotic palms have the potential to serve as a new economic crop, specifically for *Bactris* palm material for the production of palm hearts and fruits. For *Jessenia*, *Oenocarpus*, *Euterpe*, and *Babasu*, the understanding of agronomic requirements needs further studies for a better understanding of the optimum requirements. Through research and development, establishment and improvements of these crops can be explored for understanding their agronomic needs and yield potential as plantation monocrops and also as intercrops in the oil palm plantation in Malaysia.

Keywords: *Babasu*, *Bactris*, *Euterpe*, *Oenocarpus*, *Jessenia*.

INTRODUCTION

The plant world is rich in unexploited or underutilised species with potential economic value. The current plantation crops, such as oil palm, rubber, and cocoa, were introduced during the colonial era as botanical curiosities, and these turned out to be billion-dollar industries. This success has provided impetus to the MPOB to search for other species which are natural sources of vegetable oils and other products. In 1982 and 1989, the Malaysian Palm Oil Board (MPOB) had a collection of exotic palms from South America, which is the centre of the natural distribution of palm species. The expedition team collected five exotic palm germplasms involving *Bactris gasipaes*, *Jessenia batau*, *Oenocarpus mapora*, *Orbignya speciose*, and *Euterpe oleracea* (Rajanaidu et al, 2017).

Peach palm or *Bactris gasipaes*, was known for its nutritional fruits as well as the excellent taste of palm heart and starchy fruits (Clement et al, 2004). This species has great potential for diversification, agroforestry, and intercropping in Malaysia. The other species, *Oenocarpus-Jessenia* complex, has oil, which closely resembles olive oil and is a very valuable food source for beverages, pulp, palm hearts, and as a medium for cultivating protein-rich insect larvae for human consumption (Balick,1985). Babassu is an important source of edible oil and charcoal. The kernels of the palm produce oil that is similar to coconut oil in composition and applications. It is also used as a substitute for food, fuel, and lubricant (Anderson and Balick, 1988). *Euterpe oleracea*, also known as the acai fruit, is primarily used to make acai liquid, which is extracted from the mesocarp and is recognised for its good-quality palm hearts (Schauss, 2006). Generally, these species are primarily valued for their production, edible fruits, and other excellent nutritional quality products.

Over time, information on the genetic constitution of a crop is essential to understand the genetic structure of these native populations and, therefore, for the design of conservation strategies, improvements, and sustainable management (Kageyama et al., 2003). The exotic palms have the potential for cultivation as new economic crops in Malaysia. Thus, the knowledge of the variability between natural exotic palm germplasms is vital for plant breeding structure, preservation and adequate management. In addition, it allows a better understanding of how the selection is working, therefore, a widely used way to detect these variability is through studies of the genetic structure of natural populations (Estopa et al, 2006). Molecular markers have been used frequently in genetic variability studies (Ramos et al, 2021).

Among the molecular markers used in the studies about crop improvement, microsatellites or Simple Sequence Repeat-SSR stand out, which are codominant and specific to each species, with possible transferability to other species, with possible transferability to other species of the same genus (Faleiro, 2007). SSRs are markers widely used to estimate genetic parameters of populations, gene flow patterns and kinship, being abundant and well distributed across the plant genome. According to Manel et al. (2003), the use of molecular data in studies of population and landscape genetics, contributes to the analysis of the gene flow rate, genotype distribution, genetic adaptation, adaptive differentiation and speciation.

Despite the abundance studies of oil palms, the potential and utilisation of exotic palms have rarely been investigated and utilised in Malaysia. Due to the limited understanding of the other palms species, the potential and benefits of the palms on the current commodity remain unconvinced. In fact, little research has been conducted on the conservation status of these palm species. Thus, the aim of this study was to verify the genetic diversity of MPOB-exotic palm germplasm collection using SSR markers for comparative study with *E.guineensis.*

MATERIALS AND METHOD

Planting materials.

Five species of MPOB-exotic palm germplasm collections, *Bactris gasipaes*, *Jessenia batau*, *Oenocarpus mapora*, *Orbignya speciose*, and *Euterpe oleracea* were collected from several sites in Peru and Colombia (Figure 1) and planted at four MPOB Research Stations in Peninsular Malaysia: MPOB Research Station Kluang, MPOB Research Station Hulu Paka, MPOB Research Station Keratong, and MPOB Research Station Bagan Datuk.

Figure 1: The exotic palms were sampled in Peru and Colombia in 1982 and 1989

DNA (Deoxyribonucleic Acid) extraction

The molecular characterization was carried out on a total 163 leaf samples. This included 30 samples from each five species except for *Babassu*, which had 13 samples. Leaf samples of *E.guineensis* (DxP) were also included for comparison. The leaf sample was cut into small pieces and then ground with sand in liquid nitrogen until it became a fine powder. Four-day DNA extraction was carried out using the modified CTAB (cetyl trimethyl ammonium bromide) method. The concentration and purity of DNA were determined by measuring the absorbance at λ = 260 nm, 280 nm, and 350 nm using a spectrophotometer (Thermo Scientific^{TM} Multiskan^{TM} GO). All the samples were diluted to 50 ng/μl and stored at 20 °C for subsequent PCR amplification.

Genotyping by multiplex PCR for amplification of microsatellite markers

A total of 12 highly polymorphic and reproducible markers were selected for this study. The information of the 12 markers was presented in *Table 1*. The markers were developed at the Genomics Unit of ABBC-MPOB. Multiplex PCR protocol was conducted for genotyping the MPOB-exotic palm germplasm due to the large number of samples. For multiplex PCR reaction, a combination of four primers was designed. Every forward primer was M13-tailed and labeled with one of four florescent M13-dyes, viz., NED, FAM, VIC, and PET, to identify the multiplexing of the four markers for scoring of band pattern. Different dye colours distinguished the markers and related alleles in the output data where the band sizes overlapped. The total reaction of each polymerase reaction was 10 μl, comprising 50 ng of genomic DNA, 6.625 μl of MilliQ water, 1×PCR standard buffer (NEB, USA), 0.2 μl of 10 mM deoxynucleotide triphosphates (dNTPs) (NEB, USA), 0.025 μl of each of the M13-tailed forward primers and untailed reverse primers for every primer pair, 0.025 μl dye, and 0.1 μl of Taq DNA polymerase (5 U/μl) (NEB, USA). PCR was performed in a Perkin Elmer 9600 Thermocycler following an initial denaturation temperature at 95 °C for 3 minutes, followed by 35 cycles at 95 °C for 30 seconds, primer annealing for 30 seconds, and an extension temperature of 72 °C for 30 minutes, terminated by a final extension at 72 °C for 2 minutes. The amplified PCR products were resolved using 1 % agarose gel and run in a horizontal electrophoresis system to check the band sizes using a 100 bp ladder. The DNA fragment on agarose gel was documented using Gel Imager® (GelDocTM XR, Bio-Rad Lab. Inc., Hercules, CA, USA). Four PCR products for different primers labeled with different fluorescent dyes were pooled and multiplexed with the new PCR plate. The pooled PCR products (2 μl) were combined with 7.84 μl of formamide (Applied Biosystems, Foster City, CA) and 0.16 μl of the Gene Scan 500 (-35, -250, -340) LIZ size standard (Applied Biosystems, Foster City, CA). The samples were inserted in a 96-well PCR microplate and heated for 3 minutes. The sample plate was kept at 4°C before automated capillary electrophoresis using an ABI 3730 DNA Genetic Analyzer.

Table 1: List of selected polymorphic SSR markers for MPOB-exotic palm germplasm

Microsatellite ID	M13 +Forward primer	Reverse primer	TA (°C)	ES (bp)
sMo00036	CACGACGTTGTAAAACGACGCATTTGTAACTTTGTTGTTCTACC	CTTGAGGTAAGCCAGACATCC	52	231
sMo00061	CACGACGTTGTAAAACGACTTTGGGAGATTTGGTGTAAGTC	AGGAAGGTAACAGAGAAAGCAA	56	200
sMo00080	CACGACGTTGTAAAACGACGATGGCTTATATGTCCTTAGCTTT	CccccaaGATGTATTCTATGAC	55	150
sMo00085	CACGACGTTGTAAAACGACGTTAGTAGCAGTTGTCAGGATGG	AGGAGACGATCAAAAATCAGG	56	175
sMo00090	CACGACGTTGTAAAACGACAATCTTCCTGCGGTTTCACT	gagccaagaaatggatgAATAA	56	198
sMo00121	CACGACGTTGTAAAACGACCAGGCAAGAGGTTGGTATTT	GATCAAAGTTCTTCACCCATTT	54	198
sMo00186	CACGACGTTGTAAAACGACAGCAGTGGGAGCAAAAGAAA	AGTAGCCATGAGTCGCAACC	58	142
sMo00192	CACGACGTTGTAAAACGACCCCTCTGCTCTAGCCTTCCT	TGTGAGTGGGAACCATGCTA	58	243
sEg00018	CACGACGTTGTAAAACGACAGGAGTCGGTCAAAAACTCT	CTATTATGGGTGTGATCTGTAGT	52	200
sEg00020	CACGACGTTGTAAAACGACCAGGAATCTATATCACGTCCA	ACAATCAAAGTGACAGGAAAA	54	179
sMg00014	CACGACGTTGTAAAACGACGAAACCATGAAGAACAACAATA	AGATTCCGTTGCTTTAACTG	52	208
sMo00028	CACGACGTTGTAAAACGACTTTCAAGAGTAGGAGGTCTGGTT	CAGGAGAGTTTGGATTGTGTG	54	262

Statistical Analysis

SSR data were analyzed using Genemapper 4.1 software to identify the allele sizes of each marker. Electropherogram profiles (sample plots) were generated, and allele sizes for the markers were exported as data table for genotyping. Genetic diversity parameters such as allelic frequency, number of alleles per locus (N_a), effective allele number (N_e), observed heterozygosity (H_o), expected heterozygosity (H_e), percentage of polymorphism (%P), and number of private alleles were calculated using GenAlex 6.5 software. DARwin software was used for cluster analysis using the neighbor-joining (NJ) method to evaluate the genetic relationships among five exotic palm collections and *E.guineensis* (DxP) as a comparison.

RESULT AND DISCUSSION

The estimates of genetic variability parameters of the exotic palms were represented in Table 2. The number of different alleles (N_a) detected across six palms species ranged from 3.25 to 6.33, with an average of 5.28 different alleles. Among the palms, *Jessenia* and *Euterpe* exhibited the highest number of different alleles at 6.333 and 6.250, respectively, while *Babasu* had the least number of different alleles at 3.250. On the other hand, significant variation was observed between the number of effective alleles (N_e), estimated as the inverse of homozygosity, and the observed alleles. The number of effective alleles varied from 2.323 to 2.985 and *Euterpe* had the highest number of effective alleles (2.985).

Diversity indices provides important information regarding the rarity and commonness of species in a community. Shannon's information index (*I*) ranges from 0.837 (*Babasu*) to 1.235 (*Euterpe*), with *Euterpe* as the highest palm. The lowest observed heterozygosity (H_o) was observed in *Babasu*, by reason of small sample sizes analysed while the highest was recorded in oil palm. The expected heterozygosity (H_e) among the palms species varied from 0.471 to 0.585 while the average H_e was 0.526. The highest heterozygosity (H_e) was recorded in *Euterpe*. The lowest genetic diversity was observed in *Babasu*.

The Chi-square test was employed in the determination of the differences between the observed (H_o) and the expected (H_e) heterozygosity of exotic palms. There was no significant difference between observed heterozygosity and Hardy-Weinberg heterozygosity. The percentage of polymorphism among the palms varied from 91.67% to 100% with an average of 98.61%, indicating a high level of polymorphism among the palms and wide genetic variations among them. The entire 12 amplified SSR markers were polymorphic with the highest level (100%) across the palms observed in all palms except *Bactris*. This results showed that most of the palms have high genetic diversity.

Table 2: Allelic diversity indices in MPOB-exotic palm germplasm

Exotic palm	**N**	**Na**	**Ne**	**I**	**Ho**	**He**	**%P**	**Chi² test**
Jessenia	30	6.33	2.55	1.14	0.38	0.54	100.00	0.05
Bactris	29	5.33	2.64	1.06	0.36	0.51	91.67	0.04
Oenocarpus	24	5.33	2.70	1.09	0.43	0.55	100.00	0.03
Babasu	12	3.25	2.32	0.84	0.33	0.47	100.00	0.04
Euterpe	26	6.25	2.99	1.24	0.39	0.59	100.00	0.07
Oil Palm	29	5.16	2.60	0.98	0.49	0.50	100.00	0.00
Mean	25	5.28	2.63	1.06	0.40	0.53	98.61	

N_a: number of different alleles; N_e: number of effective alleles; *I*: Shannon's information index; H_e: Nei's gene diversity index; H_o: number of observed alleles; %*P*: percentage of polymorphism.

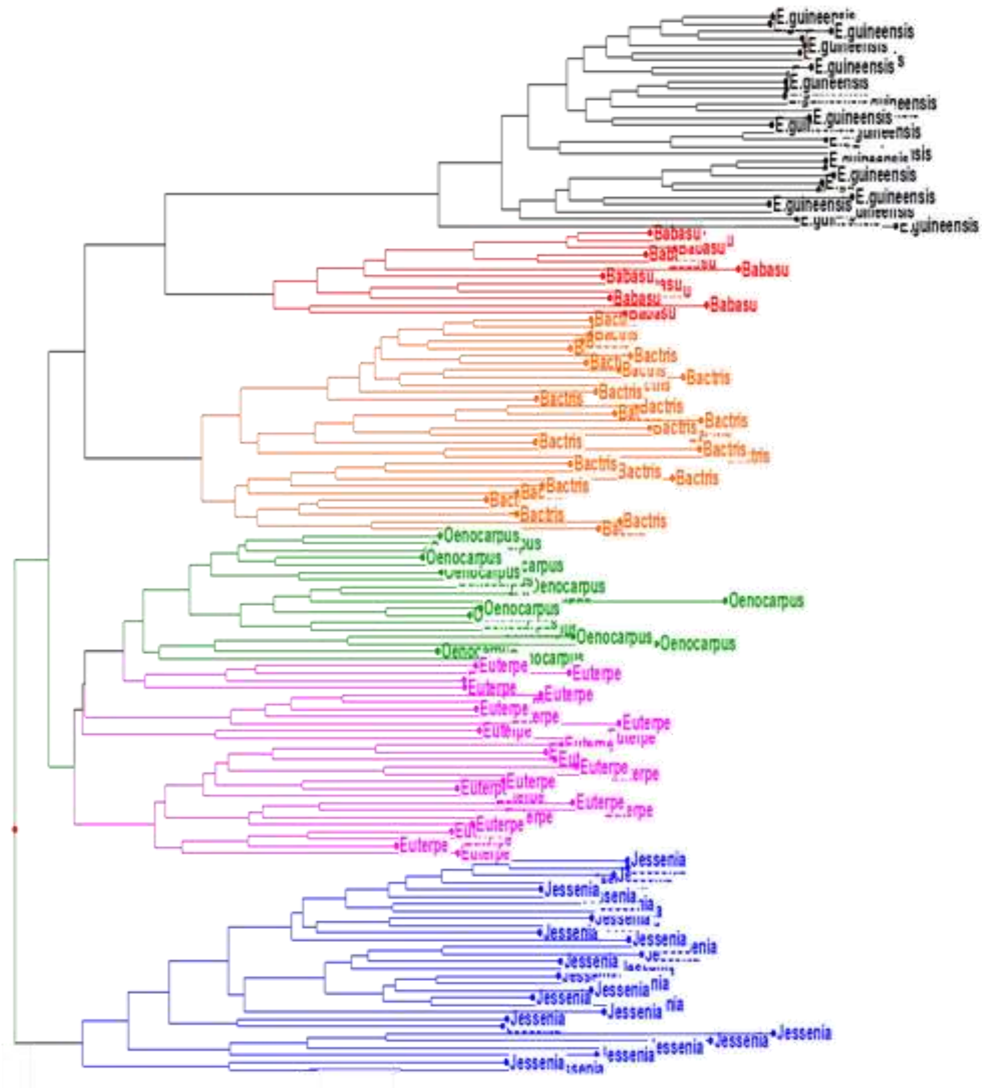
The Nei's unbiased measures genetic identity and genetic distance among the exotic palms were calculated according to the allele frequency of each locus and the results were summarised in Table 3. The genetic distance between the exotic palms species ranged from 0.239 to 2.405, while the genetic identity of exotic palms species ranged from 0.090 to 0.787. The genetic distance between the *Jessenia* and *E.guineensis* was the largest (2.405) and the genetic similarity was the smallest (0.090). The genetic distance between *Oenocarpus* and *Euterpe* were the closest (0.239) and the genetic similarity was the largest (0.787).

Table 3: Nei's unbiased measures of genetic identity and genetic distance among the exotic palm species

	Jessenia	Bactris	Oenocarpus	Babasu	Euterpe	Oil Palm
Jessenia	***	0.322	0.564	0.202	0.445	**0.090**
Bactris	1.134	***	0.560	0.440	0.547	0.293
Oenocarpus	0.572	0.579	***	0.480	**0.787**	0.201
Babasu	1.600	0.821	0.733	***	0.459	0.288
Euterpe	0.811	0.603	**0.239**	0.779	***	0.402
Oil Palm	**2.405**	1.228	1.603	1.246	0.910	***

Dataset of 12 SSR markers used in the study of MPOB-exotic palms germplasm were analysed using DARwin software, and the dendrogram was constructed the result obtained (*Figure 5*). The dendrogram grouped the exotic palms into two major clusters. Cluster I is the largest cluster, comprising five palm species, and consists of three subclusters. The first subcluster consists of *E.guineensis* and *Babasu*, followed by *Bactris* palm, and the third subcluster consists of *Euterpe* and *Oenocarpus*. The clustering of *Euterpe* and *Oenocarpus* may be attributed to the fruit character. The physical characteristics of the fruit are closely comparable. Meanwhile, *Jessenia* has formed a distinct cluster and is grouped in isolation, indicating that the genetic divergence of the *Jessenia* palm is the greatest thus revealing a certain genetic differentiation.

Figure 5 Neighbour-Joining (NJ) tree constructed for SSR dataset based on MPOB-exotic palms germplasm



CONCLUSION

Generally, the MPOB-exotic palms have potential to serves as new economic crop specifically for *Bactris* palm material for the production of palm heart and fruits. For *Jessenia*, *Oenocarpus*, *Euterpe*, and *Babasu*, the understanding of agronomic requirements needs further studies for a better understanding of the optimum requirements. The genetic background of each exotic palm has been clearly interpreted by genomes studies, assisting a thorough understanding of species evolution, as well as facilitating the assessment of diversity and interbreeding with other species. Hence it serves as a great tool for conservation genetics. Through research and development, establishment and improvements of these crops can be explored for understanding their agronomic needs and yield potential as plantation monocrops and also as intercrops in the oil palm plantation in Malaysia.

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DEVELOPMENT OF VERMICELLI FORTIFIED WITH POMEGRANATE PEEL POWDER

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ABSTRACT

Pomegranate peel contains dietary fiber ranging from 33 to 62%. This study aims to develop a high fiber vermicelli by fortification with pomegranate (*Punica granatum*) peel powder. Formulations 1 and 2 consist of 5% and 10% of pomegranate peel powder (PPP), respectively. Control was prepared without PPP. Proximate analysis, antioxidant assay, and sensory evaluation were performed on the control and samples. The dietary fiber content of formulation 1 (18.88±0.08%) and formulation 2 (25.53±0.28%) were significantly higher than that of the control (6.14±0.12%). Formulation 2 contained significantly higher total phenolic content (69.62±2.01 mg GAE/100g), total flavonoid content (44.07±1.81 mg QE/100 g) and exhibited significantly higher DPPH radical scavenging activity (21.40±0.87%) when compared to the control which contained TPC (0.86±0.20 mg GAE/100 g), TFC (0.47±0.37 mg QE/100 g) and DPPH radical scavenging activity (1.07±0.71%). The result of sensory evaluation showed that vermicelli fortified with 5% pomegranate peel powder (Formulation 1) had the highest acceptance index of 84%. In conclusion, the product serves as a good source of dietary fiber and antioxidants, and with satisfactory sensory quality. This project aligns with the sustainable development goals - promoting good health and environmental protection.

Keywords: Pomegranate Peel, Antioxidant, Dietary Fiber, Vermicelli.

031-027

IMPACT OF GENOTYPE AND FERMENTATION DURATION ON THE QUALITY OF MALAYSIAN COCOA BEANS

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ABSTRACT

The economic value of cocoa beans is determined by their quality, with high-quality beans commanding higher market prices. Various factors contribute to bean quality, including genetics, agricultural practices, environment, and post-harvest processes. This study aimed to determine the specific fermentation duration required by six Malaysian cocoa clones (PBC 123, QH 1003, BR 25, KKM 5, MCBC 7, and MCBC 5) to produce the best quality beans. Cut test, fermentation index (FI) analysis, and pH measurements were conducted to assess the quality of Malaysian cocoa beans fermented for different durations (0, 24, 48, 72, 96, 120, 144, and 168 h). Cut test analysis revealed that each clone required a different fermentation duration to achieve good-quality beans. For MCBC 7, BR 25, PBC 123, and KKM 5 fermentation durations of 96, 120, 144, and 168 hours yielded 66.7%, 63.5%, 71.5%, and 71.8% completely fermented beans, respectively. However, QH 1003 and MCBC 5 did not produce good-quality beans, even after 168 hours of fermentation. This indicates the impact of different genetic characteristics inherent to each Malaysian cocoa clone on the completion of the fermentation process. Additionally, the pH analysis showed that beans could be produced within a favorable pH range of 5.35 to 5.85 can be obtained, which disapproved previous claims of low pH values and excessive acidity in Malaysian beans. These findings emphasize the importance of understanding the characteristics of different cocoa clones and optimizing the fermentation process to enhance the flavor and quality of Malaysian cocoa beans. However, the FI analysis tended to overestimate the fermentation degree, indicating the completion of fermentation as early as within 72 to 96 hours. This discrepancy raises questions regarding the reliability of the FI method in assessing the completion of the fermentation process and its usage in cocoa bean grading.

Keywords: *Theobroma Cacao*, Cut Test Analysis, Fermentation Index (FI), Cocoa Flavor Enhancement, Cocoa Bean Grading.

032-028

EFFECT OF VARIOUS SEED PRIMING TREATMENTS ON GERMINATION AND ROOT DEVELOPMENT OF CHILLI (*CAPSICUM ANNUUM* L.) SEEDLINGS

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ABSTRACT

Seed priming is a technique used to enhance seed performance by hydrating and drying them before germinating. This process improves metabolic processes, resulting in higher germination rates, better seedling growth, and increased crop yield. Seed priming has become a crucial method for alleviating the impact of abiotic stress particularly in chilli cultivation. This study investigated the effects of diverse priming treatments on the germination characteristics and root morphology of chilli seedlings. In this study, several priming treatments with 1% potassium nitrate (KNO_3), 1% gibberellin (GA_3), 1% brassinolide, and water were conducted on seeds chilli (461) and compared with unprimed seeds (control). The results showed that the germination percentage of primed seeds significantly improved compared to non-primed ones; seeds germinated quickly and had a higher germination index, especially for those primed with 1% of KNO_3 and GA_3 solution. Consequently, the priming treatment with KNO_3 and GA_3 was superior in the seedling biomass and had the highest seedling vigour index. Compared to the unprimed seeds, KNO_3 seedlings had a significantly higher root length (95.04 vs. 64.81 cm), root surface area (18.94 vs. 12.01 cm^2), root tips number (655.33 vs. 301.25) and root fork number (1317 vs. 905). In general, all priming treatments were superior to the control treatment, which gave the lowest germination and root characteristics.

Keywords: Germinability, Gibberellin, Brassinolide, Pepper.

033-029

A COMPARATIVE STUDY ON GROWTH AND YIELD: HYDROPONIC AND CONVENTIONAL FARMING SYSTEM OF *Abelmoschus esculentus*

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ABSTRACT

This study entails comparing the growth and yield responses of okra between root dipping hydroponic and conventional farming methods. The plant growth parameters assessed include plant height and number of leaves, while for plant yield, the parameter assessed was fruit weight. Yield measurements were taken upon crop maturity. The results indicate that both farming systems had a significant effect on plant height and yet had no significant effect on the number of leaves and fruit weight. Thus, the findings suggest that soilless farming using the root dipping technique offers an alternative approach to cultivating okra, providing a potential source of income for communities interested in okra farming.

Keywords: Conventional Farming, Hydroponic Farming, Okra, Plant Growth.

INTRODUCTION

Malaysia's population growth has been steady from the mid-20th century to the present. Besides that, the world's population has experienced unprecedented growth, too, surging from approximately 4 billion in 1960 to over 8 billion in 2023. This rapid increase has significantly impacted food demand. As the population increased, food consumption surged (Pomoni, D.I., Koukou, M.K., Vrachopoulos, M.G. & Vasiliadis, L., 2023). The agricultural sector faces pressure to boost productivity and sustainability amidst these changes.

Okra (*Abelmoschus esculentus*), also known as lady's fingers, is a very nutritious vegetable, and it is cultivated widely throughout the year. It has been a high demand vegetable in the market. Mostly, okra plants are planted in an open field or in a conventional farming system. Okra plant has not been extensively investigated for cultivation in hydroponic farming systems (Olutola, O.O., Elijah, A.A. & Femi A.D., 2020). However, the limited availability of land and resources calls for an urgent solution to agricultural challenges.

Conventional farming refers to traditional agricultural practices where crops are grown in soil. This approach has been the predominant method of farming for many years and is still widely practised today. However, this farming system has drawbacks, including environmental degradation from chemical use, soil erosion, heavy water usage and loss of biodiversity (Pomoni, D.I. et al., 2023). Furthermore, weather variability such as extreme temperature, precipitation and uncertain weather patterns poses a significant challenge to conventional farming, impacting crop growth and yield.

A hydroponic farming system is the practice of growing plants without soil, using nutrient-rich water solutions to deliver essential minerals directly to plant roots. Hydroponic systems offer several advantages, including faster plant growth, higher yield and the ability to grow crops in areas with poor soil quality or limited space. Moreover, it uses water more efficiently than conventional farming (Arumugam, T., Sandeep, G. & Maheswari D.M., 2021). However, more research is needed to see how well okra plants grow in hydroponic systems under different weather conditions to determine if this method is suitable and sustainable.

The goal of this study is to compare the growth and yield of okra plants using two farming systems: the hydroponic farming system and the traditional open field conventional farming system.

OVERVIEW AND BOTANICAL CHARACTERISTICS OF OKRA PLANTS

Okra, also known as lady's finger, gumbo, or bendi, is a popular warm-season vegetable grown in the tropics and temperate Asia. It belongs to the Plantae kingdom and Angiosperms phylum. Okra is classified as an eudicot with two cotyledons in embryos and belongs to the Malvaceae family, which includes cotton and hibiscus (Lamont, W. J. Jr., 1999).

The okra plant, a semi-woody, fibrous, herbaceous annual plant, is larger and rougher than its close relative, cotton. It grows 3 to 6 feet tall and forms deep, dense, shallow food roots. Its leaves are alternating, palmate, and have small stipules. The plant produces flower buds at the axils of each leaf, which develop into five large, eye-catching yellow petals with dark maroon or royal purple patches. The lowest buds open shortly after sunrise and mid to late afternoon (Lamont, W. J. Jr., 1999).

NUTRITIONAL CONTENT AND HEALTH BENEFITS OF OKRA

Okra is a highly nutritious vegetable grown in temperate and subtropical regions. Its immature pods are favoured for their nutritional content and unique flavour. Okra seeds provide unsaturated fatty acids and essential amino acids, while leaves are used in cooking. Okra is low in calories and rich in protein, vitamins, minerals, and dietary fibre. Protein comes from high okra seeds, while vitamins A and C are essential. Okra also contains calcium, potassium, iron, and zinc. Extracts from okra can be used as food additives (Gemede, H. F., Ratta, N., Haki, G. D., Woldegiorgis, A. Z., & Beyene, F., 2015).

CONVENTIONAL FARMING

The conventional farming system, which heavily relies on synthetic fertilizers and herbicides, is a cash-flow grain, a row-crop farming model that uses a simple crop rotation and high external energy inputs to achieve high-yield crops and labour efficiencies (Gold, 1999). This system includes intensive cropping practices that use higher input levels to meet specific chemical, or nutritional requirements set by the end market (Bastakoti, N., Dhital, S. & Gurung, B., 2011).

HYDROPONIC FARMING

Hydroponic systems or soilless cultivation is a method in horticulture that uses nutrient solutions to supply water and essential minerals to plants without traditional soil. These systems can be categorized into two types: open systems, which do not recycle additional nutrient solutions, and closed systems, which collect and reuse excess nutrient that flows away from the plant roots within the system (Maucieri, C., Nicoletto, C., Zanin, G., Xiccato, G., Borin, M., & Sambo, P., 2020).

Soilless culture systems are a viable solution to soil-borne diseases in greenhouse cultivation. These techniques, which use substrates other than soil, offer several benefits over traditional methods. They eliminate pathogens, ensure growth and yield are unaffected by soil type or quality, enhance growth regulation through precise nutrient supply, and improve quality produce through effective management of temperature, humidity, and pest control (Arumugam et al., 2021).

DESCRIPTION OF STUDY AREA

The experiment was conducted at the University College of Agrosciences Malaysia in Melaka due to its convenient access to farming lands and adequate water resources. It is situated in a spacious residential farming area near the experimental field. The soil in that area is Batu Lapan Series that has a yellowish-brown colour, clayey loam texture, and moderate-grade structure. The soil also has moderately deep layers with lateritic shale flakes, and has well-drained and a cation exchange capacity of 5-10 cmol per kilogram besides low base saturation.

METHODOLOGY AND DATA COLLECTION

This study involves two methods: conventional farming and hydroponic farming. Conventional farming involves planting okra plants in designated planting beds, with two soil zones for each set of ten plants. The hydroponic passive system cultivates okra seedlings in twenty nutrient-rich pots with hydrokorrels as growing media.
The soil-based treatment involves removing debris improving the land with compost and organic materials, adding dolomite, and conducting a soil test to determine the current pH status before adjusting it to a suitable value. The okra plants require regular maintenance such as fertilization, watering, weeding and pest control. The hydroponic treatment used a more regulated setting, placing the plants in a nutrient-rich water pot. The pH, fertilizer concentration, water levels lighting, and frequent nutrient changes were observed.

The F1 - hybrid okra green beauty seeds were sown in a seedling tray using peat moss, with each hole filled with one seed. The seeds were watered daily and germinated for a few days, with shading provided to prevent wilting. After three weeks, the seeds were transplanted when they reached a height of 5 cm to 10 cm. Care was taken to prevent damage to the roots and to avoid bright sunlight exposure. Only healthy plants were selected for transplantation, and shading was provided to avoid wilting.

Okra plants are ready for harvest 60-80 days after sowing, with pods exhibiting firm texture, glossy appearance, and greenish coloration. Harvesting was conducted periodically to stimulate new growth and promote productivity, aligning with best practices.

Growth metrics like plant height and number of leaves were recorded weekly for three weeks after transplantation. The weight of the okra fruits was collected during the harvesting period.
The experiment compares the conventional or soil-based farming and hydroponic systems for the growth and yield of okra. Data from these two treatments and plots were analyzed by using SPSS statistics software, two-sample comparisons, and one-way ANOVA analysis for meaningful descriptions.

RESULT AND DISCUSSION

PLANT HEIGHT AND LEAF COUNT AS PARAMETERS OF HYDROPONIC AND CONVENTIONAL PLANTING SYSTEMS

This study compares the growth and yield responses of okra plants with those of hydroponic and conventional farming systems. The plant growth parameters assessed include plant height and number of leaves for plant growth. One-way ANOVA analysis was performed to assess the effect of hydroponic and conventional farming systems on the plant growth of okra. The results indicate that both farming systems significantly affected plant height. Pairwise comparisons were conducted using the Bonferroni test to identify significant differences in plant height on different planting systems.

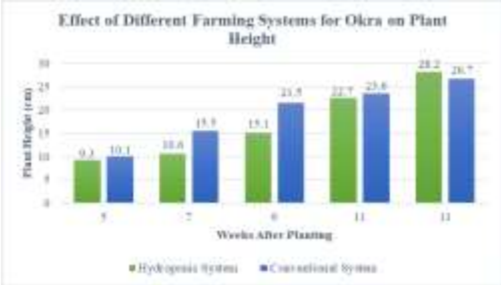
Table 1: Mean for Plant Height and Leaves Count of Okra Plant Grown in Conventional and Hydroponic Planting Systems

Planting System	Plant height (cm)	Leaves count
Hydroponic System	28.18^{a}	12.50^{a}
Conventional System	26.72^{b}	12.35^{a}

Means that do not share an alphabetic letter are statistically significant (P = 0.05)

Referring to Table 1, the Bonferroni at a 5% significance level for the plant height highlighted that the most significant differences were observed in the hydroponic system, which had the highest mean of 28.18 cm. In contrast, okra planted using the conventional method had the lowest mean value, which is 26.72cm. This result showed a similar trend with to the finding of Olutola, O.O. et. al. (2020). Besides that, this finding further supports Arumugam, T. and his friends' (2021) findings on the benefits of hydroponic systems, which can grow plants faster and larger.

Figure 1: Graph of growth rate in height of okra plant



Generally, plant height is a key indicator for plant health, and growth potential and influencing overall yield production. Figure 1 shows that the plant height increases as the plant ages. All plants in the hydroponic and conventional farming systems grew. Hydroponically grown okra may be advised in terms of physiological characteristics of the plant since the plant height from this planting technique demonstrated the best outcome.

Besides plant height, number of leaves is a crucial parameter in plant growth analysis because it directly influences photosynthesis, the process by which plants convert light into energy (Evans G.C., 1972, pg 13). As the number of leaves assessed, the result shows that these two farming systems had no significant effect on the number of leaves of okra plant ($p = 0.222$). Table 1 shows that the number of leaves of okra planted in hydroponic system had higher mean value of 12.50, and conventional system shows a lower number of leaves with the mean value of 12.35. The hydroponic and conventional farming system did not influence leaf development (Gashgari, R., Alharbi, K., Mughrbil, K., Jan, A., Glolam, A., 2018).

Figure 2: Graph of growth rate in leaves count of okra plant

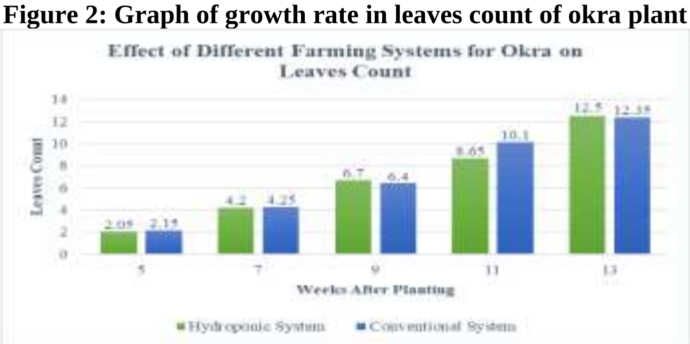
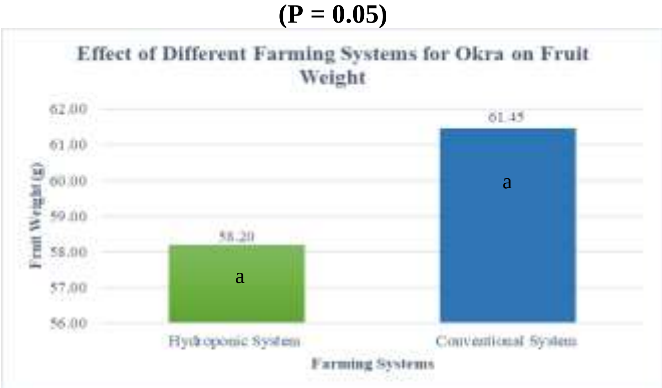


Figure 2 shows the growth rate in the number of leaves of okra plant. The number of leaves increased as the plant aged.

OKRA FRUIT WEIGHT

Figure 3: Yield components of fruit weight of Okra as influenced by two different farming systems and means that do not share a letter are significantly different (P = 0.05)



One-way ANOVA was conducted to investigate the impact of different farming systems on the fruit weight of okra. The analysis revealed no statistically significant effect of varying farming systems on fruit weight, with a p-value of 0.455. This indicates that the choice of farming systems had no significant influence on the fruit weight of okra. The analysis revealed that the highest fruit weight value was recorded in the conventional farming system (61.45g). Following closely, the hydroponic farming system with the fruit weight recorded was 58.2g.

CONCLUSION

As the global population continues to expand, there is a rising demand for food production. However, conventional or traditional farming systems may not meet this growing need. Consequently, the development of new farming and cultivation techniques is essential to mitigate potential food crises in the future. This study's primary focus was to compare the impact of different farming systems on the growth performance and yield of okra plants. The assessment was based on two (2) parameters; plant height and the number of leaves for plant growth and fruit weight parameter for the plant yield.

According to this study, there is a substantial difference in plant height between hydroponic and conventional farming systems for okra. The hydroponic approach produces higher plants. However, for the other two parameters, which are leaves count and fruit weight, both farming systems show about the same level of efficacy. This finding suggests that hydroponic techniques show better comparable plant growth and yield results.

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036-031

THE IMPACT OF LED LIGHT SPECTRA ON THE VEGETATIVE GROWTH, PHYSIOLOGY TRAITS AND STOLON PRODUCTION OF TWO STRAWBERRY CULTIVARS (*FRAGARIA* × *ANANASSA* CV. FESTIVAL AND *FRAGARIA* × *ANANASSA* CV. SNOW WHITE)

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ABSTRACT

Strawberry from the Rosacea family has a great demand for premium fresh produce market in Malaysia due to its flavour, aroma, and nutritional value. However, most of the supply are imported since the local supply is only obtained from the highlands. This creates a lengthy chain of carbon footprints and lead to increase the end price. Application of plant factory with LED lights, enable the cultivation of strawberry in lowland. This is because its ability to control environmental parameters such as light, temperature, and humidity. The objective of this study is to investigate the vegetative growth, physiology traits and stolon production of two strawberry cultivars *Fragaria* × *Ananassa* cv. Festival and *Fragaria* × *Ananassa* cv. Snow white under exposure of four different LED light formulations consist of; blue (B), red (R), green (G) and far-red (FR). The 69R24B7G light treatment significantly ($P<0.05$) increased the number of strawberry stolon at 46.5% compared to control treatment, this light formulation suitable to be used for producing planting materials in the plant factory. It was also discovered that the W (Full spectrum/ control) can potentially be used for early growth in the vegetative stage for strawberry growth. The addition of G spectral to the light formulation has a positive effect on strawberry vegetative growth parameters compared to the light treatments without the G spectral. This correlates with the rate of nett photosynthesis and chlorophyll fluorescence (Fv/Fm). It was also discovered that using the FR spectrum during the vegetative growth stage of strawberries is ineffective because it has a negative effect on the growth and physiological traits.

Keywords: Led Light, Strawberry, Plant Factory, Vegetative Grow, Vertical Farming.

029-033

ADVANCEMENTS IN BIOLOGICAL CONTROL STRATEGIES FOR BACTERIAL HEART ROT DISEASE IN PINEAPPLE: A REVIEW

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ABSTRACT

Bacterial heart rot disease poses a significant threat to pineapple cultivation worldwide, causing substantial economic losses. In recent years, the development and implementation of biological control strategies have emerged as promising alternatives to conventional chemical methods for disease management. This comprehensive review evaluates the latest advancements in biological control approaches targeting bacterial heart rot disease in pineapple. It explores various biocontrol agents, including bacteria, fungi, and bacteriophages, highlighting their mechanisms of action and efficacy in disease suppression. Additionally, the review discusses the integration of biocontrol agents with cultural practices and other management strategies to enhance their effectiveness in sustainable disease management. Furthermore, this review critically evaluates the challenges and future prospects of implementing biological control in pineapple farming. It emphasises the necessity for ongoing research and innovation to overcome limits and enhance biocontrol options for effectively managing bacterial heart rot disease. By synthesizing current knowledge and identifying research gaps, this review aims to provide valuable insights for researchers, growers, and policymakers seeking to combat bacterial heart rot disease and promote sustainable pineapple production.

Keywords: Bacteria Heart Rot, Biological Control, Pineapple.

1.0 INTRODUCTION

Pineapple (*Ananas comosus L. Merr)* is recognized as the third most important tropical fruit following bananas and citrus (Coppens d'Eeckenbrugge et al., 2011). Primarily consumed fresh, it belongs to the *Bromeliaceae* family, which encompasses approximately 2,000 species. This tropical fruit is notable for its high nutritional content, appealing flavor, and significant export potential. In Malaysia, pineapples constitute a major source of income for numerous smallholder farmers. In 2019, Malaysia's exports of pineapple-related products amounted to RM419 million. The Asia-Pacific region is the largest pineapple producing region, accounting for 41% of global production in 2021. Top producers include Indonesia, the Philippines, and India (Pineapple Market Trends., 2023)

Bacterial heart rot disease is a devastating disease that shows a substantial menace to pineapple farming on a global scale. The disease is attributed to pathogenic bacteria, specifically *Dickeya zeae*, and can result in significant financial losses for pineapple cultivators (Tajudin et al., 2018). According to Sapak et al. (2021) the pineapple sector is still dealing with the disease known as "bacterial heart rot," which is caused by *Dickeya zeae*. The six species of *Dickeya*, formerly classified as *Erwinia chrysanthemi*, include *D. dianthicola, D. dadantii*, *D. zeae*, *D. chrysanthemi*, *D. dieffenbachia*, and *D. paradisiaca* (Aeny et al., 2020). The virulence of *E. chrysanthemi* strains are associated with their capacity to produce pectinase, an enzyme that can degrade plant cells.

The initial phase of disease progression is characterised by the presence of waterlogged wounds and decay, as indicated by the brownish coloration of the leaves. Eventually, this will lead to the detachment of the stem and the emission of unpleasant odours (Veléz-Negrón, Y. I. et al., 2023). The presence of water-soaked lesions on the white basal portion of leaves in the central whorl is indicative of bacterial heart rot disease in pineapple. As the infection progresses, these lesions expand into the leaf's chlorophyll-rich region and transition from an olive-green colour to a brown shade. The leaves swell as they are inflated with a foul-smelling gas generated by bacterial fermentation of the leaf tissue (Reinoso et al., 2021). Dickeya zeae bacteria utilise this medium to metabolise sugars into lactic acid and establish their development pattern, facilitated by the abundant presence of carbohydrates in the fruits (Reinoso et al., 2021).

Traditional control strategies, which mostly depend on chemical pesticides, have achieved very limited success in treating this disease. Consequently, there is an increasing demand for alternative, environmentally-friendly ways that can be maintained in the long term. Biological control measures have become increasingly popular in managing bacterial heart rot disease in pineapple, replacing chemical ones. Biological control agents, such as bacteria, fungi, and bacteriophages, have demonstrated the ability to effectively inhibit the growth and dissemination of the pathogens responsible for the disease (Reinoso et al., 2021). These biocontrol drugs utilise a range of mechanisms, including antibiosis, competition, and induced systemic resistance, to efficiently manage the disease.

This review aims to assess the most recent improvements in biological management techniques for treating bacterial heart rot disease in pineapple. This text delves into the wide array of biocontrol agents, examining their many modes of action and evaluating their effectiveness in suppressing diseases. In addition, the research examines the incorporation of biocontrol agents with cultural practices and other management measures to optimise their efficacy in sustainable disease control. Moreover, the review rigorously assesses the difficulties and potential opportunities of using biological control methods in pineapple cultivation. It emphasises the necessity for continuous research and innovation to overcome constraints and enhance biocontrol alternatives.

2.0 MATERIAL AND METHOD

This study used the Preferred Reporting Items for Systematic Reviews (PRISMA) methodology, drawing data from three databases: Web of Science, SCOPUS, and Science Direct. After then, every finding undergoes further screened for eligibility and exclusion. The review process consists of multiple stages, namely identification, screening, eligibility, and data abstraction and analysis.

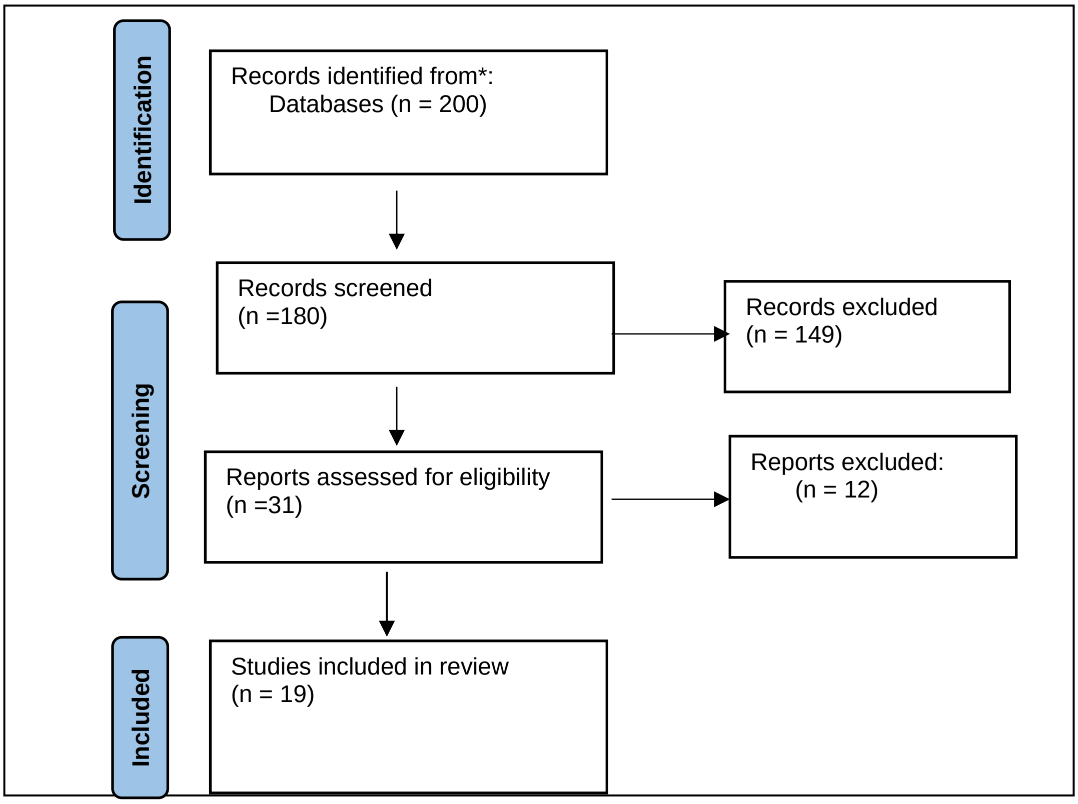
2.1 Database sources

A total of three databases were used to identify the source of 200 research papers. Further modifications to the criteria led to a removal of duplicate articles, resulting in the final total of 180 articles. During the screening stage, a total of 180 papers were evaluated. This process required thoroughly examining all journal articles and choosing only the ones that were pertinent to the matter at hand. After the title screening, the number of articles was decreased to 149. Following the abstract screening, the number was further cut to 31 articles. This publication includes articles that examine the use of biological methods to control the bacterial heart rot disease. Figure 1 illustrates the process of screening articles using a flow diagram. Non-indexed journals serve as repositories of literature that is published in journals not included in prominent bibliographic indexes like Web of Science, SCOPUS, or Science Direct. Non-indexed journals were not utilised as the main data source in this research, but rather as a supplementary source to complement data from indexed articles.

2.2 Systematic review literature process

The methodology employed for this study is a systematic literature review (SLR). A systematic literature review (SLR) involves the identification, evaluation, and synthesis of empirical data that fulfils specific qualifying criteria related to a particular research question.

Figure 1. The figure shows the SLR screening process.



2.2.1 Identification

Identification of information sources. The inquiry began with a literature search for the terms “bacterial heart rot”, “biological control” or “biocontrol”, “disease”, and “pineapple”. The initial relevance of the manuscript is determined based on the title of each document. The title suggests that the article discusses matters concerning the use of biological control for bacterial heart rot disease in pineapple. We have gathered the full reference of the article, which contains the author, year, title, and abstract, for further research. This is accomplished by utilising three databases: Web of Science, SCOPUS, and Science Direct. These databases are commonly consulted by scholars from diverse fields.

2.2.2 Screening

This stage is crucial for identifying all relevant studies on the topic of biological control for bacterial heart rot disease in pineapple. Additionally, this stage consists of multiple steps. The initial screening involves searching through various article journals using search engines such as Web of Science, SCOPUS, and Science Direct. Keywords like "bacterial heart rot" and "biological control" for pineapple are used, resulting in a collection of 200 articles. Subsequently, a second screening stage is conducted, where all the journal articles are reviewed and only those related to the topic are selected, resulting in 180 articles being screened. However, 149 sources are excluded after a screening of their title content as they are deemed irrelevant to the topic. The remaining thirty-one articles then undergo a third-level screening to determine their eligibility for inclusion in the study.

2.2.3 Eligibility

The third stage is an eligibility evaluation for 31 chosen articles. The titles, abstracts, and main contents of all articles were extensively reviewed to ensure that the articles met up the inclusion criteria and were suitable for use in the current study to achieve the research objectives. As a result, a total of 5 articles were removed due to duplication and lack of empirical data. Finally, a total of 19 articles were selected for further analysis.

2.2.4 Data extraction and analysis

The articles will undergo review, evaluation, and analysis in accordance with the eligibility criteria. This report will provide a comprehensive analysis of the outcomes. The evaluations will be conducted on specific papers that are relevant to and tackle the research problem. The studies were gathered to identify relevant themes and sub-themes for the current research. The data were extracted and categorised into relevant themes and sub-themes by analysing the titles, abstracts, and complete body content of the articles. A comprehensive review was undertaken by synthesising several research designs, including qualitative, quantitative, and mixed approaches. In order to achieve the goals of the study, data extraction is carried out by thoroughly reading the entire articles. The information was cited using Mendeley. The retrieved data categories were synthesised using both qualitative and quantitative methods for data analysis. The data were obtained from secondary sources by gathering and extracting information from another survey that was published in a peer-reviewed academic journal. Ultimately, the researchers compared publications authored by different individuals to achieve the intended outcomes.

3.0 RESULT

3.1 Bacterial biocontrol agents

Several researches have explored the capacity of bacterial biocontrol agents to effectively control bacterial heart rot disease in pineapple. *Bacillus cereus* is a well-researched bacterial biocontrol agent that has shown impressive effectiveness in suppressing the growth of the pathogen *Dickeya zeae*, both in laboratory settings and in living organisms (Husin N et al., 2022).

Bacillus cereus is thought to inhibit the disease by producing antimicrobial compounds, competing for resources and space, and generating systemic resistance in the host plant (Husin N et al., 2022). The research demonstrated that *Bacillus cereus* had the capacity to suppress the growth of the BHR pathogen, as evidenced by an inhibition zone with a diameter of 18.10 ± 0.36 mm in in vitro experiments. In greenhouse testing, the application of *Bacillus cereus* resulted in a significantly decreased disease severity index of 0.04 ± 0.01 compared to the positive control treatment using only the pathogen (0.53 ± 0.04) (Husin N et al., 2022).

In addition to *Bacillus cereus,* many bacterial species such as *Pseudomonas spp*. and Serratia spp. have shown promise as biocontrol agents in the fight against bacterial heart rot disease (James et al., 2015). The bacteria possess the capacity to impede the growth of harmful microorganisms, produce siderophores to acquire essential nutrients, and induce systemic resistance in pineapple plants, leading to a decrease in the severity of the disease (James et al., 2015).

3.2 Fungal biocontrol agent

Fungal biocontrol compounds have been studied for their potential in controlling bacterial heart rot disease in pineapple. Various fungal species, including *Trichoderma* spp. and *Gliocladium* spp., have been found to be highly effective in inhibiting the growth of *Dickeya zeae* and other bacterial diseases, as shown by recent studies (Weifeng Z et al., 2020) (Anuar, I. S. M. et al 2024). The fungus can antagonize the infection by employing mechanisms such as antibiosis, resource competition, and the secretion of lytic enzymes that break down the pathogen's cell wall (Weifeng Z et al., 2020).

Trichoderma spp., for example, has shown the ability to generate antibiotics that hinder the growth of Dickeya zeae, thus lessening the intensity of bacterial heart rot disease. *Gliocladium* spp. have demonstrated antagonistic characteristics against *Dickeya zeae*, such as the synthesis of enzymes that degrade the pathogen's cell wall and the release of chemicals that impede bacterial proliferation (Husin N et al., 2022). Moreover, these fungi have the ability to surpass the disease to obtain resources, such as nutrients and space, therefore reducing the pathogen's capacity to infect the plant.

Research has shown that these fungal biocontrol agents are effective in controlling bacterial heart rot disease in pineapple. A study conducted by Weifeng Z et al. (2020) shown that the application of *Trichoderma harzianum* effectively decreased the intensity of bacterial heart rot disease in pineapple plants, as compared to untreated control plants (Husin N et al., 2022).

Aeny et al. (2020) conducted a study which shown that *Gliocladium virens* successfully inhibited the growth of *Dickeya zeae* in pineapple plants, leading to decreased disease severity and enhanced plant well-being. In general, the utilisation of fungal biocontrol agents presents a hopeful strategy for the management of bacterial heart rot disease in pineapple. These agents have the potential to offer enduring protection against the pathogen and decrease the need on chemical pesticides (Pérez-Rodríguez et al., 2021).

3.3 Bacteriophage Biocontrol Agents

Bacteriophages, often known as phages, are being used as a new method of biocontrol to manage bacterial heart rot disease in pineapple. Phages are viral agents that selectively attack and kill bacterial cells, rendering them a focused and ecologically sound substitute for chemical means of control (Hamzah et al., 2021). Scientists have identified and described phages that have the ability to attack and kill *Dickeya zeae*, the microorganism responsible for bacterial heart rot disease (Hu et al., 2022). Phage-based biocontrol techniques have demonstrated encouraged outcomes in reducing disease severity and enhancing pineapple yield (Drenth et al., 2004).

Research has shown that phages may effectively reduce *Dickeya zeae* populations in laboratory conditions and on the surfaces of potato tubers. This suggests that they have the potential to be used as biological control agents in agricultural fields (Czajkowski et al., 2014). Phages have demonstrated promising efficacy as a biocontrol agent against bacterial heart rot disease in pineapple. Using phage cocktails to pineapple plants can effectively inhibit the spread of disease by diminish the bacterial population. This methodology provides a viable and ecologically conscious substitute for conventional chemical control approaches. Phage-based biocontrol techniques are a new and effective way to manage bacterial heart rot disease in pineapple. Phages provide various benefits compared to conventional control approaches, such as precise targeting, ecological compatibility, and the ability to respond to bacterial resistance. Additional study is required to thoroughly investigate the potential of phages in biocontrol and to create efficient phage-based products for commercial purposes.

4.0 DISUSSION

Although there have been significant developments in biological management measures for treated bacterial heart rot disease in pineapple, there are still various issues that must be addressed to ensure the general adoption and long-term viability of these systems. An important obstacle is the variable effectiveness of biocontrol agents in real-world settings, which can be affected by many environmental parameters, including temperature, humidity, and soil properties (Aristoteles et al., 2011) (Anuar, I. S. M. et al 2024). Continued research is necessary to improve the formulation, application techniques, and integration of biocontrol agents with other management strategies in order to boost their dependability and efficiency in various pineapple production conditions.

Another obstacle lies in the inadequate comprehension of the complex interactions between the biocontrol agents, the pathogen, and the pineapple plant. Additional investigation is necessary to better understand the processes by which biocontrol agents work, the factors that affect their effectiveness, and the possibility of synergistic interactions between diverse biocontrol techniques (El-Saadony et al., 2022). (Joy, P. et al., 2013) In order to overcome these difficulties and fully exploit the capabilities of biological control in managed bacterial heart rot disease in pineapple, a multidisciplinary strategy is required. This involves the cooperation of researchers, pineapple farmers, and politicians to promote the creation, experimentation, and widespread use of efficient and environmentally-friendly methods for controlling pests.

5.0 CONCLUSION

Bacterial heart rot disease poses a significant threat to pineapple cultivation worldwide, and the development of effective and sustainable control strategies is crucial. This review has highlighted the advancements in biological control approaches, including the use of bacterial, fungal, and bacteriophage-based biocontrol agents, as promising alternatives to conventional chemical methods.

Integration biocontrol chemicals with cultural methods and using resistant pineapple cultivars has demonstrated the ability to improve the overall efficiency of disease management strategies. Continued research and innovation are required to tackle the issues related to the inconsistent effectiveness of biocontrol agents in practical applications and to enhance our understanding of the complex interactions between the biocontrol agents, the pathogen, and the host plant.

This review seeks to give helpful insights for academics, producers, and policymakers by synthesising existing knowledge and identifying research gaps. Its goal is to battle bacterial heart rot disease and promote sustainable pineapple production. Constant efforts in this direction will be vital in cultivating resilient and ecologically sustainable resolutions to protect the pineapple sector and ensure its ongoing sustainability.

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038-036

SCREENING RAPD PRIMERS FOR SEX-SPECIFIC IDENTIFICATION OF DIOECIOUS GAC PLANTS (*MOMORDICA COCHINCHINENSIS*)

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ABSTRACT

Gac (Momordica cochinchinensis) is a dioecious plant, with the sex of the plant remaining unknown until its flowering stage. DNA markers, such as Random Amplified Polymorphic DNA (RAPD), offer an alternative method for determining the sex of Gac plants. However, there are currently no validated RAPD primers specifically designed for identifying the sexes of Gac plants. Thus, this study was carried out to screen publicly available RAPD primers to differentiate between the sexes of Gac plants. DNA extraction was initially performed, followed by a quality assessment. Subsequently, PCR was conducted using ten selected primers, and the resulting DNA profiles were visualized using a documentation system. Among the fourteen primers tested, two demonstrated the ability to differentiate between male and female Gac plants, as evidenced by distinct banding patterns in the results. Specifically, the RAPD primers OPC05 and OPO08 utilized in this study successfully distinguished gender. This finding demonstrates a promising method for identifying the sex of Gac plants. There is potential for the application of DNA markers, such as RAPD, to be utilized in diagnostic kits or assays for sex identification. This may contribute to the advancement of crop production, particularly by aiding farmers in early-stage sex identification of Gac plants.

Keywords: Gac, Molecular, Gender.

043-040

DEVELOPMENT OF FUNCTIONAL COOKIES USING RICE BRAN POWDER

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ABSTRACT

Rice bran is a nutrient-rich byproduct of the rice milling process. It is a good source of dietary fiber, minerals, and antioxidants. This study aimed to develop functional cookies using the underutilized rice bran powder (RBP). The main ingredients used for preparing the control cookies include high-gluten flour, butter, egg white, chocolate, and vanilla extract. High-gluten flour in Formulation 1 (F1) and Formulation 2 (F2) were partially substituted with 25% and 50% of RBP, respectively. The cookies were evaluated by 50 panelists using a 7-point hedonic scale. The overall acceptability scores for control (5.28), F1 (5.08) and F2 (5.30) cookies were not significantly different. F2 cookies had the highest acceptance index of 76%. F2 sample contained 40.87% carbohydrate, 34.77% fat, 12.58% protein, 4.77% crude fiber, 3.29% ash, and 3.71% moisture. The crude fiber content of F1 (2.50%) and F2 (4.77%) samples was significantly higher than that of the control (0.76%). The addition of RBP increased the crude fiber of F1 and F2 samples by 3-fold and 6-fold, respectively. Ash content of F1 (2.24%) and F2 (3.29%) samples were significantly higher when compared to the control (1.19%). Furthermore, F1 and F2 samples exhibited DPPH radical scavenging activity of 40.48±2.90% and 50.55±0.40%, respectively; and both values were significantly higher than that of the control (27.35±0.61%). In conclusion, rice bran powder could be utilized for the development of functional cookies. The products serve as a good source of fiber, minerals and antioxidants. This project aligns with the sustainable development goals - promoting good health and reduction of food waste.

Keywords: Rice Bran, Functional Cookies, Antioxidants, Fiber.

INTRODUCTION

Functional foods are foods that provide health advantages beyond their nutritional characteristics. The global functional food market size was valued at USD 297.9 billion in 2022 and is expected to reach USD 525.9 billion by 2031 with a CAGR growth rate of 6.5% during the forecast period from 2023 to 2031 (Straits Research, 2023). Rising consumer interest in nutritional supplements is a key driver for the market's growth. For the past few decades, there has been a paradigm shift in consumers' perception of health care, switching from disease treatment to disease prevention. Many consumers are willing to pay a higher price for healthy products such as functional foods to reduce the risk of various diseases. The concept of functional foods is widely accepted as consumers can take in health-promoting ingredients through delicious foods.

Cookies have gained popularity as a snack food among children and adults owing to their convenience and palatability. However, most cookies in the market tend to be high in refined sugar and saturated fat, lack nutrients, and are generally considered unhealthy. In this study, an attempt was made to develop healthier cookies by using rice bran powder as a functional ingredient.

Rice bran is a nutrient-rich byproduct of the rice milling process. It is a good source of dietary fiber, minerals, vitamins, essential fatty acids, and antioxidants. Rice bran contains 11-17% protein, 11-21% fat, 34-62% carbohydrates, 20-29% dietary fiber, and 8-10% minerals (Choi et al., 2011; Kumari et al., 2017; USDA, 2020). It contains an array of bioactive phytochemicals such as flavones and flavonols, phenolics, flavonoids, cinnamic and phenolic acids, phytic acids, and anthocyanins (Zhou et al., 2004; Ghasemzadeh et al., 2018; Limtrakul et al., 2019; Kalschne et al., 2020). Rice bran bioactive components such as g-oryzanol, phytosterols, tocopherols, and amino acids have demonstrated antioxidant, antibacterial, anti-inflammatory, cancer chemoprevention, decreased cholesterol absorption, and development of hypoallergenicity (Baskar et al, 2010; Khan et al., 2011; Borresen & Ryan, 2014; Manzoor et al., 2023). Despite its nutritional and therapeutic value for human health, a huge amount of rice bran generated is used mostly in livestock feed. Hence, this study aimed to develop cookies by partially substituting high-gluten flour with underutilized rice bran powder (RBP) to enhance the nutritive values.

MATERIALS AND METHODS

Preparation of Cookies

The main ingredients for preparing the control cookies include 40% high-gluten flour, 15% unsalted butter, 14% egg white, 10% rice bran oil, 9% chocolate chips, and 4.5% sugar. Minor ingredients were vanilla extract, skim milk powder, and rosemary. High-gluten flour in Formulation 1 (F1) and Formulation 2 (F2) were partially substituted with 25% and 50% of rice bran powder (RBP), respectively. RBP is gluten-free, it was combined with high-gluten flour in the formulations to give the desired texture. To prepare the cookies, butter, rice bran oil, and sugar were mixed with egg white to form a batter. Next, the pre-mixed dry ingredients were added to the batter and mixed, then vanilla extract and chocolate chips were added and mixed evenly to form a dough. The dough was cut, shaped, and baked at 150ºC for 20 min.

Physicochemical Analysis

The proximate composition of the cookie samples was carried out according to AOAC official methods of analysis. Color profile of the samples was determined by using the Lovibond spectrocolorimeter after calibration and recorded as L*, a* and b* values. The texture (hardness) of the cookie samples was measured using a TX. XT2 Texture Analyzer (Stable Micro Systems Ltd., Surrey, United Kingdom). All measurements were done in triplicate.

Extraction of Sample for Antioxidant Assay

Sample extraction was carried out by the method described by Stoica et al. (2022) with slight modification. A 5.0 g of each cookie sample was extracted using 100 mL of 80% methanol. The mixture was sonicated in an ultrasonic water bath (not more than 50ºC, 1 h), then centrifuged at 5000 rpm for 10 mins. The supernatant was transferred into a rotary evaporator to remove the solvent. The extract was refrigerated until further analysis. Each antioxidant assay was carried out in triplicate.

2,2-diphenyl-1-picrylhydrazyl (DPPH) Assay

The DPPH free radical scavenging activity of the extracts was determined according to AOAC Official Method (AOAC 2012.04). DPPH reagent was dissolved in methanol to obtain a 0.06 mM methanolic DPPH solution. A 3.9 mL of DPPH solution was mixed with 0.1 mL of extract and incubated in a dark room for 30 min at 30°C. After incubation, the absorbance was measured at 517 nm by using the UV-Vis spectrophotometer. The analysis was done in triplicate. A standard curve was established using various concentrations of ascorbic acid (0, 20, 40, 60, 80, 100 ppm). The calculation for DPPH radical scavenging activity (%) was as below.

$$DPPH\ radical\ scavenging\ activity\ (\%) = \frac{Abs\ blank - Abs\ sample}{Abs\ blank} \times 100\%$$

Determination of Total Phenolic Content (TPC)

TPC of the samples was analysed according to the AOAC Official method (AOAC SMPR 2015.009). Folin-Ciocalteu (FC) reagent was prepared by diluting it with distilled water in a ratio of 1:10 and kept in a dark place. 400 µL of sample extract was added into a test tube containing 2.0 mL of FC reagent and incubated for 5 mins, then 1.6 mL of 7.5% sodium carbonate was added, vortexed and incubated for 1 h in darkness. The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. A standard curve was established using various concentrations of gallic acid (0, 20, 40, 60, 80, 100 ppm). TPC was calculated using the formula below.

$$TPC\ (mg\ GAE/g) = \frac{C \times DF \times V}{W}$$

Where,
C = concentration of gallic acid obtained from standard curve (ppm),

DF = dilution factor,
V = volume used for extraction (L),
W = weight of sample used for extraction (g).

Determination of Total Flavonoid Content (TFC)

TFC of each sample was analysed by the method described by Sulaiman and Balachandran (2012). A 1.0 mL of sample extract was added into a test tube containing 4.0 mL of distilled water and 300 µL of 5% sodium nitrite. The mixture was incubated for 5 mins at room temperature, and 300 µl of 10% aluminum chloride hexahydrate was added into each test tube and incubated for another 5 mins. Then, 2.0 mL of 1 M of sodium hydroxide was added into each test tube and the absorbance was measured at 510 nm using a UV-Vis spectrophotometer. A standard curve was established using various concentrations of quercetin (0, 25, 50, 100, 125, 150, 200 ppm). TFC was calculated using the formula below.

$$TFC\ (mg\ QE/g) = \frac{C \times DF \times V}{W}$$

Where,
C = concentration of quercetin obtained from standard curve (ppm),
DF = dilution factor
V = volume used for extraction (L),
W = weight of sample used for extraction (g).

Sensory Evaluation

Sensory attributes (color, aroma, taste, texture, aftertaste and overall acceptance) of the cookies were evaluated by 50 untrained panelists (age 20-24, male 26% and female 74%) using a 7-point hedonic scale (1: dislike very much to 7: like very much). A preference test was also conducted on the samples. A briefing on the evaluation procedure and sensory attributes was given to the panelists. The 7-point hedonic scale is commonly used for affective sensory evaluation to determine the acceptability of a product. The Acceptance Index (AI) of cookies was calculated using the formula: AI = (Mean overall acceptance score/7) × 100%.

Statistical Analysis

All data in this study were shown as mean ± standard deviation of three replicates of each sample. The data were analyzed by analysis of variance (ANOVA) and the means were compared using Tukey's HSD test. The significance was defined at $p<0.05$. The data analysis was performed using IBM SPSS software version 25.0.

RESULTS AND DISCUSSION

Physicochemical Analysis

The color profile of the cookie samples is shown in Table 1. L* represents lightness from black to white on a scale of 0 to 100; while positive values of a* and b* represent red and yellow, respectively; and negative values of a* and b* represent green and blue, respectively. As the levels of rice bran powder (RBP) increased, the cookies became darker (L* value decreased) and the redness (a* value) and yellowness (b* value) increased due to the brown color of the RBP. A similar observation was also reported by Sapwarobol et al. (2021).

The hardness of cookies was recorded as the force used to break the samples. Based on Table 1, the hardness of the control was significantly higher than that of the F1 and F2 samples which contain 25% and 50% RBP, respectively. The decrease in hardness in F1 and F2 cookies could be due to relatively high fiber content in RBP. Fiber has water retention and water holding capacity resulting in higher water content in the dough which might interfere with the drying process during baking, causing less crispiness and hardness of texture (Xiao et al., 2024). In this study, the gluten-free RBP was combined with high-gluten flour in the formulations to yield a firmer dough, improving the texture of the products to a considerable extent.

Table 1. Color and hardness measurement of cookies

Samples	Control	Formulation 1	Formulation 2
L*	43.2 ± 0.7 [a]	30.3 ± 1.5 [b]	25.1 ± 0.4 [c]
a*	6.4 ± 0.3 [b]	7.4 ± 0.2 [ab]	8.3 ± 0.8 [a]
b*	9.2 ± 0.8 [c]	13.8 ± 0.7 [b]	17.5 ± 0.8 [a]
Hardness (kg)	2.38 ± 0.20 [a]	1.47 ± 0.11 [b]	1.16 ± 0.14 [b]

Each value is the mean of triplicate ± standard deviation. Means with different letters within the same row are significantly different ($p < 0.05$).

Proximate Composition

When compared with the control, rice bran cookies (F1 and F2) showed a significant increase in moisture, protein, fat, crude fiber, and ash but there was a significant decrease in carbohydrates (Table 2). The moisture of control (2.43%), F1 (2.77%), and F2 (3.71%) samples significantly increased in ascending order. As mentioned, rice bran is rich in dietary fiber which has high water retention and water holding capacity, thereby increasing moisture content of the cookies. According to a forum of the International Food Safety and Quality Network (IFSQN, 2018), it was stated that soft cookies contain 7-9% moisture while crunchy cookies contain 2.5-4.5% moisture. The moisture of F1 and F2 samples was within 2.5-4.5%, indicating that the rice bran cookies had acceptable crunchiness. Rajini et al. (2021) reported that cookies prepared from wheat-breadfruit (60:40) composite flour contained 4.97% moisture and microbial counts were within the acceptable range up to 12 weeks of storage. Hence, the rice bran cookies (F1 and F2) with less than 4% moisture shall have satisfactory microbial stability.

Table 2. Proximate composition of cookies

	Control	Formulation 1	Formulation 2
Moisture (%)	2.43 ± 0.01 [c]	2.77 ± 0.04 [b]	3.71 ± 0.01 [a]
Protein (%)	12.37 ± 0.03 [b]	12.51 ± 0.02 [a]	12.58 ± 0.05 [a]
Fat (%)	29.84 ± 0.11 [c]	32.46 ± 0.16 [b]	34.77 ± 0.08 [a]
Crude Fiber (%)	0.76 ± 0.02 [c]	2.50 ± 0.06 [b]	4.77 ± 0.24 [a]
Ash (%)	1.19 ± 0.01 [c]	2.24 ± 0.01 [b]	3.29 ± 0.00 [a]
Carbohydrates (%)	53.79 ± 0.13 [a]	47.62 ± 0.20 [b]	40.87 ± 0.28 [c]

Each value is the mean of triplicate ± standard deviation. Means with different letters within the same row are significantly different ($p < 0.05$).

Crude fiber is a component of the proximate analysis, and it measured the amount of insoluble non-digestible cellulose, hemicellulose, and lignin in plant-based foods. The addition of RBP increased the crude fiber of F1 and F2 samples by 3-fold and 6-fold, respectively (Table 2). This is attributed to the high crude fiber of the RBP (20.27%) which was pre-determined in this study. On the other hand, dietary fiber includes both the soluble and insoluble portions of plant-based foods. Rice bran contained 21.17% insoluble dietary fiber and 2.17% soluble dietary fiber (Manzoor et al., 2023). The components of the insoluble fiber in rice bran include arabinoxylans, cellulose, and hemicellulose, while soluble fiber includes pectin and beta-glucan (Sapwarobol et al., 2021). Thus, the functional rice bran cookies could serve as a potential source of dietary fiber which may promote health benefits such as reducing the risk of Type II diabetes, cardiovascular disease, lowering blood glucose and blood lipid levels, and improving bowel movement (Timm et al., 2023).

The ash content of F1 (2.24%) and F2 (3.29%) cookies was significantly higher when compared to the control (1.19%). The increase in ash is mainly attributed to the addition of RBP. Crude rice bran contained 9.98% ash, including minerals (per 100g): 1680 mg phosphorus, 1480 mg potassium, 781 mg magnesium, 57 mg calcium, 18.5 mg iron, 14.2 mg manganese, and 6.04 mg zinc (USDA, 2020) which are important for physiological functions.

With regards to fat content, F1 (32.46%) and F2 (34.77%) cookies contained significantly higher fat than that of the control (29.84%), mainly due to the addition of RBP. Rice bran contains 20.8% of total fat (USDA, 2020). Rice bran oil contains a range of fats, with 47% of its fats monounsaturated, 33% polyunsaturated, and 20% saturated. Rice bran oil is rich in essential fatty acids (EFA) omega-6 (linoleic acid, C18:2), and omega-3 (alpha-linolenic acid, C18:3) at a ratio of approximately 15:1 (Orthoefer, 2005). There is convincing evidence that replacing saturated fatty acids with polyunsaturated EFA decreases the risk of coronary heart disease (FAO, 2010). EFAs are indispensable and they play a crucial role in the brain, heart, and joint functions. (FAO, 2010). Omega-3 EFAs are important in metabolic processes including the nervous and immunological systems (Calder, 2006), as well as having anti-hypertensive, anti-atherogenic, anti-carcinogenic, and anti-obesity properties (Simopoulos, 2008; Riediger et al., 2009). Thus, incorporating RBP into cookies provides a convenient way to increase EFA intake among consumers for health improvement.

The protein levels of the control (12.37%), F1 (12.51%), and F2 (12.58%) cookies are quite similar. This is because the high-gluten flour used for the control and rice bran powder used for F1 and F2 cookies had about the same amount of protein. According to USDA (2020), high-gluten flour contains 12.5-14.5% protein, while rice bran contains 13.4% protein. Rice bran protein is a good source of essential amino acids including lysine, leucine, methionine, and phenylalanine. The carbohydrates of the cookies were calculated by difference. When compared to the control, the carbohydrates of F1 and F2 samples were reduced by 11.5% and 24.0%, respectively (Table 2), suggesting RBP cookies with lower glycemic index. The reduction was due to the carbohydrate content in high gluten flour was 70%, while that of rice bran was only 49.7% (USDA, 2020). Overall, the partial substitution of high-gluten flour with RBP improved the nutritive values of the cookies.

Antioxidant Properties

Based on Table 3, F1 and F2 samples exhibited DPPH radical scavenging activity of 40.48% and 50.55%, respectively; and both values were significantly higher than that of the control (27.35%). Similar trends were observed for total phenolic content and total flavonoid content. Previous studies by Kong et al. (2012) reported that pasta supplemented with rice bran had higher polyphenols, flavonoids, anthocyanins, and higher antioxidant activity, which aligned with the present findings. The radical scavenging capacity of rice bran cookies could be attributed to various antioxidants such as g-oryzanol (39-63%), phenolic acids (33-43%), anthocyanins (18-26%) present in rice bran (Liu et al., 2023; Laokuldilok et al., 2011). Rice bran contains phenolic compounds such as gallic acid, syringic acid, cinnamic acid, protocatechuic acid, and ferulic acid (Andriani et al., 2022); and flavonoid compounds such as such as luteolin, quercetin, catechin, myricetin, and apigenin which possess antioxidant activities (Ghasemzadeh et al., 2018). Additionally, these phytonutrients also demonstrated health benefits such as antiviral, anticancer, and anti-inflammatory (Ullah et al., 2020).

Table 3. Antioxidant properties of cookies

Samples	Control	Formulation 1	Formulation 2
Free Radical Scavenging Activity (%)	27.35 ± 0.61 [c]	40.48 ± 2.90 [b]	50.55 ± 0.40 [a]
Total Phenolic Content (mg GAE/g)	0.634 ± 0.004 [c]	1.188 ± 0.001 [b]	1.294 ± 0.004 [a]
Total Flavonoid Content (mg QE/g)	0.030 ± 0.001 [c]	0.035 ± 0.000 [b]	0.043 ± 0.003 [a]

Each value is the mean of triplicate ± standard deviation. Means with different letters within the same row are significantly different ($p < 0.05$).

Sensory Evaluation

The cookies were evaluated by 50 panelists using a 7-point hedonic scale. The scores of various sensory attributes are displayed in Table 4. Except for texture, there was no significant difference among the cookie samples in terms of color, aroma, taste, aftertaste, and overall acceptance. F2 cookies with 50% RBP had the highest acceptance index (AI=76%), followed by the control (AI=75%) and F1 sample (AI=73%), suggesting panellists' satisfaction with rice bran cookies. Preference test results also showed that F2 sample was the most preferred. Further research is warranted in developing more palatable rice bran cookies with improved texture.

Table 4. Sensory scores of cookies

Attributes	Control	Formulation 1	Formulation 2
Color	4.94 ± 1.449 [a]	5.14 ± 1.262 [a]	5.44 ± 1.091 [a]
Aroma	5.42 ± 1.012 [a]	5.34 ± 1.022 [a]	5.34 ± 1.062 [a]
Taste	5.18 ± 1.257 [a]	4.92 ± 1.140 [a]	5.16 ± 1.037 [a]
Texture	5.18 ± 1.335 [a]	4.46 ± 1.054 [b]	4.48 ± 1.249 [b]

Aftertaste	5.08 ± 1.192 [a]	5.08 ± 1.158 [a]	5.20 ± 1.161 [a]
Overall Acceptance	5.28 ± 0.991 [a]	5.08 ± 1.066 [a]	5.30 ± 0.974 [a]

Each value is the mean of 50 observations ± standard deviation. Means with different letters within the same row are significantly different ($p < 0.05$).

CONCLUSION

Rice bran powder could be utilized for the development of functional cookies. The products serve as a good source of fiber, minerals, and antioxidants. This project aligns with the sustainable development goals - promoting good health and reduction of food waste.

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IN-SILICO SCREENING FOR POTENTIAL NATURAL COMPOUNDS AGAINST ACETYLCHOLINESTERASE, CAUSING ALZHEIMER DISEASE

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ABSTRACT

Alzheimer's disease (AD) is a neurodegenerative disorder marked by cognitive decline, memory issues, and behavioral changes. It involves the buildup of amyloid-beta plaques and neurofibrillary tangles in the brain, leading to synaptic dysfunction and neuron loss. Acetylcholinesterase, a key enzyme in AD, regulates acetylcholine levels important for cognitive function. However, there is a growing interest in exploring natural products as potential inhibitors against acetylcholinesterase. In this study, we aimed to explore natural products as potential inhibitors against acetylcholinesterase, a key enzyme implicated in Alzheimer's disease progression. 16 plant-derived compounds were selected and subjected to molecular docking simulations using the YASARA tool. Additionally, the YASARA docking protocol was validated by super positioning between the deposited crystal structure of Acetylcholinesterase in complex with the inhibitor, which was obtained from PDB and the simulated docked Acetylcholinesterase with the same inhibitor. Among the 20 natural compounds docked, zeaxanthin exhibited the highest binding energy of 14.70 kcal/mol with acetylcholinesterase, forming 3 hydrogen bonds, 23 hydrophobic interactions, and 7 π-π stacking interactions. The molecular docking protocol was successfully validated as the superposition value of root mean squared deviation (RMSD) was 0.330 Å which is below 2.0 Å. Therefore, zeaxanthin shows the best potential candidate for inhibiting Acetylcholinesterase.

Keywords: Alzheimer’s Disease, Acetylcholinesterase, Phytochemicals, Molecular Docking, YASARA.

INTRODUCTION

Alzheimer’s disease is a progressive neurodegenerative disorder characterized by memory loss, cognitive decline, and behavioural changes (Sosa-Ortiz et al., 2012). It is the most common cause of dementia in the elderly population and poses a significant burden on the healthcare system worldwide (Alzheimer's Association, 2021). One of the key enzymes involved in the progress of Alzheimer's disease is cholinesterase, which plays a critical role in the breakdown of acetylcholine, a neurotransmitter essential for memory and cognitive function (Lleó et al., 2006). The relationship between acetylcholinesterase and Alzheimer's disease is significant because the decreased availability of acetylcholine contributes to the cognitive decline and memory impairment characteristic of the disease. Inhibitors of acetylcholinesterase, such as donepezil, rivastigmine, and galantamine are synthetic drugs commonly used as a treatment for Alzheimer's disease. These medications work by boosting acetylcholine levels in the body through the inhibition of its breakdown, which leads to some improvement in cognitive symptoms. However, these cholinesterase inhibitors are associated with several adverse effects, including nausea, vomiting, diarrhoea, and bradycardia, which can limit their use (Hossein, et al., 2023). Apart from that, the inhibition mechanism between the functional group of these compounds towards the active residues of acetylcholinesterase is still unclear. Apart from that, some of these synthetic drugs have consistently shown only modest benefits on cognition after consumed for long term (Zhang et al., 2022).

Therefore, this study aims to address these critical issues by investigating the potential of the natural products as inhibitors towards acetylcholinesterase via molecular docking analysis. Hence, this study shall contribute to the development of the natural product discovery against Alzheimer’s disease.

METHODOLOGY

Molecular docking simulations

YASARA, a bioinformatic tool that implements AutoDock program, was used to conduct these molecular docking simulations. This docking process was performed using a pre-made script that had been downloaded from the link, dock_run.mcr. Initially, the 3D structure of the acetylcholinesterase protein (PDB Id: 4EY4) was downloaded in PDB format from the Protein Data Bank database (https://www.rcsb.org/) (Tallei et al., 2020). Meanwhile, for the phytochemicals compounds, their 3D structures were downloaded from Pubchem database (https://pubchem.ncbi.nlm.nih.gov/) (Tallei et al., 2020). According to Son et al. (2019), the most likely active site of acetylcholinesterase is located from amino acids 202 to 447. Hence, the ligand structures were positioned within the active site range to focus docking with the selected 20 phytochemicals: chamazulene, eucalyptol, menthol, menthone, aloin, anthocyanins, valerenic acid, linalyl acetate, citral, zeaxanthin, leptodactylone, tetrandrine, chrysophanol, physcion, celastrol, tingenone, iguesterin, hirsutenone, wogonin, and cepharanthine. These phytochemicals with the prepared receptor, acetylcholinesterase, were then docked together. The binding energy and the molecular interactions of each ligand were observed and tabulated.

RESULTS AND DISCUSSION

Molecular docking analysis

Twenty phytochemicals as listed above, as well as the target receptor, acetylcholinesterase, were used for the docking studies. The binding affinities and molecular interactions between each ligand and the receptor for the 10 best-fitted phytochemicals were investigated in detail and enumerated in Table 1.

Table 1: Results of docking between acetylcholinesterase and the 10 best-fitted phytochemicals

Comp. No.	Phytochemicals	Binding Energy (kcal/mol)	Contacting Receptor Residues	No. of Hydrogen Bond	No. of Hydrophobic Interaction	No. of π-π Stacking
1	Iguesterin	10.63	**TRP A 286** HIS A 287 LEU A 289 GLN A 291 GLU A 292 SER A 293 VAL A 294 **PHE A 295** ARG A 296 PHE A 297 **TYR A 337** PHE A 338	-	11	5
2	Physcion,	10.08	**TRP A 86** ASN A 87 PRO A 88 **GLY A 120 TYR A 133 GLU A 202 SER A 203 TYR A 337** TYR A 341 **HIS A 447** GLY A 448	2	14	4
3	Wogonin	10.64	**TRP A 86** ASN A 87 PRO A 88 **GLY A 120 GLU A 202 SER A 203 TYR A 337** TYR A 341 **HIS A 447** GLY A 448 TYR A 449 ILE A 451**ASN A 487** CYS A 488	2	7	1
4	Cepharanthine	12.07	**TRP A 286** HIS A 287 **PHE A 295** ARG A 296	-	13	3

5	Celastrol	12.44	**TRP A 286** HIS A 287 VAL A 294 **PHE A 295**	3	9	4
6	Hirsutenone	12.52	**TRP A 86** TYR A 119 **GLY A 120 TYR A 133 GLU A 202 SER A 203 TRP A 286** SER A 293 VAL A 294 **PHE A 295** ARG A 296 PHE A **297 TYR A 337** PHE A 338 TYR A 341 **HIS A 447**	4	13	7
7	Tetrandine	11.47	TYR A 124 **TRP A 286** VAL A 294 **PHE A 295**	-	13	4
8	Tingenone	11.07	**VAL A 288** LEU A 289 4 **PHE A 295** ARG A 296 PHE A 297 **TYR A 337** PHE A 338	-	9	4
9	Aloin	13.05	**TRP A 286** LEU A 289 **PHE A 295** ARG A 296 PHE A 297 **TYR A 337**	3	7	6
10	Zeaxanthin	14.7	**TRP A 86** TRP A 117 TYR A 119 **GLY A 120 TYR A 133 GLU A 202 SER A 203** GLN A 250 LEU A 254 **TRP A 286 PHE A 295** PHE A 297 **TYR A 337** PHE A 338 TYR A 341 GLY A 342 **HIS A 447**	3	23	7

Note: Contacting receptor residues in bold are the active site residues of acetylcholinesterase.

From the results in Table 1, iguesterin, physcion, hirsutenone, tetrandrine, tingenone, aloin, and zeaxanthin showed remarkable binding affinity with acetylcholinesterase, which ranged between 10.63 kcal/mol to 14.70 kcal/mol. Moreover, the intermolecular hydrogen bonding results in a stronger complex formation and more precise docking outcomes. The strong geometric limitation brought about by the presence of hydrogen bonds also helps to improve the ligand's displacement (Wu et al., 2012). Based on the research done by Asamitsu et al. (2000), Harel et al. (1993) and Rhodes & Crabbe (2005), Trp86, Tyr133, Gly121, Glu202, Ser203 and His287 were identified as the major amino acid involved in the protein-ligand bindings which could also be seen from Table 1. Moreover, all 10 phytochemicals showed hydrophobic interactions with the acetylcholinesterase. Furthermore, referring to Table 1, all 10 phytochemicals have π-π stacking with the aromatic ring of acetylcholinesterase.

Best-fitted phytochemical

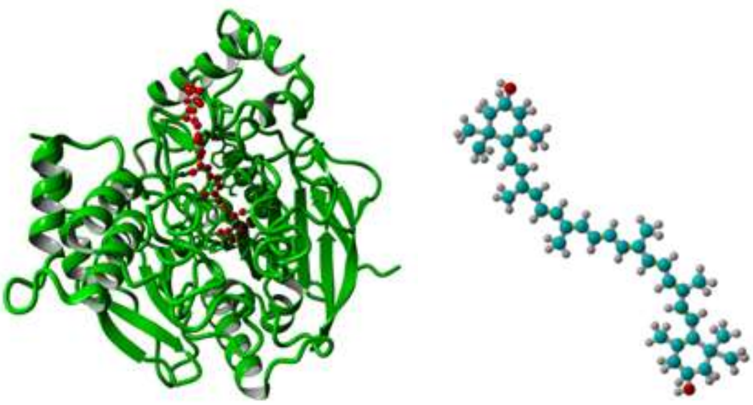
Zeaxanthin, the best-fitting compound among the 10 compounds, was chosen for further analyses based on its high binding energy, the presence of more than one hydrogen bond, hydrophobic interaction and π-π stacking with acetylcholinesterase.

Binding energy

Figure 1 shows the docking of zeaxanthin towards the acetylcholinesterase. Zeaxanthin obtained a 14.70 kcal/mol docking score against acetylcholinesterase, which is considered a high binding affinity compared to the other phytochemicals. YASARA, used in this study has a built-in protocol for calculating binding energies (Derek et al., 2015). The following equation is used to compute the binding energy that results:

Binding Energy = EpotRecept + EsolvRecept + EpotLigand + EsolvLigand – EpotComplex – EsolvComplex

Energy in the YASARA binding energy function was calculated as the difference between the total potential and solvation energies of the separated compounds as well as the total potential and solvation energies of the complex. Hence, a more favourable binding in the context of the selected force field is indicated by the higher positive binding energies obtained in the results (Aamir et al., 2018). Based on the in-silico research carried out by Mohamed et al. (2021), YASARA was used to screen for the potential reactivator against malathion-inhibited human AChE. High binding energy, which was 6.45 kcal/mol, formed between the docking of 4-hydroxybenzohydrazide and malathion-inhibited human AChE showed a stable and strong receptor-ligand interaction. In short, high binding energy values have been demonstrated in the literature to indicate a well-fitted protein and ligand complex structure.



(A) (B)

Fig. 1. (A) Docking of zeaxanthin towards acetylcholinesterase. Green colour represents acetylcholinesterase while red colour denotes zeaxanthin; (B) 3D compound structure of zeaxanthin. Light blue colour represents carbon, red colour signify oxygen, and grey colour indicates hydrogen.

CONCLUSION

In this study, the potential inhibitors were screened based on their binding affinity, molecular interactions, and the distance of the nucleophilic attack of the phytochemicals towards the acetylcholinesterase by using the YASARA. The hydrogen bond, hydrophobic interaction, van der Walls interactions, and the conformational entropy of the ligand were each identified with a distinctive physicochemical contribution to the binding free energy. Thus, zeaxanthin, which obtained a high binding energy value and consists of multiple molecular interactions with the residues of acetylcholinesterase, can fit well and bind more tightly to the active site of this protein. In short, this study has narrowed down the searching for a new potential phytochemical, zeaxanthin which could be used as a lead compound for developing acetylcholinesterase inhibitors. In vitro and in vivo studies are needed to further confirm the inhibition mechanism of zeaxanthin towards acetylcholinesterase.

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045-042

ADAPTATION AND SELECTION STUDY OF SHALLOT (*ALLIUM CEPA* L. VAR. *AGGREGATUM*) VARIETIES FOR LOWLAND CULTIVATION USING MINERAL SOIL

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ABSTRACT

Malaysia imports shallots/onions from India at 62%, followed by the Netherlands (15%), China (9%), Pakistan (8%), Thailand (2%), and Egypt (2%). In 2022, MARDI Serdang Headquarters conducted a study on the adaptation and screening of shallot (*Allium cepa* L. var. *aggregatum*) varieties in the lowlands, using mineral soil. This study comprised 9 shallot varieties from abroad, coded as SA 01, SA 02, SA 03, SA 04, SA 05, SA 06, SA 07, SA 08, and SA 09. The preliminary study determined that the best variety for further study was SA 04, which had the highest yield which is 23.11g and the lowest is SA 06 which is 4.1g per clump. However, although SA 04 has a high weight yield per clump, the amount of few bulbs will reduce earnings if planted by farmers. As a result, a selection index has been developed to make shallot selection an opportunity for future research. Apart from yield, other factors will be considered in the selecting process. The availability of planting material is the primary criterion for choosing. According to the selection index, two varieties obtained the highest-ranking values which are SA 08 and SA 02. Despite being ranked ninth, the SA 07 variety was also chosen selection variety based on the first index selection factor, planting material availability. Other varieties, especially the SA 04, are no longer available and cannot be assessed. SA 08, SA 02 and SA 07 varieties were chosen for planting in another location, particularly in Pontian, Johor, using peat soil and BRIS soil in Bachok, Kelantan.

Keywords: Shallot, Adaptation, Mineral Soil, Selection Index

1. INTRODUCTION

Shallot (*Allium cepa* L. *aggregatum* synonym *Allium ascalonicum* L.) which is formed in clusters of small onions. The onion plant originates from the West Asian region including Turkey, Iran, the Arabian Peninsula and the Sinai Peninsula (Shigyo and Kik 2008). Onion is the largest vegetable commodity in the consumption and production chain in the world. The edible portion of shallot is its bulb, which is made of many layers of inner fleshy and expanded leaf bases, covered by the outer dry membranaceous scaly leaves (Pareek et al. 2018; Teshika et al. 2018). It is a vegetable that works as a flavor enhancer in most dishes and is also a vegetable that has medicinal properties.

Malaysia fully imports fresh onions (large, small and white) for domestic needs. The total import of onions in 2021 for all three types of onions (large, small and white) is 622.2 thousand Mt worth RM 1,477.6 million (Trademap, 2023). Large onions are the most imported, which is 71.5% (445.1 thousand Mt) followed by garlic, 22.1% (137.6 thousand Mt) and small onions 6.3% (39.5 thousand Mt). Per capita consumption of onions in Malaysia shows that large onions are used the most (13.6 kg/year), followed by small onions (1.2 kg/year) and garlic (0.2 kg/year). Although the overall consumption of large onions is more, but from the aspect of value and return on sales, small shallots are higher where the import price is worth RM 1,880 / Mt compared to the import price of large onions RM 1,759 / Mt (Suhana et. al, 2023). The use of shallots is more significant for cooking decoration, especially to produce fried shallot products in Malaysia. It is believed that small shallots produce a sweeter taste than large shallots.

In December 2020, there was a crisis in the supply of imported onions, especially from India due to export restrictions due to a major flood disaster in that country. Due to this, the selling price has tripled from RM 3/kg to RM 16/kg for several months (until February 2021) (Noorazura, 2021). Later, India announced a 40% increase in onion export duty until December 31, 2023 as a result of price instability and domestic inflation. This situation has caused a reduction in the supply of imports from India and has resulted in an increase in import prices by 40-50%. India has also limited onion exports by imposing a minimum export price of US$800 (RM3,823) per metric ton due to the dwindling supply of onions and to ensure sufficient supply for domestic consumers in the country.

Therefore, MARDI has been mandated by the Malaysian government to carry out shallot research in order to overcome the issue of price and supply of the country's uncertain onion which affects the national food supply security issue. MARDI started conducting shallot research in the 1980s, however, the results of the study at that time found that the cultivation of shallots in the country was uneconomical due to high planting costs, less profitable farm sales prices and high disease and pest attacks. This has resulted in further studies not being continued. However, due to the current situation due to inconsistent supply, high import costs, unstable world onion market prices and increasing domestic demand involving national food security and safety issues, MARDI has considered re-implementing shallot research (Wan Rozita et. al 2022; Wan Rozita et. al. 2023).

2. MATERIAL AND METHODS

2.1 Plant Materials and the Preparation of the Plot

The study of shallot production in lowland Malaysia begins with the study of adaptation and evaluation of shallot varieties obtained of the collection of genetic material from various countries, seed companies and international research agencies involving the Asian Vegetable Research and Development Center (AVRDC) and The Indonesian Agency for Agricultural Research and Development (IAARD). A total of 9 varieties have been successfully collected and screened early at the Horticulture Research Center, MARDI Headquarters, Serdang, Selangor. These varieties are then screened and evaluated through an adaptation study in mineral soil, MARDI Headquarters, Serdang in 2022.

The study was carried out from February 2022 until May 2022 and was arranged in the form of Randomized complete block design (RCBD) with 4 replications. Each replication is represented by 25 selected trees. Plant spacing is 15 cm x 15 cm between trees and between rows. The boundary width is 1.2 m x 50 m using silver shine plastic as mulch (Figure 2). Watering is done once a day. Harvesting is done when 70% of the population shows the upper part of the leaves starting to dry. Data was taken on 10 randomly selected trees. Data analysis using SAS software version 9.3.


SA 01


SA 02


SA 03


SA 04


SA 05


SA 06


SA 07


SA 08


SA 09

Figure 1: Nine (9) shallot varieties selected in the adaptation study on mineral soil, at the Vegetable-Vegetable Research Plot, MARDI Headquarters, Serdang

2.2 Plant growth performance and morphological characteristic data

Data were recorded consisting of growth performance such as days after planting (DAP), plant height at 50 days after planting (PH50), leaf number at 50 days after planting (LN50), bulb number per clump (BN), fresh weight bulbs per clump (FW), individual bulb weight after curing (BW), bulb diameter (BD), bulb length (BL), total yield per clump after curing (YLD).

2.3 Statistical analysis

All the data collected were subjected to analysis of variance (ANOVA), and the mean was separated by Duncan Multiple Range at $p \leq 0.05$ (Version 9.4. SAS Institute, USA).

3. RESULTS AND DISCUSSION

3.1 Quantitative traits for plant growth performance and yield contributing traits

Mean squares result revealed that each shallot variety was significantly different ($p < 0.01$) for the characteristics of plant height and the leaf number at 50 days after planting, bulb number per clump, individual bulb weight after curing (g) and total yield per clump after curing (g). The shallot diameter (cm) showed a significant difference at $p < 0.05$ for each variety.

Table 1: Mean squares of 7 quantitative traits for plant growth performance, yield and its contributing traits

Ciri	Mean square		
	Variety (df = 8)	Rep	Error (df = 98)
Plant height at 50 days after planting (PH50)	304.04**	102.92	137.96
Leaf number at 50 days after planting (LN50)	1028.25**	249.41*	132.79
Bulb number per clump (BN)	157**	5.39	14.65
Individual bulb weight (BW)	788.84**	134.15*	48.47
Bulb diameter (BD)	2631.3*	1435.39	853.37
Bulb length (BL)	795.73**	91.35	67.04
Total yield per clump (YLD)	2639.66**	2305.89*	1367.04

Values are presented as means. Values with different superscripts are statistically significantly different based on Duncan's multiple range test at 5%.

Table 2: Genotype means values for plant growth performance and yield contributing traits

Var	**Mean**						
	PH50	**LN50**	**BN**	**BW**	**BD**	**BL**	**DWP**
SA 01	36.55^{bcd}	20.38^{c}	5.36^{c}	9.48^{c}	23.54^{b}	33.89^{c}	48.58^{b}
SA 02	36.52^{bcd}	24.65^{ab}	6.76^{b}	11.34^{bc}	25.2^{b}	30.17^{de}	52.41^{ab}
SA 03	38.93^{ab}	18.38^{cd}	4.38^{c}	12.37^{bc}	26.09^{b}	34.96^{b}	50.36^{b}
SA 04	40.25^{a}	13.74^{ef}	2.88^{d}	23.11^{a}	33.59^{ab}	38.30^{a}	62.83^{a}
SA 05	36.96^{abc}	22.35^{bc}	4.88^{c}	15.12^{b}	27.67^{ab}	32.69^{cd}	42.79^{bc}
SA 06	33.24^{de}	22.03^{bc}	6.64^{b}	4.1^{d}	17.37^{b}	22.57^{f}	35.34^{cd}
SA 07	31.24^{e}	26.83^{a}	9.61^{a}	4.72^{d}	17.37^{b}	22.07^{f}	29.15^{d}
SA 08	35.72^{bcd}	16.15^{de}	4.31^{c}	14.77^{b}	27.42^{ab}	30.82^{de}	41.69^{bc}

SA 09	34.21^{cde}	11^{f}	2.35^{d}	13.25^{ab}	43.72^{a}	29.32^{e}	30.08^{d}

Values are presented as mean. Values with different superscript within the same column are significantly different $p < 0.05$, based on DNMRT.

PH50: Plant height at 50 days after planting (DAP), LN50: Leaf number at 50 days after planting (DAP), BN: Bulb number per clump, BW: Bulb weight, BD: Bulb diameter, BL: Bulb length, YLD: Total yield per clump

As a result of the preliminary study, the shallot variety code SA 04 showed the highest yield performance compared to the other varieties (Table 2). SA 04 will be selected for further study. Nevertheless, the selection of a variety is not only based on total yield data but also other components to emphasize such as the availability of plant material. In addition, although SA 04 has a high yield, the number of bulbs per clump is too few and this will affect the profit for farmers if it is used as a planting material for further planting. The accepted diameter size of shallot in the market is between 25-35 mm. While the best number of shallots per clump is between 3-5. Therefore, to facilitate the selection of potential varieties, a selection index needs to be developed. Nine (9) shallot varieties studied after the drying process are shown in Figure 3.

Figure 3: Picture of nine shallot varieties used in adaptation and screening for lowland cultivation

3.2 Selection Index

A selection index has been developed to help make selection of potential shallots for further study. The main characteristics that help in the shallot selection index are the main components such as the availability of plant material, bulbs weight per clump, bulb number per clump, individual bulb weight, bulb diameter, bulb color, leaf number, plant height and bulb length. The main characteristics are given the top position with the greatest ranking. A score of 1- 4 shows which is 1 being the worst value up to 4 being the best value based on the average for each feature (Table 3). The highest score in the selection index table is the availability of plant material which is 0.2 and the lowest is the bulb length which is 0.02. The availability of plant material is the main selection feature to ensure continuous supply and be able to meet consumer demand if introduced in the market. The ranking value of the variety studied is shown in Table 4.

Table 3: Weights and scores developed for the selection of shallot varieties

Criteria	Weightage	Score								
		Variety								
		SA01	SA02	SA03	SA04	SA05	SA06	SA07	SA08	SA09
Availability planting material	0.2	1	3	1	1	1	3	3	4	1
Total yield per clump (g)	0.18	3	3	3	2	4	2	1	2	1
Bulb number per clump	0.16	4	3	4	4	1	3	2	4	4
Individual bulb weight (g)	0.13	2	3	3	3	4	1	1	3	3
Bulb diameter (mm)	0.11	2	2	3	3	4	1	1	3	3
Bulb colour	0.09	2	2	2	3	1	3	2	2	1
Leaf number	0.07	3	4	3	1	4	3	2	3	1
Plant height (cm)	0.04	2	2	2	3	2	1	1	2	1
Bulb length (mm)	0.02	3	3	4	4	3	1	1	3	2

Table 4: Ranking of shallot varieties for selection

Criteria	Score								
	Variety								
	SA01	SA02	SA03	SA04	SA05	SA06	SA07	SA08	SA09
Availability planting material	0.2	$\boxed{0.6}$	0.2	0.2	0.2	0.6	$\boxed{0.6}$	$\boxed{0.8}$	0.2
Total yield per clump (g)	0.53	0.53	0.53	0.36	0.71	0.36	0.18	0.36	0.18
Bulb number per clump	0.62	0.47	0.62	0.62	0.16	0.47	0.31	0.62	0.62
Individual bulb weight (g)	0.27	0.4	0.4	0.4	0.53	0.13	0.13	0.4	0.4
Bulb diameter (mm)	0.22	0.22	0.33	0.33	0.44	0.11	0.11	0.33	0.33
Bulb colour	0.18	0.18	0.18	0.53	0.18	0.27	0.09	0.18	0.09
Leaf number	0.2	0.27	0.2	0.07	0.27	0.2	0.13	0.2	0.07
Plant height (cm)	0.09	0.09	0.09	0.13	0.09	0.04	0.04	0.09	0.04
Bulb length (mm)	0.07	0.07	0.09	0.07	0.07	0.02	0.02	0.07	0.04
Total	**2.38**	**2.82**	**2.64**	**2.71**	**2.64**	**2.2**	**1.62**	**3.04**	**1.97**
Ranking	**6**	$\boxed{\mathbf{2}}$	**5**	**3**	**4**	**7**	**9**	$\boxed{\mathbf{1}}$	**8**

2.3 Varietal Recommendations

Based on the ranking in Table 4, two varieties give the highest value, namely SA 08 and SA 02. These two varieties have been selected as potential varieties and will be used in further research to get more accurate performance data. In addition to those two varieties, the SA 07 variety has also been selected for further study even though SA 07 is ranked number 9 for the selection index value. This is due to the first selection criteria which is the availability of plant material which is given priority. Other shallot varieties were found were not available the collection, especially the SA 04 variety.

2.4 Evaluation Study of Three Selected Shallot Varieties in Mineral Soil

The three selected varieties, namely SA 02, SA 07 and SA 08, were replanted for the second round of evaluation in the second season in mineral soil. The study was carried out from July 2022 until October 2022. The finding data were shown in Table 5.

Table 5: Genotype means values for plant growth performance and yield contributing traits for three selected varieties.

Variety	**Mean**						
	PH50	**LN5**	**BN**	**BW**	**BD**	**BL**	**YLD**
SA 02	26.5^c	25.15^b	5.07^b	7.20^b	22.37^b	26.60^b	34.55^b
SA 07	30.7^b	33.95^a	9.82^a	3.70^b	16.82^c	21.25^c	35.05^b
SA 08	35.77^a	13.65^c	3.40^b	26.52^a	38.08^a	40.75^a	46.15^a

Values are presented as means. Values with different superscript within the same column are significantly different p < 0.05, based on DNMRT.

PH50: Plant height at 50 days after planting (DAP), LN50: Leaf number at 50 days after planting (DAP), BN: Bulb number per clump, BW: Bulb weight, BD: Bulb diameter, BL: Bulb length, YLD: Total yield per clump

As a result of the performance evaluation study of the 2nd planting round conducted, the SA 08 variety gave the highest yield data based on the recorded morphological data. In this season's planting, the environmental weather conditions with less rain were produce better quality of the shallot bulbs. The quality of shallot obtained is the same as the imported onion in the market which is 25-35cm in size, bright red and attractive, fresh and tolerant to insect attacks and diseases, and capable of yielding around 4 - 6 tons/hectare.

CONCLUSION

As a result of a study on the adaptation and selection of shallot varieties that are suitable for planting in mineral soils in Malaysia, MARDI has launched three (3) shallot varieties adapted to low-lying soils that have been selected and have potential after a series of screenings, namely SA 08, SA 02 and SA 07 with the new commercial names of BAW-1, BAW-2 and BAW-3 each, in conjunction with the Show Tech MARDI 2023 event. The launch was completed by the Minister of Agriculture and Food Security (KPKM), the Honorable Datuk Seri Haji Mohamad bin Sabu on 25 August 2023 ago (Figure 5 and Figure 6). MARDI is now actively commercializing seed production and developing 15 seed entrepreneurs to expand cultivation in the country to ensure food security is guaranteed and can improve the national economy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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047-045

EFFECT OF BAY LEAF (*SYZYIUM POLYANTHUM* (WIGHT). WALP.) TEA ON HEALTHY ADULTS' BLOOD GLUCOSE

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ABSTRACT

Bay leaf (*Syzygium polyanthum* (Wight). Walp.), a common ingredient in cooking, is known to regulate blood sugar levels. This study aimed to develop bay leaf tea, evaluate its physicochemical composition and proximate value, gauge its acceptance in the community, and test its impact on blood sugar. Bay leaf tea was developed to complete this study using the freeze-drying technique for 96 hours at a temperature of -85°C. Small pieces of ground bay leaves are placed in a tea bag (2 g) and stored in an airtight container for storage. Bay leaf tea's phenolic content was tested using the Folin-Ciocalteu method, and its colour was assessed using a calorimeter device as part of a physicochemical analysis. Moisture and ash content were compared to bay leaf tea powder for proximate analysis. Thirty-three participants took part in the bay leaf tea acceptance test, which was designed to gauge how well they accepted the process of developing bay leaf tea. Adult participants were chosen for the study based on several criteria, including between the ages of 20 and 30, healthy, and not pregnant. According to the study's protocol, the participants were given a finger prick to determine their blood glucose levels before consuming bay leaf tea. After that, for 30 minutes before the second blood glucose reading, the subject is not permitted to consume any food or liquids. Although 46 subjects in total took part in the study, only 33 of them complied with its protocol and eligibility requirements. Subjects drank 200 mL of bay leaf tea, a temperature of 80°C to test the effectiveness of the beverage. The study's findings demonstrate a significant difference between blood glucose readings taken before and after consumption of bay leaf tea.

Keywords: Bay Leaf, *Syzyium Polyanthum,* Herbal Tea, Blood Glucose.

048-046

PRODUCTION OF LIPIDS RICH IN DOCOSAHEXAENOIC ACID (DHA) BY *AURANTIOCHYTRIUM* sp SW1 USING PINEAPPLE PEEL SKIN WASTE AS AN ALTERNATIVE CARBON SOURCE

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ABSTRACT

Thraustochytrids such as *Aurantiochytrium* sp. has attracted the attention of researchers and industry due to its ability to produce high amount of polyunsaturated fatty acids (PUFA) especially docosahexaenoic acid (DHA, C22:6 ω-3). Nevertheless, the need to use expensive substrates, such as refined carbon sources during the cultivation process has contributed to the increase in the commercialization cost of DHA from thraustochytrids. Therefore, in this study, pineapple peel waste (PPW), which is one of the main wastes of the agricultural industry in Malaysia has potential to be used as a cheaper alternative carbon source for the production of DHA from *Aurantiochytrium* sp. SW1. This study provides crucial insight to obtain the optimal amount of sugar from relatively complex recalcitrant PPW via pretreatments such as autoclave, acidic and alkaline treatment methods with different concentrations. The results showed that the hydrolysate produced from autoclave treatment had the highest sugar content (39.6 g/L) followed by the acidic and alkaline treatments recovered sugar content in the range of 31-38 g/L total sugar respectively. Then, the ability of *Aurantiochytrium* sp. SW1 to utilize all sources of PPW hydrolysates as the sole carbon source was screened and compared. It was found that the usage of PPW hydrolysate with 100mM H_2SO_4 treatment along with additional nutrients produced the highest biomass (7.74 g/L) while the highest lipid content (60.7%) was produced when SW1 was cultured using PPW hydrolysate with autoclave treatment without added nutrients. The highest DHA production (0.68 g/L) was achieved when *Aurantiochytrium* sp. SW1 was cultured using PPW hydrolysate with 100mM H_2SO_4 treatment along with additional nutrients where it was 10-35% higher when compared to production using other PPW hydrolysates. This study can be the basis for the production of cheaper DHA from thraustochytrids by using PPW as an alternative carbon source.

Keywords: *Aurantiochytrium* sp., Docosahexaenoic Acid, Pineapple Peel Waste, Pretreatment.

050-047

HEAVY METAL RESISTANT BACTERIA FROM AGRICULTURAL SOIL

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ABSTRACT

Heavy metal pollution is an environmental issue caused by anthropogenic activities such as agricultural, industrial, and domestic activities. Accumulation of heavy metals such as chromium, lead and zinc in the environment is a threat to all living organisms and may also decrease soil productivity. Bioremediation approaches using native bacteria represents a inexpensive and effective solution to treat such contaminated areas. Thus, this study aimed to isolate heavy metal-tolerant soil bacteria to widen the scope for bioremediation. The soil samples were collected from three different sites of an abandoned oil palm estate and screened with nutrient agar supplemented with 1 mM of chromium, lead and zinc respectively. A total of 15 isolates were isolated: six from zinc plates, five from chromium plates and four from lead plates. All the isolates were rod-shaped, with ten being Gram-positive while others were Gram-negative. Metal minimum inhibitory concentration test was performed by using the plate dilution method with increasing concentrations of heavy metals from 1 to 50 mM. The S3C1, S1C3, S3C5, S2C13, and S3C14 isolates showed the strongest tolerance to the three tested metals, with MIC values of 20 mM for zinc, 10 mM for lead and 3 mM for chromium. Molecular characterisation of these isolates using 16S rRNA sequencing identified all of them as *Bacillus* sp. In short, the five isolated *Bacillus* spp. tolerant to zinc, lead and chromium and have the applicability potential in the bioremediation of heavy metal contaminants. Future studies should focus on optimizing bacterial growth in different concentrations of heavy metals, and studying the efficiency of heavy metal removal.

Keywords: *Bacillus* Species, Chromium, Lead, Zinc, Metal MIC.

INTRODUCTION

Heavy metals are naturally occurring metallic elements with a density of at least 5 g cm^{-3}. which include chromium (Cr), cadmium (Cd), copper (Cu), iron (Fe) and lead (Pb)and Zinc (Zn). Heavy metal pollution of soil and water resources is a rising environmental issue. The distribution of abnormally high levels of heavy metals in the environment results from natural phenomena such as weathering and volcanic eruptions as well as anthropogenic activities, including industrial, agricultural, and domestic activities (Tchounwou et al., 2012). Accumulation of heavy metals in oil palm and vegetable farm soils have been shown to correlate to high level of Cr, Cu, Pb and Zn in palm oil and vegetable (Ismail et al., 2020; Olafisoye et. al., 2020). Long-term human exposure to heavy metals can cause irritation of the mucosa, headaches, stomach aches, dizziness, vomiting and diarrhea; excessive accumulation in the tissue may cause liver and kidney damage and even death (Sidhu, 2016; Royer and Sharman, 2020). Hence, eliminating heavy metal pollution from the environment is necessary for sustainable development.

Conventional heavy-metal treatment methods include chemical precipitation, ion exchange, oxidation/reduction, membrane filtration, reverse osmosis, and evaporative recovery. However, some of these methods increase the use and disposal of industrial chemicals, causing secondary environmental pollution and are sometimes economically non-viable (Sidhu, 2016). Thus, a cost-effective technology to treat heavy metals pollutants is in demand. Biological remediation is an effective, economical, eco-friendly technique of applying biological organisms such as bacteria, algae, fungi, earthworms, or plants to remove the pollutants from the ecosystem (Verma & Sharma, 2017; Sharma, 2020).

Several mechanisms developed by heavy-metal tolerant bacteria make them excellent candidates for bioremediation of heavy metal pollutants (Gautam, 2016). For example, *Bacillus cereus* was found to remove the Cr (VI) by reducing it (Dong et al., 2013). *Pseudomonas aeruginosa* exhibited high efficiency in removing Cr, while *Providencia rettgeri* was found to have the ability to immobilise Zn and Cd (Disi et al., 2022). However, limited reports are available for bacterial removal of Cr, Pb and Zn from the environment. Thus, there is an on-going demand for new heavy metal tolerance strains employing different metabolic mechanisms.

Therefore, the current study aimed to isolate and characterize bacterial strains that are tolerant to heavy metals (Pb, Zn, and Cr). Morphological and molecular characterisation, minimum inhibitory concentration (MIC) were studied to explore the potential of isolated bacteria for the removal of heavy metal pollutants from the environment. This study widens the scope for research on bacterial-base bioremediation by exploring new potential bacterial strains for bioremediation of toxic heavy metals pollutants.

METHODOLOGY

Sample collection

Soil samples were collected from three different positions (2°48'59.4"N 101°46'03.7"E; 2°48'36.3"N 101°46'03.4"E; 2°48'43.5"N 101°46'00.7"E) of an abandoned oil palm estate near Bukit Melati, Nilai, Malaysia. Soil from the depth of 20cm were collected using a soil sampling tube and transferred to the laboratory in sterile containers and maintained at 4 °C until further processing.

Isolation of heavy metal tolerant bacteria

Soil samples were diluted 10^{-1} and 10^{-2} in ddH2O and mixed well. Next, 100µL from each dilution was spread on three nutrient agar (NA) plates, each supplemented with 1mM of $Pb(NO_3)_2$, $K_2Cr_2O_7$, and $ZnSO_4$, and incubated overnight at 37 °C. The morphologically distinctive colonies were selected, streaked for pure culture and maintained on NA slants for further study.

Morphological characterisation of bacteria

The colony morphology (colour and size) and cell shape, size arrangement and of the isolates and their differentiation into Gram negative or Gram positive bacteria were determined.

Determination of minimum inhibitory concentrations of heavy metals

Minimum inhibitory concentrations (MIC) of Pb, Cr and Zn for each isolate were determined using the agar dilution technique. NA with increasing concentrations of each heavy metal (1mM, 3mM, 5mM, 10mM, 20mM, 30mM, 50mM) were prepared. The bacterial isolates were cultured overnight in nutrient broth on a shaker incubator with agitation at 150 rpm and a temperature of 37 ºC. The OD_{625} absorbance of the bacterial isolate overnight cultures was standardised to 0.08-0.1. Then, 10 µL of overnight culture of each isolate grown in metal supplemented nutrient broth was spotted on the prepared heavy-metals plates, which were then left to dry in a laminar flow. The plates were incubated at 37 °C for 48 hours and growth results were recorded. The metal tolerant MIC was defined as the highest concentration of the heavy metal that showed visible growth of bacteria.

Molecular characterisation by 16S rDNA sequencing

Selected strains were further characterised by 16S rDNA sequencing. Single colonies of the bacterial strains NA were suspended in 100 µL of ddH2O by vortexing and treated in boiling water for 2 min. After centrifugation at 10,000 rpm for 5 min, the supernatant was used for PCR amplification. Primers for bacterial 16S rDNA amplification were 27F (5'-AGA GTT TGA TCM TGG CTC AG-3') and 1492R (5'-TAC GGY TAC CTT GTT ACG ACT- 3'). PCR solution consists of EconoTaq® PLUS GREEN 2X MasterMix (Sigma-Aldrich) 25 µL, Primers (10 µM) 1 µL each, DNA template 5 µL, and ddH2O was added to a total of 50 µL. PCR conditions were 95°C for 8 min, and then 96°C 30 s, 55°C 30 s, 72°C 1.3 min for 30 cycles, and finally 72°C for 15 min. The PCR products were used for sequencing after being verified by agarose gel electrophoresis. Sequencing services were provided by Apical Scientific Sdn Bhd. (Kuala Lumpur, Malaysia). All 16S rDNA sequences obtained were subject to BLAST analysis (Madden, 2002) to identify similar sequences. The neighbor-joining phylogenetic analysis was also performed, in MEGA X software (Kumar, 2018).

RESULTS AND DISCUSSION

Isolation and phenotypic characterisation of metal tolerant bacteria

A total of 15 bacteria strains were isolated from the various metal supplemented NA plates. After further sub-culturing for pure cultures and morphological characterisation, identical isolates were excluded. Eventually, 15 strains (6 from Zn plates, 5 from Cr and 4 from Pb) were cultured for further characterisation. The colony and Gram characteristics of these isolates are shown in Table 1. While the colony morphology vary vastly among the isolates, 10 out of the 15 isolates were Gram positive rods. Isolation of metal tolerant Gram positive rods have been commonly reported from natural environment and polluted sites (Bharagava & Mishra, 2018; Chaturvedi, 2011).

Table 1. Macroscopic and microscopic characteristics of isolates from metal supplemented media

Isolates	Colony morphology	Gram-staining result	Metal Supplement
S3C1	Circular shaped, entire margin, raised elevation, large colony, smooth shiny surface, white colour, opaque	Gram positive rods	Zn
S2C2	Circular shaped, entire margin, convex elevation, large colony, smooth dull surface, pale yellow colour, opaque	Gram negative rods	Zn
S1C3	Circular shaped, curled margin, raised elevation, large colony, rough dull surface, milky colour, opaque	Gram positive rods	Zn
S1C4b’	Circular shaped, entire margin, raised elevation, moderate colony, smooth shiny surface, milky colour, opaque	Gram positive rods	Zn
S1C4b’’	Rhizoid shaped, filamentous margin, flat elevation, large colony, rough dull surface, milky colour, opaque	Gram positive rods	Zn
S3C5	Circular shaped, curled margin, raised elevation, large colony, rough shiny surface, milky colour, opaque	Gram positive rods	Zn
S2C6	Circular shaped, undulate margin, convex elevation, moderate colony, smooth shiny surface, milky colour, translucent	Gram positive rods	Cr
S3C7	Filamentous shaped, entire margin, flat elevation, large colony, smooth shiny surface, milky colour, translucent with transparent edge	Gram negative rods	Cr
S3C8b	Circular shaped, curled margin, crateriform elevation, large colony, rough shiny surface, milky colour, opaque	Gram negative rods	Cr
S1C9	Circular shaped, curled margin, convex elevation, moderate colony, smooth dull surface, milky colour, opaque	Gram positive rods	Cr
S2C10	Spindle shaped, curled margin, crateriform elevation, large colony, rough shiny surface, milky colour, opaque	Gram negative rods	Cr
S2C12	Circular shaped, entire margin, raised elevation, large colony, rough shiny surface, white colour, opaque	Gram positive rods	Pb
S2C13	Circular shaped, entire margin, crateriform elevation, large colony, smooth shiny surface, milky colour, opaque	Gram positive rods	Pb
S3C14	Circular shaped, entire margin, raised elevation, large colony, smooth shiny surface, milky colour, opaque	Gram positive rods	Pb
S1C15	Irregular shaped, lobate margin, raised elevation, large colony, smooth shiny surface, milky colour, opaque	Gram negative rods	Pb

Minimum inhibitory concentrations of heavy metals

The extend of tolerance of the isolates to Cr, Pb and Zn was determined by testing the MIC of heavy metal using the agar dilution technique. Higher MIC values indicate higher tolerance of the bacteria isolates toward the metal. As shown in Table 2, the tolerance for Cr was from 1 to 3 mM, Zn was 10 to 20 mM while all isolates showed the same tolerance to Pb, at10 mM. In particular, 5 strains, namely S3C1, S1C3, S3C5, S2C13 and S3C14, showed the highest tolerance to all the metals tested. *Cellulosimicrobium sp*. reported by Bharagava and Mishra (2018) was able to tolerate

800mg/L (equivalent to 15.4 mM) of Cr (VI) ions. *Bacillus circulans* strain MN1 isolated by Chaturvedi (2011) exhibited tolerance to Cr (VI) ions up to 1110 mg/L which is equivalent to 21.3 mM, higher levels than those shown in this study. On the other hand, the 5 strains are tolerant to 10 mM (equivalent to 2072 ppm) of Pb, a level higher than the 800 ppm of *Pseudomonas aeruginosa* BC15 reported by Raja et al. (2006). The results suggest that these strains showed good potential for heavy metal bioremediation applications. Thus, strains S3C1, S1C3, S3C5, S2C13 and S3C14 were selected for molecular characterization.

Table 2. Metal Minimum inhibitory concentration (MIC) of bacterial isolates from discontinuous plantation soil sediment

Bacterial isolates	**Minimum Inhibitory Concentration**					
	Zinc (Zn^{2+})		**Lead (Pb^{2+})**		**Chromium (Cr^{6+})**	
	mM	**mg/L (ppm)**	**mM**	**mg/L (ppm)**	**mM**	**mg/L (ppm)**
E. coli	10	653.8	10	2072	1	52
S3C1	20	1307.6	10	2072	3	156
S1C3	20	1307.6	10	2072	3	156
S1C4b’	10	653.8	10	2072	1	52
S3C5	20	1307.6	10	2072	3	156
S3C8b	10	653.8	10	2072	3	156
S2C10	10	653.8	10	2072	3	156
S2C13	20	1307.6	10	2072	3	156
S3C14	20	1307.6	10	2072	3	156
S1C15	10	653.8	10	2072	3	156

Molecular characterisation by 16S DNA sequencing

The 16S rRNA sequences of the bacterial isolates S3C1, S1C3, S3C5, S2C13, and S3C14 were isolated and amplified through PCR. The PCR products (Fig. 1) were sequenced and the sequencing result was used to generate a phylogenetic tree. From the phylogenetic tree (Fig. 2), the strain S3C1, S1C3, S3C5, S2C13, and S3C14 were in the same clade as other *Bacillus* spp. which is separate from a *Clostridium* sp. Therefore, the five bacterial strains were confirmed to belong to *Bacillus* spp. The result is consistent with the gram-staining result as *Bacillus* spp. are Gram-positive, rod-shaped bacteria. However, due to the high similarities between the 16S rRNA gene sequences among different *Bacillus* spp., a polyphasic approach is necessary for species identification. Biochemical characterisation and molecular characterisation using alternate gene targets (rpoB, vrrA and 16S-23S spacer regions) can be performed in combination for this purpose Blackwood et al. (2004). Alternatively, whole-genome sequencing can be performed to provide a more specific identification.

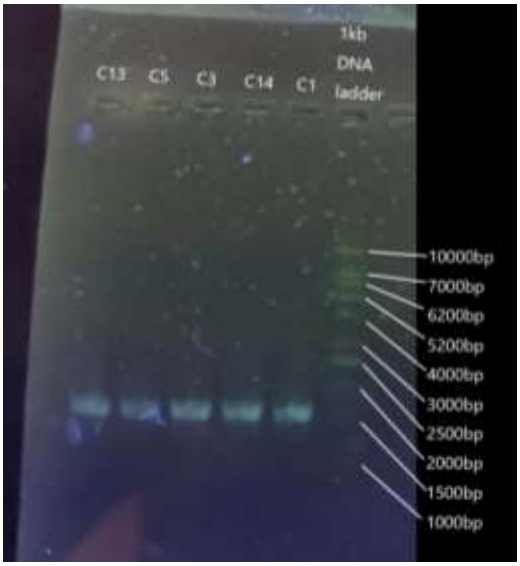

Fig. 1. Amplification of 16S rRNA gene. Lanes M: VC 1 kb ladder (Vivantis, Malaysia). Strains: S3C1 (C1), S1C3 (C3), S3C5 (C5), S2C13 (C13), and S3C14 (C14)..

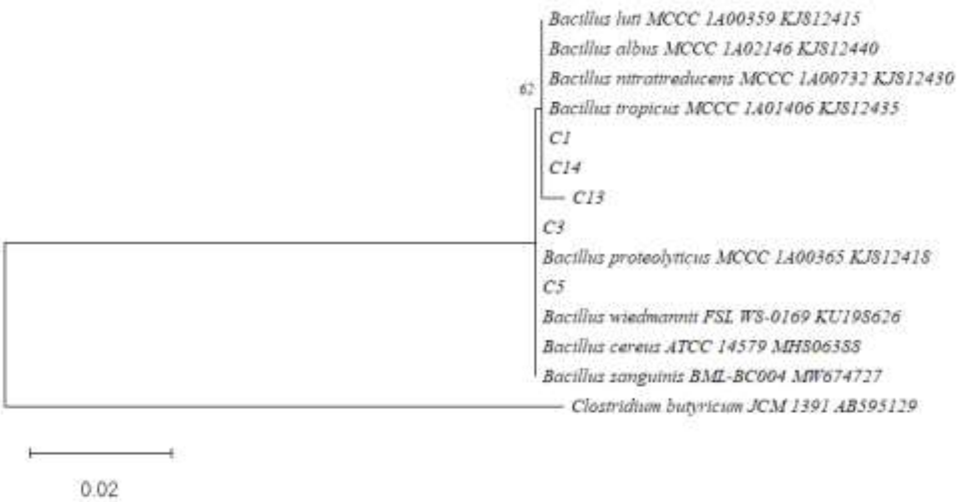

Fig. 2. Neighbour-joining distance dendrogram of the 16S rDNA sequences. Strains S3C1 (C1), S1C3 (C3), S3C5 (C5), S2C13 (C13), and S3C14 (C14). Scale bar, 0.02= 2% dissimilarity.

Bacillus spp. has been widely applied in the bioremediation of heavy metals pollution. *Bacillus circulans* EB1 isolated from the contaminated soil sample by Yilmaz (2003) was applied for metal remediation in a simple bioreactor or in-situ bioremediation. Its zinc biosorption capacity was up to 22 mg/L and is capable to remove a significant amount of zinc when it is actively growing. The MIC of the S3C1, S1C3, S3C5, S2C13, and S3C14 against zinc was found to be 20 mM, which is comparable to the MIC value of 22 mM observed for zinc in *Bacillus criculans* EB1. Pandey & Shirvastava (2018) reported that *Bacillus* sp. JMM-4 isolated from soil sample was found able to reduce lead levels in lead-contaminated soil and therefore was applied for lead removal from contaminated soil. *Bacillus cereus* isolated from the treated tannery effluent by Singh et al. (2013) showed a maximum Cr (VI) reduction of up to 72% at an initial Cr (VI) concentration of 1000 ug/ml. It is applied in the bioremediation of chromium-contaminated tannery wastes. Therefore, the *Bacillus* app. isolated from this study could be further studied for their potential in heavy metal bioremediation.

CONCLUSION

Five bacterial trains that confer high tolerance to Cd, Cu and Zn have been isolated from abandoned plantation soil and identified. In future work, the mechanisms of heavy metals tolerance can be explored to gain insight into how these bacteria contribute to the detoxification of pollutants in the environment. Further, these strains can be studied for their potential in heavy metal bioremediation. Gaining more knowledge about how they work will formulate appropriate bioremediation strategies.

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002-048

THE USE OF COMPOST AS A PEAT BLOCK MEDIA COMPONENT FOR THE PRODUCTION OF QUALITY *Capsicum annuum* L. SEEDLING

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ABSTRACT

The quality of the seedlings and future crop productivity are directly and significantly impacted by the growing medium, making it one of the fundamental needs for obtaining the highest yield and profit. Peat moss is commonly used as a basic medium for planting seeds in nurseries because it is easy to get and supplements the soil to make the medium more porous and provide an appropriate supply of nutrients for the seedlings. However, challenges with the supply chain and product shortages began in 2020, and severe peat moss shortages have persisted to the present. Incorporating compost in peat blocks (free standing compress media) could reduce peat use and promote new markets for this product. Hence, a study was conducted to evaluate the effect of green waste compost (GWC) as a component of peat block on the growth of Capsicum annuum seedlings. Five combinations were used, i.e., T1: peat moss (Control), T2: peat moss: compost GL (1:4), T3: peat moss: compost GL (2:3), T4: peat moss: compost SS (1:4) and T5: peat moss: compost SS (2:3). A complete randomized design with four replications was used in this experiment. The germination percentage (94%), number of leaves (11), stem diameter (2.1 cm), root length (16.2 cm), leaf fresh (3.31 g), and dry weight (0.45 g) of *Capsicum annuum* seedlings grown in 60 and 80% compost SS were comparable to those grown in peat moss (control). These findings suggest that compost SS is a feasible alternative to peat for growing *Capsicum annuum* seedlings.

Keywords: Compost, Peat Moss, Peat Block, *Capsicum annuum* Seedling.

INTRODUCTION

Capsicum annuum L., known as chilli is an economic crop from the Solanaceae family. It has been used in many different cuisines worldwide, especially in Asian countries like Bangladesh, Malaysia, and India (Singh et al., 2021) for its hot flavour. Chilli is rich in vitamin C as well as other nutrients, including calcium, magnesium, folate, potassium, thiamin, iron, copper, and other vitamins like A, B6 and K. Chilli are known for their hot flavour and numerous health advantages, primarily because of the bioactive component capsaicin (Swapan et al., 2017).

In Malaysia, the cultivation of chilli has been done using the conventional method and fertigation system (Yaseer et. al. 2016). According to the Department of Statistics Malaysia, local chilli production cannot meet market demand due to economic and cultural value. Between 2016 and 2020, Malaysia imported 66,295 metric tonnes of chillies, an increase of 49,069 metric tonnes (Department of Statistics Malaysia [DOSM], 2021). With the growing demand from consumers for quality chilli, better cultivation techniques are required, especially in the early phases of plant growth. The use of suitable growing media or substrates for the sowing of seeds directly affects the germination, development and functional rooting system.

Peat is the most commonly used substrate component of growing media for seed germination and cultivation of horticultural crops. Peat has the ability to hold water and create an ideal environment for seed germination (Nurgul et al., 2018). This is due to several of its favourable chemical characteristics, such as its high cation exchange capacity (CEC), as well as its high porosity and water holding capacity (WHC), slow degradation ratio, and low bulk density (Bohlin and Holmberg, 2004). However, the use of peat in horticulture has grown over the past few decades, which has led to an increase in expenses and raised concerns about the material's availability in the near future because of environmental constraints (Fascella, 2015). This has led to a growing interest in finding alternative, more sustainable media components.

Compost, derived from the controlled aerobic decomposition of organic materials, presents a viable alternative to peat. It is rich in organic matter and nutrients, promotes beneficial microbial activity, and improves soil structure (Tahseen et al., 2020). Moreover, using compost in seedling production aligns with sustainable agricultural practices by recycling organic waste and reducing dependence on non-renewable resources. This study explores the feasibility and effectiveness of incorporating compost into peat block media for the production of quality *Capsicum annuum* L. seedlings. The objectives are to assess the growth performance and quality of chilli seedlings grown in media containing various proportions of compost compared to traditional peat-based media. By evaluating parameters such as seedling height, biomass, root development, and overall vigour, this research aims to determine the optimal compost-to-peat ratio that maximizes seedling quality while minimizing environmental impact.

MATERIALS AND METHODS

Trial set-up and media preparations

The experiments were conducted in a nursery at the Horticulture Research Centre. The peat block method was used in this experiment, whereby peat block is a planting medium that has been compacted and shaped into a specific form, such as a cube. It functions as both a container for planting and a medium for seed germination and seedling growth, eliminating the need for trays and plastic pots. Peat block media were followed by treatment combinations.

Treatments

Five medium combinations were used, i.e., T1: peat moss (Control), T2: peat moss: compost GL (1:4), T3: peat moss: compost GL (2:3), T4: peat moss: compost SS (1:4) and T5: peat moss: compost SS (2:3). Compost GL derived from green waste while compost SS derived from oil palm waste. The physical properties of these growing media are shown in Table 1. Following the experiment, the following data were recorded after 30 days: the physical properties of the growing medium, plant growth, including height, stem diameter, canopy, leaf number and spad value. After taking it from the growing medium and washing off the loose medium, the plant root measurement was analysed using a WinRHIZO root scanner. After that, the plant samples were oven-dried for 72 hours at 60°C to determine their dry weight.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using SAS software version 9.4. Data were processed by two-way analysis of variance and mean separations were performed through the least significant difference (LSD) test at the 5% level of significance.

RESULTS AND DISCUSSION

Physical properties of the growing media

Table 1 shows the main physical properties of the different growing media used in this study. Bilderback et al., 2005 defined an ideal substrate as having 50–85% porosity, 45–65% water capacity, 10–30 air capacity and 0.19–0.7 g cm^{-3} bulk density. Most of the media used in the study fall within the definition. However, low air capacity was found for all treatments except T5. Consequently, media will drain fast, imposing drought stress on the plant, demanding frequent irrigation.

Table 1 Physical properties of the growing media

Treatments	Total porosity (%)	Water capacity (%)	Air capacity (%)	Bulk density (g cm^{-3})
T1	60.72	55.83	4.89	0.26
T2	59.57	54.96	4.60	0.29
T3	52.09	47.48	4.60	0.36
T4	67.34	62.73	4.60	0.28
T5	68.20	55.54	12.66	0.26

Seedling growth

The effect of different growing media on the seed germination percentage of chilli seedlings is shown in Figure 1. The germination percentage (94%) of *Capsicum annuum* seedlings grown in T3: peat moss: compost GL (2:3), T4: peat moss: compost SS (1:4) and T5: peat moss: compost SS (2:3) were comparable to those grown in peat moss (control) after seven days. The maximum germination (100%) was recorded after 13 days of sowing for all the treatments.

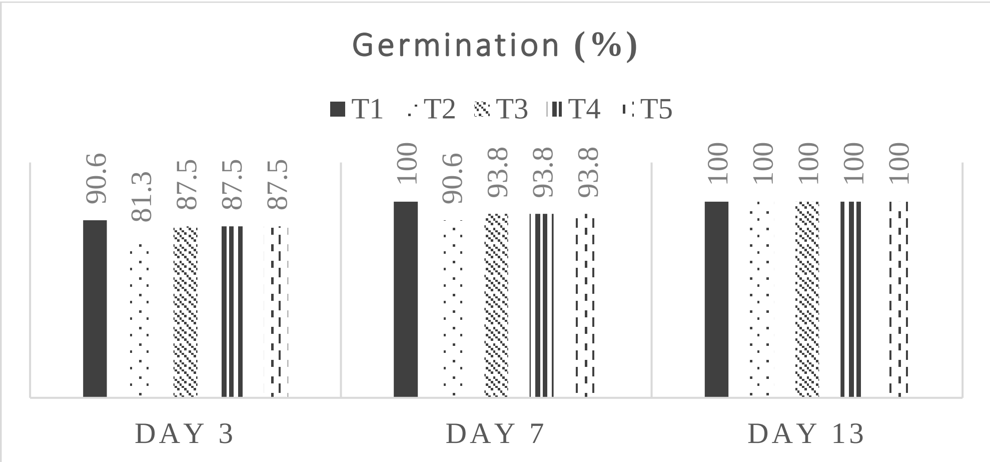

Figure 1 Effect of different growing media on seed germination percentage of chilli seedlings

Effect of different growing media on seedling yield and root-to-shoot ratio

It is shown in Table 2 that different growing media affect the fresh and dry weight of the shoot and root of chilli seedlings 30 days after sowing. The minimum fresh weight of the shoot (0.43g) and root (0.16g), dry weight of the shoot (0.06g) and root (0.03g) were recorded under treatment T2 (peat moss: compost GL, 1:4). Meanwhile, the maximum fresh weight of the shoot (3.31g) and root (0.71g), dry weight of the shoot (0.45g) and root (0.34g) were recorded under treatment T5 (peat moss: compost SS, 2:3). As for the root-to-shoot ratio of chilli seedlings, a minimum and significantly lower root-to-shoot ratio (0.06 and 0.07) was observed in treatments T2 and T3 over other treatments. However, a higher root-to-shoot ratio (0.28 and 0.34) was found in treatments T4 i.e., peat moss + compost SS (1:4) and T5 i.e., peat moss + compost SS (2:3) which was found to be statistically on par with T1 i.e., peat moss.

Table 2 Effect of different growing media on yield and root to shoot ratio of chilli seedlings

Treatments	Fresh Weight (g/plant)		Dry Weight (g/plant)		Root-to-Shoot Ratio
	Shoot	Root	Shoot	Root	
T1	2.47 b	0.68 ab	0.26 bc	0.08 b	0.30 a
T2	0.43 c	0.16 c	0.06 d	0.03 c	0.06 b
T3	0.97 c	0.27 bc	0.14 cd	0.07 c	0.07 b
T4	2.64 ab	0.71 a	0.34 ab	0.09 b	0.28 a

T5	3.31 a	0.71 a	0.45 a	0.15 a	0.34 a
$LSD_{0.05}$	***	*	**	**	*

Within each column. ns=no statistically significant difference; means followed by different letter are significantly different according to the LSD test at *p≤0.05, **p≤0.01 and ***p≤0.001

Effect of different growing media on growth parameters

Table 3 showed that plant height, leaf number, plant canopy and spad value were significantly influenced by the use of different growing media 30 days after the sowing of chilli seedlings. The maximum plant height (16.03, 16.21 and 14.69 cm, respectively), leaf number (11, 11 and 10, respectively), plant canopy (14.26, 14.99 and 13.42 cm, respectively) and spad value (36.64, 36 and 31.99, respectively) were recorded with treatments T4 i.e., peat moss + compost SS (1:4) and T5 i.e., peat moss + compost SS (2:3) which was found to be statistically at par with T1 i.e., peat moss. Meanwhile, the minimum plant height (7.35 and 8.39 cm, respectively), leaf number (6 and 7, respectively), plant canopy (8.48 and 8.72 cm, respectively) and spad value (25.23 and 27.63, respectively) were recorded with treatments T2 and T3.

Table 3 Effect of different growing media on seedlings growth

Treatments	Plant Height (cm)	Leaf Number	Plant Diameter (cm)	Plant canopy (cm)	Spad Value
T1	14.69 a	10.22 a	2.08 ab	13.42 a	31.99 b
T2	7.35 b	6.94 b	1.12 b	8.48 b	25.23 c
T3	8.39 b	7.06 b	1.27 b	8.72 b	27.63 c
T4	16.03 a	11.53 a	2.88 a	14.26 a	36.64 a
T5	16.21 a	11.16 a	2.08 ab	14.99 a	36.00 ab
$LSD_{0.05}$	***	***	**	***	***

Within each column. ns=no statistically significant difference; means followed by different letter are significantly different according to the LSD test at *p≤0.05, **p≤0.01 and ***p≤0.001

Effect of different growing media on root growth

Based on Table 4, chilli seedlings in T5 showed the highest root length (122.30 cm), surface area (23.91 cm^2), average diameter (0.65 mm) and root volume (0.39 cm^3) which is on par with seedlings in T1. Meanwhile, T2 and T3 recorded the lowest values of root length (23.82 and 24.68 cm), surface area (3.48 and 3.14 cm^2) and root volume (0.03 cm^3), respectively. Hence, applying compost derived from oil palm waste has improved root growth. This might be the result of ideal growing conditions for the seedling, especially for healthy root system development in media where less root coiling occurs. In addition, more root growth allowed seedlings to absorb more nutrients, which in turn led to higher leaf growth and increased photosynthesis, which increased the fresh and dry weight of the seedlings (Meena et al., 2017).

Table 4 *Effect of different growing media on the root growth parameters*

Treatments	Root length (cm)	Surface area (cm^2)	Avg Diam (mm)	Root Volume (cm^3)
T1	149.60 a	25.20 a	0.54 ab	0.34 b
T2	23.82 c	3.48 b	0.44 b	0.03 c
T3	24.68 c	3.14 b	0.42 b	0.03 c
T4	115.01 b	28.05 a	0.71 a	0.54 a
T5	122.30 ab	23.91 a	0.65 ab	0.39 ab
$LSD_{0.05}$	**	*	*	*

Within each column. ns=no statistically significant difference; means followed by different letter are significantly different according to the LSD test at *p≤0.05, **p≤0.01 and ***p≤0.001

CONCLUSION

The *Capsicum annuum* seedlings grown in T4 i.e., peat moss + compost SS (1:4) and T5 i.e., peat moss + compost SS (2:3) were comparable to those grown in T1 i.e., peat moss (control). These findings suggest that compost SS is a feasible alternative to peat for growing *Capsicum annuum* seedlings. These treatment combinations were helpful in reducing about 60 to 80% the use of peat moss. The findings of this study could contribute significantly to sustainable horticultural practices, providing growers with practical insights and guidelines for using compost as a peat substitute in seedling production. Ultimately, this research endeavours to support the agricultural industry's shift towards more eco-friendly and resource-efficient cultivation methods, ensuring the long-term viability and health of chilli crops and the environments in which they are grown.

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052-049

EXTRACTION OF CELLULOSE FROM OIL PALM LEAVES

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ABSTRACT

The researchers from worldwide have been investigated and studied on cellulose throughout decades, it is no longer used as the ingredients for paper and fibres manufacturing. Nowadays, it is widely used in different fields such as veterinary food, materials for clothing, cosmetics, pharmaceutical and biomedical. Oil palm produced a huge number of agricultural wastes, of which the most common wastes are empty fruit bunch (EFB), oil palm trunk (OPT) and palm oil milled effluence (POME) which always capture attention of the researchers in their studies for various applications. However, there are still lack of studies on extraction of cellulose from oil palm leaves (OPL). OPL are also abundant wastes as pruning is required every six months for immature trees or once a year for mature trees. In this study, cellulose extracted from the dried leaves of oil palm (*Elaeis guineensis*) which collected from Nilai and Seremban areas. The cellulose is extracted by using bleaching process with acidic and alkaline treatment alternate sequences for 5 cycles. Formic acid and sodium hypochlorite are used to prepared buffer solution in the bleaching process. In each cycle, ratio of 1:20 samples to solution is undergone water bath at 80ºC for 1 hour. Lignin has been successfully removed after the treatment shown by the FTIR screening. The physical structure of the samples become finer under observation of light-emitted microscope. This study showed simple and inexpensive method to extract cellulose. The products provide insight further studies of nanocellulose which nanocellulose are having high potential for application in various fields.

Keywords: Bleaching, Lignin, Nanocellulose, Pruning.

1.0 INTRODUCTION

Cellulose refers to polysaccharide that found in the cell walls of plants. In past, its traditional applications were focusing in paper and fiber manufacturing, the ancient Egyptians, Chinese, and Greeks developed methods to produce paper from plant fibre and cellulose-rich materials, such as papyrus and mulberry park (JMC Admin, 2022). Besides that, cellulose fibre that fabricated from cotton, linen and hemp have spun into yarns and woven to produce fabrics for clothing and other textiles. In the ancient civilizations, contractors started to build structures using timber framing, wattle and daub, and other techniques that incorporated cellulose-rich materials such as willow branches ad saplings were used in wattle and daub construction, straw or reeds were incorporated into clay or mud to reinforce the mixture for higher strength of mudbrick, thatches that made from dried grass or reeds were used as roofing materials (Shika et al., 2015). Cellulose in the form of dietary food also has been consumed by human since prehistoric time to promote digestive health and satiety (Joanne, 2013).

Cellulose has indeed been a subject of extensive research and development beyond its traditional usages. Nowadays, the application of cellulose which are derived from various biomasses and agricultural wastes explored various innovative studies such as biocomposites. For instance, cellulose combined with polymers like PLA (polylactic acid) or PHB (polyhydroxybutyrate) to create biodegradable and sustainable materials for packaging either food or non-food industries for longer keeping period, higher strength and good water vapour barrier (Kalpani et al., 2023). In automotive industries, natural fibres that are commonly used as reinforcing materials often depends on source proximity. In construction, cellulose incorporated in polymer to make biopolymer materials for cladding purposes. Cellulose can also be chemically modified to produce cellulose-derivative biopolymers, such as cellulose acetate and cellulose nitrate, with desirable properties for cladding and various applications. Nevertheless, cellulose also derivates as adhesive, explosives, thickening agent for food and moisture-proof coating.

Oil palm produced a huge number of agricultural wastes, of which the most common wastes are empty fruit bunch (EFB), oil palm trunk (OPT) and palm oil milled effluence (POME) which always capture attention of the researchers in their studies for various applications. Extraction of cellulose from oil palm primarily aimed at separating the cellulose fibers from the lignin and other components of the palm biomass which includes empty fruit bunches (EFBs), fronds, and trunks. However, there are still lack of studies on extraction of cellulose from oil palm leaves (OPL) or fronds. OPL are also abundant wastes as pruning is required every six months for immature trees or once a year for mature trees.

In this study, cellulose is extracted from the dried leaves of oil palm (*Elaeis guineensis*). These fronds are abundant near areas of Nilai University and oil palm plantation in Kampung Labu, Seremban. The extraction method has been optimized to achieve better quality of cellulose. The samples can be furthered studied to apply in various industries and fields.

2.0 METHODOLOGY

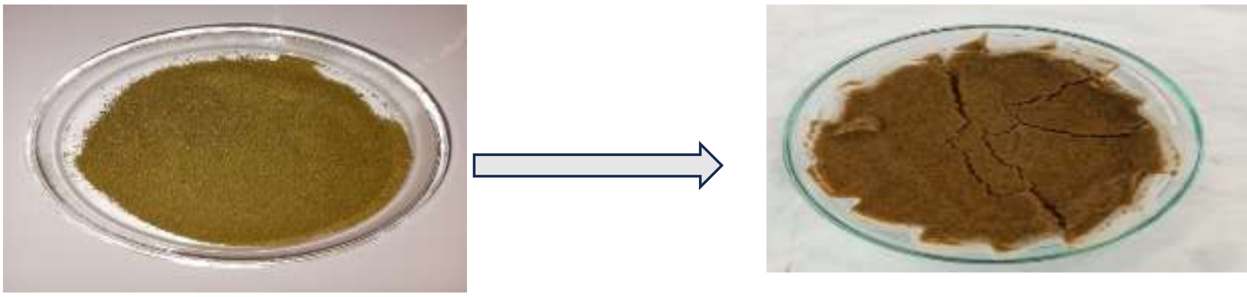
The oil palm leaves collected from areas nearby Nilai University and at Sime Darby Sdn Bhd oil palm plantation. The palm fronds will be teared from the leaves and cut to small pieces (approximately 1cm x 1cm) and washed with water for 10 min to remove dust, dirt, wax, and impurities. The samples were then gone through drying in oven at 70^{o}C overnight to remove moisture content. Then, dried samples were grinded into powder form. A solution of 30ml of chloride acid ((HmbG® Chemicals) and 5ml of glacial acetic acid (CH_3COOH) was added into the conical flask to make a total of 500ml solution with a biomass to acidic solution ratio of 1:7 (wt%) and was allowed to boil for 1, 3 and 6 hour(s) using water bath (Techne Tempunit TU-16D) at 80^{o}C. Subsequently, the flask was allowed to cool down to room temperature and the mixture was filtered through Buchner funnel with the aid of a vacuum pump (PVIV-VAC-PUMP-220). Finally, the biomass bleached and washed using bleaching process that modified from Hatika et al., 2014 for 5 stages, DEDED which D refers to acidic treatment and E refers to alkaline treatment. The buffer solution was prepared which consists of 27g NaOH, 73g of acetic acid and enough amount of distilled water up to total volume solution of 500ml in stage D. Then, 1.7% of sodium chlorite solution was prepared. The ratio of buffer solution: sodium chlorite solution: distilled water is 1:1:1. On the other hand, 2% of NaOH solution was prepared for stage E. In both D and E stages, the ratio between the powder to the solution was 1:20 by weight (wt. %) and conducted at 80°C for 2 h in water bath. After bleaching process, the powder was washed with excessive distilled water to neutralize and dried at 70°C for overnight. The homogenization was done using homogenizer (DAIHAN-brand® HG-15D) at a speed of 1000rpm under the suspension. The characterization of samples was done using optical microscope and Fourier Transmittance Infrared Spectroscopy (FTIR).

3.0 RESUTS AND DISCUSSION

Figure 1 shows the fresh collected oil palm leave and preparation for cleaning and cutting. There was no physical change or no change of colour on the powder after oven-drying and grinding into powder form. Cellulose was extracted by using acidic treatment method as displayed in Figure 2. There was no obvious change between the extracted cellulose from raw material except the colour turned to darker. In this stage, the samples are expected to have high lignin content. The bleaching process is aimed to remove lignin and change the samples to be brighter colour which will be better in further usages.

The physical structures changed obviously after chemical treatment and bleaching process. These were observed under light emission microscope as indicates in Figure 3. From the observation under magnification of 100 times, the size of the powder became smaller and finer. This indicates that the lignin successfully removed after bleaching process with 5 stages of acidic and alkaline alternatively treatment.

Figure 1: Collected oil palm leaves



(a) (b)

Figure 2: (a) Cleaned raw materials that grinded into powder; (b) Chemical treated sample with chlorite acid and glacial acetic acid

The appearance of the powder also became white at stage 5. This result agrees with the previous study of Nasid et al. 2021. The removal of lignin will be benefit to the cellulose as lignin will weaken the mechanical properties of cellulose (Vivek et al., 2022) meanwhile the physical appearance in term of colour will be easier to be controlled when cellulose mix with other materials as additive. In addition, the samples free from lignin and hemicellulose can be transformed to nano-sized through homogenization. Nanosized cellulose has a wide usage in recent advancement technologies (Muhamad et al., 2021).

Refer to FTIP transmission in Figure 4, the peaks which are appear from 1509 – 1609cm^{-1} are corresponding to C=C aromatic skeletal vibration of lignin, thus, it only appears on raw oil palm leave. Nevertheless, raw materials also show C=O stretching from 1701 – 1747 cm^{-1} that indicated content of hemicellulose and lignin whereas the peaks are much shorter in cellulose. From the FTIR transmission as displays in Figure 4, it indicates the removal of lignin between raw material powder and bleached cellulose. This is strengthening the expectation from the observation under light emission microscope that the physical structure of the samples became shorter and finer after removal of lignin and hemicellulose (Vivek et al., 2022).

Figure 3: Images which observed under light emission microscope with x100 magnification for: (a) raw materials in powder form; (b) samples after chemical treatment and (c) samples after bleaching.

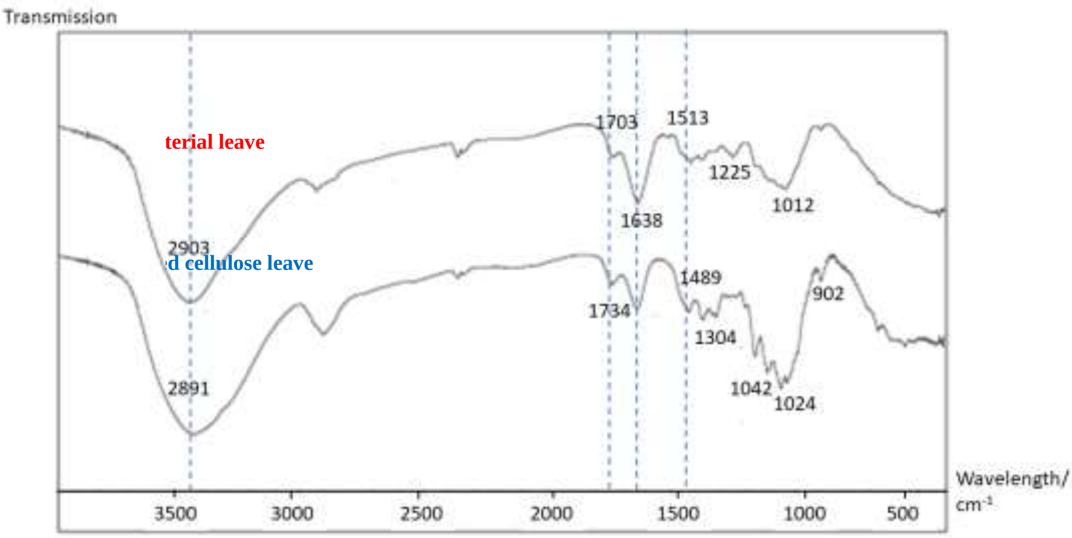


Figure 4: Comparison of FTIR transmission between raw oil palm leave and extracted cellulose from oil palm leave.

4.0 CONLUSION

This study demonstrated a pulping process which cellulose and lignin can be extracted using chemical treatment and bleaching process. The optimum condition for the chemical treatment is 1:7 ratio of solution to samples and water bath at 70ºC for 3 hours and follows by 5 stages with acidic and alkaline treatment alternatively. The method used in this study is simpler when compared to other methods of extraction of cellulose. It can be further study as nanocellulose through homogenizations that can be applied in major fields such as medicine and renewable source of energy.

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049-050

DEVELOPMENT OF CELLULOSE-BASED EDIBLE COATING FROM PINEAPPLE WASTES (CROWN, PEEL AND LEAVES)

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ABSTRACT

Pineapples, the third most significant cultivated tropical fruit, consist of 70% non-edible parts. The amount of waste is rising in proportion to the rising pineapple production. Their high cellulose content enables the production of Carboxymethyl Cellulose (CMC). CMC has a better water solubility and coating forming compared to cellulose. Limited studies have compared different parts of pineapple waste for their potential in producing edible coatings for fruits. Therefore, this study aims to extract cellulose and transformed into CMC-based edible coatings from local pineapple waste, specifically leaves (PL), peel (PP), and crown (PC). Also, to compare the effectiveness of the prepared coating. CMC was successfully extracted from all three pineapple parts, with PL having the highest yield (107.88%) due to higher cellulose content (19.92%). Mandarin oranges coated with PLCMC, PPCMC and PCCMC were observed for weight loss, pH, ascorbic acid and titratable acid concentration over 28 days of storage periods. Weight loss increased significantly ($p<0.05$) throughout the storage periods with PCCMC observed the lowest weight loss (34.99%). pH of all have no significant difference ($p>0.05$) across the storage periods. PCCMC being the best among other treatments group has no significant decrease on its ascorbic acid concentration and titrability acid in mandarin orange which prolong the shelf life which highlighted PCCMC being the best treatment group than PLCMC and PPCMC. By utilizing pineapple waste to produce cellulose for the edible coating reduces fruit waste and also maintain the quality and freshness of mandarin oranges.

Keywords: Pineapple Wastes, Carboxymethyl Cellulose (CMC), Edible Coating.

INTRODUCTION

Fruit waste poses a considerable environmental challenge in Malaysia which lead to a detrimental impact on ecosystems and waste management systems. Manikam (2020) reported an upward trend in the generation of municipal solid waste (MSW) in the country, with projections estimating a rise from 7.34 million tons in 2005 to an estimated 10.9 million tons by 2020. Specifically, around 60% of waste is from food waste which includes fruits and vegetables. The high consumption of fruits and vegetables, coupled with the industrial processing involved in producing canned fruits, fruit juices, and flavorings, contribute to the accumulation of fruit waste residues (peels, seeds, and pulp). These residues have been recognized as valuable sources of nutrients, including dietary fibers (cellulose), vitamins, minerals, as well as bioactive compounds (Pathania & Kaur, 2022). Therefore, there is a rising awareness of the potential to transform fruit waste into valuable products or applications, to utilize these fruit waste more effectively.

Edible coatings are typically made from polysaccharides, proteins, and lipid-based raw materials. Edible coatings serve as a semi-permeable physical protective barrier between the food and its surrounding environment to reduce moisture loss, and weight loss in the food, thereby maintaining its freshness and quality (Adetunji et al., 2013). This extension of shelf life allows for longer storage and transportation times, enabling food to reach consumers in a fresher and safer condition. Cellulose is one of the popular polysaccharides that are commonly used in the production of these edible coatings (Kocira et al., 2021). The native cellulose exhibits a very low water solubility property and is less suitable as a material for forming edible coatings. However, carboxymethyl cellulose (CMC), among various chemically modified forms of cellulose, exhibits favourable properties for coating applications. CMC offers improved water solubility and coating-forming capabilities, and is generally odourless and tasteless, flexible, transparent, and non-toxic which makes it a prime candidate for developing edible coatings (Dhall, 2013; Owusu, 2022). CMC can be produced via fruit waste such as papaya peels (Rachtanapun et al., 2007), durian rinds (Rachtanapun et al., 2011), and pineapple crown (Bhattacharjee et al., 2020). In light of this, edible coatings made of cellulose may help achieve sustainability goals by minimising the negative effects of food loss on the environment, maximising the use of available resources, and reducing food waste. These align with the Sustainable Development Goal (SDG), which calls for responsible production, consumption, and handling of food waste.

Pineapple (*Ananas comosus*) is a tropical/subtropical fruit and is one of the top commodity production quantities in the world. Pineapples are extensively cultivated in various tropical regions including Malaysia, Thailand, Philippines, Costa Rica, South Africa, and China (Rico et al., 2020). This is due to their high nutritional value, rich in essential vitamins, minerals, dietary fibre, and enzyme with digestive and anti-inflammatory properties (Chaudhary et al., 2019). The composition typically consists of peels (29–42%), core (9–20%), and little quantities of crown and stem (Prado & Spinace, 2019; Rico et al., 2020). Previous studies have indicated that pineapple holds promise as a potential substrate to be incorporated into making edible coating (Banerjee et al., 2018; Rodsamran & Sothornvit, 2019). Few studies recorded that the crown, peel, and leaves are rich sources of cellulose containing 56%, 32.4%, and 41.15% of cellulose respectively, from which CMC can be derived (Banerjee et al., 2017; Dahunsi, 2019).

While previous research has focused on utilising pineapple crowns for the production of edible coatings, there is a lack of studies comparing different parts of pineapple waste for their potential to produce edible coatings for fruits. Specifically, few researches were conducted on developing CMC edible coatings from local pineapple peel and leaves from Malaysia. Therefore, this study will determine the feasibility of the extracted CMC from the crown, peels, and leaves of locally grown pineapples. The main objective of this study was to prepare CMC based edible food coating from pineapple waste (crown, peel, and leaves). While the sub-objectives were to (i) prepare CMC from difference parts of plants and (ii) compare the efficacy of the various CMC-based edible coating to prolong the shelf life of mandarin oranges.

MATERIALS AND METHODS

Extraction of Cellulose from Pineapple Waste (Crown, Peel and Leaves)

The extraction of cellulose was done using method by Bhattacharjee et al., (2020) with some modification. The pineapple wastes (PW) including crown, peel and leaves, were cut into small pieces and washed with running tap water followed by distilled water to remove the dirt. Then, the samples were dried in an oven at 60°C until a constant weight was reached. After that, the pineapple samples were ground into powder with a grinder and preserved in an airtight container for further analysis.

Delignification

Delignification process was done by soaking 5 g of the dried powder of pineapple wastes in 100 mL distilled water at 80°C for 2 hours. The water-soluble impurities were removed from the pineapple waste through filtration with a suitable cotton cloth filter. The residue was collected and treated with sodium chlorite ($NaClO_2$) solution (7.5% w/v, pH = 3.8–4) at 75°C for 2 hours with intermittent mixing. The suspension of $NaClO_2$ was filtered again to collect the residue. The residue was washed with ethanol (95% v/v) and distilled water alternately for five times each to remove the soluble salts from the treated pineapple waste. The washed residue was then dried in oven at 50°C until a constant weight was reached.

Alkalization and purification

The delignified dried residue of pineapple wastes was treated with 10% w/v KOH in the ratio of 1:10 at 27°C for 8 hours. After the alkalization treatment, the final residue of pineapple waste was collected through filtration and washed with ethanol 95% (v/v) and distilled water several times until the filtrate turns neutral. The resulting pineapple wastes cellulose (PWC) was dried in oven at 50°C for until a constant weight was reached.

Synthesis of CMC

For the transformation of CMC, approximately 2.5g of each PWC was treated with 50 mL isopropanol (100% v/v) and 5 mL sodium hydroxide (NaOH) (30% w/v) at 27°C for 1 hour under constant stirring at 100 rpm. The alkaline PWC mixture was then treated with 3 g of chloroacetic acid for 90 minutes at 27°C and 100 rpm in an incubated shaker. Then, the solution mixture was heated in an oven at 45°C for 3 hours. The solution was filtered through a suitable cotton cloth and the residue was collected. The collected residue was then soaked in 300 mL of absolute methanol overnight to remove undesired salts. The final pH of the solution was adjusted to 7 with glacial acetic acid.

Residue was washed with ethanol and methanol respectively to remove remaining undesired salts. The final residue was dried completely at 60°C in an oven and referred as PWCMC. Particularly, pineapple crown CMC (PCCMC); pineapple peel CMC (PPCMC) and pineapple leaves CMC (PLCMC) in subsequent experiments.

Efficacy of Pineapple Waste CMC

Formulation of pineapple waste CMC

The five different edible coating formulations reported by Bhattacharjee et al., (2020) was followed with slight modification. The treatment groups including positive and negative control were prepared by using the prepared CMC (PCCMC, PPCMC and PLCMC), Commercial Carboxymethyl Cellulose as positive control (PC) and negative control (NC). The coating experiment was based on the procedure by Forato et al. (2016). First, 0.5 g of respective CMCs was dissolved in 25 mL distilled water in 100 mL conical flask to form a clear solution. Next, glycerol (2% in v/v), tween 80 (~ 0.1% in v/v) and essential oils (4% in v/v) were subsequently added and stirred at 9500 rpm at 70°C until a homogeneous solution was obtained. The mandarin oranges were bought from the nearby market and were chosen according to similar size, shape, and appearance.

Application of pineapple waste CMC on fruits (mandarin oranges)

The prepared coating solutions were cooled to 25°C and applied over the mandarin oranges using dipping method. The coated mandarin oranges were kept on a basket and were analyzed in term of their weight loss, pH changed, ascorbic acid and titratable acid after the coating dried up at ambient condition (25°C, 70%–80% humidity). Total 15 coated mandarin oranges from each treatment were kept over 28 days at 25°C.

Chemical Analysis of Coated Mandarin Orange

Pulps from coated mandarins were homogenized in a grinder and then centrifuged for 20 minutes for 40,000 rpm. The supernatant phase was collected for pH, ascorbic acid and titratable acidity. Weight loss, pH, ascorbic acid and titratable acidity were determined at the beginning (Day 3) and intervals of 7 days during the experiments. The weight loss of coated mandarin oranges was determined by weighing the uncoated mandarin oranges on day zero and weighing the coated mandarin oranges on Day 3, 7, 14 and 28. A digital pH meter (Hanna instrument pH211) was used to measure the pH of orange juice samples and was calibrated using commercial buffer solutions. The vitamin C content of the extract was measured using the method of Papoutsis et al., (2016). The absorbance was measured at 695nm using a spectrophotometer. Meanwhile, the total titratable acid was determined by using the method of Toğrul and Arslan (2004) withslight modification. Briefly, 0.1 M NaOH was titrated until the colour changes from light orange to pink. Results were expressed as g citric acid/100 g fruit.

RESULT AND DISCUSSION

Extraction of Cellulose from Pineapple Waste

Three types of pineapple wastes (PC, PP and PL) were used to extract the cellulose. The samples were designated as Pineapple Crown Cellulose (PCC), Pineapple Peel Cellulose (PPC) and Pineapple Leaves Cellulose (PLC). The dark colour (brownish) of the PW powder indicated the presence of lignin, which was then removed through sodium chlorite ($NaClO_2$) treatment. Delignification process was important in extracting cellulose by breaking down the molecules with chromatographic groups in lignin. This procedure not only removes the impurities but also whitens the pulp (Agarwal et al., 2020). Delignified PW was then treated with potassium hydroxide (KOH) to remove hemicellulose to produce pure cellulose. PWC appeared white in colour after drying in its final form.

Percentage yield of PWC

The percentage yield of PWC obtained are presented in Table 1. The results indicated that the percentage yield of PCC was 19.37%, slightly lower than the reported 28.72% in the study by Bhattacharjee et al. (2020). The result value can be affected by various factors such as geographical variation, cultivation practice and varieties can contribute to difference in cellulose yield. Additionally, the percentage yield from PPC was found to be 13.62% whereas PLC was 19.92%. The order of the cellulose content was arranged as PPC < PCC < PLC. The findings were aligned with the most trend observed in previous studies where the leaves tend to have the highest percentage of cellulose compared with other parts of PW (Bhattacharjee et al., 2020; Dahunsi, 2019; Pereira et al., 2022).

Table 1. Percentage yield of PWC from differences parts of pineapple.

Pineapple parts	Mean ± SD (g)	% yield of PWC
PCC	0.581±0.052	19.37%
PPC	0.681±0.137	13.62%
PLC	0.996±0.055	19.92%

Percentage yield of PWCMC

The percentage yield of PWCMC are shown in Table 2. The percentage yield of PLCMC, PPCMC and PCCMC were 107.88%, 104.45% and 106.53% respectively. The yield exceeding 100% was due to the overall increase in the molecular weight of the anhydrous glucose resulting from the substitution of carboxymethyl group derived from chloroacetic acid (Bhattacharjee et al. 2020).

Table 2. Percentage yield of PWCMC from difference parts of pineapple.

Pineapple parts	Weight of PWC used (g)	After carboxylation (g)	% yield of PWCMC
PLCMC	2.5	2.697	107.88%
PPCMC	2.0	2.089	104.45%
PCCMC	1.5	1.598	106.53%

Chemical analysis of PWCMC-coated mandarin oranges

Mandarin orange, are perishable and prone to spoilage due to its abundance during peak harvesting seasons and rapid ripening. The rate of weight loss increased significantly ($p<0.05$) throughout the storage period for all treatment (Figure 1a). Generally, all the coated samples has lower weight loss compared to the uncounted samples. From all the coated samples, the lowest weight loss was observed in PCCMC (34.99%) after 28 days of room temperature storage. However, there was no significant difference ($p>0.05$) between the weight loss of PCCMC, PPCMC, PLMC and PC. As expected, NC had the highest weight loss (67.70%) over 28 days of the storage periods. As the fruit further ripen, the respiration rate will increase thus increasing the weight loss (Wang et al., 1993). By edible coating from PW, it could efficiently reduce the weight loss, thus slowing down the respiration process and delay spoilage.

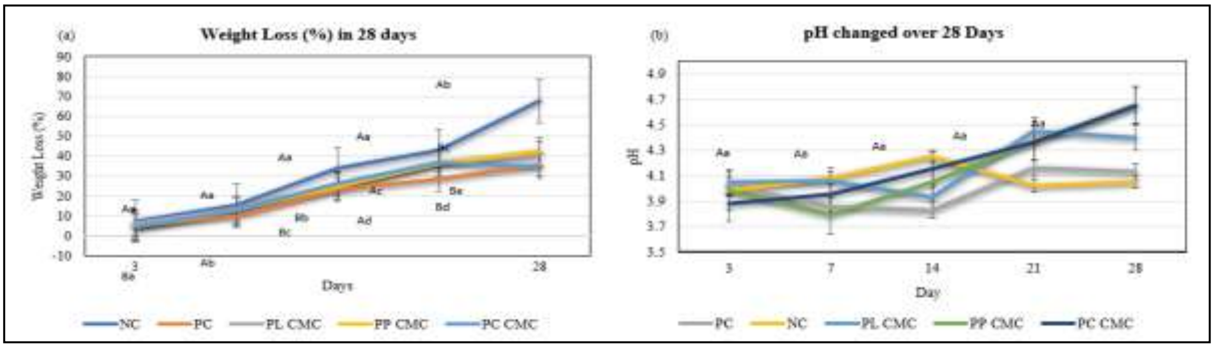

Figure 1. (a) Weight loss in percentage (%) over 28 days. (b) pH observation over 28 days. The upper-case letter refers compared the value across the group while the lower-case letter compared the value within the same group.

There is no significant difference ($p>0.05$) of pH across difference treatment group. The pH of mandarin oranges of all treatment groups ranged from 3.79 to 4.65 throughout the storage periods (Figure 1b). The increase of pH also indicates the loss of organic acid (ascorbic acid) in mandarin oranges. As the mandarin oranges overripe, the pH can increase significantly. The increase of pH can be linked to the decrease in the concentration of ascorbic acid and acid titratability (Ijaz et al., 2017). When the edible coating slows down the rate of respiration, it also slows down the rate of organic acid break down which lead to a slower rise in pH.

Meanwhile, the ascorbic acid (AA) content is related to the antioxidant effect of the fruit. As Figure 2a shows, PC (197.10 ug/mL) has the highest peak of increase of AA concentration at day 14 followed by NC (164.24 ug/mL) and PLCMC (146.55 ug/mL). Fruits can experience oxidative stress that due to factors such as mechanical damage or potential microbial spoilage, leading to an increase in reactive oxygen species (ROS) production. In response, fruits may increase the production of antioxidants such as ascorbic acid as a defence mechanism to mitigate damage caused by ROS induced oxidation stress. Fruits with high levels of ascorbic acid can effectively scavenge excessive ROS generated during stress thus enhance the tolerance to oxidative stress (Venkatesh & Park, 2014). Therefore, an increase in AA content over time could be a response to oxidative stress experienced by the fruit during storage. This increase in AA helps to protect the fruit's cells from oxidative damage and maintain their quality. Ascorbic acid is a potent antioxidant that helps to neutralize harmful free radicals generated during oxidative stress (Yimcharoen et al., 2019). However, there were a decrease of PC (123.60 ug/mL), NC (110.25 ug/mL) and PLCMC (101.24 ug/mL) after day 21 could be due to the further degradation of AA. The PLCMC remain the lowest AA concentration at day 3 to day 28, although the AA has an increase at day 14. PPCMC and PCCMC did not show significant decrease in AA concentration which implying that the edible coatings were effective as a physical barrier which lowered the oxygen permeability, in turn reduced the loss of AA (Freire et al., 2005). The present findings are consistent with the findings of Oluwaseun et al. (2013), which noted that coating carrots had a beneficial effect on slowing down the amount of AA.

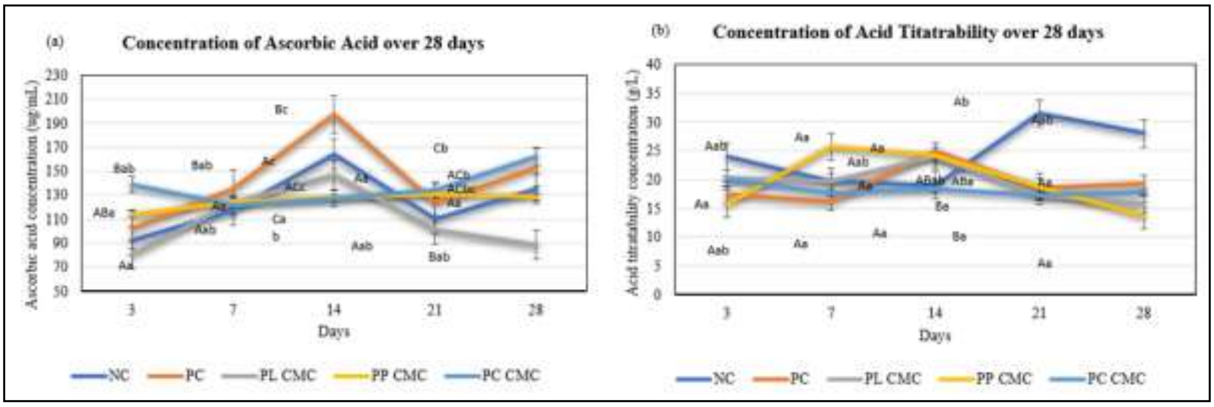

Figure 2. (a) Concentration of ascorbic acid over 28 days. (b) Concentration of titratability over 28 days. The upper-case letter refers compared the value across the group while the lower-case letter compared the value within the same group.

Citric acid content is closely related to TA, as citric acid contributes to the fruit's sour taste. During the storage period, the NC showed a sharp increase in TA at day 21 (31.5 g/L) (Figure 2b). In contrast, all other treatment groups, except for the NC group, did not shown a significant difference ($p>0.05$) in TA throughout the storage period. This explained that there is a positive impact of the edible coating in maintaining the organic acid content and acidity of the fruit. Fruit flavour is greatly influenced by titratable acid (TA), which is a measurement of the total quantity of organic acids contained in a fruit (Tyl & Sadler, 2017). A decrease in organic acid content can result in the reduction of TA and an increase in pH.

CONCLUSION

In conclusion, the cellulose produced from PW has successfully undergo the transformation from cellulose to CMC. The results of chemical analysis shown that the mandarin oranges coated with the PCCMC showed better result in term of the ability to reduced weight loss, maintain the pH level, retained ascorbic acid content and titratability acid compared to other coating treatment over the 28 days of storage periods.

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054-052

GRAFTING AS A STRATEGY TO IMPROVE TOMATO YIELDS IN THE LOWLANDS

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ABSTRACT

The practice of grafting vegetables was first documented in Japan around 1920 and spread to neighboring countries such as Korea, China, and others. According to Lee and Oda (2003), commercial tomato grafting began in the early 1960s and become an important cultivation practice for the tomato crop in many parts of the world. Grafting involves joining together different plant tissues, typically from different individuals or varieties. This technique serves as a tool for investigating a range of traits, including disease resistance, tolerance to environmental stresses, and the development of fruit qualities. Grafting facilitates the breeding process by allowing breeders to focus on developing rootstocks with specific resistance traits. The study was conducted in lowland areas under a protective structure. Tomato var. Baccarat (*Solanum lycopersicum*) was grafted onto brittle (*Solanum melongena*) and pea (*Solanum torvum*) eggplant rootstocks using the splice grafting technique. The use of brittle and pea eggplant rootstocks significantly increased the yield of tomato var. Baccarat in lowlands by 82% and 60%, respectively, without affecting fruit quality (TSS content, vitamin C). The vitamin C content (ascorbic acid) of tomatoes grafted onto brittle eggplant rootstock was significantly higher (28 mg/100 g) than that of non-grafted plants (25 mg/100 g) and comparable to tomatoes grafted onto pea eggplant rootstock (26 mg/100 g). Incorporating brittle and pea eggplants as rootstocks enhanced plant resilience to temperature stress in the lowlands and influenced the overall performance of the crops. Grafting can promote the exchange of signaling molecules, hormones, and nutrients between the scion and rootstock, leading to synergistic effects that enhance overall plant growth and performance.

Keywords: Tomato, Eggplant, Grafting, Rootstock, Scion.

055-053

NOVEL INSIGHTS INTO THE EFFECTS OF *IN VITRO* CONDITIONS ON MICROPROPAGATION AND SUBSEQUENT *EX VITRO* ACCLIMATISATION OF *DENDROBIUM* HYBRID

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ABSTRACT

Micropropagation of *Dendrobiums* has been achieved using various explants on media supplemented with growth regulators. However, the plantlets have different growth stages and growth rates. Due to sudden changes in environmental conditions, these plantlets may not survive after *ex-vitro* transfer. To provide enough materials for acclimatisation in the greenhouse and field conditions, it is crucial to devise a method to form plantlets *in vitro* that are morphologically uniform with well-developed leaves and established root systems. With its significant findings, the present study compared the effects of various supplements and light sources on plantlets during *in vitro* cultivation and *ex-vitro* acclimatisation. Young seedlings of *Dendrobium* cv Sonia, approximately 0.5 to 1.0 cm, were cultured onto ½ MS medium supplemented with BAP, NAA, 10% (v/v) coconut water, 10% mashed banana, and 10% (v/v) beetroot juice. The cultures were kept in the culture room and the greenhouse for 16 weeks. Among the treatments, the medium supplemented with 1.0 mg/L NAA helped increase root length significantly ($P<0.05$) in plantlets. Cultures with controlled temperature and lighting had better PLBs and shoot development in the culture room. In contrast, cultures in the greenhouse exposed to natural day and night conditions had better root development. After two weeks of acclimatisation, plantlets kept in the greenhouse had a higher survival rate and grew well compared to plantlets kept in the culture room.

Keywords: *Dendrobium*, *In Vitro* Culture, Greenhouse, Acclimatisation.

INTRODUCTION

Mass propagation methods have been developed and established to meet the growing demand for orchids, ornamental plants, and many other high-value plants. However, the micropropagation technique is limited in developing countries due to the high cost of the media components and operations. Our research, which has practical implications, showed that using white sugar as a carbon source can reduce 94% of the cost compared to sucrose. Another experiment showed that food-grade agar can be used as a gelling agent in a media to minimise the total cost by 32 to 76%. However, the plantlets have different growth stages and growth rates and do not have well-developed root systems. Due to sudden changes in environmental conditions, these plantlets may not survive after *ex-vitro* transfer.

Our research, with its unique focus on devising a method to form plantlets *in vitro* that are morphologically uniform, with well-developed leaves and established root systems, offers a fresh perspective. The success of the micropropagation method hinges on producing plants through tissue cultures at low costs and with a high survival rate. This urgency and relevance underline the novelty and importance of our research and its potential impact on plant biology.

The present study compared the effects of various supplements and light sources on plantlets during *in vitro* cultivation and *ex-vitro* acclimatisation.

MATERIALS AND METHODS

Plant Materials

Young seedlings of *Dendrobium* cv. Sonia were initiated and maintained. These young seedlings were used to generate the stock cultures as a source of explants. The seedlings were subcultured and maintained on MS (Murashige and Skoog, 1962) media supplemented with 8 g/L agar, 1 g/L activated charcoal, 20 g/L sucrose, 10% (v/v) coconut water and 10% (v/v) mashed banana. The subculturing was repeated every 4-5 weeks to get sufficient stock of explants to initiate the experiments.

Preparation of Extracts

Fresh coconut water was collected one day before media preparation from young coconuts purchased from local stores in the Nilai area to prepare extracts. The water was carefully filtered to remove any impurities from the coconut husk. Fresh bananas were also bought from local stores one day before media preparation. These bananas were sliced into small pieces and mashed before being added to the media. Similarly, fresh beetroots were purchased from local stores one day before media preparation. The beetroots were processed using a food processor to extract the juice, which was then added to the media.

Preparation of Culture Media and Culture Conditions

We used 50ml of 1/2 MS medium for our experiments throughout the study. The medium was supplemented with 30 g/L sucrose, 8 g/L agar, 2 g/L activated charcoal, and different supplements (0.5 mg/L BAP, 0.1 mg/L NAA, 1.0 mg/L NAA, 10% (v/v) coconut water, 10% (v/v) mashed banana, or 10% (v/v) beetroot juice). The medium was adjusted to pH 5.7 ± 0.1 with 1M NaOH or 1M HCl before autoclaving at 121ºC for 15 minutes under 1 kg cm^{-2} pressure. A total of 50 ml autoclaved medium was poured into each glass jar.

All cultures in the culture room were incubated at 25 ± 2ºC under 12/12 (light/dark cycle), and irradiance was provided by white, fluorescent tubes (Philips, Thailand) of 24 $\mu molm^{-2}s^{-2}$. All cultures in the greenhouse were incubated under shaded sunlight of 35 $\mu molm^{-2}s^{-2}$ with 35 ± 2ºC and 30 ± 2ºC day/night temperature.

Effect of Supplements and Culture Conditions

The effect of supplements on the growth and development of *in vitro* cultures was studied by culturing 0.5 grams of 0.5 cm to 1.0 cm young seedlings on 1/2 MS medium supplemented with 30 g/L sucrose, 8 g/L agar, 2 g/L activated charcoal, and different supplements (0.5 mg/L BAP, 0.1 mg/L NAA, 1.0 mg/L NAA, 10% (v/v) coconut water, 10% (v/v) mashed banana, or 10% (v/v) beetroot juice). The cultures were kept at 2 culture conditions to evaluate the effect of different culture conditions on the growth and development of cultures:

[1] controlled conditions in the culture room with 25 ± 2ºC temperature and 12/12 (light/dark cycle) and irradiance provided by white, fluorescent tubes of 24 $\mu molm^{-2}s^{-2}$.
[2] natural condition in the greenhouse with 35 ± 2ºC and shaded sunlight of 35 $\mu molm^{-2}s^{-2}$.

Acclimatisation of Cultures

Twelve cultures from each treatment were subcultured for 1 month before acclimatisation. A total of 5 rooted cultures from each treatment were washed, treated with fungicides (Mancozeb, 25 g/L) and rooting hormone (Spectra rooting powder, 10 g/L), and then transferred in an orchid potting mixture (wood charcoal and coconut husk) for acclimatisation in the greenhouse for 2 weeks. The plantlets were misted with water twice per day. The fertiliser (100 ml/L) was sprayed once per week. All *ex-vitro* experiments were set up in a completely random design.

Assessment of Growth and Statistical Analysis

Fresh weight, number of shoots per culture, number of shoots with roots, number of shoots without roots, height of shoots, number of roots per culture, number of roots per shoots, and length of roots of cultures were recorded after 16 weeks in culture. All *in vitro* experiments were set up in a completely random design. Each treatment consisted of 5 cultures and was replicated four times. The data was presented as mean ± standard error (SE) values of 8 cultures. All data was subjected to Analysis of Variance (ANOVA), and means were compared using Tukey's test at $p < 0.05$.

RESULTS AND DISCUSSION

Through visual observation, there was a mixture of elongated shoots and PLBs in all the cultures that were kept in the culture room, but there were more elongated shoots than PLBs. The cultures in the greenhouse also contained a mixture of elongated shoots and PLBs, but there were more elongated shoots than PLBs through visual observation (Figure 1 and Figure 2). When the cultures in the culture room were compared with those in the greenhouse, the greenhouse cultures had shoots with more extended and broader leaves. However, there were generally fewer shoots and PLBs produced in all the treatments kept in the greenhouse.

Effect of Supplements on Fresh Weight

The growth of *Dendrobium* cv. Sonia plantlets were established on 1/2 semi-solid MS medium that was supplemented with 30 g/L sucrose, 8 g/L agar, 2 g/L activated charcoal, and different supplements (0.5 mg/L BAP, 0.1 mg/L NAA, 1.0 mg/L NAA, 10% (v/v) coconut water, 10% (v/v) mashed banana, or 10% beetroot juice). The results showed that the plantlets multiplied further from an original clump of 0.5 grams of shoots after 16 weeks of cultivation.

The addition of cytokinin (0.5 mg/L BAP), auxin (0.1 – 1.0 mg/L NAA), and complex organic additives (10% coconut water and 10% mashed banana), however, increased the number of PLBs and shoots produced per culture. The mean fresh weight of cultures ranged from 5.31 g to 9.01 g (Table 1). The PLBs accounted for more than 40% of the fresh weight, the shoots without roots for 15 – 27%, and the rooted shoots for 22 – 35% of the fresh weight, suggesting that the treatments produced more PLBs than shoots. The lowest responding cultures were observed on medium containing 10% beetroot juice, while the highest were on medium containing 0.1 mg/L NAA. However, the differences over the control were not significant.

Table 1: Effect of supplements on mean fresh weight of culture, PLBs, shoots without roots and shoots with roots of *Dendrobium* cv. Sonia, after 16 weeks of culture on 1/2 MS medium

Treatments	Supplements	Mean fresh weight of culture (g ± SE)	Mean fresh weight of PLBs (g ± SE) (%)	Mean fresh weight of shoots without roots (g ± SE) (%)	Mean fresh weight of shoots with roots (g ± SE) (%)
T0	Control	7.01 ± 0.77[a]	3.27 ± 0.61[a] (46.65)	1.32 ± 0.34[a] (18.83)	2.42 ± 0.40[a] (34.52)
T1	0.5 mg/L BAP	8.08 ± 1.40[a]	4.36 ± 1.13[a] (53.96)	1.76 ± 0.35[a] (21.78)	1.97 ± 0.32[a] (24.38)
T2	0.1 mg/L NAA	9.01 ± 0.89[a]	5.29 ± 1.02[a] (58.71)	1.71 ± 0.25[a] (18.98)	2.01 ± 0.24[a] (22.31)
T3	1.0 mg/L NAA	7.60 ± 0.98[a]	4.21 ± 0.96[a] (55.39)	1.17 ± 0.20[a] (15.39)	2.22 ± 0.32[a] (29.21)
T4	10% Coconut Water	7.32 ± 1.06[a]	3.69 ± 0.88[a] (50.40)	1.43 ± 0.26[a] (19.54)	2.21 ± 0.31[a] (30.19)
T5	10% Mashed Banana	7.62 ± 1.67[a]	3.67 ± 1.30[a] (50.13)	1.68 ± 0.42[a] (22.05)	2.28 ± 0.33[a] (29.92)
T6	10% Beetroot Juice	5.31 ± 1.08[a]	2.29 ± 0.74[a] (43.12)	1.45 ± 0.32[a] (27.31)	1.57 ± 0.23[a] (29.57)

**Means ± SE followed by the different letters within a column significantly differ at the $P<0.05$ by Tukey's multiple range test. Sixteen cultures were used in each treatment.

Effect of Supplements on Number and Height of Shoots

Shoots with and without roots were carefully separated from the cultures in all treatments. The mean number of shoots per culture is presented in Table 2. Shoot growth and development were observed in the control group after 16 weeks in an *in vitro* culture. Adding cytokinin, auxin, and complex organic additives has slightly increased the mean number of shoots produced in cultures. Likewise, the mean number of rooted shoots in these treatments was higher than in the control. However, NAA at 1.0 mg/L and coconut water at 10% appeared to reduce the mean number of shoots with and without roots. The regenerated shoots have a height of approximately 0.3 to 4.3 cm. Adding 10% mashed banana in the culture medium produced taller shoots.

Table 2: Effect of supplements on the mean number of shoots per culture, number of shoots with roots and number without roots of *Dendrobium* cv. Sonia, after 16 weeks of culture on 1/2 MS medium

Treatments	Supplements	The mean number of shoots with roots	Mean number of shoots without roots	Height of shoots (cm)
T0	Control	20.44 ± 2.79^{a}	23.31 ± 4.12^{a}	0.30 – 2.50
T1	0.5 mg/L BAP	20.25 ± 2.30^{a}	34.38 ± 6.97^{a}	0.30 – 2.40
T2	0.1 mg/L NAA	20.00 ± 2.73^{a}	32.44 ± 4.19^{a}	0.30 – 2.60
T3	1.0 mg/L NAA	18.25 ± 3.07^{a}	18.06 ± 2.51^{a}	0.30 – 3.50
T4	10% Coconut Water	18.25 ± 2.50^{a}	21.81 ± 2.97^{a}	0.30 – 2.60
T5	10% Mashed Banana	22.31 ± 4.53^{a}	29.25 ± 6.68^{a}	0.30 – 4.30
T6	10% Beetroot Juice	19.06 ± 2.97^{a}	26.31 ±5.36^{a}	0.30 – 2.00

**Means ± SE followed by the different letters within a column significantly differ at the P<0.05 by Tukey's multiple range test. Sixteen cultures were used for each treatment.

Effect of Supplements on Number and Length of Roots

After 16 weeks of culture in 1/2 MS basal medium supplemented with 3% sucrose and 2 g/L activated charcoal, rooted shoots produced approximately 2.73 roots with a mean length of 0.85 cm (Table 3). Adding a low concentration of BAP and NAA and 10% organic additives did not significantly affect the mean number of roots produced in cultures. The mean number of roots per shoot ranged between 2.44 to 2.90. However, adding a higher concentration of NAA (1.0 mg/L) significantly increased the mean length of roots compared to the control. On the other hand, 10% of beetroot juice in the culture medium significantly inhibited the growth of roots. The mean length of roots in beetroot-containing medium was 0.72 cm. On the other hand, NAA significantly increased the length of roots of *Dendrobium* species (Goswami *et al.*, 2015). Moreover, medium supplemented with 1.0 mg/L NAA significantly increased the length of roots and root number of *Alstroemeria* cv. Furgo compared to medium supplemented with 0.1 mg/L NAA (Seyyedyousefi *et al.*, 2013), which agreed with the results of the present experiment.

Table 3: Effect of supplements on mean number of roots per shoot and length of roots of *Dendrobium* cv. Sonia, after 16 weeks of culture on 1/2 MS medium

Treatments	Supplements	Mean number of roots per shoot (number ± SE)	Mean length of roots (cm ± SE)
T0	Control	2.73 ± 0.25^{a}	0.85 ± 0.02^{bc}
T1	0.5 mg/L BAP	2.44 ± 0.25^{a}	0.92 ± 0.02^{ab}
T2	0.1 mg/L NAA	2.53 ± 0.30^{a}	0.93 ± 0.02^{ab}
T3	1.0 mg/L NAA	2.90 ± 0.30^{a}	0.96 ± 0.02^{a}
T4	10% Coconut Water	2.87 ± 0.34^{a}	0.88 ± 0.02^{abc}
T5	10% Mashed Banana	2.79 ± 0.39^{a}	0.83 ± 0.02^{c}
T6	10% Beetroot Juice	2.72 ± 0.22^{a}	0.72 ± 0.02^{d}

**Means ± SE followed by the different letters within a column significantly differ at the P<0.05 by Tukey's multiple range test. Sixteen cultures were used for each treatment.

Pyati (2022) revealed that micropropagation of some important medicinal and ornamental *Dendrobiums* has been achieved using various explants on media supplemented with growth regulators. BAP is the most used cytokinin to induce regeneration and proliferation. In shoot proliferation, there is a synergistic effect between cytokinins, auxins and organic supplements (e.g. coconut water and banana extract). The beneficial effect of coconut water in enhancing the growth of orchids *in vitro* may be correlated to the fact that coconut water contains sugars, amino acids, minerals, vitamins, and phytohormones (Yong *et al*., 2009).

In the present study, medium supplemented with 10% mashed banana increased the fresh weight of PLBs and shoots. This observation is consistent with the findings of other researchers. Fresh weight of PLBS and shoots of *Dendrobium* cv. Sonia was increased in the medium supplemented with banana extract (Obsuwan and Thepsithar, 2014; Islam *et al.*, 2016). Similarly, 10% Sabri banana pulp gave a better performance on fresh weight of PLBs, several shoots and leaves per explant in *Dendrobium* orchid (Akter *et al*., 2007), while 10% Mas (AA) banana pulp increased PLB proliferation in *Phalaenopsis violacea* orchid (Gnasekaran *et al*., 2010). Bananas contain a high content of sucrose concentration (Kaur and Bhutani, 2012), iron, potassium, vitamins B6 and B2 and tryptophan (Gnasekaran *et al.*, 2010), Vitamin C or ascorbic acid, provitamin A (β-carotene, α-carotene, β-cryptoxanthin), and mineral composition (Wall, 2006), which may contribute to the increase in the number of shoots in *Dendrobium* cv. Sonia cultures.

Ascorbic acid is an antioxidant that can prevent oxidation and is involved in cell division and elongation (Smirnoff, 1996). Ascorbic acid was found to increase the number of shoots of banana cv. Cavendish (Ko *et al.*, 2009). Beetroot juice contains high levels of inorganic nitrate, sugars, betalains and oxalic acid (Wruss *et al*., 2015). These complex compounds may have contributed to the shoot formation in *in vitro* cultures. In sweet potatoes, a 1/2 MS medium that was supplemented with 6-benzyl adenine (BA) (2.0 mg L-1) and oxalic acid (100 mg L-1) yielded the greatest shoot proliferation (Yaser Hassan Dewir et al., 2020).

Effect of Different Culture Conditions on Growth and Development of *In Vitro* Cultures

As shown in Table 4, there were no significant differences in mean fresh weight and number of shoots produced in cultures maintained for 16 weeks in the culture room and the greenhouse. However, the conditions in the culture room significantly promoted the growth of PLBs and shoots without roots in the cultures. The mean fresh weight of PLBs and shoots without roots in the culture room was 4.87 g and 2.12 g, respectively. The mean fresh weight of PLBs accounted for 57.09%, while the shoots without roots were 24.85% of the mean fresh weight of the culture. Consequently, the mean number and dry weight of shoots without roots were significantly higher in the cultures kept in the culture room than in the greenhouse. On the other hand, the mean fresh weight of PLBs and shoots without roots in the greenhouse accounted for around 44.06% and 13.95% of the mean fresh weight of the culture. As such, the mean number and dry weight of shoots without roots in the greenhouse cultures were significantly lower than those in the culture room.

The greenhouse's higher temperature and light intensity conditions appeared to have induced rooting in *in vitro* cultures. The average temperature in the greenhouse was 35 ± 2 ºC during the day and 30 ± 2 ºC at night, while light intensity was 35 $\mu molm^{-2}s^{-2}$. In most studies on *Dendrobium* micropropagation, the temperature ranged from 22 ºC to 29 ºC, with an average of 25 ± 2 ºC. The photoperiod was 10-16 hours with 30 $\mu molm^{-2}s^{-2}$ to 60 $\mu molm^{-2}s^{-2}$ illumination intensities (Teixeira da Silva et al., 2015). Interestingly, the mean fresh weight of rooted shoots was 2.65 g, accounting for 42.00% of the mean fresh weight of the cultures, while the mean fresh weight of PLBs was 2.78 g (44.06% of the mean fresh weight of the culture). These results indicated that the greenhouse conditions had balanced the formation between PLBs and rooted shoots in the cultures. Moreover, the mean number of roots per shoot and length of roots were significantly higher in cultures kept in the greenhouse. The mean number of roots per shoot was 3.35, with a mean length of 0.95 cm.

Table 4: Effect of different culture conditions on the growth and development of *Dendrobium* cv. Sonia, after 16 weeks in culture

Growth parameters	Culture Room	Greenhouse
Mean Fresh Weight of Culture (g ± SE)	8.53 ± 0.86^a	6.31 ± 0.88^a
Mean Fresh Weight of PLBs (g ± SE) (%)	4.87 ± 0.63^a (57.09%)	2.78 ± 0.63^b (44.06%)
Mean Fresh Weight of Shoots without Roots (g ± SE) (%)	2.12 ± 0.15^a (24.85)	0.88 ± 0.10^b (13.95)
Mean Fresh Weight of Shoots with Roots (g ± SE) (%)	1.41 ± 0.06^b (16.53%)	2.65 ± 0.27^a (42.00%)
Mean Number of Shoots per Culture	48.82 ± 5.03^a	43.79 ± 2.71^a
Height of Shoots (cm)	0.30 – 4.30	0.30 – 3.50
Mean Number of Shoots with Roots	16.73 ± 1.26^b	22.86 ± 1.21^a
Mean Number of Shoots without Roots	32.09 ± 3.97^a	20.93 ± 2.00^b
Mean Number of Roots per Shoots	2.07 ± 0.1^b	3.35 ± 0.07^a
Mean Length of Roots (cm ± SE)	0.68 ± 0.03^b	0.95 ± 0.04^a

**Means ± SE followed by the different letters within a column significantly differ at the $P<0.05$ by Tukey's multiple range test. Eight cultures were used for each treatment.

Acclimatisation of Plantlets

The full-grown shoots with roots obtained from shoot cultures were kept in the culture room, and the greenhouse was transferred to pots containing wood charcoal and coco peat, which gradually hardened off in the greenhouse. After 2 weeks of acclimatisation in the greenhouse, it was found that the survival rate was lower in plantlets from the culture room compared to plantlets from the greenhouse. Approximately half of the plantlets from the culture room died, while most of the plantlets from the greenhouse survived. New roots started developing, while old roots became longer and turned green in plantlets from both cultures. The shoots and leaves were not growing much in plantlets from the culture room, but the shoots and leaves in plantlets from the greenhouse were growing longer and bigger after 2 weeks. Visual morphological abnormalities were observed in plantlets from the culture room but not in the greenhouse. Overall, plantlets from the greenhouse grew better than those from the culture room (Figure 3).

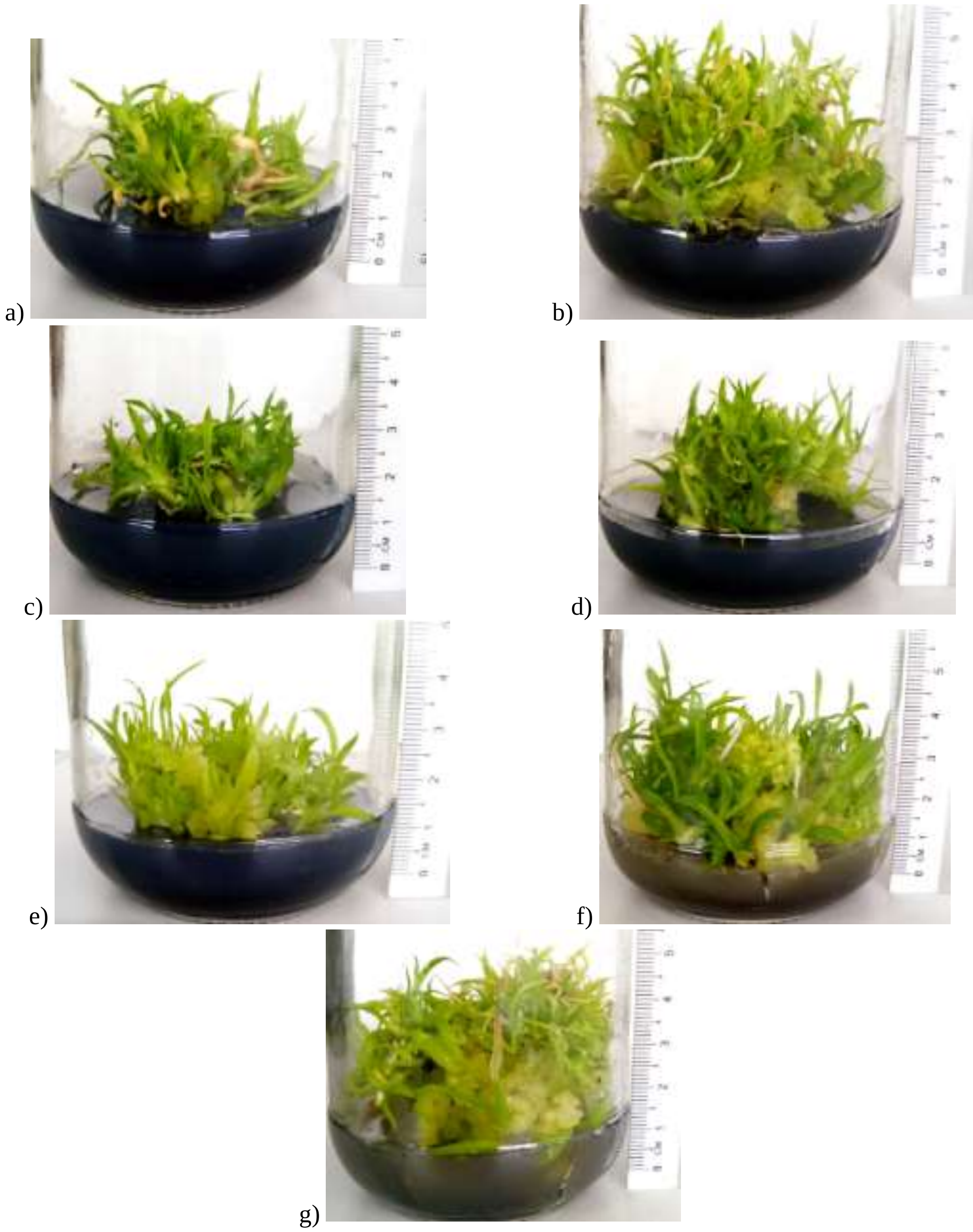

Figure 1: *Dendrobium* cv. Sonia plantlets after 16 weeks of cultivation on 1/2 MS medium supplemented with different supplements in the culture room: a) control; b) 0.5 mg/L BAP; c) 0.1mg/L NAA; d) 1.0 mg/L NAA; e) 10% coconut water; f) 10% mashed banana; g) 10% beetroot juice

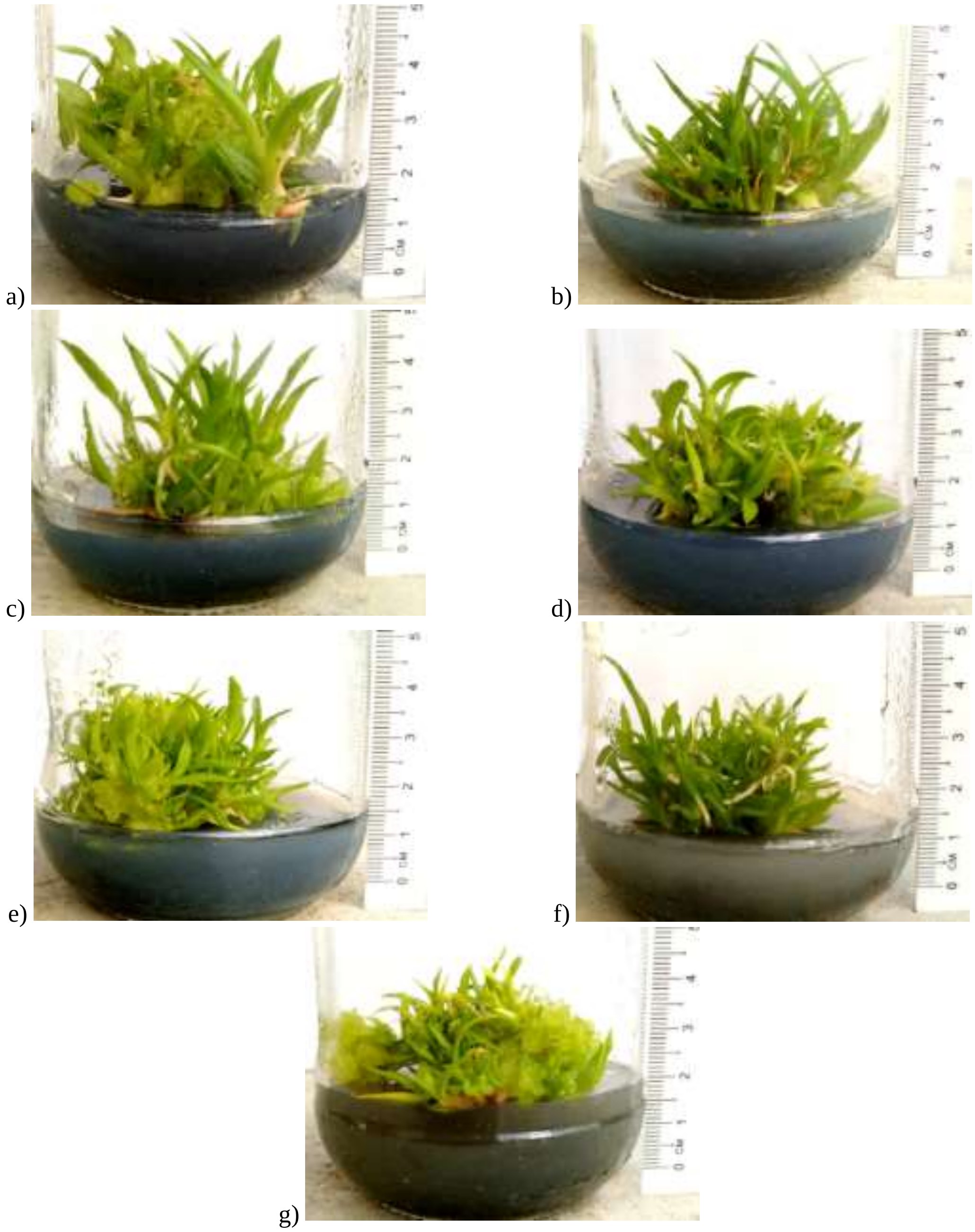

Figure 2: *Dendrobium* cv. Sonia plantlets after 16 weeks of cultivation on 1/2 MS medium supplemented with different supplements in the greenhouse: a) control; b) 0.5 mg/L BAP; c) 0.1mg/L NAA; d) 1.0 mg/L NAA; e) 10% coconut water; f) 10% mashed banana; g) 10% beetroot juice

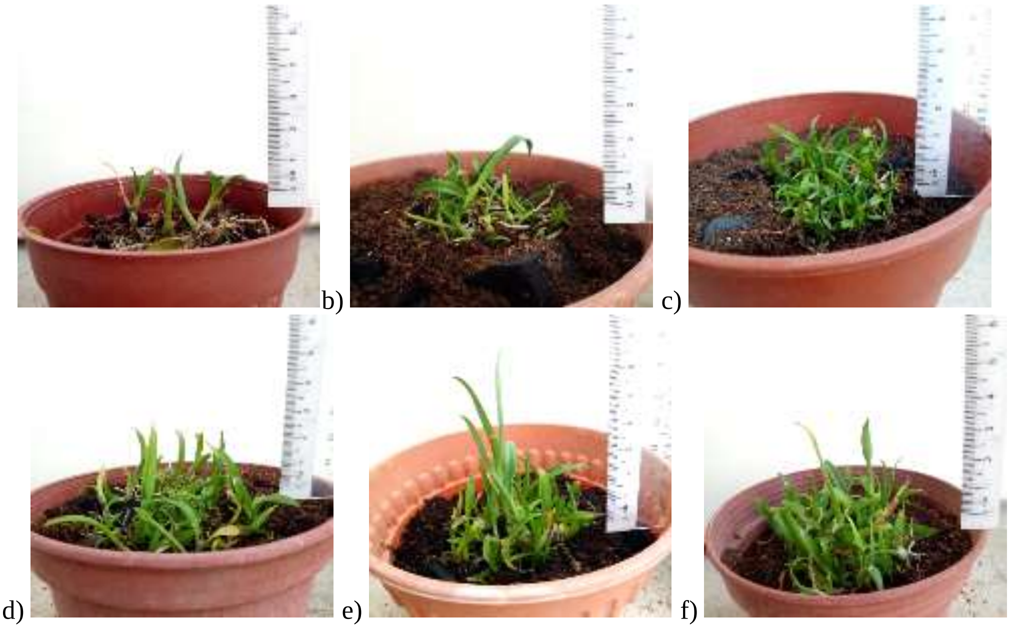

Figure 3: *Dendrobium* cv. Sonia plantlets after 2 weeks of acclimatisation in the pots containing wood charcoal and coco peat in the greenhouse:
a – c. Plantlets derived from the culture room
d – f. Plantlets derived from the greenhouse

CONCLUSION

The medium supplemented with 1.0 mg/L NAA helped increase root length significantly in plantlets. Cultures with controlled temperature and lighting had better PLBs and shoot development in the culture room. In contrast, cultures in the greenhouse exposed to natural day and night conditions had better root development. After two weeks of acclimatisation, plantlets kept in the greenhouse had a higher survival rate and grew well compared to plantlets kept in the culture room.

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REGULATING ASYNCHRONY IN *DENDROBIUM* CV. SONIA *IN VITRO* CULTURES

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ABSTRACT

Dendrobium is one of the most popular orchid types cultivated in the floriculture industry. *In vitro* cultures of Dendrobium cv. Sonia have been successfully initiated and maintained in the plant tissue culture laboratory at Nilai University. However, the cultures grew asynchronously, producing plantlets at different stages of growth. Asynchrony is considered disadvantageous as it hampers seedling maintenance and transplanting. The present study was carried out to evaluate the role of explant type and NAA in regulating synchronous shoot development to obtain rapid production of plantlets for acclimatisation. The findings of this study have significant implications for the field of plant tissue culture and orchid cultivation, as they provide insights into how to minimise asynchrony *in vitro* cultures, thereby enhancing the efficiency and productivity of orchid propagation. The explants (protocorm-like-bodies or PLBs and shoots) were cultured in ½ MS medium supplemented with banana homogenate and NAA (1.0, 3.9, and 5.0 mg/L). The findings revealed that PLBs would multiply *in vitro* and produce cultures at different stages of growth. PLBs that were in contact with the culture medium continued to produce PLBs, while PLBs that were not in contact with the culture medium germinated. It appeared that asynchrony would be minimised *in vitro* culture if the shoots of at least 0.5 cm were segregated and cultured into fresh medium. These shoots would elongate, producing well-developed shoots and roots. Moreover, larger explants developed further, producing rooted shoots rather than PLBs and young shoots. ½ MS medium containing 5.0 mg/L NAA resulted in the highest shoot and root length.

Keywords: *Dendrobium*, *In Vitro* Culture, Asynchrony, Explant Segregation.

INTRODUCTION

Dendrobium is one of the most popular orchid types cultivated in the floriculture industry. *In vitro* cultures of *Dendrobium* cv. Sonia have been successfully initiated and maintained in the plant tissue culture laboratory at Nilai University. However, the cultures grew asynchronously, producing plantlets at different growth stages. Though asynchrony is generally a function of the plants' biology, physiology, biochemistry and adaptation to various environments, it is considered disadvantageous as it hampers seedling maintenance and transplanting. This nonuniformity of plant growth and development also reduces the researchers' or the commercial facility's ability to obtain consistent results.

With its unique ability to offer precise control over the physical and chemical environment, tissue culture presents opportunities for cell synchronisation in plant tissues. Our research is part of a more considerable effort to enhance the efficiency of *in vitro* propagation for various orchid species, particularly *Dendrobium* cv. Sonia. The present study evaluated the role of explant type and NAA in regulating synchronous shoot development to obtain rapid production of plantlets for acclimatisation.

MATERIALS AND METHODS

Plant Materials

The shoot cultures of *Dendrobium* cv. Sonia, meticulously initiated and maintained in the laboratory, were used as a source of explants for the study. Three types of explants, protocorm-like bodies, 0.5-1.0cm, and 1.5-2.0cm young shoots, were chosen for the study.

Preparation of Culture Media and Culture Conditions

½ MS medium (Murashige & Skoog, 1962) was used in the experiments. It was supplemented with 30g/L sucrose, 2.0g/L activated charcoal, 10% (v/v) banana homogenate, NAA (1-5 mg/L) and 0.7% agar (Sigma Aldrich, USA).

Fresh ripe bananas were bought on the day of media preparation from local stores to prepare the banana homogenate. The skin-peeled bananas were sliced into small pieces and completely crushed using a pestle and mortar before adding to the medium. The pH of the culture medium was adjusted to 5.8 with 0.1N NaOH or HCl before adding agar into the medium. The medium was autoclaved at 121 ℃ for 15 minutes under 1kg cm^{-2} pressure. Autoclaved medium (50mL) was poured into GA7 containers (Magenta Corp., Chicago, USA).

The explants were cultured in 50mL of the culture medium. Six shoots and 2.0g of PLB explants were cultured per GA7 container. The explants were placed upright in the medium, with the lower half dipped inside the agar. The GA7 containers were sealed with two layers of cling wrap. All cultures were kept under cool, fluorescent light (12h photoperiod) at 25 ± 2°C under 12/12 (light/dark cycle) in the culture room for 16 weeks.

Assessment of Growth and Statistical Analysis

The present study investigated 3 explant types (PLBs, 0.5-1.0 cm shoots, and 1.5-2.0 cm shoots). Each explant type had 5 cultures and was independently repeated 5 times under the same conditions. The cultures were observed weekly, and growth parameters were gathered after 16 weeks of *in vitro* culture. The parameters recorded included the number and length of shoots per culture and the length of roots.

RESULTS AND DISCUSSION

Effect of Explant Types

The protocorm-like-bodies (PLBs) and young shoots of *Dendrobium cv.* Sonia were cultured in ½ MS medium supplemented with 30g/L sucrose, 10% banana homogenate, different concentrations of NAA (1.0mg/L, 3.0mg/L, and 5.0mg/L) along with 2g/L activated charcoal and 7g/L agar. During the 16 weeks, the 3 types of explants multiplied and developed into a culture composed of a mixture of shoots at different stages of growth (Figure 1). Most explants produced PLBs, shoots without roots, and shoots with roots at varying amounts (Table 1).

Table 1. Percentage of cultures with PLBs and shoots after 16 weeks.

Explant type	Percentage of cultures with PLBs	Percentage of cultures with shoots without roots	Percentage of cultures with rooted shoots
PLBs	100	100	78
0.5-1.0 cm shoots	71	100	100
1.5-2.0 cm shoots	10	70	100

When PLBs were used as the original explant, 100% of the cultures produced PLBs after 16 weeks in culture. In addition, 78% and 100% of the PLB-derived cultures produced rooted shoots and shoots without roots, respectively (Table 1). The cultures that made the least PLBs and shoots without roots were derived from 1.5-2.0 cm shoot explants. The larger explants developed further to produce rooted shoots than PLBs and young shoots. These findings suggest that the size and type of the explant play a significant role in the development of the culture, which can be crucial for enhancing the efficiency and productivity of orchid propagation.

The conversion of PLBs usually occurs 7 – 15 days after culture and can be determined by the first leaf primordium's colour change from light green to dark green. The conversion of PLBs into plantlets takes place at different timings, and there are various stages of growth in a culture. The PLBs in contact with the culture medium tend to divide and continuously produce PLBs, whereas the PLBs away from the culture medium are developed into shoots.

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Figure 1. Mixed culture consisting of PLBs and shoots

Synchrony of Growth

Table 2 and Figure 2 show that besides the explant type, the concentration of NAA in the culture medium also affected synchronous growth in *Dendrobium* cultures. The addition of NAA in the culture medium influenced different stages of micropropagation in plant tissue culture, which enhanced micropropagation. Shoot development can be completed without NAA or by adding a higher concentration (5.0 mg/L) of NAA to the culture medium. Culture media supplemented with a low concentration of NAA promoted shoot formation, while a high concentration of NAA initiated roots. When the concentration of NAA is increased to 5 mg/L, the explants grow taller, with longer roots ranging from 0.2 to 3.6 cm.

Table 2. Height of shoots and length of roots of *Dendrobium* cv. Sonia, after 16 weeks in culture.

Explant type	Treatment #	NAA concentration (mg/L)	Mean no. of shoots per culture	Height of shoots (cm) range	Length of roots (cm) range
	T0	0	51.6	0.5 - 1.8	0.1 - 2.0
PLBs	T1	1.0	9.40	0.5 - 1.5	0.4 - 1.0
	T2	3.0	2.80	0.5 -1.7	0.5 - 1.1
	T3	5.0	12.25	0.5 - 1.2	0.1 - 2.4
	T4	0	14.00	0.5 - 1.5	0.1 - 1.5
0.5-1.0 cm shoots	T5	1.0	16.75	0.5 - 2.2	0.2 - 2.4
	T6	3.0	13.20	0.5 - 1.5	0.4 - 1.7
	T7	5.0	17.80	0.5 - 2.0	0.2 - 3.0
	T8	0	21.25	0.5 - 2.4	0.2 - 2.6
1.5-2.0 cm shoots	T9	1.0	15.00	0.5 - 2.3	0.3 - 2.5
	T10	3.0	10.20	0.5 - 2.5	0.4 - 2.6
	T11	5.0	9.20	0.6 – 3.6	0.2 - 3.6

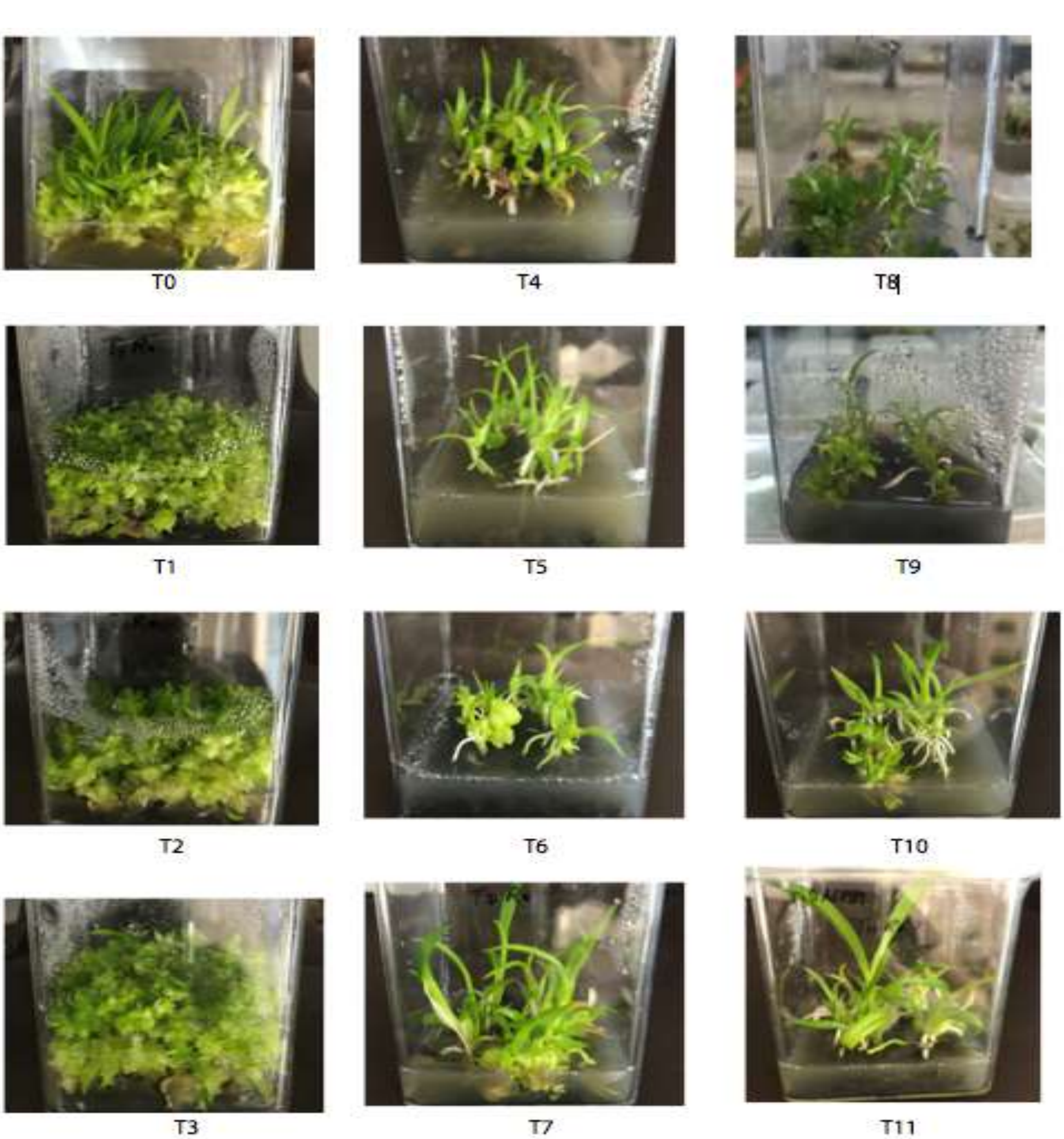

Figure 2: The growth of *Dendrobium* cv. Sonia, after 16 weeks in culture

Because asynchronous growth does occur in most plant tissue culture systems, synchronising tissue growth has been obtained by controlling the chemical microenvironment and manipulating the cell cycle stage at the beginning of the culture period. According to King (1980), repeated synchronous division may be induced by adding auxin to quiescent cells or starving and regrowing plant cell cultures. Cell division synchrony is associated with discontinuous biosynthetic events in several such cases.

For example, Nishida et al. (1992) established a system of synchronous cell division to suspension cultures of cells of *Catharanthus roseus* L. cv. Little Pinky by auxin starvation, followed by its readdition. When cells in the stationary phase were transferred to a fresh medium free of 2,4-dichlorophenoxyacetic acid (2,4-D), cells were arrested preferentially at the G_1 phase. After 2 days in the 2,4-D free medium, the readdition of 2,4-D induced the synchronous division of cells.

In cotton, friable clumps of cells selectively collected over filter mesh 40 and subjected to one cycle of Myo-inositol starvation have induced highly synchronised embryogenesis in the culture. The protocol gave 100% of embryos at the globular stage, out of which more than 80% developed into bipolar torpedo-stage embryos (Kumar &Tuli, 2004). Similarly, in another cotton cultivar, Jing-Lin Cao et al. (2008) reported combining suspension culture and solid culture (with filter paper) to improve somatic embryogenesis frequency and synchronous development of mass somatic embryos. Sieving embryonic calli a few times before placing them on the solid medium containing 2.46 μmol L^{-1} indole-3-butyric acid (IBA) and 0.70 μmol L^{-1} kinetin resulted in a more synchronised development of embryos. About 70.2% for globular, 52.3% for torpedo-shaped, and 73.0% for cotyledonary embryos were obtained during the culture.

In our study with *Dendrobium*, asynchrony would be minimised *in vitro* culture if the shoots of at least 0.5 cm were selected, segregated and cultured into a fresh medium containing 5 mg/L of NAA.

CONCLUSION

Explant type and NAA improved synchronous growth of *Dendrobium cv.* Sonia shoots. Culturing PLBs and young shoots on half-strength MS, with or without the addition of NAA, results in the successful formation and proliferation of PLBs and shoot development. Moreover, larger explants developed further, producing rooted shoots rather than PLBs and young shoots. ½ MS medium containing 5.0 mg/L NAA resulted in the highest shoot and root length. Asynchrony would be minimised *in vitro* culture if the shoots of at least 0.5 cm were selected, segregated and cultured into a fresh medium containing 5 mg/L of NAA.

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051-055

A PRELIMINARY INVESTIGATION ON THE POTENTIAL OF AGRO-BASED LIGNOCELLULOSIC RESIDUES AS REINFORCEMENT MATERIAL FOR BIODEGRADABLE POLYMERS

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ABSTRACT

Biodegradable plastics have garnered widespread attention as an alternative to petroleum-based plastics which accumulate in landfills and result in greenhouse gas (GHG) emissions. Owing to its good film-forming properties, alginate has been widely used in the food industry as a packaging material. Unfortunately, alginate-based biopolymers have poor water vapor barrier and low. Blending these biopolymers with nanofillers such as cellulose isolated from lignocellulosic sources could improve its functionality. In this study, mango peels and lettuce leaf powders were investigated as potential nanofillers in alginate-based biopolymers. Here alginate-based biopolymer films were blended with 5% and 10% concentrations of crude cellulose extracted from powdered mango peel and lettuce leaf. Cellulose was extracted through alkaline hydrolysis by heating raw powdered mango peel and lettuce leaf in 5% potassium hydroxide at 90°C for 2 hours, followed by heating them in 1% sodium chlorite at 70°C for 1 hour. The bioplastic was then reinforced with calcium chloride crosslinking to produce calcium alginate (CA). This process enhanced bioplastic appearance, smoothness, and rigidity while reducing thickness. Up to 16.32% degradation of mango peel-derived CA bioplastic (10% cellulose) and 18.93% degradation of lettuce-derived CA bioplastic (10% cellulose) was observed on Day 7. The high-water vapor transmission rate (WVTR) of SA bioplastics suggests their suitability for applications such as wound dressings, while enhanced WVTR of CA bioplastics is ideal for food packaging, ensuring food stability and prolonging shelf life. This study highlights the diverse potential applications of reinforced films across various industries.

Keywords: Biodegradable, Food Packaging, Lignocellulosic Waste, Nanofillers, Reinforced Bioplastics.

056-056

EVALUATION OF POSTHARVEST QUALITY OF MORINGA OLEIFERA LEAVES DURING STORAGE IN DIFFERENT STORAGE TEMPERATURES AND PACKAGING

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Moringa oleifera or known as *merungai* and *kelor* in Malaysia and Indonesia is a perennial crop which grow well in tropical and subtropical regions. It is called a superfood tree because it contains various benefits for medicine and health. The leaves contain full essential amino acids, rich in protein, vitamins, minerals, potassium, iron, calcium and an amount of polyphenols which contribute to biological activities such as antibacterial, antifungal, antiinflammatory and antiviral. In traditional medicine, it is used for preventing cardiovascular, liver disease, inflammation, skin and digestive disorders. In order to maintain the quality of Moringa leaves, postharvest handling of the leaves such as storage technique plays an important role. This present study aims to determine optimal storage temperature and suitable packaging for Moringa leaves storage to maintain its quality. Moringa leaves were harvested manually, collected in basket before transferred to packaging house, sorted and cleansed. The leaves were packed in two types of packaging; polyethylene (PE) 0.04mm and polypropylene (PP) 0.04mm and stored in three different storage temperatures; 5°C, 8°C and 10°C. Leaf respiration rate (CO2), total phenolic content, total soluble solids content, moisture content and colour were measured every week throughout the storage period. The results of the study found that storage of Moringa leaves in 5°C temperature packed in polyethylene (PE) plastic could delay the senescence process up to 35 days storage as indicated by lower respiration rate, lower total soluble solids content, higher total phenolic content and stable colour values throughout storage period compared to storage in 8°C and 10°C and polypropylene (PP) plastic.

Keywords: Storage, Temperature, Packaging, Polyethylene, Polypropylene.

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THE ULTIMATE DECISION OF ALFONSO DE ALBUQUERQUE WHETHER TO CONSTRUCT A FORTRESS OR NOT IN MALACCA (1511)

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ABSTRACT

This article attempts to examine the ultimate decision of Alfonso de Albuquerque, who was in the 1500s', the Captain-General and Governor of India, as to whether to construct or not a Portuguese Fortress in the spice-rich port of Malacca in 1511. The article's research was conducted using quantitative (secondary) resources with regards to some historical texts, peer-reviewed journals and papers depicting Alfonso de Albuquerque's sojourn in the Indian Continent and Malay Archipelago in the early 16th century (Khusyari; JA et. al., 2012).

Half of the pepper supplies from Malacca were leaked through Cairo, Alexandria, and Venice before reaching Portugal. The Arabs could sail straight from there, Malacca to Bab-el-Mandeb1 and elude the Portuguese Indian Fleet in Goa and Cochin. With Malacca in Moslem hands, the Portuguese spice trade monopoly would never be complete (Elaine S. et. al., 1936). The conquest of Malacca would be a great step towards the ruin of Islam; and Malacca conquered would be the fairest jewel in D. Manuel's crown.2 Malacca held an important role in the European expansionist imagination. Tom Pires said, "Whoever is Lord of Malacca has his hands on the throat of Venice." (Scott-Ross 1971, pp.28). English texts from the 16th century onwards demonstrate a desire to capture the port, and the cultural position its name held as a symbol of wealth and the exotic (Gulliver K., et. al., 2009).

Keywords: Malacca (Melaka), Alfonso de Albuquerque, Goa, Cochin, Spices, D. Manuel, Sultan Mahmud Shah, King Manuel.

LITERATURE REVIEW

Background of Malacca (or Melaka)

The Kingdom of Malacca was founded by a Sumatran Prince, Parameswara in 1400. He embraced Islam and adopted the Islamic name, Raja Iskandar Shah in 1414. Malacca is in a favorable position in bridging West and East Asia, it was naturally a base market for goods from all corners of Asia (Jacobs 2006; pp 202). Nevertheless, Malacca prospered because of its facilities, port charges and taxes, which attracted traders, and not because of its ability to control the shipping and trade passing through the Straits of Malacca (Kwa 1990; pp 107-135).

In 1511, Malacca fell to the Portuguese under Alfonso de' Albuquerque. The Portuguese realized that Malacca was the vital gateway between the East and West. A Portuguese adventurer, Tom Pires, wrote "whoever is Lord of Malacca, has his hands on the throat of Venice." (Scott-Ross 1971, pp.28). Since Malacca was the most important key to control the trading from the East and West Asia, the Portuguese were keen on occupying the city. The attack on Malacca was triggered by the imprisonment of some 'infidel' Portuguese by the Bendahara. Malacca under the Portuguese rule, was not as busy as it had been since most Moslem traders preferred to trade in Acheh.

Who was Alfonso de Albuquerque?

Alfonso de Albuquerque was the first European since Alexander the Great who dreamed of establishing a Portuguese Empire in India, or rather in Asia, governed from Europe (Hetal S, 1897). He was well-known for his great administration skills and military strategies and was dubbed as the 'Great', the 'Caesar of the East' and the 'Lion of the Seas' (Nogueiria Francisco M, et. al., 2016). He was appointed as the second Viceroy of Portuguese India after capturing Goa in 1510.

Alfonso was born in 1456 in the district of Alhandra in Portugal, to Goncola de Albuquerque and P.Leonor de Menesses. His father served the King of Portugal, D. Alfonso the Fifth, and therefore, Alfonso received his early education in the royal court. He displayed a great interest in Mathematics and Classical Latin. He began his military career in 1471 and accompanied Alfonso the Fifth in the conquests of Tangier, Arafa and Arzila in Africa. At the request of the future Portuguese Monarch, King John the Second, he fought in the wars against Castile in Spain (Nogueiria Francisco M, et. al., 2016).

Alfonso advanced the three-fold Portuguese grand scheme of combating Islam, spreading Christianity, and securing the spice trade by establishing a Portuguese Asian Empire (Ooi, 2004, pp17). He conquered Goa, India and was the first European of the Renaissance period to raid the Persian Gulf, and he led the first voyage by a European Fleet into the Red Sea (Stevens et. al., 1711, pp113). He is generally considered a highly effective military commander (Diffie, Winius and Shafer, 1977, pp239-260) and "probably the greatest naval commander of the age" (Ricklefs, 2020), given his successful strategy, he attempted to close all the Indian Ocean naval passage to the Atlantic, Red Sea and to the Pacific.

He was appointed Head of the "fleet of the Arabian and Persian Sea" in 1506 (Aubin J., Dec 1985). Many of the conflicts in which he was directly involved took place in the Indian Ocean, in the Persian Gulf regions for control of the trade routes, and on the Indian coasts. It was his military brilliance in these initial campaigns that enable Portugal to become the first global empire in history (Erickson and Goldstein, 2012). He led the Portuguese forces in numerous battles, including the conquest of Goa in 1510 and the capture of Malacca in 1511. During the last five years of his life, he turned to administration (Bandelier, 1907), where his role as the second governor of Portuguese India were crucial to the longevity of the Portuguese Empire. He oversaw expeditions that resulted in the establishment of diplomatic ties with Thailand, Myanmar, the Moluccas, and Timor. He laid the path for European trade with China's Ming Dynasty (Vilhena, Maria da Conceicao, et. al., 2001).

Establishment of trade ties with Malacca

The first Portuguese references to Malacca appear after Vasco da Gama's return from his expedition to Calicut that opened a direct route to India around the Cape of Good Hope. It was described as a city that was "40 days journey from India, where clove, nutmeg, porcelains, and silks were sold" (João Paulo de Oliveira e Costa, Vítor Luís Gaspar Rodrigues (2012). Since then, King Manuel had showed an interest in contacting Malacca (José Damião Rodrigues, Pedro Aires Oliveira (2014)). In 1505, Dom Francisco de Almeida was dispatched by King Manuel the First as the first Viceroy of Portuguese India, tasked to discover its precise location.

Dom Francisco, however, unable to dedicate resources to the enterprise, sent only two undercover Portuguese envoys in August 1506, Francisco Pereira and Estevao de Vilhena, aboard a Muslim merchant's ship. The mission was aborted once they were detected and nearly lynched on the Coromandel Coast, narrowly making it back to Cochin by November (Costa JP, Rodrigues VL, 2012). Unimpressed with Almeida's lack of results, in April 1508, King Manuel dispatched a fleet directly to Malacca, composed of four ships under the command of Diego Lopez de Sequeira. He received royal orders specifically instructing him to obtain permission to open a trading post diplomatically and trade peacefully, not to respond to any provocations and not to open fire unless fired upon him.

Sequeira's Arrival in Malacca

The Portuguese expedition arrived in Malacca in September 1509 and immediately Sequeira sought to contact the Chinese merchants in the harbor. They invited him onboard one of their trading junks and received him very well for dinner and arranged a meeting with the Malaccan ruler, Sultan Mahmud Shah. The Sultan promptly granted the Portuguese authorization to establish a 'feitoria' and provided a vacant building for commercial purpose. Wary of the threat that the Portuguese posed to their interests, however, the powerful merchant communities of Muslim Gujaratis and Javanese convinced Sultan Mahmud and the 'Bendahara' to betray and capture the Portuguese (Costa JP, Rodrigues VL, 2012).

Sequeira in the meantime was so convinced of the Sultan's amiability that he disregarded the information that Duarte Fernandes, a Christian who spoke Parsi, obtained from a Persian innkeeper about the ongoing preparations to destroy the fleet, confirmed even by the Chinese merchants (Castanheda FL, et. al., 1979). He was playing chess aboard his flagship when a few Malaccan ships, disguised as merchants, ambushed the Portuguese ships (Barros JD, 1553, Decades de Asia). The Portuguese repelled every boarding attempt but faced with the sheer number of Malaccan ships and unable to land any forces to rescue their countrymen who were trapped in the 'feitoria', Sequeira made the decision to sail back to India before the monsoon started and left them completely stranded in Malacca. Before departing, he sent a message to the Sultan and the 'Bendahara' in the form of two captives with an arrow through their skulls as a testimony to what would happen to them should any harm come to the Portuguese (Barros JD, 1553, Decades de Asia).

Preparations to avenge and conquer Malacca

Upon reaching Travancore (present day Kerala and Tamil Nadu) in April 1510, Sequeira heard that Alfonso had succeeded Dom Francisco de Almeida as Governor of Portuguese, India. Fearful of reprisals from Albuquerque for previously supporting Almeida, Sequeira promptly set sail beck to Portugal (Barros JD, 1553, Decades de Asia). At that same time in Lisbon, King Mauel dispatched another smaller fleet under the command of Diego de Vasconcelos to trade directly with Malacca, based on the assumption that de Sequeira had been successful in establishing commercial ties with the Malacca. Alfonso had in the meantime received messages from the captives at Malacca, written by the factor Rui de Araujo, and sent through envoys of the most powerful merchant of Malacca, a Hindu named Nina Chatu who interceded for the Portuguese. Araujo detailed the Sultan's military force, the strategic importance of Malacca as well as their atrocious captivity. Hence, Alfonso was fully aware that for Vasconcelos to proceed to Malacca with such a meagre force was suicide and managed to convince him to reluctantly aid him in capturing Goa later that year instead.

Having given the finishing touches to the Goa administration, Alfonso collected his troops for an expedition against Malacca. He set sail for Malacca in April 1511, with 18 ships and 600 men at arms besides slaves.

The Attack on Malacca

By 1st July 1511, the Portuguese Armada arrived at Malacca, firing their guns, and displaying battle arrangements, which caused great commotion in the harbor. Alfonso declared that no ship should set sail without his permission and immediately he tried to negotiate the safe return of the remaining prisoners still trapped in Malacca. As Alfonso considered the Sultan's conduct to have been treasonous, he demanded that the prisoners be returned without a ransom as a token of good faith, but Sultan Mahmud Shah replied with vague and evasive answers and insisted that Alfonso sign a peace treaty beforehand. In reality, the Sultan was buying time to fortify the city and call back the fleet, whose Admiral the Portuguese identified as 'Lasemane' (or Laksamana, literally 'admiral').

Alfonso in the meantime kept receiving messages from his prisoner, Rui de Araujo, informed him of the Sultan's military strength, through a local Portuguese collaborator, Nina Chatu. The Sultan could muster 20,000 men, which included Turkish and Persian bowmen, thousands of artillery pieces, and 20 war elephants; but he noted that the artillery was crude and lacking enough gunners. Alfonso himself would later report to the King that only 4,000 of those were battle-ready (de Castanheda et. al., 1979).

The Sultan on his part was not too intimidated by the small Portuguese contingent. Alfonso would later write to King Manuel that, to his great consternation, the Sultan had somehow managed to correctly estimate the total number of soldiers aboard his fleet with a margin of "less than three men" (Bulhao Patora, de Mendonca HL et. al., 1884). Thus, he remained in the city organizing its defense, "not realizing the great danger he was putting himself into" (Ballao. A, et. al., 1923). After weeks of stalled negotiations, by the middle of July, the Portuguese bombarded the city. Startled, the Sultan promptly released the prisoners and Alfonso then took the chance to further demand a heavy compensation of 800,000.00 cruzados (i.e., 1,080,000 grams of gold) and authorization to build a fortress. The Sultan refused. Presumably, Alfonso had already anticipated the Sultan's response at that point.

Alfonso's hesitation in establishing a fortress in Malacca

When a few days had elapsed, Alfonso, seeing that the Sultan had not sent him any reply, though he had already tasted the power and capability of the Portuguese, became anxious. Alfonso was forcibly compelled for a second time, that he might have to risk his men by launching an attack on Malacca with the hope of taming the Sultan's pride; and ***he had not in the land any means of building a fortress*** (Alfonso's unintentional desire of building a fortress in Malacca) which it was his chief intent to do, neither could Ruy de Araujo give any advice on these events.

Another instance in which Alfonso was hesitant to establish a Portuguese fortress in Malacca was when he gathered his captains and revealed that an assault would take place in the morning of 25th July 1511, the Day of Santiago. "The Governor accordingly got ready, to assault the town. ***But he warned all the 'fidalgos' (Portuguese nobility) that if he succeeded, he would build a fort. Unless they were prepared for this, it was not worthwhile to risk a single man to gain Malacca"***. Alfonso's soldiers were exhausted from their battle against Sultan Mahmud Shah's forces. They had to fight beneath the hot equatorial sun for eight hours. His men neither had eaten nor slept and he could not risk anyone of them to fetch supplies on board their vessels. Without refreshment or rest, his men could not be expected to construct barricade and keep off the enemy at the same time. Having observed all this, Alfonso decided to postpone the final assault to another day. He set fire to one part of the city, permitted those who desired to do a little plunder, and then withdrew on board. Much of the Sultan's artillery was carried off.

Albuquerque appears to have expected Sultan Mahmud to surrender after this. All the Sultan did, however, was to send a messenger deprecating the Portuguese pugnacity, since he had given up the prisoners. Meanwhile, the Captains became bored. When anything hung fire, they quickly lost interest. ***Besides, the idea of fortress building was depressing. Suppose Malacca was conquered, some pessimists gloomily remarked it would be after such a struggle and there would be so many wounded that men would be more fit to go to bed than to start fortress building***.

Alfonso assembled them in council. He explained at length the many reasons why he thought Malacca must win. For the sake of the pepper trade alone, it was most necessary. Half of the pepper that still leaked through Cairo, Alexandria and Venice came from Malacca. The Arabs could sail straight from there to Bel-el-Mandeb. With Malacca in Moslem hands, the Portuguese monopoly would never be complete. The conquest of Malacca would be a great step towards to ruin Islam, and with Malacca conquered would be the fairest jewel in Dom Manuel's crown. of the world had ever see, "I have put the case before you", he concluded, "tell me your views. ***But he emphasized, he would not take Malacca as a gift unless they were prepared to build the strongest fortress that part you think fit, and they answered sweetly, "you know the best?"*** "Make yourself clear upon one point," said Alfonso. "Will you build that fort or will you not?" To which they recklessly replied that they would build two forts if necessary. "Very well," said Alfonso, "now we can proceed," and the captains, full of enthusiasm, entreated him to hurry up.

Also, it was reported to Alfonso that ***there were some among the captains who were in the habit of saying that they did not think it of service to the King of Portugal for them to maintain the city nor to build a fortress within it***. On being apprised of this, he ordered them to be called to his ship, with all the 'fidalgos' and cavaliers of the fleet, and said to them, "Sirs, you will have no difficulty in remembering that we decided upon attacking the city, it was with the determination of building a fortress within it, for so it appeared to all to be necessary, and after having captured it, ***I was unwilling to let slip the possession of it yet, because ye all advised me to do so, I left it and withdrew; but being ready, as you see, to put my hands upon it again once more, I learned that you had already changed your opinion; now this cannot be because the Moors have destroyed the best part of us***. (By analyzing this statement, Alfonso adhered to the earlier advice of his council members as not to establish a fortress in Malacca, However, their opinions altered which compelled Alfonso to change his mind to conquer and establish a fortress in Malacca).

Research Questions

a) Was Alfonso de Albuquerque hesitant to capture and establish a fortress in Malacca?
b) What were the influential factors which changed his mind to besiege and establish a fortress in Malacca?
c) Who influenced Alfonso de Albuquerque to go ahead with the final assault on Malacca?

Research Aims

a) The main aim (or purpose) of the research was to find out (or uncover) why Alfonso de Albuquerque was hesitant to capture and establish a fortress in Malacca.
b) The second purpose of the research was to unearth what were the influential factors which changed Alfonso de Albuquerque's mind to besiege and establish a fortress in Malacca.
c) The final purpose of the research was to uncover who influenced Alfonso de Albuquerque in launching his final besiege on Malacca.

FINDINGS

a) Alfonso de Albuquerque was the first European since Alexander the Great who dreamed of establishing a Portuguese Empire in India, or rather in Asia, governed from Europe (Hetal S, 1897). He was well-known for his great administration skills and military strategies and was dubbed as the 'Great', the 'Caesar of the East' and the 'Lion of the Seas' (Nogueiria Francisco M, et. al., 2016).

b) Sultan Mahmud Shah (1488 – 1511), the last Malaccan Sultan, promptly granted the Portuguese authorization to establish a ‘feitoria’ and provided a vacant building for commercial purpose. Wary of the threat that the Portuguese posed to their interests, however, the powerful merchant communities of Muslim Gujaratis and Javanese convinced Sultan Mahmud and the ‘Bendahara’ to betray and capture the Portuguese (Costa JP, Rodrigues VL, 2012).

c) Alfonso de Albuquerque emphasized, that he would not take Malacca as a gift unless they were prepared to build the strongest fortress that part of the world had ever see, “I have put the case before you”, he concluded, “tell me your views. Do you think fit,” and they answered sweetly, “you know the best?” “Make yourself clear upon one point,” said Alfonso. “Will you build that ford or will you not?” To which they recklessly replied that they would build two forts if necessary. “Very well,” said Alfonso, “now we can proceed,” and the captains, full of enthusiasm, entreated him to hurry up.

d) The capture of Malacca defended by an army of 30,000 men, by 1,100 Portuguese soldiers was a most brilliant feat, but characteristically, Alfonso de Albuquerque did never receive even any verbal acknowledgment from his King for the service.

CONCLUSION

The Portuguese rules Malacca for the next 130 years (1511 – 1641), when it was captured by the Dutch. The research clearly revealed that Alfonso de Albuquerque was quite hesitant to besiege Malacca and establish a fortress therein, until he had to consult with his military commanders and ‘fidalgos’ to seek their views. Albuquerque’s capture of Malacca in 1511 gave the Portuguese the major distributing centre for Indonesian spices, as well as a naval base which controlled the bottleneck between the Indian Ocean, the Java Sea and the South China Sea, for the alternative route through the straits of Sunda was rarely used. This research welcome further research and analysis by future historians specializing in European Maritime Powers from the 15th to the 18th century.

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046-060

LOCAL CLIMATE MODELING USING DOWNSCALED REGIONAL CLIMATE MODEL (RCM) FOR CAMERON HIGHLANDS, MALAYSIA

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ABSTRACT

Cameron Highlands is currently being impacted by climate change resulting from global warming, which is affecting crucial economic sectors such as agriculture and tourism. As one of Malaysia's primary vegetable producers, this region plays a vital role in the country's agricultural industry. This study employs a downscaled regional climate model (RCM) to project future climate conditions in Cameron Highlands, specifically focusing on temperature, relative humidity, and precipitation patterns under the Shared Socioeconomic Pathway 2 (SSP2) scenario with Representative Concentration Pathway 4.5 (RCP 4.5). Based on the findings from the RCM, there is a projected steady increase in maximum temperatures by 0.4 to 0.8°C by the end of the century. Moreover, the future precipitation pattern under SSP2-4.5 reveals a slight upward trend throughout the 21st century, with an estimated annual increase ranging from 350 to 845 mm by the end of the century. Relative humidity is projected to experience changes ranging from 0.1% to 2.9%, with the highest projected value reaching 90.8%. These changes in temperature, precipitation, and humidity can have significant implications for agricultural activities in Cameron Highlands. Therefore, the objective of this study is to offer valuable insights to local authorities and stakeholders, enabling the development of effective adaptation and mitigation strategies to counteract the adverse effects of climate change in Cameron Highlands.

Keywords: Climate Modeling, Cameron Highlands, SSP2 Scenario, Agriculture, Climate Change.

063-061

CULTIVATING GROWTH: INTEGRATING AGRICULTURE INTO MALAYSIA SCHOOL CURRICULA

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ABSTRACT

The promising trend to integrate agriculture in school curricula sparks more than just a pedagogical shift. The Malaysia Education Philosophy requires the learners to think critically and innovatively. By incorporating agriculture in school curriculum, it enables creative problem solving as well as cultural awareness that are essential for competition due to strategic position of our nation globally. In addition, adopting agriculture in education is in keeping with the government's goal to develop well-informed, environmentally aware, and analytically inclined citizens who will promote sustainable development and support their country's growth agenda. Current view of situation demonstrates legislators and educationists concur with this idea of embedding agriculture in learning institutions. This paper explores the inseparable relationship between educational strategies and agricultural knowledge claiming that such integration is no longer a matter of choice but a necessity. Indeed, the inclusion agricultural education can bring forward a new generation of change enablers, ready to address the challenges of food production and sustainable land use. Thus, strategic policy adjustment along with reliance on the community's potential for participation can provide a solution to foster future generations that are competent with sustainable practices.

Keywords: Agricultural Program, Sustainable Development, School Curricula, Experiential Learning, Educational Policy.

INTRODUCTION

The integration of agriculture into educational curricula is an emerging global trend with significant implications for preparing the next generation for future challenges and opportunities. This approach includes school gardens, urban and rural farming, and experiential learning, recognizing the critical nexus between agriculture, the environment, and food systems. "Learning by doing" enhances traditional classroom learning and supports the development of practical skills (Williams & Dixon, 2019; Berezowitz et al., 2019). Over the years, integration of sustainable agriculture into education has become a growing focus in order to address global concerns while spreading the idea of environmental conservation. In order to highlight Sustainable Development Goals (SDG), some learning institutions emphasize the importance of sustainable agriculture as topic in Biology subject (Kunze & Büssing, 2022). Through this education does not only boost learners' comprehension of sustainability but also promotes the needed shift for the future well-being of the environment. To add, Mouser et al. (2019) point out three components that foster the efficiency of agriculture in education which are the experience in the classroom, development of leadership by participation in farming projects, and experiential learning through supervised agricultural programmes. Students are exposed to practical skills which equip them for potential careers in agriculture. The need to cultivate generations who has agricultural literacy has been highlighted by Sterling et al. (2021) It revolves around the development of professionals that are capable of making well-informed decisions about agriculture, food and nutrition which are related to building a more sustainable future.

In Malaysian context, the education plan agrees with the idea of having agriculture programs in teaching and learning. Some of the objectives in the Malaysia Education Blueprint 2013 - 2025 are to ensure a comprehensive, entrepreneurial, and values-based approach to education. The goal of this strategy is to cultivate holistic individuals who is conscious about global issues without compromising their national identity (Ministry of Education Malaysia, 2013). This can be made possible by integrating agriculture activities in education as early as primary schools. To support this, Pellizzi & Febo (1994) noted that the inclusion of agriculture in education promotes the development of new ideas, originality, and a sense of responsibility towards society and the environment. Thus, it is undeniable that relation between increasing population growth and preservation of traditional farming techniques makes agricultural education in Malaysia important. The development of these initiatives corresponds to Malaysia's goals for sustainability and addresses the growing demand for an education system that promotes environmental awareness. As the country progresses, agriculture education holds the potential to foster standards of sustainability and awareness of the environment in next generations (Normazian et al., 2021).

PROBLEM STATEMENTS

Accordingly, this paper seeks the view of writers on several questions as stated:

1) How can the integration of agriculture projects or activities in the school curriculum impact students' skills as per the Malaysia Education Philosophy?
2) In what ways can embedding agricultural projects or activities in school curricula promote competencies on sustainability among Malaysian students?
3) How can the integration of agricultural projects and activities in the school curricula prepare students to address future challenges related to food security and sustainable land use?
4) What strategic policy adjustments are required to successfully incorporate agricultural projects or activities in schools to foster a generation competent in sustainable practices?

METHODOLOGY

In order to address the problem statements stated earlier, this paper is an attempt to explore the connection between Malaysia education and agriculture projects. This includes the reasons why schools and other learning institutions in Malaysia should adopt and adapt agricultural activities, such as urban garden or community farm projects, in the curricula. Articles reviewed were obtained online, via Google Scholars search engine. The thematic search focuses on related keywords such as: school gardens, school farm, garden-based education, school-based agricultural education, agricultural programs in schools, food security, sustainability, urban gardens, community gardens and students' engagements. Consequently, 22 articles (2019 – 2024) were analysed to look at the impacts and the need of agricultural activities in Malaysia school curricula, as well as necessary policy adjustments to incorporate agriculture activities in school curricula.

INTERSECTION BETWEEN THE MALAYSIA EDUCATION BLUEPRINT AND AGRICULTURE PROJECTS.

The "Malaysia Education Blueprint 2013-2025" is a thorough education plan designed by the Ministry of Education to revolutionize the education system in Malaysia. The blueprint aims to address potential opportunities and challenges in the education sector. Essential aspects such as quality, access, equity, efficiency and unity have become the focus in preparing Malaysian students to face the demands of the 21st century (Ministry of Education Malaysia, 2013). Thus, active learning strategies such as experiential learning, Problem-Based Learning (PBL) and STEM are relevant to the principles mentioned in the blueprint. These learning strategies can be exercised through practical agriculture activities or projects in education.

The Malaysia National Education Philosophy (NEP), which enshrines the Ministry's and Government's vision of education, emphasizes the holistic development of individuals to achieve their fullest potential in a balanced and harmonious manner. Integral to the education blueprint, the NEP advocates for an education system that focuses on the development of intellectual, emotional, spiritual, and physical. It also promotes responsible, knowledgeable, and ethical individuals while developing well-rounded individuals.

Accordingly, one crucial aspect that students will encounter is related to the development of transferable skills which are important for students to survive not only in the workplace but also in facing global issues. These skills can be found listed in the NEP which include critical thinking, employability skills, effective communication, computational thinking, advanced problem-solving skills, strong reading comprehension, proficient writing skills, and effective language learning strategies. To support this idea, Chandra Vadhana and Zakkariya (2022) mentioned the need to strongly possess such skills among students to assist them in navigating the modern workplace and globalized environment.

The perspective above is aligned with the inclusion of agriculture elements in the school curricula and such soft skills can be fostered through school-based agricultural projects or activities which provide learners a means to participate in hands-on experiential learning. For instance, urban school garden or community farm managed by students has the potential to immerse learners in an experiential learning setting as they are given the opportunities to actively interact and reconnect with the practical and authentic elements of the real world.

To add more, school-based agricultural projects as suggested above are commonly based on constructivism and have the potential to encourage accountability among learners, thus promoting the principles of experiential learning. encompasses intellectual, spiritual, emotional, and physical development of the learners (Skelton et al., 2020; Normazian et al., 2021; Lohr et al., 2022). The experiential learning setting of school-based garden or farm projects is further explained through Experiential Learning Cycle by Kolb (1939). Aligned with the NEP, this learning theory focuses on the process of converting experience into impactful learning and prepares the learners to face the real world. Students' experience, encompassing their ideas, emotions, and surroundings, influences their learning process on the process of learning. Therefore, agricultural projects carried out in school settings offer a practical avenue for experiential learning through hands-on gardening activities. The direct engagement with these activities (e.g: planning a garden, costing, planting, harvesting, maintaining and composting) allows students to immerse themselves in concrete real-world experiences, which later on foster a deeper connection to the subject matter (Klemmer et al., 2005). Moreover, through reflection on the gardening practices, observation of the plant growth and evaluation the outcomes of their actions promotes the Reflective Observation stage in Kolb's cycle. Coincidentally, the Abstract Conceptualisation of the cycle is met through reflective process when the critically think about their experiences and the theories and principles involved in the agricultural projects.

The incorporation of agriculture projects into the curriculum becomes the platform for meaningful, authentic and contextually relevant learning experience for learners. Agricultural projects such as school garden, community urban farm and compost projects are useful in cultivating essential skills for the 21st century which include teamwork, problem-solving, critical thinking and environmental awareness (MckKibben, 2021). Moreover, the hands-on nature of agriculture projects also supports differentiated instruction, catering to diverse learning styles and needs. Agricultural projects organized in learning institutions supports the aims of the Malaysia Education Blueprint as the activities encourage the use of Project-Based Learning (PBL) and STEM education. These projects offer students actual opportunities to apply STEM principles, hence reinforcing their understanding through hands-on experiences (MckKibben, 2021). These efforts support the development of critical thinking, problem-solving, and practical skills, which are in line with the overall objectives of the blueprint to raise the quality of education and upgrade the country's educational system (Intan et al., 2022). Therefore, agricultural and farming initiatives in educational institutions have a crucial impact on advancing project-based learning (PBL) and STEM education. These initiatives contribute to achieving the objectives outlined in the Malaysia Education Blueprint and nurturing individuals who are well-rounded and equipped with the necessary skills to meet the demands of the 21st century.

THE CONNECTION TO SUSTAINABLE DEVELOPMENT GOALS.

The Sustainable Development Goals (SDG) were launched by the United Nations (2019) with 17 goals related to sustainable development. Goal 2: Zero Hunger aims to ensure food security, eradicate hunger, improve nutrition and encourage sustainable agriculture practice around the world. Therefore, students must be equipped with the knowledge and skills to address sustainability challenges and contribute to creating a more sustainable world (Kilai Riksaning Ayu et al., 2024). Programs which are related to agriculture such as school gardens and farm projects, contribute to promoting the goal stated earlier. These programs are relevant with the aim to end world hunger, as stated in Goal 2 of SDG. Valuable lessons and skills can be imparted to learners through these projects. Educational institutions become the avenue to teach about sustainability, such as to foster biodiversity, conserve valuable resources as well as minimize activities that can harm the environment. (Purcell et al., 2019). However, these agriculture projects impact more than just hands-on learning opportunities for the students. Mperejekumana et al. (2023) confirmed that they are taught to practice farming that allows food security form a long-term basis.

Tasks such as maintaining gardens, cooking, and managing food waste systems encourage involvement on campus. As they become the avenue to community connection and experiential learning, it also aid in sustainability awareness by promoting meaningful understanding of healthy and sustainable food practices (Purcell et al., 2019; Siwaraju & Mahmud, 2022) This evidently promotes food security for the future. These agricultural initiatives also help to handle issues on food security through supporting local economies thus reducing dependence of importing food from other countries. As the food is cultivated locally, it contributes to the goals of sustainability and resilience in food systems (Purcell et al., 2019; Green et al., 2020; Burton et al., 2023; Kempler, 2024).

Thus, school-based agricultural projects are ideal for learners as it does not only promote the development of transferable skills. By growing their own food, learners contribute to promoting local produce, increase food security and also develop consciousness about the food they choose to eat. By embedding these principles within the education system (i.e. the curricula) Malaysia can cultivate future generations that are more conscious about sustainable food production and consumption patterns. Agriculture programs carried out by schools or universities evidently will add in supply to the food production of national farm produce. Consequently, the dependence on food imports will be reduced as the citizens are relying more on the food produced locally.

CASES OF AGRICULTURE PROGRAMS IN EDUCATION SETTINGS

In numerous schools across the globe, various agriculture projects have been brought forth to empower learning and community involvement. For instance, Australian primary schools have been using vegetable and herb gardens as important learning resources in different academic spheres such as social and emotional aspects, nutritional knowledge on food, environmental balance enhancement. (Burton et al., 2023). These gardens offer opportunities for practical skills development, cross-curricular activities and hands-on learning.

Additionally, the presence of school gardens provides not only academic rewards but also paves the way for empowerment, sustainability and cohesiveness within the society. Initiatives like The Green School Programme in Fiji advocate for schools to be community-based institutions that promote conventional wisdom on farming and agriculture that is sustainable as well. Their primary aim is to ensure that schools are not only healthy, but they also empower people within the communities (Katz, 2024). Meanwhile, in the United States, school gardens are integrated into national education programs (Lavrinoviča, 2021). This integration encourages a sense of pride, belonging and ownership among students leading to improved connection to the school.

The inclusion of agriculture programs in education settings does not only involve learners and education community. In Cambodia, research was conducted to evaluate the consequences of parental engagement in upscaling agricultural technologies from school gardens to domestic farms. The idea of this program is to make school garden projects more beneficial beyond school settings so that apart from students, their relatives as well as the society at large can equally benefit from practical agricultural education and nutrition regime training (Ader & Richards, 2023). Through engagement of parents in such projects, schools can propagate knowledge extension in farming to a larger audience thus promoting sustainable farming practices and community empowerment.

Malaysia has not lagged in urban allotment schemes including community-based agriculture programs. Examples of significant community garden systems in Putrajaya are the “Bumi Hijau” and “Program Kebun Komuniti” which involve stakeholder engagement while advocating for urban farming initiatives aimed at sustainable agricultural practice and food security (Nazanin et al., 2020; Milah & Rosmiza, 2021). Without a doubt, these efforts hold the possibility of school and higher education involvement as an initiative to include agriculture elements in Malaysia education curriculum. For example, schools could incorporate urban farming and community gardening in some parts of their curriculum. Another option is to have weekly gardening as part of a lesson or any related assignment. Colleges can as well teach sustainable farming, gardening including organic methods whose results are to the benefit of a community. Furthermore, some of these programs call for trainings, workshops or seminars on urban sustainable farming targeted at the public in areas such as agriculture on a small scale.

DISCUSSION AND CONCLUSION

The integration of agriculture programs into the Malaysian educational system brings numerous benefits, at which experiential learning is enriched and students are fully prepared for the challenges that lie ahead in the future. For this integration to happen, adjustments should be made to the relevant policies. Insights and evidence from relevant references should be attained before new policy suggestions can be proposed. One policy to be mandated is to include hands-on learning experiences such as garden maintenance or farm visits (Ingram et al., 2018). Moreover, sufficient resources and financial aid allocation need to be planned for education institutions not only to kick start the agriculture program, but also to sustain it. (DiBenedetto et al., 2018). In order to develop and implement agricultural programs effectively, collaborations among stakeholders should be made. This includes government entities, NGOs and community members. Consequently, these policies and schemes should be reviewed after each cycle in order to evaluate the effectiveness and relevance of the integration.

Another aspect to be considered is the need to assess the impact of school-based agricultural projects on students. In order to evaluate the total effectiveness of these projects in schools, it is crucial to measure the extent to which students acquire transferrable skills, environmental awareness and readiness to support sustainable development. Thus, it is necessary to utilize a combination of quantitative and qualitative measurements. For example, evaluation of knowledge acquisition may be accomplished by utilizing quizzes or interviews by teachers, reflective journals may be assigned for students to express their understanding and personal experiences on relevant issues and focus groups may encourage dialogues among students. Furthermore, by maintaining logs of participation may assist in observing their active involvement in the projects.

Additionally, behavioural surveys and questionnaires may be utilized to assess students' perspectives on sustainable goals, food security and their individual dedication to implementing such practices. These surveys may concentrate on behaviours such as composting, minimizing food waste, promoting local food systems, and comprehending the carbon footprint associated with food choices. It is also beneficial to monitor students' participation in extracurricular activities, groups, or initiatives related to sustainable gardening and food sustainability. Higher levels of dedication and actual application of sustainable practices can be shown by participation rates and the extent of involvement. This can be observed through their efforts in assuming leadership positions, founding community gardens, or engaging in farm-to-table initiatives.

To conclude, the integration of agriculture programs into the Malaysian educational curriculum can be considered as necessity as it is in relevance with the aims of the Malaysia Education Blueprint as well as the Sustainable Development Goals. Through the integration, students will be equipped with essential knowledge and transferable skills needed to survive in their future endeavour. Inevitably, new challenges will arise as we continues to advance in the era of globalization. Hence there will always be the need for policy amendments in the future. But at least we can be at ease knowing that the future generations will be competent in practicing sustainable living.

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PRE-SOWING TREATMENT FOR IMPROVING THE GERMINATION OF *Coffea liberica* SEEDS

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ABSTRACT

Coffea liberica seeds can be dormant for three to four months followed by erratic germination despite having optimal light, moisture and temperature in the germination medium. It could be attributed to the mucilage residue covering the endocarp and germination inhibiting compounds within the seeds. Removal of mucilage is laborious, impractical and probably lethal when the endocarp is damaged, exposing the seeds with only papery seed coat to microbial infestations. The current experiment found that air drying of the seeds at room temperature of 28±2 °C for one week prior to sowing facilitated germination. The desiccated seeds sprouted as early as 18 days after sowing in moistened sand sized 0.2-2.0 mm in enclosed plastic boxes under laboratory condition. Most other desiccated seeds germinated within six to eight weeks. There was a germination rate of 82.5% following ambient desiccation as a pre-sowing treatment for the seeds. On the other hand, the control non-desiccated seeds had a 77.5% germination rate but these seeds germinated no earlier than 80 days. In addition, the seeds had fungal growth on the mucilage residue during the initial 10 days when sown non-desiccated in the moistened sand. Removal of the fungi using slow-running tap water was needed to save the seeds. On the other hand, soaking the non-desiccated seeds in tap water for one week was beneficial in overcoming the fungal problem but this treatment was not apparent in speeding up the seed germination process; the seeds generally germinated after 70 days with soaking as pre-sowing treatment. Future work could be focused on the physiological changes during seed desiccation to further optimize this technique for enhancing the germination of this beverage crop.

Keywords: Desiccation, Dormant, Inhibition, Microbial, Mucilage.

INTRODUCTION

Among the widespread types of coffee plant, *Coffea liberica* is cultivated in many countries in Southeast Asia, particularly in Malaysia (Ismail *et al*., 2014; Duaja *et al*., 2019; Haile & Kang, 2019; Mubarak *et al*., 2019; Halim-Lim *et al*., 2022). Despite the numerous attempts that have been made to develop efficient vegetative and micro-vegetative propagation systems, the vast majority of *C. liberica* is still regenerated directly from seeds and propagated mostly through the seedlings. However, the seeds have a sluggish and non-uniform germination in terms of time, which makes it difficult to acquire a large number of uniform and robust seedlings at the time of transplanting (da Silva, 2002; Eira *et al*., 2006; Kawabata *et al*., 2022). The emergence period of the seeds could extend beyond 90 days depending on the germination medium and temperature. *Coffea liberica* seeds also frequently show problems related to changes in their internal morphology, such as the occurrence of deformities, damage caused by tissue degeneration, and injuries. All these issues can result in a loss of the physiological capacity of the seeds within (Rosa *et al*., 2005; Trujillo *et al*., 2019).

The germination of *C. liberica* seeds is influenced by a variety of environmental conditions, including temperature, light, pH level and germination medium condition (Ahn *et al*., 2021). When mature berries with a minimum moisture content (MC) of 60% are collected from the trees (Adnan *et al*., 2017), their seeds enclosed in endocarp tolerate postharvest desiccation to about 15%. The seeds have been documented as intermediate (Ellis *et al*., 1990). During desiccation, the endocarp covering the seeds could be modified to allow for effective imbibition and germination. On the other hand, mucilage as a viscous liquid residue on the endocarp could also be hindering the seed germination. It may also contain some biocompounds that could affect seed germination. Soaking has been beneficial for chemical change or microbial degradation that reduces or converts these compounds into other simpler molecules and thus, seed germination can be initiated earlier. Past research by Gebreselassie *et al*. (2010) indicated that the removal of the seed coat followed by soaking in pure water for 72 hours was an effective method to increase the germination of *C. liberica* seeds. The germination percentage and speed that describe the physiological quality of the seeds are important for the potential fitness of the crops in the subsequent field planting (Sudrajat *et al*., 2015). Thus, the objectives of this study were to determine *C. liberica* seed germination following ambient air drying and soaking for varying periods in tap water. The treatments are practical and inexpensive for any farmer. Treatments that facilitate seed germination would be useful in the propagation of *C. liberica* by seeds.

MATERIALS AND METHODS

Test Material

A total of 260 fully ripe *C. liberica* berries were collected from the field in Jasin Campus of Universiti Teknologi MARA in the end of March 2023. The pericarp of each berry was removed manually and the two seeds in it were taken out. The seeds were cleaned by rubbing off the mucilage on the endocarp using a scrubber. Then, the seeds were washed with running tap water, pat dried and air dried overnight to remove the excessive surface water on the seeds before the seeds were used as test materials. A total of 480 seeds of uniform size were selected for experimentation.

Study Location

Experimentation was carried out in the Crop Science Laboratory, Faculty of Plantation and Agrotechnology in the Jasin Campus, Universiti Teknologi MARA.

Preparation of Germination Medium

Sand was sieved to obtain that sized 0.2 to 2.0 mm. A series of washing and rinsing cycles with tap water was conducted on sieved sand particles. After undergoing a thorough washing process, the sand was subsequently exposed to direct sunlight in order to achieve complete drying. Following this, rectangular plastic containers were each filled with 300 g dry sand, in preparation for seed germination at a subsequent phase. The systematic and thorough process was meant to achieve the sanitation and optimal level of cleanliness in the sand, thereby creating an ideal setting for the successful germination and growth of seeds.

Pre-sowing Treatment of Seeds

Some pre-sowing treatments on the seeds were studied for enhancing the seed germinability as shown in Table 1. The brief descriptions of the treatments were as follows.

- The non-desiccated seeds were those after overnight air drying as mentioned above.
- Desiccated seeds were obtained after continued air drying for one week under ambient conditions at 28±2 ºC in the laboratory.
- Both the non-desiccated and desiccated seeds were subjected to soaking with tap water for zero, one or two weeks, respectively.

Table 1: Pre-sowing treatment

Treatment	Air drying for 1 week under ambient condition in laboratory	Soaking with tap water
No desiccation (Control)	No	No
No desiccation, soaking for 1 week	No	Yes – 1 week
No desiccation, soaking for 2 weeks	No	Yes – 2 weeks
Desiccation	Yes	No
Desiccation, soaking for 1 week	Yes	Yes – 1 week
Desiccation, soaking for 2 weeks	Yes	Yes – 2 weeks

For each soaking treatment, the seeds were immersed in 175 mL tap water in a 320 mL plastic bottle. The amount of water has been calibrated as sufficient to cover the seeds in the bottle. Throughout the seed-soaking procedure, each bottle was meticulously capped loosely and subjected to gentle swirling in every six hours. The cap was securely fastened for a brief duration of a few seconds during swirling. The seed soaking procedure was carried out under conditions of low light, while having a rather consistent ambient temperature in the laboratory. In the end of the soaking procedure, the seeds were briefly washed before sowing.

Each treatment, as in Table 1, was replicated four times based on a completely randomized design (CRD). There were 10 seeds per replicate.

Seed Germination

The experiment employed rectangular plastic containers with their lids for seed germination test. Each container of 1000 mL was filled with 300 g cleaned and dried sand as mentioned above. A volume of 50 mL tap water was applied to each container, ensuring sufficient moist in the sand.

After desiccation and seed soaking treatments, the treated seeds were initiated into the germination process through their placement at a depth of 1 cm in the moistened sand in the rectangular plastic containers. Seed sowing was carried out based on a completely randomized design (CRD).

Data Collection

Seed MC, pH of suspension during seed soaking and seed germination were recorded in this experiment. A weighing method was utilized to ascertain the MC of non-desiccated and desiccated seeds in this study. For each type of seeds, 40 seeds in four replicates were used for the determination of seed MC. At the outset, ten seeds were meticulously positioned within a pair of moisture dishes devoid of any contaminants and moisture. The moisture dishes containing the seeds were weighed using an analytical balance. Then, the seeds in the moisture dishes were transferred to an oven. The oven was adjusted to a temperature of 103±2 °C and allowed to function for a period of 16±1 hours. The covers of the moisture dishes were opened to allow the moisture removal from the seeds in them during drying procedure.

After the completion of the drying process, the covers of the moisture dishes were closed. Then, the moisture dishes containing the oven dried seeds were taken out from the oven and subsequently cooled for a duration of one hour in a desiccator. Following the cooling process, the weight of the seeds in the moisture dishes was recorded again using the analytical balance. Lastly, the oven-dried seeds were removed from the moisture dishes and the weight of the moisture dishes was taken. The seed MC was subsequently determined as a fresh weight percentage (%) and rounded to one decimal place, employing the following formula:

$$\%\, MC = \frac{(M_1 - M_2)}{(M_1 - M_3)} \times 100$$

where,
M_1 = the weight of the moisture dishes and seeds before oven drying (g).
M_2 = the weight of the moisture dishes and seeds after oven drying (g).
M_3 = the weight of the moisture dishes after oven drying (g).

For obtaining the pH of the suspension in each bottle, pH was assessed daily or every other day using a tabletop pH meter in order to monitor any pH variations during the seed soaking procedure.

The evaluation of seed viability entails the quantification of the potential of plant growth. The germination rates of seeds after their respective treatments, as in Table 1, was assessed by counting them daily or every alternate day, provided that they showed a radicle longer than 5 mm in the moist sand. Germination rate and mean germination time (MGT) were respectively calculated as follows.

$$Germination\ rate = \left(\frac{\sum_{i=1}^{k} n_i}{N}\right)$$

$$MGT = \frac{(\sum_{i=1}^{k} n_i\ x\ d_i)}{N_g}$$

where,
n_i = number of germinated seeds on the i^{th} day after sowing (not the accumulated number)
d_i = i^{th} day after sowing
N = total number of sown seeds

N_g = total number of germinated seeds
k = last day of germination test

Statistical Analysis

In a laboratory setting, the effectiveness of seed germination following treatments was assessed using a completely randomized design (CRD) with four replicates. MCs of non-desiccated and desiccated seeds were compared using independent sample t-test. Non-parametric analysis by means of Kruskal-Wallis test was carried out with the seed germination rates and MGT. Mean comparison of treatments was performed by using Mann-Whitney U test.

RESULTS

Seed Moisture Content (MC) following Ambient Air Drying

The MC of non-desiccated seeds, as measured at 0.59 g H_2O/g FW (fresh weight), exhibited a statistically significant disparity through an independent sample t-test when compared to desiccated seeds, which displayed a MC of 0.18 g H_2O/g FW (Figure 1).

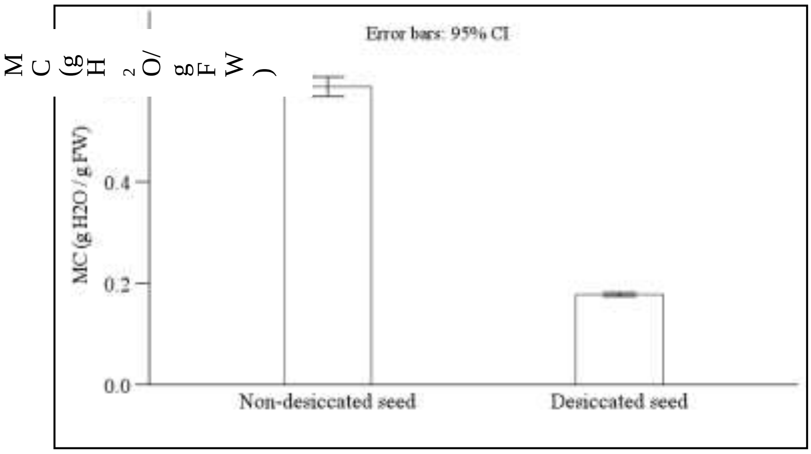

Figure 1: MC of non-desiccated and desiccated seeds (P<0.05)

pH Changes during Seed Soaking

It is noteworthy that the immersion of seeds resulted in a notably reduced suspension pH. The pH of the non-desiccated seed suspension changed from 4.16 to 3.64 indicating that some organic acids were released during soaking (Figure 2). Likewise, the desiccated seed suspension also turned acidic, having pH change from 4.6 to 3.73.

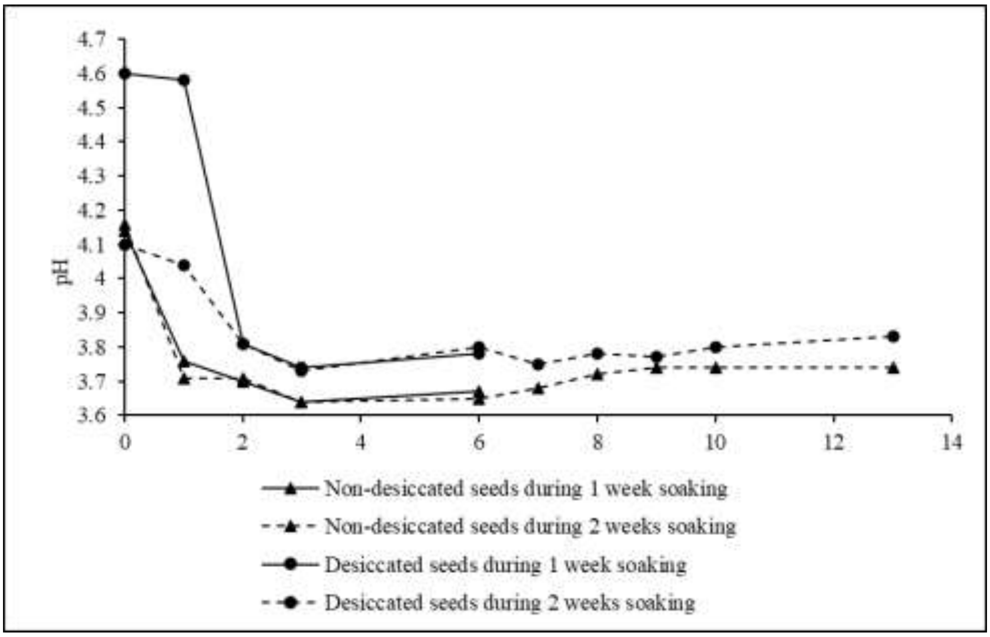

Figure 2: pH changes during soaking of desiccated seeds

Seed Germination

The germinability of the non-desiccated seeds was 77.5% in moistened sand (Figure 3). At the outset, it was observed that the non-desiccated seeds displayed fungal infestation on the mucilage residue. However, this concern was successfully addressed through the implementation of a one-week soaking for the seeds. The soaked seeds had comparable germinability of 80%. However, a prolonged immersion period of fourteen days did not yield any supplementary advantages in relation to the germination capacity of the seeds.

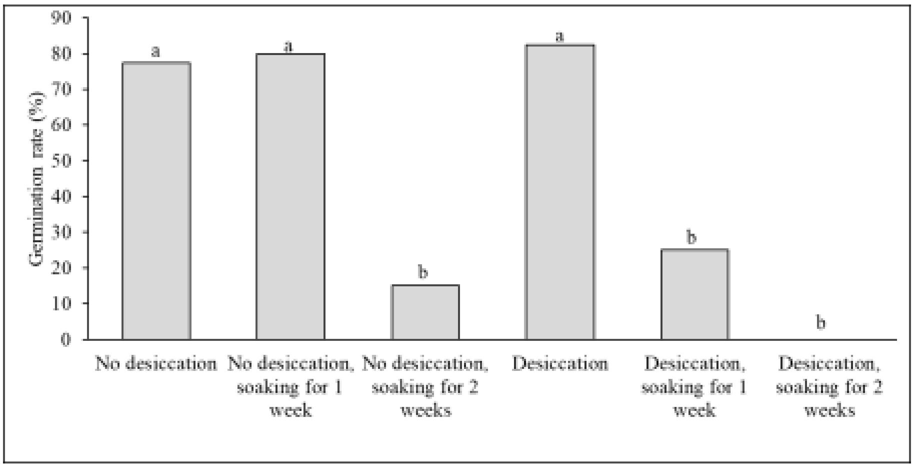

Figure 3: Germination rate following desiccation and soaking of seeds

On the other hand, the germinability of desiccated seeds was observed to be similar to that of the non-desiccated seeds; they had a germination rate of 82.5% (Figure 3). After the desiccation procedure, seed soaking for one week or two weeks could introduce any undesirable microbes to the seeds through the changed seed coat, inducing the death of the seeds.

The utilization of desiccation and soaking for *C. liberica* seed holds the potential to yield notable advantages in terms of promoting and expediting seed germination. In the current experimental study, it was observed that the non-desiccated seeds did not germinate earlier than 80 days (Figure 4). Nevertheless, a captivating discovery surfaced when the seeds underwent a week-long immersion process. The soaked seeds exhibited a shortened period of germination in comparison to their untreated counterparts, as they sprouted about 10 days earlier. Moreover, the incorporation of desiccated seeds into the investigation produced even more encouraging outcomes. The desiccated seeds exhibited a notable variation in the duration of germination. While the earliest germination was noted at 18 days after sowing, the majority of desiccated seeds initiated sprouting within a span of 45 to 70 days. Thus, desiccation could be a beneficial method for enhancing early seed germination and may have implications for diverse agricultural and horticultural applications.

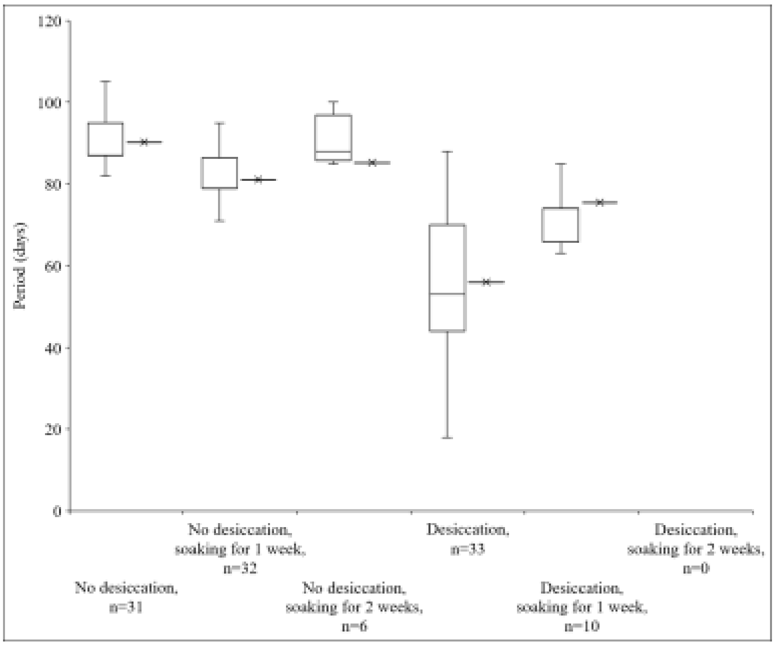

Figure 4: Quartiles and whiskers of germination period following desiccation and soaking of seeds; x indicates MGT

DISCUSSION

The results presented in this study offer significant insights into the impact of desiccation and soaking on the germination rate of *C. liberica* seeds. The focus of the discussion can be directed towards the comparison and evaluation of germination rates observed in the treated seeds, as well as the implications these treatments had on seed viability and the prevention of fungal infestation.

To begin with, it is worth noting that the germination rate surpassed 75% for both groups, indicating that the impact of desiccation down to MC of 0.18 g H_2O/g FW on the overall germinability of *C. liberica* seeds was not significant. According to the observations derived from the examination of desiccation's effectiveness, it has been determined that this process plays a crucial role in facilitating the germination of seeds (Baskin & Baskin, 2020). Nevertheless, it is crucial to acknowledge that non-desiccated seeds initially exhibited fungal infestation on the mucilage residue after sowing in the moist sand. The resolution of this matter was effectively achieved by implementing a seven-day period of seed soaking, highlighting the significance of this procedure in mitigating fungal contamination and promoting ideal seed sprouting.

Subsequently, a comparative analysis of the observed germination periods among treatments indicated that desiccation could be a practical technique for overcoming the dormancy in *C. liberica* seeds. Non-desiccated seeds exhibited a distinct germination period of above 80 days. Nevertheless, upon subjecting the seeds to a week-long soaking process, it was observed that the duration of germination was shortened to 70 days. This suggests that the process of soaking the non-desiccated seeds can accelerate the germination process.

Moreover, the inclusion of desiccated seeds in the investigation yielded even more promising results. The seeds that had undergone desiccation displayed significant variability in the length of time required for germination, with the majority of seeds initiating sprouting between 45 and 70 days. This suggests that desiccation may serve as a favorable approach for promoting early seed germination. Prior research has also documented comparable results, underscoring the beneficial impacts of desiccation on the processes of seed germination and initial seedling development (Berjak & Pammenter, 2008).

In summary, the findings indicate that both desiccation and soaking techniques exhibit promises in facilitating the successful germination of *C. liberica* seeds. Nevertheless, desiccation seems to present benefits in terms of accelerated germination, as a larger proportion of seeds sprouted within a reduced time frame when compared to the non-desiccated seeds. Seed desiccation might be useful in a variety of agricultural and horticultural contexts for accelerating the germination of seeds. For the orthodox or desiccation-tolerant seeds, desiccation reduces moisture, stops the development of microbes and enzymatic processes, and keeps seeds viable for future germination. However, *C. liberica* seeds do not tolerate very low MC while desiccation down to a certain level could modify the seed coat and speed up seed germination. More studies should be done to learn how and why desiccation affects seed viability and how it can affect seedling emergence in *C. liberica* seeds in the long run.

CONCLUSION

Based on the results obtained, desiccating *C. liberica* seeds before sowing improved their germination rates. Soaking the non-desiccated seeds for a week before planting reduced the danger of fungal infection without impairment in seed germinability. It is imperative to acknowledge that prolonging the soaking duration of seeds beyond one week did not yield any supplementary advantages in relation to germination. These findings serve as a reference for seed management practices, facilitating the achievement of optimal planting material availability for this beverage plant species.

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067-063

GENETIC DIVERSITY AND POPULATION STRUCTURE OF IOI GROUP OIL PALM (*ELAEIS GUINEENSIS*) BREEDING MATERIALS BASED ON MICROSATELLITE MARKERS

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ABSTRACT

This study presents a comprehensive analysis of genetic variation and population structure within IOI's oil palm populations utilizing 24 Simple Sequence Repeats (SSR) markers. Examination of 627 individuals from 63 crosses revealing a total of 109 alleles, averaging 6.167 alleles per SSR locus. Heterozygosity analysis revealed high observed heterozygosity (Ho) and expected heterozygosity (He), consistent with previous studies, indicating high heterozygosity levels. Marker polymorphism assessment showed moderate polymorphism, with an average PIC value of 0.54. Genetic differentiation analysis displayed significant divergence among populations, with an average Fst value of 0.425. Population structure analysis identified two major clusters, primarily influenced by different improvement programs. Structure Analysis identified two optimal genetic clusters (K = 2) corresponding to Dura-dominated and Tenera/Pisifera-dominated clusters, with further sub-clustering within the latter. Our findings contribute to understanding the genetic diversity and population structure of oil palms, vital for effective breeding and conservation strategies. We explore the significance of SSR markers and motifs in oil palm genetic analysis, drawing from a comprehensive review of research literature and empirical data. The selection of SSR markers, guided by their polymorphic nature and genomic distribution, aims to provide researchers with optimal markers for comprehensive genetic analysis in oil palm. Through the selection of 24 diverse SSR markers covering all chromosomes (except 7), this study addresses technical limitations while ensuring informative markers across the oil palm genome. Our study provides valuable insights for researchers in selecting an optimal combination of SSR markers to maximize genetic information within the oil palm research community. The study highlights the need for standardized genotyping procedures to preserve oil palm genetic diversity. A comprehensive genetic database would support research and guide

informed decisions on breeding strategies, conservation efforts, and management practices, ensuring the industry's long-term sustainability.

Keywords: Oil Palm, Genetic Diversity, Population Structure, Simple Sequence Repeat (SSR) Markers, Genotyping.

INTRODUCTION

Genetic diversity in oil palm resources (OPGR) is crucial for developing high-yield, pest-resistant cultivars. Integrating genomics and phenomics in ex-situ collections enhances our understanding of oil palm's molecular foundations, improving conservation, management, and utilization of OPGR. Strategic breeding efforts using molecular techniques rely on diversity assessment to select resilient, productive varieties.

Established in 1983, the IOI Group has significantly contributed to the plantation industry, with approximately 176,925 hectares of oil palm planted as of the 2023. Renowned as one of the world's largest integrated palm oil producers, IOI manages 98 estates, 15 palm oil mills, and diverse resource-based manufacturing businesses (refining, oleochemical and specialty oils and fats sub-segments) and four research and development ("R&D") centres across Malaysia and Indonesia. IOI's involvement in oil palm research dates back to 1990, marked by the acquisition of Dunlop Estates Berhad and the subsequent addition of Unipamol Group in 2003 (Donough and Chia, 2005). The Oil Palm Genetics Laboratory (OPGL), housing breeding materials from Dunlop and Pamol, forms a comprehensive collection of breeding crosses as described by Donough and Chia (2005), along with germplasm materials acquired over the years.

Understanding the genetic diversity and population structure of oil palm breeding materials is vital for selecting resilient and productive varieties. Microsatellites or SSRs are favoured for their multi-allelic nature, specificity, and low cost (Wang *et al.* 2008). They play a crucial role in genetic diversity studies, evolutionary analysis, constructing linkage maps, and marker-assisted selection (Jonah *et al.*, 2011; Kalia *et al.*, 2011). This study aims to review and select SSR markers for genetic diversity and population structure analysis in the company's oil palm. Previous studies have explored oil palm genetic diversity extensively, with 267 papers available since 2000. Most authors utilized between 8 to 20 SSR markers, primarily from (French Agricultural Research Centre for International Development) (CIRAD) and Malaysian Palm Oil Board (MPOB) sources. However, despite the wealth of research, there is a notable gap in the literature regarding the compilation and review of marker sets used in these studies. This paper addresses this gap by not only reviewing SSR marker studies but also by systematically selecting and evaluating them.

Three publicly accessible oil palm SSR databases exist: MPOB's OPSRI (http://opsri.mpob.gov.my/, Rosli *et al.*, 2022), OpSatdb (https://ssr.icar.gov.in/index.php, Kalyana Babu *et al.* 2019), and CIRAD's TropGENE-DB (https://tropgenedb. cirad.fr/tropgene/JSP/index.jsp, Hamelin *et al.*, 2012). Numerous studies have explored genetic diversity in oil palm using various molecular markers particularly microsatellite makers. Billotte *et al.* (2001, 2005) pioneered oil palm SSR research, testing 99 primers. Subsequent studies, such as those by Bakoumé *et al.* (2009, 2015) and Zulkifli *et al.* (2012), employed SSR and genic-SSR markers, respectively, to assess genetic diversity in wild germplasm collection of different oil palm species of MPOB. Durand-Gasselin *et al.* (2009) utilized 12 SSR markers for ID checking in oil palm variety selection and production processes at CIRAD. Thongthawee *et al.* (2010) suggested using four loci for planting error detection and 7-8 for confident pollination error detection in an oil palm breeding population, based on their parentage analysis with 8 SSR markers.

Seng *et al.* (2014) developed an SSR markers panel for clonal fidelity and breeding legitimacy, while Budiman *et al.* (2019) evaluated genetic diversity in company oil palm breeding populations using 16 SSR markers. Sunilkumar *et al.* (2020) used nine oil palm-specific SSR primer pairs to assess genetic variability in germplasm accessions of The Indian Council of Agricultural Research (ICAR). Gan *et al.* (2021) examined genetic diversity using 107 SSR markers in a Nigerian-based germplasm, identifying a core collection for conservation. Magaña-Álvarez *et al.* (2023) reported using twenty CIRAD SSR and seven RAM markers to evaluate genetic diversity in 151 oil palm accessions from all regions of Mexico. These diverse studies collectively contribute to our understanding of oil palm genetic diversity using various marker types and populations.

The present paper aims to 1) Review and select SSR markers published in studies related to genetic diversity and population structure analysis in oil palm 2) Assess the genetic diversity and determine the genetic structure of IOI's oil palm breeding materials using the chosen SSR markers 3) Develop an SSR markers panel tailored for the diverse range of breeding and clonal materials. This paper also aims to contribute to the broader understanding of oil palm genetics, providing valuable insights for future breeding efforts and conservation strategies.

MATERIALS AND METHODS

OIL PALM BREEDING CROSSES, ORTETS AND RAMETS SAMPLING

Leaf Sampling involved collecting a total of 726 leaf samples from parental palms and their offspring, representing 63 breeding crosses. These samples were gathered from the IOI Research Centre in Gemencheh, Negeri Sembilan, and IOI Pamol in Kluang and Sabah. The sampled palms covered IOI's breeding materials, encompassing both duras lineage (OPGL LBP, including pure and mixed lines from Ulu Remis, Serdang Avenue, Socfin, Klanang Bharu, DOA, MPOB, Angolan, and Nigerian Dura) and pisiferas lineage (AVROS, NPM, Ekona and Binga). For consistency in analysis, 15 palms per cross were randomly sampled, with the actual number of palms (progenies) varying based on breeding objectives and trial design. Additionally, leaf samples were collected from the ramets of dura clones, which are slated for commercial semiclonal DxP seed production. Limited samples were also taken from the ramets of pisifera clones, as pisifera cloning is relatively uncommon. These detailed collections aim to provide a comprehensive representation of genetic diversity within the breeding programme, ensuring a robust analysis for the project.

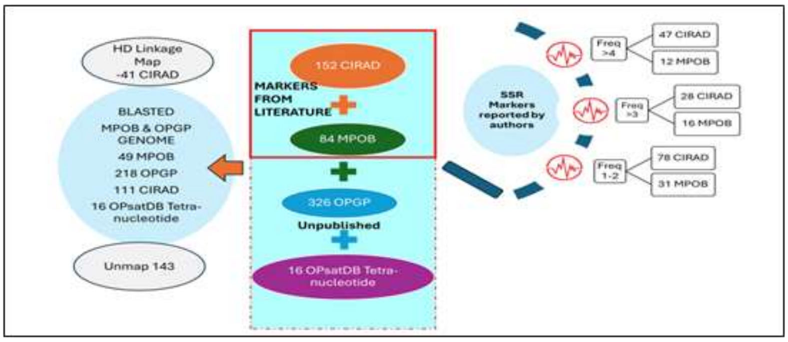
DNA EXTRACTION AND QUANTIFICATION

Mature leaves were collected, and total genomic DNAs were extracted using the FavorPrep™ Plant Genomic DNA Extraction Mini Kit (Favorgen, Taiwan). To assess both quantity and quality, the extracted DNA was quantified using a DeNovix spectrophotometer. Additionally, DNA quality was confirmed through gel electrophoresis on a 0.8% agarose gel. The genomic DNAs were stored in fridge at 4°C for further analysis and -80 °C in the freezer for long term storage.

SSR MARKER COMPILATION, FILTRATION AND SELECTION

The process of SSR marker selection commenced with an extensive compilation from 70 published papers, resulting in a total of 236 markers. This compilation included 152 markers from CIRAD, 84 from MPOB, and an additional 326 mapped markers obtained from Oil Palm Genome Project (OPGP, https://umr-agap.cirad.fr/en/research/main-projects/opgp). Tri- and tetra-nucleotide motif markers from OPsatDB (Kalyana Babu *et al.*, 2019) were also incorporated (Figure 1). Desktop filtration of SSR markers involved several steps: 1) **Frequency-Based Categorization:** All compiled markers were categorized based on the frequency reported in the literature. 2) **Genomic Comparison:** Markers were blasted against the MPOB genome and compared with the OPGP genome (unpublished). 3) **SSR Motif Analysis:** Evaluation of SSR motifs based on specified criteria. Following the filtration process, a total of 48 markers with diverse motifs were shortlisted, with representation from each chromosome. To validate the efficacy of these markers, a panel of 90 samples representing nine crosses, including parental trees, underwent screening. Subsequently, 28 markers exhibiting a high level of polymorphism were chosen for genotyping across all samples in the project.

Figure 1: Source and distribution of SSR Markers - 1) Successfully blasted to oil palm genome, 2) Manually aligned to OPGP high density linkage map, and 3) Remaining unidentified. Markers gathered from literature are categorized and grouped based on the frequency reported by various authors.



MICROSATELLITE GENOTYPING

DNA fragments were amplified through polymerase chain reaction (PCR) using 48 SSR primers for preliminary screening and 24 for genotyping all samples. PCR reaction performed in 11 µl final volumes with mixture of 20 ng of genomic DNA, 5µl exTEN 2X PCR Master Mix (1st Base, Singapore), 0.02 µM of forward and 0.08µM reverse primers and Fluorescent-Labelled M13 Primer, and Nuclease-Free Water. The PCR was done on a Veriti™ 96-Well Thermal Cycler (Applied Biosystems™, USA) using the two-steps PCR protocol. The first step consists of initial denaturation for 5 minutes at 95°C followed by 30 cycles of 30s at 94°C, 45 seconds at the annealing temperature and 72°C for 45s, followed by the second step of 8 cycles at 94°C for 30s, 53°C for 45s and 72°C for 45s, and a final incubation at 72 °C for 10 minutes. The reaction products were checked by agarose gel electrophoresis. Fragment analysis of the all SSR loci was conducted using the 96-capillary ABI Prism 3730 XL DNA Analyzer (Applied Biosystems Inc, USA) with the GENESCAN-500 (Liz) size standard by Apical Scientific Sdn Bhd, Malaysia.

DATA CLEANING PROCESS

The initial step in data analysis involved thorough data cleaning to align it with software input requirements. This included identifying and addressing missing data by cross-referencing with electropherograms. Furthermore, the data underwent normalization based on the marker's motif (dinucleotide, trinucleotide, and tetranucleotide). Upon completion of data cleaning, a comprehensive global analysis was conducted, utilizing all input data without excluding any data points. Allele scoring data, provided by the third-party genotyping provider (First Base Laboratories, Malaysia), were meticulously organized for analysis using Microsoft Office Excel. Subsequently, the appropriate data files were prepared to facilitate subsequent analyses, including genetic diversity assessments, population parameters, and population structure analyses, using various specialized software tools. This systematic approach ensures the reliability and accuracy of the genetic analysis performed on the oil palm breeding materials.

GENETIC DIVERSITY ANALYSIS

The genetic diversity analysis encompassed several steps, starting with the manual cleaning and normalization of genotyping data based on marker motifs. Once the dataset was prepared, various software tools were employed to explore different aspects of genetic diversity. The data analysis consisted of allele frequency analysis, microsatellite polymorphism analysis and genetic diversity analysis. In the allele frequency analysis, POPGEN v1.32 (Yeh *et al.*, 1999) software was utilised to obtain results for the number of different alleles per locus (*Na*), number of effective alleles (*Ne*), and Shannon's Information Index (*I*) (Lewontin, 1972). The observed heterozygosity (*Ho*) and expected heterozygosity (*He*) were calculated using GeneAlEx v6.503 software (Peakall and Smouse, 2012). For the microsatellite polymorphism analysis, Cervus v3.07 (Kalinowski *et al.*, 2007) was utilized to analyse the informativeness of all the SSR markers using the Polymorphic Information Content (*PIC*) value (Botstein *et al.*, 1980). The genetic diversity analysis involves multiple parameters. In this study, the parameters used were Fixation Index (*F*), F-statistics (*Fis*, *Fit* & *Fst*), Gene Flow (*Nm*), The Analysis of Molecular Variance (AMOVA) (Excoffier *et al.* 1992) and Principal Coordinate Analysis (PCoA) were analysed using GeneAlEx 6.503 software (Peakall and Smouse, 2012). In addition, phylogenetic analysis using unweighted pair group methods with arithmetic mean (UPGMA) via MEGA software (Kumar *et al.*, 2018) and population genetic structure analysis using STRUCTURE 2.3.4 (Pritchard *et al.*, 2000) was done as well. The approach by Evanno *et al.* (2005) was used to select the appropriate K clusters for the studied populations.

RESULT & DISCUSSION

MICROSATELLITE GENOTYPING AND DATA CLEANING

In this project, 630 samples from 63 oil palm crosses were screened using 48 SSR markers. After removing markers with missing data, poor quality, unsuccessful amplification, or monomorphic patterns, 24 markers were retained. Four markers (mEgCIR0832, mEgCIR0894, sMo00130, and mEgIOIPBSB20) failed to amplify, leaving a gap in chromosome coverage as mEgCIR0894 was the only marker for linkage group 7. Post-cleaning, the remaining 24 markers produced 31,500 data points with only 3 missing, which were deemed insignificant and removed.

Deviations from Mendelian genetics in oil palm breeding can arise due to contamination and illegitimacy. Although methods and quality control procedures have been described in the literature (Donough *et al.,* 1993; Chin, 1999; Rao and Kushairi, 1999), the effectiveness of past practices remains uncertain. Additional factors such as technical limitations in genotyping, assortative mating, gene interactions, genetic drift, gene flow, inbreeding, and natural selection can also lead to deviations from expected patterns. Despite these complexities, the study did not exclude possible illegitimate progenies, and data were analyzed without specific exclusions.

A review of 70 publications on oil palm genotyping with SSR markers highlights a significant lack of consensus on optimal design and adoption strategies. Determining the ideal number of microsatellite loci remains challenging due to variation in marker selection based on specific analysis objectives. In this study, 24 highly polymorphic markers from three SSR databases were used for IOI breeding materials, emphasizing the effectiveness of high PIC markers. Recent studies demonstrate varied approaches and marker sets across regions, reflecting diverse research priorities and methodologies within the oil palm genomics community. The complexity of the oil palm genome and genetic diversity necessitates specific protocols for different varieties, with ongoing efforts required to develop standardized procedures adaptable to technological advancements. Collaboration among researchers, breeders, and stakeholders is crucial for establishing common procedures and achieving consensus in oil palm genetic characterization.

ANALYSIS OF SSR MARKER VARIATION AND HETEROZYGOSITY LEVELS

In this study, 24 SSR markers was conducted on 627 individuals derived from 63 oil palm crosses, revealing a total of 109 alleles. The allelic diversity, as depicted in Table 1 through allele frequency analysis, exhibited a range of 1 to 12 alleles per SSR locus (Na). Notably, MEgIOIPBSB05 displayed the lowest allelic count (2), while mEgCIR3292 exhibited the highest (12), contributing to an average of 6.167 alleles per SSR locus. Budiman *et al.* (2019) also reported that mEgCIR3292 generated the highest number of alleles (11 alleles) in their studies on oil palm originated from Cameroon.

The average effective number of alleles (Ne) per locus varied from 1.061 at MEgIOIPBSB05 to 6.113 at mEgCIR3886, aligning closely with Taeprayoon *et al.*'s (2015) findings, reporting an average of 5.45 alleles within the range of 3 to 8 per locus. Moreover, the outcomes for average alleles per locus surpassed those reported by Okoye *et al.* (2016). Assessing the genetic information content using Shannon's information index (I), mEgCIR3292 stood out with an index of 1.992, indicating a substantial contribution. The average Shannon's information index across all loci was 1.195, ranging from 0.133 (MEgIOIPBSB05) to 1.992 (mEgCIR3292).

Heterozygosity analysis revealed a mean Ho of 0.397 and a mean He of 0.327. Ho values ranged from 0.043 (MEgIOIPBSB05) to 0.735 (mEgCIR3886), while He ranged from 0.035 (MEgIOIPBSB05) to 0.570 (mEgCIR3886). All Ho values exceeded the corresponding He values, indicative of high heterozygosity. Additionally, comparable levels of heterozygosity were reported by Okoye *et al.* (2016) (Ho: 0.683, He: 0.625), Budiman *et al.* (2019) (Ho: 0.708, He: 0.556) and Gan *et al.* (2021) (Ho: 0.644, He: 0.576). It's noteworthy that while the values of Ho and He in this study were comparatively lower, the consistent trend of high heterozygosity remains a key feature of the genetic diversity observed in this investigation.

Table 1: Genetic Analysis Results from CERVUS Software Using 24 Selected SSR Markers in 627 Samples. Includes information on Chromosome Location, Code, Primer Name, SSR motif, number of total alleles (Na), Effective Allele (*Ne)*, Shannon's Information Index (I), Observed Heterozygosity (Ho), Expected Heterozygosity (He), and Polymorphic Information Content (PIC), frequency reported by the author and Null Allele Frequencies F(Null)

Linkage Group	Code	Primer name	SSR motif	Na	Ne	I	Ho	He	PIC	Frequency reported by the author	Null allele frequency estimate F(Null)
1	M40	sEg00038	Tri	4	2.08	0.92	0.34	0.28	0.5	4	0.2164
2	M12	mEgCIR3282	Di	8	4.35	1.69	0.59	0.43	0.7	12	0.1413
2	M2	mEgCIR0408	Tri	10	1.59	0.64	0.21	0.18	0.3	2	0.2822

3	M1	mEgCIR0173	Di	5	2.88	1.27	0.02	0.22	0.6	7	0.9434
3	M4	mEgCIR0425	Tri	3	2.39	0.95	0.45	0.34	0.5	6	0.1293
4	M11	mEgCIR3275	Di	6	3.12	1.38	0.57	0.41	0.6	4	0.0789
5	M18	mEgCIR3574	Di	9	4.14	1.69	0.46	0.42	0.7	9	0.2513
6	M16	mEgCIR3543	Di	7	2.4	1.03	0.38	0.32	0.5	8	0.2117
6	M44	sEg00154	Tri	3	2.02	0.72	0.47	0.33	0.4	5	0.0343
8	M15	mEgCIR3363	Di	8	4.12	1.54	0.09	0.3	0.7	7	0.7837
8	M25	MEgIOIPBSB05	Tetra	2	1.06	0.13	0.04	0.04	0.1	0	0.1348
9	M21	mEgCIR3886	Di	7	6.11	1.87	0.74	0.57	0.8	7	0.0644
9	M45	sMg00175	Tri	3	2.42	0.97	0.48	0.36	0.5	1	0.1059
10	M19	mEgCIR3785	Di	7	5.34	1.81	0.54	0.45	0.8	10	0.2
10	M5	mEgCIR0445	Tri	2	1.68	0.59	0.3	0.22	0.3	2	0.1478
11	M14	mEgCIR3362	Di	10	5.7	1.98	0.6	0.46	0.8	8	0.1698
12	M20	mEgCIR3825	Di	10	4.61	1.8	0.55	0.4	0.8	5	0.1772
12	M6	mEgCIR0465	Tri	4	1.76	0.71	0.42	0.31	0.4	7	0.0075
13	M42	sEg00125	Tri	3	1.08	0.18	0.06	0.05	0.1	5	0.1256
14	M17	mEgCIR3546	Di	9	5.09	1.8	0.62	0.49	0.8	13	0.1304
15	M13	mEgCIR3292	Di	12	5.87	1.99	0.47	0.43	0.8	9	0.2818
15	M3	mEgCIR0409	Tri	7	2.04	0.99	0.38	0.28	0.5	3	0.1494
16	M7	mEgCIR0782	Di	7	3.97	1.58	0.59	0.43	0.7	6	0.1213
16	M41	sEg00106	Tri	2	1.33	0.42	0.18	0.13	0.2	6	0.1587
Average				6.17	3.21	1.2	0.4	0.33	0.5		

ASSESSMENT OF MARKER POLYMORPHISM

The assessment of marker polymorphism, determined through PIC calculations using CERVUS software, provided valuable insights in this study. Results showcased a spectrum of PIC values (Table 1), with the highest observed at mEgCIR3886 (0.82) and the lowest at M25 (MEgIOIPBSB05), registering 0.06. The average PIC per locus stood at 0.54, reflecting the overall informativeness of the markers. In genetic studies, PIC values serve as crucial indicators of marker informativeness. Markers with PIC values above 0.7 are considered highly polymorphic, signifying their ability to capture extensive genetic variation. Conversely, markers with PIC values below 0.4 indicate limited allelic diversity within the studied population.

In this study, the analysis identified markers exhibiting varying degrees of polymorphism. Following the classification by Okoye *et al.* (2016), 10 markers demonstrated PIC values exceeding 0.7, indicative of high polymorphism. Conversely, 7 markers fell within the range of 0.4 < PIC < 0.7, representing moderate polymorphism, while 8 markers exhibited PIC values below 0.4, characterized as low polymorphic markers. It's noteworthy that while Okoye *et al.* (2016) utilized a PIC threshold of 0.7 for defining polymorphism, Taamalli *et al.* (2008) considered any marker with a PIC value exceeding 0.5 as polymorphic, aligning with the broader understanding of PIC interpretation. The average marker informativeness in this study was calculated at 0.52, indicating a moderately polymorphic nature. This aligns with the findings of Okoye *et al.* (2016), who reported an average PIC of 0.66. However, the presence of two markers with extremely low PIC values (sEg00025: 0.07, MEgIOIPBSB05: 0.06) contributed to the moderate informativeness observed.

GENETIC DIVERSITY AND DIFFERENTIATION

Understanding genetic diversity and differentiation at the population level is crucial for plant breeders, providing insights into the variability and distinctiveness of different populations. In this study, 3089 alleles were identified across the populations, with allele counts per population ranging from 29 (136R/1) to 83 (136R/2), averaging 49 alleles per population. The average number of alleles (Na) per population was 1.96, ranging from 1.16 to 3.32, indicating variations in allele richness. The average effective number of alleles (Ne) per population was 1.71, with values ranging from 1.16 to 2.23, reflecting the true number of alleles accounting for frequency variations. This diversity underscores the genetic variation present within the studied populations.

Heterozygosity, a measure of genetic diversity within populations, was assessed through observed heterozygosity (Ho) and expected heterozygosity (He). Ho ranged from 0.152 to 0.608, averaging 0.381, while He ranged from 0.08 to 0.459, averaging 0.314. Most populations displayed higher Ho than He, indicating greater observed heterozygosity and genetic diversity, but seven populations had lower Ho values, suggesting reduced heterozygosity. The percentage of polymorphic loci (PPB) ranged from 16% to 92%, with an average of 65%, indicating varying levels of genetic diversity. About 26 populations had PPB values below the average, indicating lower genetic diversity. The fixation index (F) ranged from -0.956 to 0.226, with an average of -0.23. Negative F values suggest excess heterozygosity, while positive values in three crosses indicate possible inbreeding or undetected null alleles. 10 crosses with F values close to zero are likely undergoing random mating, even if the values are slightly negative.

Table 2 provides insights into the genetic differentiation between populations, indicated by Fst values. With an average Fst value of 0.425, the study reveals significant genetic divergence among populations. Gene flow, measured by the effective number of migrants (Nm), averaged 0.339, suggesting a medium level of genetic exchange between populations (Wright, 1978). The inbreeding coefficient (*Fis*) was -0.162 across all loci and populations, though this negative value was statistically insignificant. The overall genetic diversity (*Fit*) was 0.331, indicating a moderate level of genetic diversity within the studied populations. Analysis of Molecular Variance (AMOVA) showed that 61% of genetic variation occurred within populations, while 39% occurred among populations. These findings highlight considerable genetic differentiation among populations and moderate gene flow, enhancing our understanding of the genetic dynamics within oil palm populations.

Comparative analyses with previous studies provide insights into the genetic structure of oil palm populations. Okoye *et al.* (2016, 2020) reported lower genetic differentiation (Fst = 0.131 & Fst = 0.150) in NIFOR crosses compared to our study. Gan *et al.* (2021) observed a wide range of genetic differentiation (Fst) among Nigerian populations, spanning from 0.07 to 0.70. Similarly, Budiman *et al.* (2019) reported high genetic differentiation (Fst=0.38) among six populations from Cameroon. Sarimana *et al.* (2021) found significant Fst values of 0.37 across six African origins. Ting *et al.* (2010) reported genetic differentiation among germplasm (FST) ranging from 0.142 to

0.645, with a mean of 0.373. Myint *et al.* (2021) assessed 26 families of MPOB-Senegal Oil Palm Germplasm and found a total genetic diversity (Fst) of 0.174.

Taeprayoon *et al.* (2015) and Budiman *et al.* (2019) conducted studies involving populations derived from various oil palm crosses, with AMOVA outcomes similar to our study, showing 67% of genetic variation among populations and 33% within populations. In contrast, Okoye *et al.* (2016) found 13% of total diversity attributed to group differences and 87% to differences within groups of NIFOR-based crosses. Myint *et al.* (2021) observed the highest genetic variation of 83% within families and 17% among families of Senegal germplasm. Gan *et al.* (2021) revealed 71% of molecular variance within populations and 29% among populations in Nigerian-based crosses.

These comparisons highlight consistent findings regarding genetic variation within and among oil palm populations across studies, indicating substantial genetic diversity within individual populations and moderate differentiation among populations. The analysis of genetic diversity, marker polymorphism, population structure, and genetic differentiation provides valuable insights into the genetic dynamics of this economically important crop. Through SSR markers and analyses like AMOVA, PCoA, and population structure, oil palm genetics have been elucidated.

Our findings revealed substantial allelic diversity and heterozygosity within studied populations, indicating a rich genetic variation useful for breeding programs. Marker polymorphism analysis identified informative markers, aiding in genetic studies and breeding applications. Population structure analysis identified distinct genetic clusters, providing insights into genetic relationships among oil palm crosses and guiding breeding strategies. The study emphasized considering both within-population and among-population genetic variation in oil palm breeding programs. Significant genetic differentiation among populations underlines the importance of carefully selecting parental materials to maintain diversity and prevent inbreeding depression. Overall, our research contributes to understanding and utilizing oil palm genetic potential for sustainable cultivation and improved productivity. Future investigations with advanced genomic techniques and larger sample sizes will further elucidate genetic complexity and enhance targeted breeding efforts.

Table 2: Summary results of analysis of molecular variance (AMOVA) using input as allelic distance matrix for F-statistics analysis

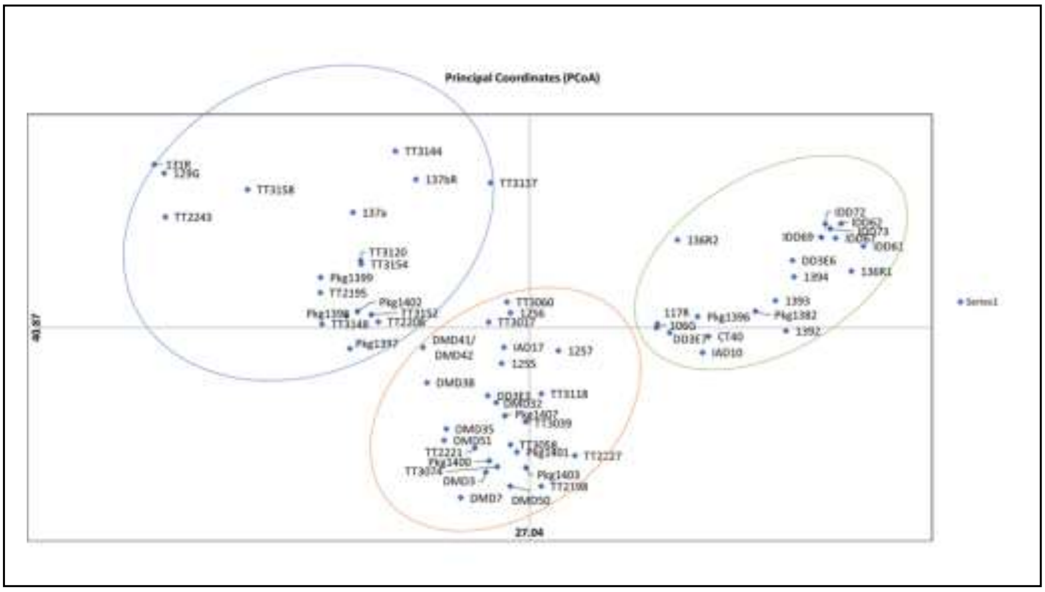
Source	df	SS	MS	Est. Var.	%
Among Pops	62	3940.333	63.554	3.021	39%
Among Indiv	564	1934.161	3.429	0.000	0%
Within Indiv	627	2984.000	4.759	4.759	61%
Total	1253	8858.494		7.780	100%

F-Statistics	Value	P(rand >= data)
Fst	0.425	0.001
Fis	-0.162	1.000
Fit	0.331	0.001
Nm	0.339	

POPULATION STRUCTURE

Population structure analysis was conducted to explore the genetic relationships and clustering among populations using PCoA and Structure Analysis. PCoA was employed to visualize the genetic similarity among populations, revealing the presence of two major clusters denoted as Cluster A and Cluster B (comprises of B.1 & B.2) (Figure 2). These clusters were primarily distinguished by the dominance of the crosess derived from Dura improvement programme in Cluster A. While the crosses from Tenera/Pisifera improvement programme mostly found in Clusters B.1 and B.2. Phylogenetic analysis corroborated the PCoA results, further validating the separation of these clusters. However, the positioning of the outgroup cross IAD17 in PCoA differed from the phylogenetic analysis, necessitating further investigation into its ancestral information.

Figure 2: The Principal Coordinate Analysis (PCoA) was conducted on 63 different oil palm crosses, utilizing a set of 24 selected SSR markers. The analysis revealed three distinct groups. Each point on the plot represents an individual cross, with the distances between points reflecting the genetic dissimilarities among them.



In Cluster B (comprising clusters B.1 and B.2), which predominantly consisted of crosses derived from the Pisifera/Tenera improvement programme, crosses from the Dura list labeled 'NPM/2' and 'DMD' were clustered within Cluster B.2 instead of Cluster A. The origins of these two Duras were from Nigerian Prospected Materials (NPM) and Non-Deli-Dura Materials (DMD), respectively, with lineages distinct from Deli Dura. The DMD materials originated from various sources, including COWAN, UFUMA, and ANGOLA. In Cluster A, it is evident that the crosses denoted as IDD* were clustered together. These crosses originated from the selected Deli-dura family D22/Pkg118 from OPGL, consisting of half KB and half UR. The remaining crosses in Cluster A were derived from OPGL Duras, including the clones. Therefore, despite being categorized as DURA crosses, the NPM/2 and DMD trials exhibited genetic distance differences. These results were consistent with PCoA, Structure, and phylogenetic analyses.

Structure Analysis determined the optimal number of clusters as K = 2 based on delta K (Figure 3). Subsequent analysis with a reduced number of K and higher burn-in rate identified K = 3 as the optimal number of clusters. To account for biological relevance, both genetic structures K = 2 and K = 3 were depicted (Figure 4). At K = 2, clear differentiation between Dura-dominated and Tenera/Pisifera-dominated clusters was observed. At K = 3, further clustering within the Tenera/Pisifera-dominated clusters was evident. These results were consistent with PCoA and UPGMA analysis, except for the positioning of the outgroup clusters in UPGMA (Figure 5). Notably, the clustering of IAD17 in Cluster B at K = 2 and Cluster B.2 at K = 3 in Structure Analysis aligned with the PCoA results.

Figure 3: Delta K results obtained from a Bayesian clustering analysis with a burn-in period of 10,000 iterations. Delta K values are plotted against the number of clusters (K), highlighting an optimal number of two genetic clusters. The peak Delta K value indicates that the data best fits into two distinct genetic groups, providing insights into the population structure and genetic differentiation among the analyzed oil palm crosses.

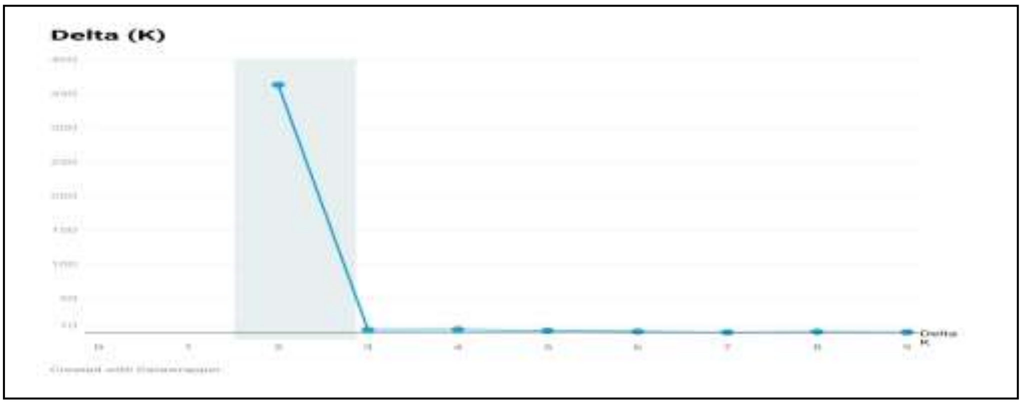


Figure 4: The Structure Bar Plots derived from the Bayesian clustering analysis visually represent the genetic composition of each of the 63 oil palm crosses. These plots illustrate how individuals are assigned to K=2 and K=3 genetic clusters, showing the proportion of each individual's genome that belongs to each identified cluster.

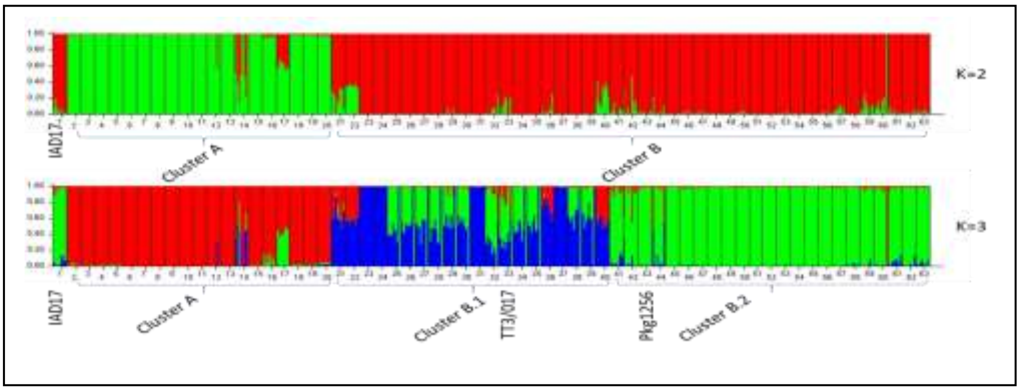
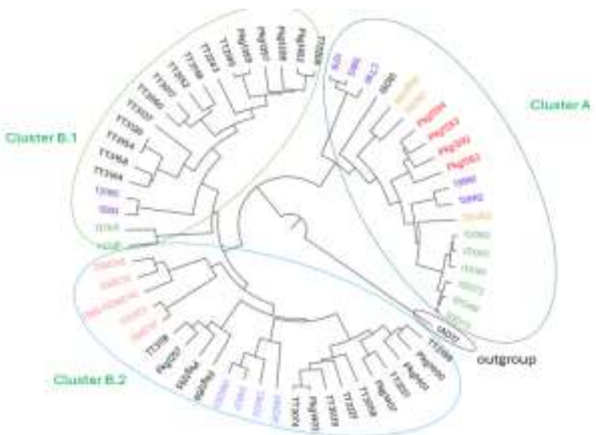


Figure 5: The UPGMA (Unweighted Pair Group Method with Arithmetic Mean) dendrogram, constructed based on genetic similarity among the 63 oil palm crosses using 24 selected SSR markers, provides a hierarchical clustering representation. This dendrogram illustrates the genetic relationships and distances among the crosses, including three main clusters and one outgroup.



SSR MARKERS AND MOTIF

MPOB and CIRAD are key institutions in developing SSR markers for oil palm research. Billotte *et al.* (2001) created oil palm genomic SSRs, while MPOB contributed genic SSRs from EST. Genomic SSRs, found in coding and non-coding regions, aid in understanding genetic diversity, with non-coding region SSRs, like those in introns and the 5' UTR, showing higher polymorphism. Trinucleotide SSRs, especially in the 5' UTR, are highly polymorphic and advantageous for genetic analysis (Wan *et al.*, 2020). Studies of Rosli *et al.* (2022) show that the 5' UTR contains more trinucleotide SSRs, which are highly polymorphic and advantageous for genetic analysis. Motif length and localization in the genome influence SSR marker polymorphism, with dinucleotide and trinucleotide motifs being more efficient for genetic analysis compared to tetranucleotide motifs in current study.

Dinucleotide markers are prevalent in oil palm research, with trinucleotide markers offering stability during DNA replication and fewer stutter bands. Genic SSRs, though less polymorphic, provide insights into gene function and conservation efforts. However, it's important to note that some SSRs located within coding regions can still be highly polymorphic, especially if they are present in non-functional regions of the protein or if they do not disrupt the protein's structure or function. Ting *et al.* (2010) reported that the study using genic-SSRs revealed a high percentage of unique alleles (78.0%) among oil palm species, indicating their conservation and potential for improving cultivated varieties. Moreover, analysis of genic-SSRs aids in identifying genetic bottlenecks of Deli dura and heterozygosity deficits in the Madagascar and Panama germplasm, guiding conservation efforts. The genic-SSRs offer improved transferability and are useful for genetic diversity analysis despite being less polymorphic than genomic SSRs (Singh *et al.*, 2008; Ting *et al.*, 2010; Chabane *et al.* 2005). The transferability facilitates synteny studies across related palm species, enhancing understanding of genomic relationships (Singh *et al.* 2008; Ting *et al.*, 2010).

Trinucleotide SSRs in this study showed lower null allele frequency (0.135) compared to dinucleotide markers (0.2734), reflecting similar trends observed previously. While trinucleotide repeats have been suggested to enhance allelic diversity within populations and improve the resolution of genetic studies, this phenomenon was not observed in the present study. Nevertheless, SSR markers containing trinucleotide repeats, especially those within coding regions, hold functional significance, rendering them valuable for functional genomic studies and association mapping. In summary, leveraging SSR markers with trinucleotide or higher order repeats offers technical advantages and broadens the applicability of genetic markers across diverse research domains.

In this study, 24 markers were selected, primarily dinucleotide genomic SSR motifs (13), with 10 trinucleotide and one tetranucleotide motif. Genomic SSRs developed by Billotte *et al.* (2001, 2005) were commonly used, with 16 dinucleotide markers employed, and only 3 excluded from the final panel. Trinucleotide SSRs (5 genomic, 12 genic) performed well, as did five genic markers, while only one tetranucleotide marker proved informative. The study prioritized SSR markers covering diverse motifs across 15 chromosomes, focusing on loci with high allele numbers, PIC, and Probability of Identity (PD) scores. Both genomic and genic SSRs were utilized to comprehensively cover the oil palm genome, aiding investigations into phylogenetic relationships and functional insights. However, determining the ideal number of microsatellite loci for analysis remains challenging, influenced by factors such as oil palm origin and analysis objectives, with this study aiming to guide researchers in selecting optimal SSR marker combinations for maximizing genetic information.

TOWARDS CONSENSUS: NAVIGATING THE COMPLEXITY OF OIL PALM GENOTYPING WITH SSR OR SNP MARKERS

A review of 70 publications on oil palm genotyping with SSR markers reveals a significant lack of consensus on the optimal design and adoption strategy. Despite extensive literature, determining the ideal number of microsatellite loci for analysis remains challenging due to the variation in marker selection based on specific analysis objectives. Researchers typically start with numerous markers from preliminary screenings and then refine their selection to suit unique materials and origins, reflecting diverse research priorities and methodologies within the oil palm genomics community.

In this study, 24 highly polymorphic markers from three SSR databases were used for IOI breeding materials. High Polymorphic Information Content (PIC) markers were particularly effective in detecting numerous alleles. Recent studies, such as those by Sarimana *et al.* (2021), Zolkafli *et al.* (2021), and Magaña-Álvarez *et al.* (2023), illustrate the varied approaches and marker sets employed across different regions, demonstrating the lack of standardized protocols. Research institutions prioritize varying numbers and types of markers, reflecting their distinct breeding programs, academic communities, and resource availability.

The complexity of the oil palm genome, with numerous repetitive sequences and high heterozygosity, complicates the development of universally applicable protocols. The genetic diversity within oil palm, driven by natural variation and breeding efforts, necessitates specific approaches for different varieties. As molecular biology and genomics advance, protocols must evolve to incorporate new techniques, ensuring accurate and efficient genetic characterization. Collaboration among researchers, breeders, and stakeholders is essential for developing and adopting standardized procedures, despite the gradual process due to the complex and diverse nature of oil palm genetics.

The absence of standardized protocols for genetic characterization reflects the multifaceted nature of oil palm genetics, requiring ongoing efforts to develop robust methodologies that can be universally applied. Establishing common procedures for multi-locus genotyping using SSR or SNP markers is crucial for characterizing and preserving oil palm genetic diversity, facilitating transferable datasets, and supporting local rediscovery and valorization efforts. This is particularly important for a national program in Malaysia, where a standardized genotyping procedure would allow the collection of genetic information from all industry members to assess diversity and inbreeding status.

Malaysia, as one of the leading producers of palm oil, would benefit from a comprehensive genetic database of oil palm cultivars. Standardized genotyping procedures ensure compatibility of genetic data from various sources, enhancing collaboration and accelerating scientific discoveries, and addressing the stagnant national oil palm yield seen for decades. Such standardized data can be compiled into valuable genetic databases for research, breeding programs, and policy-making. This program would help industry members understand their genetic information, manage genetic resources effectively, and avoid genetic bottlenecks in breeding programs. Understanding the inbreeding status of oil palm populations is critical to prevent inbreeding depression. Genotyping data can reveal the level of genetic relatedness between individuals and guide breeding programs to maintain or increase genetic diversity. Accurate genetic data enables informed decisions on breeding strategies, conservation efforts, and management practices, ensuring the long-term sustainability of the oil palm industry.

CONCLUSION

This study enhances our understanding of oil palm genetic diversity and population structure. By systematically reviewing SSR marker studies, selecting and evaluating markers, and developing a tailored SSR marker panel for IOI's breeding and clonal materials. The analysis of breeding materials supports future breeding efforts and conservation strategies. The selection and utilization of SSR markers depend heavily on research objectives, resource availability, and the nature of the analysis. SSR marker research in oil palm reveals a complex landscape influenced by motif types, genomic locations, and functional significance. Dinucleotide markers are prevalent, while trinucleotide markers, despite technical advantages, are less favoured. Tetranucleotide markers are rare due to lower abundance and efficiency. The panel includes both genomic and genic-SSR markers, enabling comprehensive genetic diversity assessments. Despite challenges in determining the optimal number of loci, consolidating frequently tested SSR markers offers guidance on motif performance and genomic distributions, aiding researchers in maximizing genetic studies. This compilation serves as a valuable resource for researchers and breeders, facilitating the development of standardized protocols and conservation efforts. Continued collaborative efforts and advancements in marker development will further shape oil palm genetics for sustainable development.

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068-064

PERFORMANCE EVALUATION OF REAL-TIME PORTABLE SOIL CONDITION MONITORING SYSTEM (REPSOIL) IN ROCK MELON FERTIGATION FARM

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ABSTRACT

In the face of escalating food demands, Malaysia's agricultural sector is pivotal, with 1.6 million people engaged in activities ranging from farming to agribusiness. The sector is ripe for transformation through smart farming technologies that promise enhanced efficiency and sustainability. This study introduces the Real-Time Portable Soil Condition Monitoring System (REPSOIL), a groundbreaking innovation designed to revolutionize soil and environment parameter monitoring. Conducted at the rock melon farm of Kolej Universiti Agrosains Malaysia (UCAM), the research evaluates REPSOIL's performance, which boasts an efficiency improvement of up to 99.9% over traditional methods. The system features advanced parameters such as a Low-Power Wireless Control Protocol for data transmission and an Extensa LX-6 microcontroller architecture for processing, ensuring rapid and accurate soil analysis. With its solar-powered energy system, REPSOIL represents a sustainable solution that aligns with Malaysia's smart farming initiatives. The study's findings demonstrate that REPSOIL's precision agriculture performance significantly improves farming operations. By enabling farmers to receive real-time data on soil conditions, REPSOIL facilitates immediate and informed agricultural decisions, thereby enhancing crop productivity and contributing to national food security. The system's performance, characterized by its efficiency and sustainability, positions it as an essential tool for the future of farming in Malaysia and beyond.

Keywords: Automated Soil Sensing, Precision Agriculture Technology, Wireless Soil Data Transmission, Sustainable Farming Monitoring.

INTRODUCTION

The quest for food security is a pressing concern in Malaysia, with the Department of Statistics Malaysia highlighting the nation's reliance on a trio of agricultural sub-sectors: crops, livestock, and fisheries (N. S. Abu *et al.*, 2022). Despite contributing 8.9% to the GDP in 2022, the agriculture sector experienced a slight decline due to factors such as reduced commodity production. Malaysia has turned to smart farming initiatives to address these challenges and bolster food security (Kassim, A.M., Sahak, S., *et al.* 2022). These initiatives aim to enhance domestic production, reduce import dependency, and employ technologies like the Internet of Things (IoT) for tasks ranging from soil pH control to long-distance farming (Rozenstein *et al.*, 2024). The government's commitment to this technological transformation is evident in its execution of pilot projects that integrate IoT in agriculture. These projects aim to resolve food security issues and ensure the safety and quality of food supplies (N. S. Abu *et al.*, 2022).

This research delves into the innovation and comparative performance assessment of the Real-Time Portable Soil Condition Monitoring System (REPSOIL), an automated device engineered to quantify soil and environment parameters. The study unfolds within the agricultural confines of a rock melon farm at Kolej Universiti Agrosains Malaysia (UCAM), where REPSOIL's capabilities are meticulously benchmarked against conventional manual soil testing techniques. The empirical evidence gathered from the comparative analysis underscores a substantial enhancement in the efficiency of soil data collection and the optimization of energy consumption attributed to the deployment of REPSOIL (Kassim, A.M., Kamarudin, N.A., *et al.* 2022). The findings illuminate the transformative potential of REPSOIL in streamlining agricultural practices through technological intervention.

Globally, developed countries have widely adopted smart farming technologies, leveraging them to enhance agricultural productivity and sustainability (Ratshiedana *et al.*, 2023). Nations like Japan, Indonesia, and China have become leaders in integrating technologies such as GPS-based guidance systems and yield monitors, with adoption rates reaching 60-80% by 2016 (Mandal, Ali, and Saha, 2021). In Kazakhstan, smart greenhouses equipped with IoT technology and AI are optimizing crop growth conditions. This widespread use of advanced agricultural technologies has resulted in increased efficiency, reduced environmental impact, and improved crop management (Melo, et.al, 2021). The success of smart farming in these countries serves as a model for nations like Malaysia, demonstrating the transformative power of such innovations in securing food supplies and advancing the agricultural sector (Ahmad Anas Yusof *et al.* 2022).

This research evaluates the Real-Time Portable Soil Condition Monitoring System (REPSOIL), a ground-breaking invention meant to transform the monitoring of soil and environmental parameters. The study, which is being carried out at Kolej Universiti Agrosains Malaysia (UCAM) rock melon farm, assesses REPSOIL's performance, claiming an efficiency gain of up to 99.9% over conventional techniques. The system has sophisticated technologies such as an Extensa LX-6 microcontroller architecture for processing and a Low-Power Wireless Control Protocol for data transmission to provide quick and precise soil analysis. REPSOIL is a sustainable solution that complements Malaysia's smart farming programs with its solar-powered energy system. The study's conclusions show that farming operations are much improved by REPSOIL's precision agriculture performance. REPSOIL helps farmers make quick and informed agricultural decisions by providing them with realtime data on soil conditions. This increases crop productivity and promotes national food security (Nawar, S., et al. 2022). The system's performance, which is distinguished by its sustainability and efficiency, makes it a vital instrument for farming in Malaysia and elsewhere in the future.

MATERIALS AND METHODS

Study Site: The research was conducted at a Kolej Universiti Agrosains Malaysia (UCAM) rock melon farm. An in-situ REPSOIL device and a handheld type 7-in-1 multi-soil and environment sensor were used to collect soil and environmental data from the rock melon polybag.

Figure 1. The rock melon farm in Kolej Universiti Agrosains Malaysia (UCAM)

As shown in Figure 2, the Real-Time Portable Soil Condition Monitoring System (REPSOIL) is a cutting-edge soil condition monitoring solution that leverages the Extensa LX-6 microcontroller architecture for robust performance. It utilizes the Low-Power Wireless Control Protocol (LPWCP) for efficient communication between the sensor nodes and the gateway, ensuring data integrity and timely delivery. The device is powered by a 1500 mAh battery, which is sustainably recharged via a 550 mA solar panel, making the system eco-friendly and reducing the need for manual battery replacement (Azam, M.A., et al. 2021). Additionally, the inclusion of a Type-C USB port offers a convenient alternative for charging. The system's design allows for the simultaneous operation of multiple sensors, drastically reducing the time required for data collection across several polybags. Figure 2 depicts the handheld type 7-in-1 multi soil and environment sensor used conventionally to collect the same data as the REPSOIL device (Kassim, Nawar and Mouazen, 2021). However, an operator needed to manually operate the device to collect the data from each polybag.

Developed in-situ REPSOIL device deployed on rock melon polybag

Developed handheld type 7-in-1 multi soil and environment sensor

Figure 2. Developed multi soil and environmental sensor for agriculture

Each sensor was deployed for 10 polybags, with the distance between each polybag being 80 cm, as shown in Figure 3. Table 1 tabulates the method for comparing both conventional and REPSOIL systems, and Table 2 displays the technical specifications of the REPSOIL device.

Table 1. Comparison method between handheld 7-in-1 soil sensor and REPSOIL system

Method Component	Handheld 7-in-1 soil sensor	REPSOIL System
Data Collection Procedure	Manual insertion of a 7-in-1 multi soil sensor into the soil within each polybag.	Automated embedding of REPSOIL sensors in each polybag prior to the start of the data collection.
Data Stabilization	Waiting period for the sensor to stabilize before recording data.	No waiting period required as REPSOIL sensors are designed for immediate data acquisition.
Data Recording	Manual recording of data readings from the sensor display.	Automatic logging of data in the system's online Dashboard for viewing and analysis.
Data Transmission	No electronic transmission, data is manually transcribed to a recording medium	Electronic transmission of data from sensors to the gateway node using LPWCP.

Energy Source for Sensors	Battery Powered, needed to be recharged or replaced.	REPSOIL sensors powered by a 1500 mAh battery and 550mA solar panel.
Sensor Technology	7-in-1 multi soil sensor technology requiring manual operation.	Advanced Extensa LX-6 microcontroller architecture enabling automated operation.
Communication Protocol	N/A	Low-Power Wireless Control Protocol (LPWCP) for efficient data communication.
Sensor Deployment	Manual placement and removal of sensors for each measurement.	Permanent installation of sensors allows continuous monitoring without manual intervention.

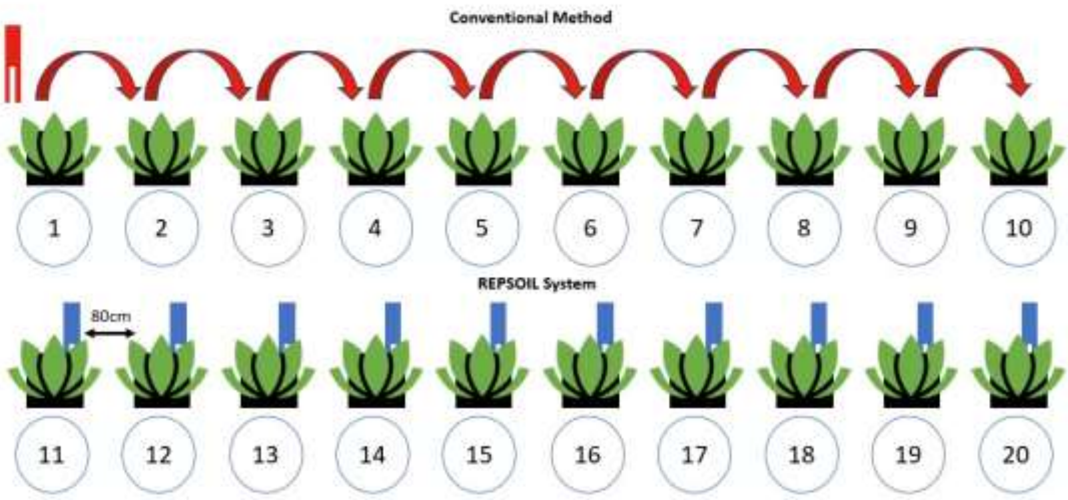

Figure 3. Experimental setup for handheld 7-in-1 soil sensor and REPSOIL system Table 2. REPSOIL technical specifications.

Specification	Details
Microcontroller Architecture	Extensa LX-6
Communication Protocol	Low-Power Wireless Control Protocol (LPWCP)
Battery Capacity	1500mAh
Solar Panel Output	550mA
Charging Port	Type-C USB
Maximum Communication Range	220 meters
Data Transmission Speed	0.63 Mbps
Transmission Delay	2.4 ms
Energy Usage per Transmission	1024 mW

PERFORMANCE EVALUATION

The performance of the REPSOIL system and handheld 7-in-1 soil sensor was evaluated. The test at UCAM Rock Melon Farm was evaluated for each method using 10 polybags. Using Equation 1, the time needed to collect data from the polybags was recorded to determine the handheld 7-in-1 soil sensor's time efficiency.

$$TTcccccccccccccccccccccccc = nn \times ttppccccppppccpp$$

(1)

Where:

- ($T_{conventional}$) is the total time for the handheld 7-in-1 soil sensor (s).
- (n) is the number of polybags.
- ($t_{polybag}$) is the time taken for one polybag (s).

For the REPSOIL system the time taken to collect data from all 10-polybag calculated using Equation 2 as all the sensors work simultaneously.

TTRRRRRRRRRRRRRR =ttcccccccccccccccccccc +ttccttcccctttttcctttttcccccc

(2)

Where:

- ($T_{REPSOIL}$) is the total time for the REPSOIL system.
- ($t_{collection}$) is the time taken to collect data from one polybag (s).
- ($t_{transmission}$) is the data transmission time (2.4 ms).

For the handheld 7-in-1 soil sensor, energy consumption is not typically measured as it involves manual labour. The energy consumed by the system used for transmitting data from 10 polybags is calculated using Equation 3.

EERRRRRRRRRRRRRR =PP×TTRRRRRRRRRRRRRR

(3)

Where:

- ($E_{REPSOIL}$) is the energy consumption for the REPSOIL system.
- (P) is the power usage per transmission (W).
- ($T_{REPSOIL}$) is the total time for the REPSOIL system (s).

RESULTS AND DISCUSSION

The study's results highlight a stark contrast in the efficiency of data collection between the handheld 7-in-1 soil sensor and the REPSOIL system as shown in Table 3. The conventional approach, utilizing a 7-in-1 multi soil and environment sensor, required a laborious 2-5 minutes per polybag for data acquisition. This translates to a total of 20-50 minutes for 10 polybags, factoring in the manual labor involved in walking to each polybag, inserting the probe, and waiting for the sensor to stabilize. In stark contrast, the REPSOIL system's automated sensors operate concurrently, taking a mere 30.0024 seconds to collect and transmit data from all 10 polybags. This data is then automatically displayed on an online dashboard for immediate viewing and analysis, as shown in Figure 6, showcasing the system's real-time monitoring and decision-making capability.

The traditional soil data collection method is time-consuming and labor-intensive, requiring significant human effort to physically interact with each polybag (Hasim, N., et al. 2012). Additionally, the 7-in-1 sensors necessitate periodic recharging or battery replacement, which adds to the operational downtime and maintenance costs. Conversely, the REPSOIL device is equipped with a built-in solar panel, enabling it to self-replenish its energy supply through solar power. This feature eliminates the need for manual recharging, thereby enhancing the system's sustainability and reducing the labor required for maintenance (Kitchen, N.R. 2008).

Precision and timeliness are paramount in agricultural decision-making, particularly when determining the need for fertilizer application or irrigation (Kassim, A.M., Sahak, S., *et al.* 2022). The REPSOIL system's ability to provide precise readings in the fastest possible time ensures that farmers can make informed decisions swiftly, optimizing plant health and yield. Field tests accentuated the REPSOIL's efficiency in data transmission. Data upload times to the cloud server ranged from 1 to 10 seconds, influenced by internet connectivity and data size. Figure 4 shows a graphical representation of the time required for data uploads to the cloud server, demonstrating REPSOIL's efficiency.

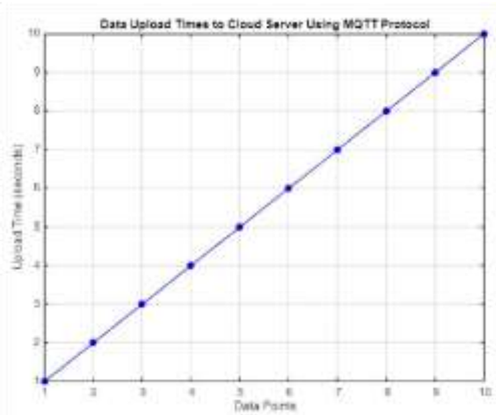

Figure 4. Data transmission efficiency.

This rapid data acquisition is crucial for effectively adjusting soil parameters. It ultimately benefits the farmer by reducing resource waste and improving crop productivity. Adopting such technology could significantly advance agricultural practices, aligning with global trends toward smart farming and precision agriculture (Kumar, P., Raghvendra, P. and Souvik, K. 2021).

Table 3. Comparison of handheld 7-in-1 soil sensor with REPSOIL system

Comparison features	Handheld 7-in-1 soil **sensor**	**REPSOIL System**
Data collection method	Manual insertion and data reading	Fully automated data collection and transmission
Data collection time (per polybag)	2-5 minutes	seconds
Data transmission time (per polybag)	N/A (manual collection)	2.4 ms
Total time for 10 polybags	20-50 minutes	30.0024 seconds
Energy usage	Variable (manual effort)	mW per transmission

Figure 5 illustrates the correlation analysis of data collection time between the handheld 7-in-1 soil sensor and the REPSOIL System, providing insights into their efficiency comparison. The graph shows a clear linear relationship between the data collection times of both methods. This means that as the data collection time increases for the handheld 7-in-1 soil sensor, there is a corresponding increase in the time required by the REPSOIL System. However, what stands out is the consistently lower data collection times of the REPSOIL System, indicated by the clustering of data points around the lower end of the graph. This highlights the REPSOIL System's efficiency and consistency in data collection compared to the handheld 7-in-1 soil sensor.

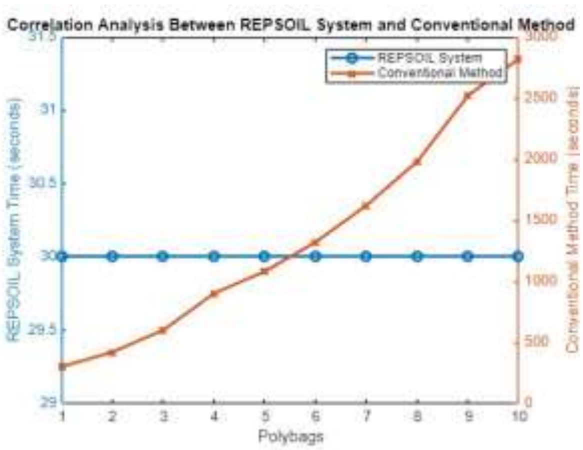

Figure 5. Correlation analysis between REPSOIL system and handheld 7-in-1 soil sensor

Figure 6 shows the Soil Monitoring IoT Dashboard, a pivotal tool for real-time monitoring and decision-making in agricultural operations. This dashboard integrates various sensor data, including pH, temperature, electrical conductivity, and humidity, providing farmers with comprehensive insights into soil conditions. Farmers can make informed decisions regarding irrigation, fertilization, and crop health management by visualizing this data in a user-friendly interface.

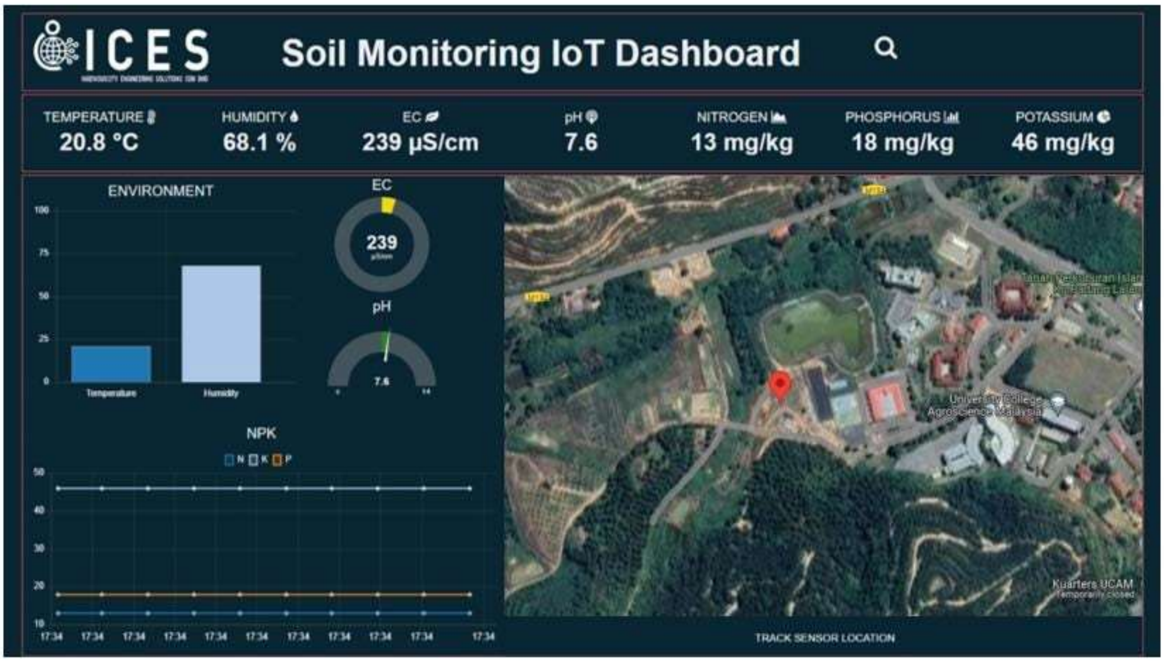

Figure 6. Soil monitoring IoT dashboard

CONCLUSION

The REPSOIL system's introduction into the agricultural landscape heralds a transformative era in soil testing methodologies. The empirical findings of this study illuminate the system's remarkable time efficiency, where the handheld 7-in-1 soil sensor's 20-50 minutes for testing 10 polybags is eclipsed by REPSOIL's brisk 30.0024 seconds. This equates to an efficiency improvement of up to 99.9%. Such a leap in productivity is not merely incremental; it is exponential, paving the way for a paradigm shift in agricultural practices. For farmers, the implications are profound: the adoption of REPSOIL can significantly enhance crop productivity by enabling rapid, data-driven decisions on fertilization and irrigation, thus contributing to the overarching goal of food security in Malaysia. By integrating this advanced system, farmers are empowered to optimize resource utilization, reduce waste, and increase yields, which are critical factors in the nation's pursuit of agricultural self-reliance and sustainability.

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068-065

ADVANCEMENTS IN AGRICULTURAL DECISION SUPPORT SYSTEMS: A DATA FUSION APPROACH

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ABSTRACT

At the forefront of precision agriculture, this paper presents an advanced decision support system that integrates a focused dataset from soil sensors measuring pH, temperature, electrical conductivity, and humidity. This research is centered on a rock melon farm, where the aforementioned soil parameters are critical for crop success. By leveraging MQTT for efficient data transmission and Grafana for intuitive data visualization, the system offers real-time insights that guide critical farming decisions. The study demonstrates how this selective data fusion approach can significantly improve the accuracy of soil health assessments, which in turn can lead to enhanced crop yields and reduced operational expenses. The findings provide valuable contributions to the field of agronomy, particularly in the cultivation of rock melons, by offering a refined method for managing agricultural inputs. The practical application of this system is exemplified in its use within a rock melon farming context, proving its versatility and effectiveness in supporting farmers with precise, data-informed strategies to maximize resource efficiency and economic returns.

Keywords: Soil Sensor, Data Fusion, Internet of Things, Precision Agriculture, Decision Support.

INTRODUCTION

Agriculture stands as a cornerstone of the global economy, tasked with the monumental challenge of feeding a projected population of 9.7 billion by 2050. This sector, vital for supplying food, fuel, and raw materials, is grappling with formidable challenges such as climate change, water scarcity, soil degradation, and pestilence, which threaten its (Rozenstein *et al.*, 2024). The escalating demand for food necessitates a significant enhancement in agriculture.

The advent of data-driven agriculture has ushered in a new era of precision farming, particularly through the utilization of soil sensors that measure critical parameters such as pH, temperature, electrical conductivity, and humidity. These parameters are pivotal in assessing soil health and informing farming decisions (Ratshiedana *et al.*, 2023). The integration of this data through advanced data fusion techniques equips farmers with actionable insights, enabling informed decisions that optimize crop management and productivity (Kumar, Raghvendra and Souvik, 2021).

Data fusion, a method that amalgamates information from various sources, has been instrumental in providing a comprehensive view of the agricultural landscape. By employing MQTT for efficient data transmission and Grafana for effective data visualization, intelligent systems can offer precise recommendations for irrigation, fertilization, and pest control (Mandal, Ali and Saha, 2021). This approach not only improves crop yields but also reduces operational costs, representing a significant leap from traditional farming methods (N. S. Abu *et al.*, 2022).

The application of data fusion in agriculture is well-established, with studies demonstrating its effectiveness in enhancing crop management. For instance, electromagnetic techniques have been used to measure soil electrical conductivity and moisture, providing valuable data for irrigation management in arid environments (Kitchen, 2008). Similarly, dielectric properties-based methods have refined soil water content measurements, essential for precision agriculture.

Beyond immediate crop management, the data collected through these systems can be analyzed by agronomists and researchers to refine agricultural practices and aid in the development of new crop varieties (Melo, Báez and Acuña, 2021).This integration of data sources assists farmers in improving yields and profits and contributes to the advancement of sustainable agricultural practices.

This paper introduces a novel decision support system that leverages data fusion, focusing on rock melon crops. The system is designed to empower farmers with the information needed to make more informed decisions, thereby improving crop yields, increasing profitability, and conserving vital resources. The methodology includes data collection, fusion techniques, real-time recommendations, system testing, data analysis, and system enhancement. The performance evaluation of the system is discussed, highlighting its impact on sustainable agriculture and its potential as a model for future agricultural decision support systems.

MATERIALS AND METHODS

System Configuration:

Figure 1 illustrates the block diagram for the system configuration proposed for agricultural decision support systems. In this system configuration, the data will flow from the input, which is located in the agricultural field, up to the output, which is located remotely, such as in the plantation office or headquarters in the urban area. The input data from the agricultural sites are collected based on multiple sensors based on soil, crops, and environmental monitoring through developed cutting-edge sensor systems (N.S. Abu *et al.*, 2022). In terms of site security, the PIR sensor and integrated CCTV camera can be used to detect any unknown movement through artificial intelligence methods and send the data to the microprocessor such as the ESP32 microcontroller (Kassim, A.M., Jaafar, et al. 2013). The battery level which is supplied inside the sensor is also very important to ensure the sustainability of real-time data collected in the agricultural field (Azam, M.A. et al. 2021).

Moreover, the connection between both areas is through the Internet of Things, where data are transferred to the cloud by using the MQTT gateway. The communication can be done by using WiFi or LoRa transmission such that it can be cover long-range and wide-range communication, which perfectly applied in the plantation and agricultural sites (Kassim, Kamarudin, *et al.*, 2022). On the other hand, the output will be displayed and monitored by the supervisor of the top management and can be viewed through the personal computer and mobile phone dashboards (Kassim, A.M., Jamri, M.S., et al. 2012). The data will be collected and stored through USB and SD cards as hardware storage and in cloud storage, such as an AWS cloud server (Hasim, N., et al. 2012).

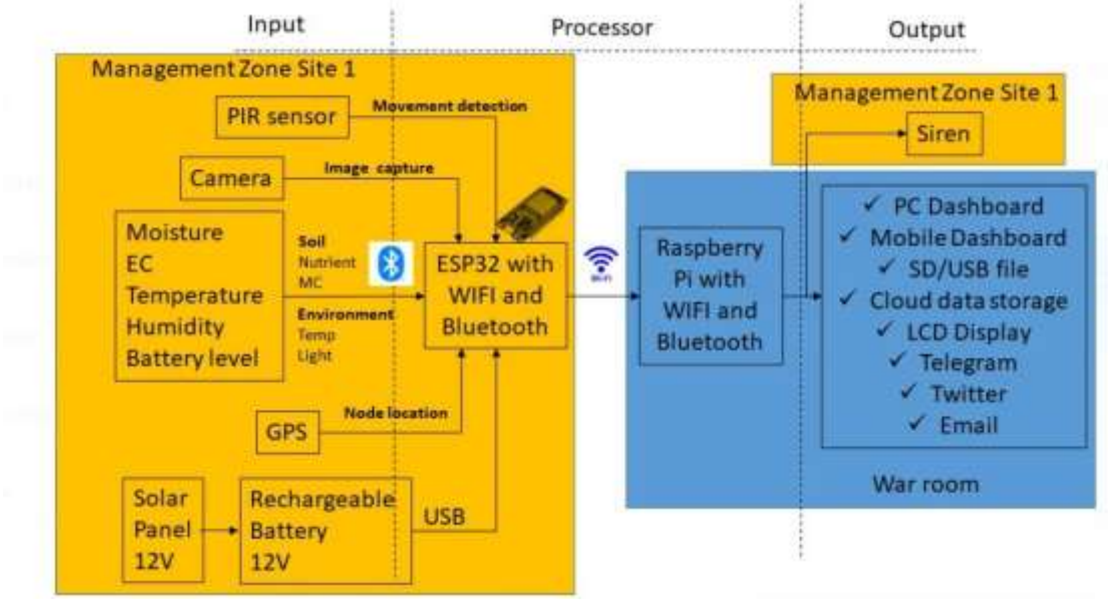

Figure 1: System configuration for agricultural decision support systems

Data Collection:

The foundational phase entailed the systematic collection of soil data, focusing exclusively on four critical parameters: pH, temperature, electrical conductivity (EC), and humidity. Data acquisition was automated through soil sensors, with operators using mobile devices to collate data from various points within the rock melon farm (Kassim, Sahak, *et al.*, 2022). The MQTT protocol was harnessed to transmit this data efficiently, ensuring real-time updates and seamless communication between the sensors and the central system (Bin Mohamed Kassim, A., et al. 2015). Figure 2 depicts the MQTT protocol's role in the data transmission process, illustrating the journey from soil sensor data collection through to the central decision support system.

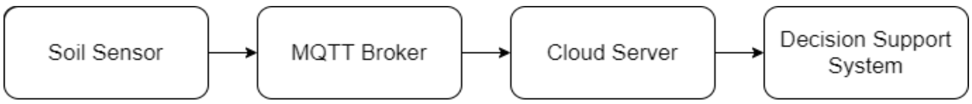

Figure 2: MQTT data transmission flow

Data Fusion:

Subsequent to data collection, the integral process of data fusion commenced. The collected multiple soil data, such as moisture and electrical conductivity (EC) with the environmental sensors for the humidity and temperature, were collected and synthesized using advanced algorithms to provide cohesive and comprehensive data and information on the soil and environmental conditions pertinent to the rock melon agricultural site (Nawar. S *et al.*, 2022). The data fusion from all the sensors was instrumental in augmenting the data's precision, facilitating more accurate agricultural decisionmaking shown in Figure 3.

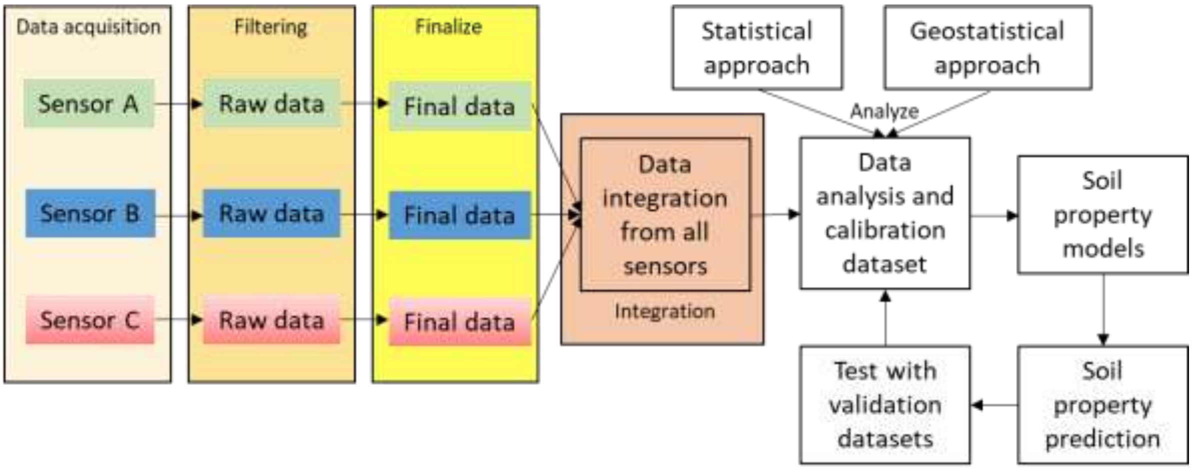

Figure 3: Data fusion for agricultural decision support system System Testing:

The system underwent rigorous testing within the operational environment of a rock melon farm. The evaluation focused on the system's capacity to yield actionable insights and its consequential influence on farming outcomes, particularly in terms of irrigation scheduling, fertilization optimization, and pest management strategies (Kassim, Nawar and Mouazen, 2021). Figure 4 illustrates the deployed soil sensor in the field, demonstrating its integration into the rock melon farming system and its role in data collection.

Figure 4: Deployed multi soil and environment sensor

Visualization and analysis:

The implementation of the data fusion-based decision support system has been instrumental in enhancing the management of rock melon cultivation (Ahmad Anas Yusof *et al.*, 2022). The system's utilization of soil sensor data pH, temperature, electrical conductivity, and humidity has provided a detailed view of the soil environment, which is crucial for the precision agriculture of rock melons (Kassim, Nawar and Mouazen, 2021). Figure 5 illustrates the Grafana dashboard interface displaying real-time data of soil pH, temperature, EC, and humidity levels. The dashboard provides a visual representation of the data trends over time, aiding farmers in making informed decisions for crop management.

Figure 5: Grafana soil parameter dashboard

The evaluation involved collecting and synthesizing soil data via the MQTT protocol, which enables real-time transmission, and Grafana for advanced data visualization. This methodology facilitated a thorough analysis of the soil parameters influencing rock melon health and productivity. The system's performance was benchmarked against conventional farming methods, showcasing significant improvements in the precision and promptness of the information delivered to farmers. A key feature of the system was the incorporation of data input by human operators, blending human expertise with sensor data. This synergy ensured that farmers' nuanced understanding of their land was integrated into the decision-making process.

RESULTS AND DISCUSSION

The implementation of the data fusion-based decision support system has been instrumental in enhancing the management of rock melon cultivation. The system's utilization of soil sensor data pH, temperature, electrical conductivity, and humidity has provided a detailed view of the soil environment, which is crucial for the precision agriculture of rock melons. The evaluation involved the collection and synthesis of soil data via the MQTT protocol, enabling real-time transmission, and Grafana for advanced data visualization. This methodology facilitated a thorough analysis of the soil parameters influencing rock melon health and productivity. The system's performance was benchmarked against conventional farming methods, showcasing significant improvements in the precision and promptness of the information delivered to the farmers.

A key feature of the system was the incorporation of data input by human operators, blending human expertise with sensor data. This synergy ensured that farmers' nuanced understanding of their land was integrated into the decision-making process. Table 1 presents the aggregated soil parameter data collected from the sensors and its correlation with the rock melon yield, illustrating the impact of precise soil data on crop management. Figure 6 shows a real-time visualization of soil sensor data, illustrating the fluctuations in soil pH, temperature, EC, and humidity over a growing season.

Table 1: Soil parameter data and correlation with rock melon yield

Soil Parameter	Average Value	Correlation yield
pH	6.5	Positive
Temperature (°C)	24	Positive
Electrical Conductivity (dS/m)	1.2	Negative
Humidity (%)	60	Positive

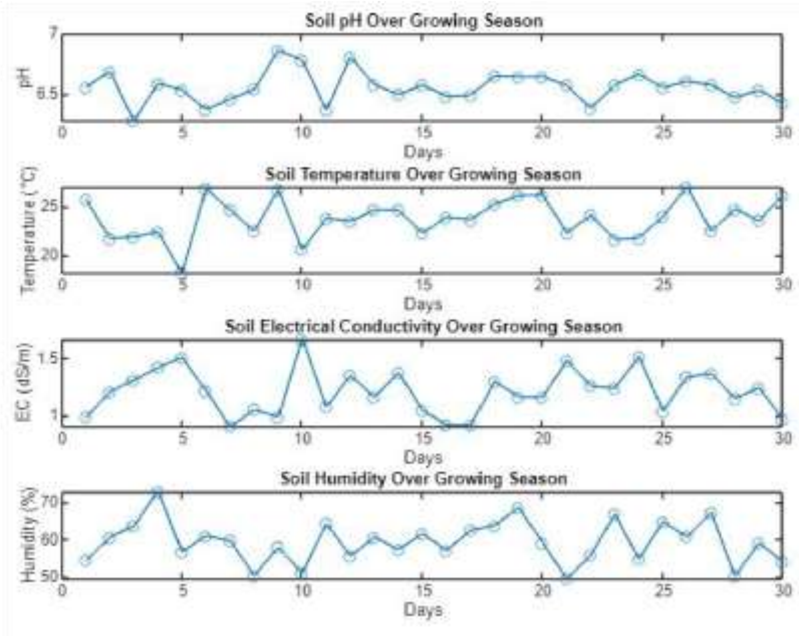

Figure 6: Real-time data visualization

In addition to the existing sensor data, a comparative study was conducted to evaluate the correlation between the sensor fusion system and the Atlas Scientific EC sensor. The study revealed a strong correlation, with an R^2 value demonstrating the sensor fusion system's accuracy in comparison to the conventional sensor. Table 2 presents a comparative average value of the average soil parameter values obtained from the sensor fusion system and the conventional sensor.

Table 2: A comparative average value of sensor fusion with conventional sensor

Soil Parameter	Sensor Fusion	Conventional Sensor
pH	6.5	6.48
Temperature (°C)	24	23.95
Electrical Conductivity (dS/m)	1.2	1.19
Humidity (%)	60	59.8

The R^2 values indicate a high degree of correlation between the measurements from the sensor fusion system and the Atlas Scientific EC sensor. The average values for pH (6.5 vs. 6.48, $R^2 = 0.89$), temperature (24°C vs. 23.95°C, $R^2 = 0.93$), electrical conductivity (1.2 dS/m vs. 1.19 dS/m, $R^2 = 0.85$), and humidity (60% vs. 59.8%, $R^2 = 0.90$) are very consistent between the two systems. This strong correlation across all parameters underscores the reliability and precision of the sensor fusion system in providing accurate soil measurements, comparable to those obtained from the Atlas Scientific EC sensor. Figure 5 shows a correlation between sensor fusion systems with conventional sensors.

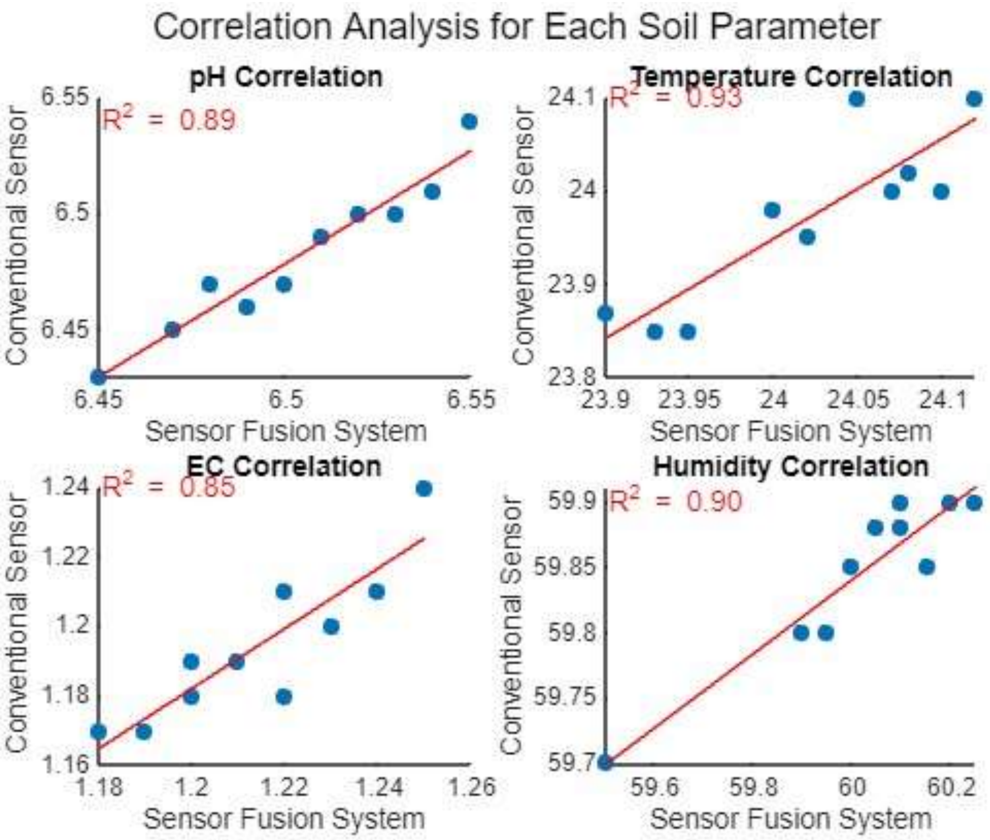

Figure 4: Correlation between sensor fusion system with conventional sensor

The findings highlight the potential of data fusion techniques to substantially improve agricultural decision support systems. By providing real-time, precise data, the system enables farmers to make more effective decisions, which could lead to increased yields, optimized resource utilization, and enhanced sustainability. Figure 6 shows a comparative analysis of decision-making efficiency before and after the implementation of the data fusion-based system.

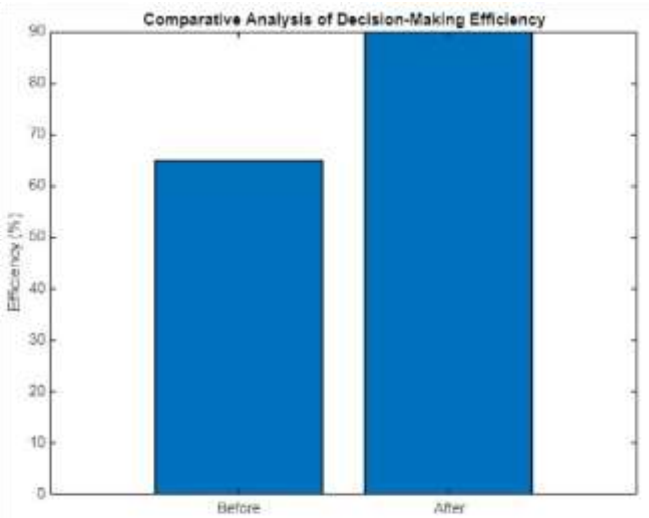

Figure 6: Impact on crop management

In summary, this research validates the application of data fusion techniques alongside modern IoT technologies to forge a responsive and intelligent agricultural management system. The confluence of human input with sensor data through MQTT and visualization via Grafana signals a promising trajectory for the future of precision agriculture in rock melon farming. The system enhances the decision-making process and contributes to the sustainable intensification of agriculture.

CONCLUSION AND FURTHER WORKS

The introduction of a data fusion-based decision support system is a significant advancement in agricultural technology, especially for rock melon farming. This system effectively synthesizes soil data, providing real-time irrigation, fertilization, pest control, and harvest timing recommendations. It brings benefits like improved crop yields, cost savings, and better resource management. Beyond the farm, it aids agronomists and researchers in developing new crop varieties and improving management techniques. This system fosters informed decision-making among farmers, leading to increased profitability and sustainable agriculture. Leveraging data-driven decisions could shape macroeconomic policies, like Malaysia's National Agrofood Policy 2.0, promoting smart agricultural practices. Future efforts should focus on interpreting fused data to maximize IoT platform capabilities. Creating user-friendly interfaces and mobile apps is essential for farmer accessibility and usability, simplifying agriculture and enhancing productivity in a data-centric landscape.

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STOP-AND-GO SOIL FERTILITY MEASUREMENT BY USING ALL TERRAIN VEHICLE FOR IN-SITU NITRATE MEASUREMENT

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ABSTRACT

Agriculture is the main source of food, and it plays an important role in economic growth. The chemical fertilizer that is not absorbed by the plant becomes chemical waste in the soil and flows into underground water, causing water pollution, soil pollution, and acidic rain. The waste of fertilizer in agriculture activities should be minimized to prevent the degradation of the environment. Hence, this project describes the design and development of on-the-go and real-time measurements for soil fertility by using an all-terrain vehicle (ATV). Conventionally, the soil sample needs to be sent to a laboratory for lab analysis, which takes about 2-3 months. Therefore, the device that can measure and analyze soil fertility is a stop-and-go device to determine fertilizer recommendations. The Ion selective electrode (ISE) for nitrate (NO_{3-}) micronutrient with the GPS device will be installed on the ATV device and can be easily changeable. This paper aims to design and develop a soil proximal sensory-based soil collecting system with GPS that can analyze the concentration of the micronutrients contained in the soil and form a fertility mapping. The percentage of error for the sensor will be evaluated before the starting the soil sampling. The soil sample will be done in palm oil field, the depth of the soil collected in 20 cm and 42 samples collected in distance of 10 m between each sample in area of 4 acres. The correlation of the soil sensor and the lab analysis will be done. In the end, the soil mapping and the recommendation map to the farmer based on the result will be developed.

Keywords: Precision Agriculture, All-Terrain Vehicle, Ion Selective Electrode, Soil Fertility, Soil Micronutrient.

INTRODUCTION

Agriculture stands as a cornerstone of the global economy, tasked with the monumental challenge of feeding a projected population of 9.7 billion by 2050. This sector, vital for supplying food, fuel, and raw materials, is grappling with formidable challenges such as climate change, water scarcity, soil degradation, and pestilence, which threaten its (Rozenstein *et al.*, 2024). The escalating demand for food necessitates a significant enhancement in agriculture.

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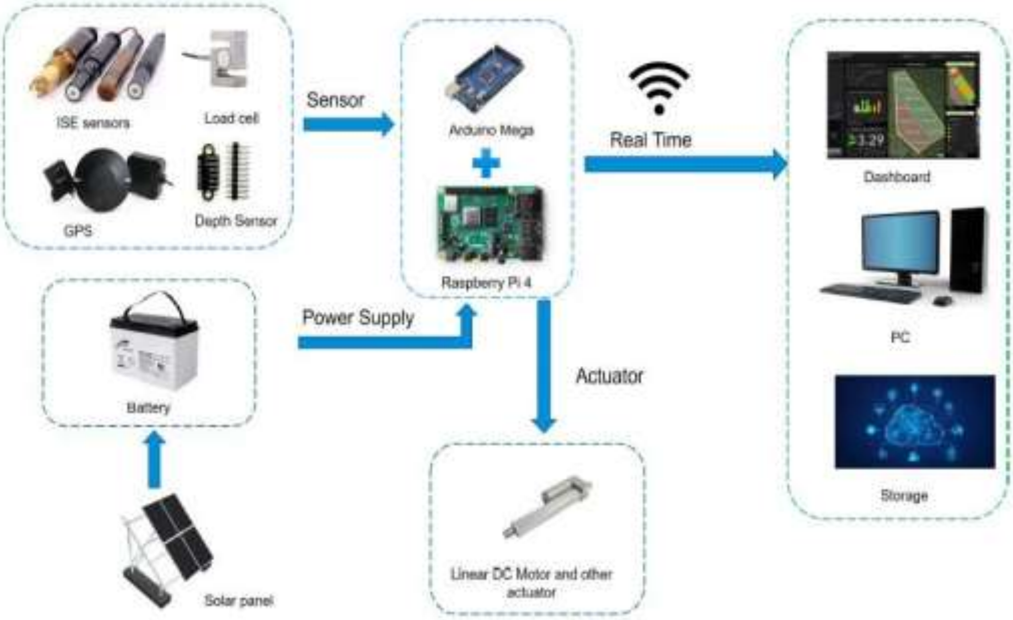

Figure 1: System configuration for agricultural decision support systems Data Collection

The foundational phase entailed the systematic collection of soil data, focusing exclusively on four critical parameters: pH, temperature, electrical conductivity (EC), and humidity. Data acquisition was automated through soil sensors, with operators using mobile devices to collate data from various points within the rock melon farm (Kassim, Sahak, *et al.*, 2022)The MQTT protocol was harnessed to transmit this data efficiently, ensuring real-time updates and seamless communication between the sensors and the central system (Bin Mohamed Kassim, A., et al. 2015). Figure 2 depicts the Stop— Measure-Go device using ATV, illustrating the journey from the soil sensor during soil collection to the central decision support system.

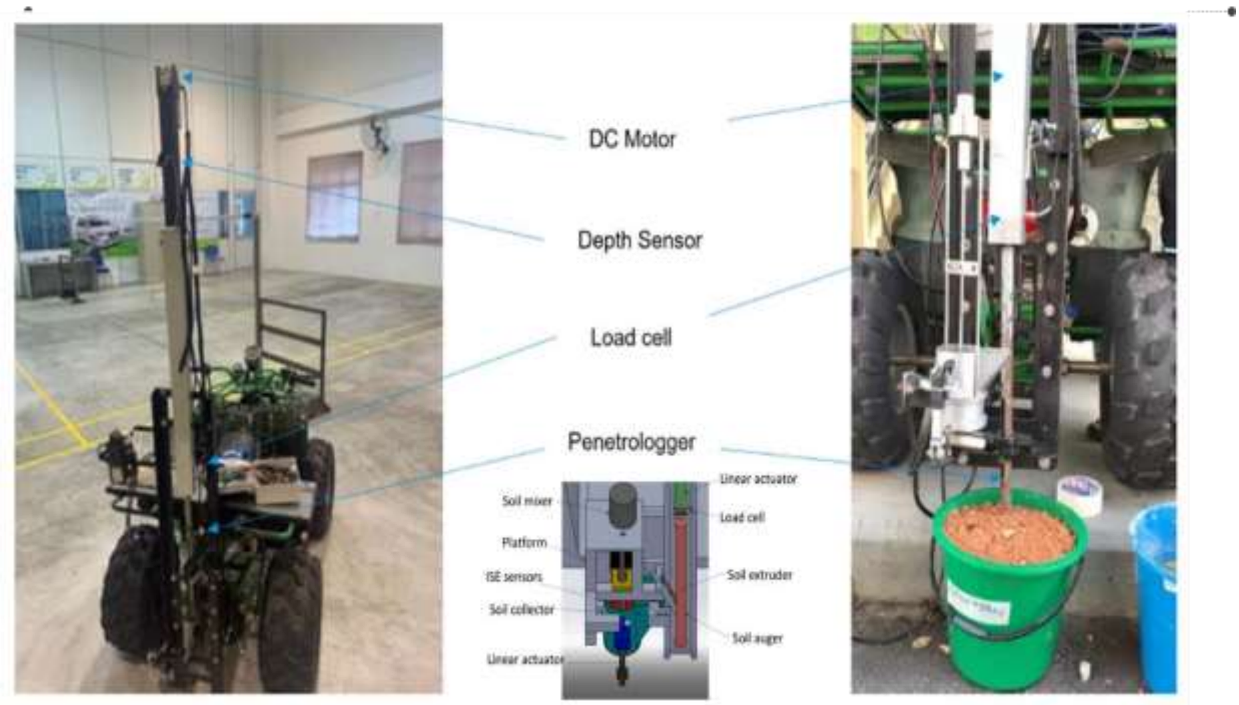

Figure 2: Stop- Measure-Go device using ATV Ion-selective electrode sensor (ISE)

Two experiments will be conducted using an ISE sensor but with a different type of detection. The detection that will be tested is the concentration of soil potassium and nitrate content. The sensor model that will be used is a Vernier ion-selective electrode. This experiment will validate the measurement accuracy of the sensor reading to develop a site-specific soil measurement system. The sensor measured the concentration of both mixtures at the same time and sent the data to the Raspberry Pi via the serial port. The Vernier ISE sensor used in this project is shown in Figure 3.

Figure 3. Ion-selective electrode (ISE)

Soil electrical conductivity (EC) measurement

An electrical Conductivity sensor will be used to measure the nutrient in the soil. The sensor that will be used is a developed multi-soil environment sensor. Figure 4 shows the diagram of the sensor. This sensor is used to validate the accuracy of the sensor and to obtain the correlation between ISE and EC sensor.

Figure 4. Developed multi soil environment sensor System Testing

The system underwent rigorous testing within the operational environment of a rock melon farm. The evaluation focused on the system's capacity to yield actionable insights and its consequential influence on farming outcomes, particularly in terms of irrigation scheduling, fertilization optimization, and pest management strategies (Kassim, Nawar and Mouazen, 2021). Figure 5 illustrates the deployed soil sensor in the field.

Figure 5: Deployed multi-soil and environment sensor on the field System Performance Analysis

After the system's design is completed, the system's analysis will be assessed to verify that the data collected is valid. A simple experiment setup will be used to conduct the evaluation. The performance of the ISE sensor and GPS was assessed.

i. Evaluation of ISE sensor

To determine the ISE sensor's error percentage, it was immersed in a known concentration aqueous solution. Low-concentrated sodium nitrate (NO3-), NaNO3 (1.00 mg/L), highconcentrated sodium nitrate (NO3-), NaNO3 (50.00 mg/L), low-concentrated potassium(K) chloride, KCl (1.00 mg/L), and high concentrated potassium(K) chloride, KCl (50.00 mg/L) were used to make the aqueous solution. The sensor will be soaked in each solution, and the readings and the sensor's percentage of error will be recorded. The experiment will be done three times to determine the valid results. The formula for the percentage error is:

Percentage of error =|(Actual value-Observed value)|/(Actual value)×100%

ii. Evaluation of GPS

For the GPS, the NMAE sentence reading in (Gpgll) format was exported to the NMAE decoder online, and the sensor's geographical location reading was shown on a Google map. Then, QGIS software will be used to generate the map based on the data that will be measured.

Data Analysis

The data will be analyzed using the linear regression approach. Linear regression is a data mining analysis approach for predicting group membership given a set of data. The relationship and structure between the input and output variables are also expressed using linear regression. Multiple linear regression predicts more than one data category than linear regression. Since there are many input variables, multiple linear regression is utilized, and it can be defined as follows:

$$YY = \beta\beta 0 + \beta\beta 1XXII + \beta\beta 2XXII2 + \in II$$

where Y is the output value, X is the observed value, 0 is the data curve, and I is the error The data from the ISE sensor and the EC measurement were compared using linear regression. However, because the units are different, some calculations must be performed before the comparison. mg/L = ppm = (μS/cm) *0.64

Soil Sampling

Soil samples were taken in the UiTM palm oil field in Jasin. A total of 42 soil samples were taken at a depth of 20 cm. The sampling area was 1 acre, and the sampling strategy was grid point sampling with a 10-meter separation between samples, as shown in Figure 6. 42 soil samples were gathered for lab testing to determine the sensor's validity. ISE sensor-based real-time and on-the-go soil nutrient analyses were also performed during sampling, and a soil nutrient table was developed.

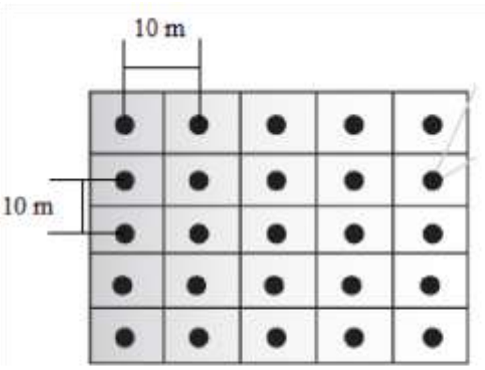

Figure 6. Soil sampling grid

RESULTS AND DISCUSSION ISE sensor analysis

To assess the ISE sensor's performance, the percentage of error was analyzed. The results are shown in Table 1 and Table 2.

Table 1: Percentage of Error of ISE soak in LOW (1 mg/L) concentration Sodium Nitrate (NO3-) solution.

Sodium Nitrate (NO3-)	LOW		
	1	2	3

Concentration (mg/L)	1.0062	0.9852	0.9544
Percentage of error (%)	0.62%	1.48%	4.56%

Table 2: Percentage of error of ISE soak in HIGH (50 mg/L) concentration Sodium Nitrate (NO3-) solution.

Sodium Nitrate (NO3-)	HIGH		
	1	2	3
Concentration (mg/L)	50.79	50.18	50.05
Percentage of error (%)	1.58%	0.36%	0.09%

Tables 1 and 2 show a low percentage of errors recorded based on the test that was carried out. This will result in the ISE sensor being suitable to be used in measuring soil nutrients. The accuracy of a sensor is the most important element in choosing a parameter for measuring data to get an accurate result. Because the economic and/or environmental risk of applying the incorrect treatment to the crop might be significant, accurate measurements are critical in determining the appropriate management treatment.

Soil Sampling and data analysis

The linear regression graph was formed by comparing the concentration of nitrate (NO_{3-}) with the EC. The graph of linear regression is shown in Figure 7. According to Figure 7, the linear regression of Nitrate (NO_{3-}) is lower than the linear regression of the EC value obtained in the field, with $R^2(NO_{3-})= 0.0243$ versus $R^2(EC)=0.3325$. Figure 8 shows the relationship between Nitrate and EC. The correlation of nitrate to EC is $R^2 = 0.9616$. The correlation for nitrate is the nearest to the value of 1, which is the most accurate.

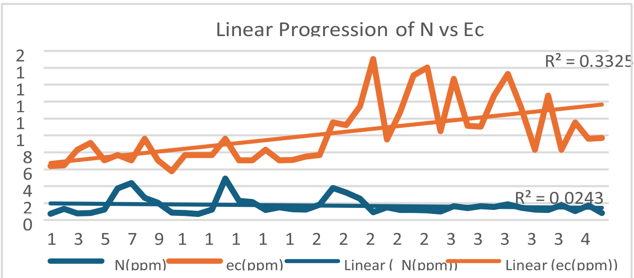

Figure 7: Linear regression relationship between Nitrate and EC

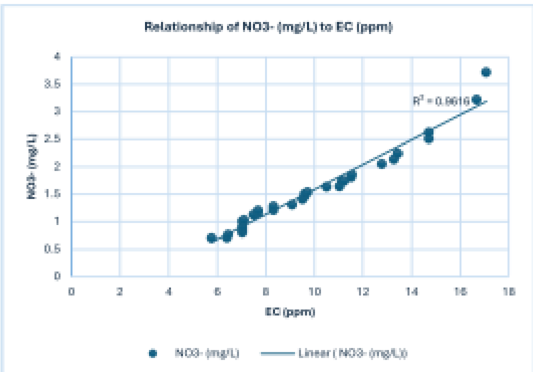

Figure 8: Relationship of nitrate to EC Soil Mapping Analysis

The data collected were used to construct the fertility mapping of the palm oil field. Figure 9 depicts the field's fertility mapping in terms of nitrate (NO_{3-}). A color legend was added to the fertility mapping of soil nutrients to identify the soil's nutrient level. Figure 9 depicts 42 samples with various color indicators. The content of nitrate ranges from 0 to 5 mg/L. This indicates that there is a lag of nitrate (NO_{3-}) in the soil, as the most optimum nitrate (NO_{3-}) for plant growth is between 10 and 20 mg/L. The system can also keep track of the information gathered through the user interface. The user interface may track the sensor's real-time GPS location, soil nutrient levels, and battery life.

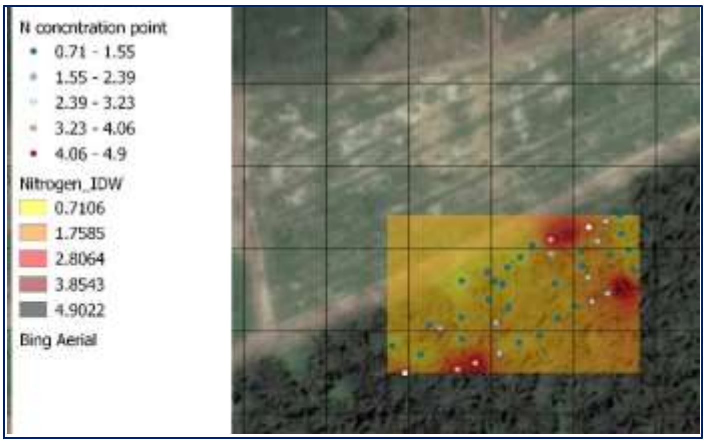

Figure 9: Fertility Mapping of Nitrate (NO_{3-})

CONCLUSION AND FURTHER WORKS

In this paper, the efficiency of the ion-selective-electrode to be developed as a site specification nutrient measurement was evaluated in this study. Nitrate ISE is used as a nutrient in soil, and the measurement of nitrate (NO3-) is used as a composition for nitrogen. Each sensor shows an accurate reading based on the test that has been carried out. However, when comparing the result for the ISE sensor with the EC, it has a different reading; EC only detects the moisture of the soil and changes it into the form of conductivity. Compared to ISE, it will directly detect the nutrients in the soil. The correlation R^2 value for the ISE sensor shows a high value which is nearest to 1. This means the sensor is accurate in reading the measurement of the soil nutrient. An accurate sensor needs to be used to develop a site specification soil nutrient measurement to replace the laboratory method for analyzing soil nutrients, which takes a long time to get the result.

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061-071

GROWTH AND YIELD RESPONSE OF GRAIN CORN DIFFERENT LEVELS N.P.K FERTILIZERS IN THE MINERAL SOIL

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ABSTRACT

The key to increasing crop productivity and production is the proper use of fertilizers in balanced and adequate amounts. Low grain corn yields are often caused by unbalanced plant nutrient management, particularly due to a lack of information on the dose responses to macronutrients in soil, especially in grain corn production. The purpose of this study was to measure the impact of different application rates of nitrogen (N), phosphorus (P), and potassium (K) on grain corn yield and agronomic efficiency when applied in balanced amounts on mineral soil for grain corn production. The experiment was arranged in a Randomized Complete Block Design (RCBD) with nine treatments and 3 replicates. The experiment was arranged in a Randomized Complete Block Design (RCBD) with seven treatments and 3 replicates. Treatment one T1 represents No fertilizer (Control), T2 = 100% rate NPK 15:15:15, T3= NPK 15:15:15 (50% rate) + 2 (N biobooster), T4= 200kg N + 40kg P + 80kg K , T5= 200kg N + 40 kg P + 60kg K , T6=160kg N + 40 kg P + 80kg K , T7=160kg N + 40 kg P + 60kg K, T8 = 120kg N + 40 kg +80 kg K and T9 : 120kg N + 40 kg +60 kg K. The result showed that there was significant difference on vegetative growth performance for all treatments. The research finding revealed that application rate of T5= 200kg N + 40 kg P + 60kg K recommended for increasing in the yield of grain corn in the highest grain yield (1116.9 kg/ha), compared with extra fertilizer on T3= NPK 15:15:15 (50% rate) + 2 (N biobooster) in grain yield (8247.0 kg/ha). Thus, conclude that the addition of increasing rate of N increases the yield.

Keywords: Grain Corn, Nitrogen, Phosphorus, Potassium, Mineral Soil.

INTRODUCTION

Grain corn has been given higher priority in Malaysia as one of the new sources of national wealth. It is due to its importance as a component in the formulation of animal feed, where Malaysia has relied heavily on corn imports for more than 50 years.The grain corn industry in Malaysia is relatively small. However, the development of the livestock industry, especially the ruminants, broilers and swine, needs millions of tons of grain corn as the main component for feed. Almost all of this raw material product was imported to cover the high demand of the Malaysian livestock industry which cost above RM 3 billion, annually (Nor et al., 2020).

The productivity of grain corn is dependent on its nutrient requirement and management, particularly that of nitrogen, phosphorus and potassium (Asghar et al, 2010). Grain corn requires nutrients such as nitrogen (N), phosphorus (P), and potassium (K) more than others, leading to symptoms of deficiency of these elements. Inadequate fertilization practices, lack of proper fertilization recommendations, and limited knowledge of nutrients beyond N, P, and K can limit crop production. Factors such as low maintenance, environmental conditions, and soil fertility levels can also contribute to low plant productivity (Nziguheba et al, 2009). Understanding the fertilizer response reference is crucial to improving the effectiveness and efficiency of fertilization in maize.

The relative response of maize to N-P-K fertilizer tends to decrease with improved soil quality. Soil type plays a significant role in various aspects, including resource capture efficiency. While fertilizers have long been known to enhance plant growth and yield, the optimal ratio of macro elements, particularly N, P, and K, needs to be determined for optimise nutrient requirement specifically for grain corn plant. This study aims to investigate nutrient requirement the ideal N:P: K ratio for maximizing growth and yield in grain corn plant.

MATERIALS AND METHODS

Study area and plant materials: The experiments were carried out at the research field at MARDI Headquarters, Serdang using a total area of 80 m × 20 m. The soil of the experimental site was the alluvial soil. Grain corn hybrid was planted at rate 17 kg/ha.

Land preparation and agronomic practices: The experimental plots were disc ploughed, harrowed, leveled and the ridged and the layout was demarcated using rope, pegs and tape. Each experimental unit has a dimension of 5 m x 5m plot and the two seeds were sown per hole at a depth of 3-5 cm with spacing of 75x20 cm. Organic fertilizer was applied 7 days before planting at the rate of 3 t/ha distributed on the ridged-plot and mixed with soil. Plants were irrigated immediately after sowing and thinned to one plant/hole after a week from sowing. Weeding was carried out manually when needed.

Fertilizer treatment: Table 1 Treatment rate NPK fertilizer application to increase the growth and yield of grain corn production.
T1 : No fertilizer (Control)
T2 : 100% rate NPK 15:15:15 + UREA
T3 : NPK 15:15:15 (50% rate) (15 DAS) + UREA + 2 (N biobooster)
T4 : 200kg N + 40kg P + 80kg K
T5 : 200kg N + 40 kg P + 60kg K
T6 : 160kg N + 40 kg P + 80kg K
T7 : 160kg N + 40 kg P + 60kg K
T8 : 120kg N + 40 kg +80 kg K
T9 : 120kg N + 40 kg +60 kg K

Experimental design and statistical analysis: Treatments were arranged in a Randomized Complete Block Design (RCBD) consisting of seven treatments with three replications. The data obtained was analyzed using ANOVA in the SAS software (Version 9.4, SAS Institute Inc. Cary, North Carolina, USA) and differences between treatment means were compared using Duncan Significant Difference (HSD) at $P \leq 0.05\%$.

Data collection: Five plants were randomly selected from each treatment. Parameters were measured during the experimental period as growth performance at 90 DAS which were plant height (cm), stem diameter (mm), feaf number, leaf length (cm), leaf width (cm), Leaf area (cm^2), leaf dry weight (g), stem dry weight (g). After harvesting at 105 DAS, data which were moisture content (%), total fruit, weight fruit w/o husk per treatment (kg), fruit length (cm), diameter fruit (mm), weight fruit per plant (g), number of grain per fruit, 1000-grain weight (g) and yield before drying (kg/ha).

RESULTS AND DISCUSSION

Effect of ratio NPK fertilizer on growth performance and dry matter of grain corn at 90 DAS

The vegetative responses in plant height (cm), stem diameter (cm), leaf length (cm), leaf width (cm), leaf area (m^2) leaf dry weight (g) and stem dry weight (g) showed significant differences ($p<0.05$) among all treatments applied for 90 days after sowing (DAS). Meanwhile, leaf number showed no significant difference ($p>0.05$) among all treatments (Table 1). The effect of NPK ratio on plant height and stem diameter as showed by the highest radial growth was recorded with T8 (242.34 cm) and (23.48 mm) while the least growth was recorded with T1 (170.5 cm) and (17.73 mm). Besides that, the effect of NPK ration on T5 showed the highest parameter in leaf width (8.77 cm), leaf area (5700.4 m^2), dry weight (44.94 g) and stem dry weight (66.62 g) the control treatment (T1). This could be attributed to a mere fact the higher of nitrogen may have caused rapid cell division and elongation. It could be attributed to the increased photosynthetic capacity of the plant by N nutrition, which improves cell elongation and cell division that increased vegetative growth. The better NPK nutrition to crop plants also has beneficial effect on plant metabolism, which affects physiological process of the crop and thereby increases the growth parameters. Usage of high level of nitrogen enhanced vegetative growth and number of leaves/plants, which increased the leaf-area index. Zafar et al. (2011) reported that increment of dry-matter yield ranged between 43– 60% due to phosphorus fertilization, while Kolawole and Joce (2009) indicated that application of NPK fertilizer increased the total dry matter of maize.

Effect of ratio NPK fertilizer on yield and yield of grain corn

The yield component responses in total fruit, weight fruit w/o husk per treatment (kg), fruit length (cm), diameter fruit (mm), weight fruit per plant (g), number of grain per fruit, 1000-grain weight (g) and yield before drying (kg/ha) showed significant differences ($p<0.05$) among all treatments in harvesting time at 105 days after sowing (DAS). Beside that, the effect of NPK ration on T5 showed the highest parameter in total fruit (47.25), weight fruit without husk per treatment (9.75 kg), weight fruit per plant (413.78 g), number of grain per fruit (507.0), 1000-grain weight (360.03 g) and yield before drying (11,1169 kg/ha) compare the all treatment. But in fruit length (19.62 cm and fruit diameter (50.36 mm) showed the higher in T9. However, the effects were more prominent at higher level of nitrogen especially, However, the effects were more prominent at higher level of nitrogen especially in T5 (200kg N + 40 kg P + 60 kg K), which provided the strength to give all yield parameters significantly better compare the all treatment. Gul et al. (2015) also reported similar findings. Their study showed that maximum yield attributes were achieved with higher nitrogen application, leading to increased grain yield. The higher nitrogen levels promoted the development of strong and dense shoot and root systems, enhancing the absorption of essential nutrients. This resulted in improved vegetative growth and grain yield due to enhanced root and nutrient absorption. The increased productivity may be attributed to the improved soil fertility conditions created by the accurate ratio of fertilizer,allowing plants to uptake more moisture and nutrients, leading to higher leaf area index (LAI) and increased dry matter production, ultimately resulting in higher biological yield. These results align with the findings of Amanullah et al. (2010), highlighting the positive impact of higher nitrogen application on corn grain yield optimization.

Table 1: Effect of ratio NPK fertilizer on yield and yield of grain corn

Treatment	Plant height (cm)	Stem diameter (mm)	Leaf number	Leaf length (cm)	Leaf width (cm)	Leaf area (cm²)	Leaf dry weight (g)	Stem dry weight (g)
T1	170.5b	17.7c	10.47b	50.77b	6.4b	1724.1c	20.81c	20.81c
T2	236.87a	19.64ab	11.83ab	70.7a	7.92a	3750.8b	34.59b	34.59b
T3	234.73a	22.39ab	13.43a	72.87a	8.22a	4663.5ab	38.93ab	38.93ab
T4	247.8a	22.95ab	12.2ab	73.8a	8.65a	4828.4ab	35.55ab	35.55ab
T5	230.5a	22.22ab	12.1ab	73.8a	8.77a	5700.4a	44.95a	44.95a
T6	235.04a	22.28ab	13.23a	69.95a	8.11a	5156.8ab	38.85ab	38.85ab
T7	240.63a	22.88ab	13.38a	74.88a	8.63a	5520.3ab	36.79ab	36.79ab
T8	242.23a	23.48a	13.63a	71.17a	8.6a	4883.3ab	35.97ab	35.97ab
T9	239.2a	23.93a	13.4a	73.7a	8.6a	4412.3ab	32.48b	32.48b
p-value	**	ns	ns	ns	*	**	**	**

Note: ns: ** Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the same letters are not significantly different at P ≤ 0.05% level according to Duncan's Multiple New Range Test (DMNRT) (Mean ± SE, n=8).

Table 2 Effect chemical fertilizer and N-Biobooster on growth and yield of grain corn

Treatment	Total fruit/trt	Weight fruit w/o husk/trt (kg)	Fruit length (cm)	Diameter fruit (mm)	Weight fruit/plant (g)	No. of Grain/ fruit	1000-grain weight (g)	Grain yield before drying (kg/ha)
T1	22b	1.75c	11.24c	38.33b	71.34c	189.9d	113.38b	2190c
T2	40a	8.6ab	18.5ab	49.68a	250.38b	489.3abc	344.69a	9985ab
T3	37ab	7.35b	17.06b	49.09a	217.89b	451.4c	323.11a	8247b
T4	38a	9.25ab	19.27ab	51.05a	276.35ab	522.3a	358.56a	11053a
T5	42.75a	9.75a	18.97ab	48.32a	413.78a	507.0ab	360.03a	11169a
T6	40.25a	9.05ab	18.7ab	49.44a	254.4b	494.25abc	349.03a	10427ab
T7	38.25a	8.5ab	17.8ab	49.4a	236.63b	464.8bc	345.08a	10009ab
T8	38a	8.00ab	19.94a	49.54a	243.11b	490.3abc	337.1a	10557ab
T9	38a	8.05ab	19.62a	50.36a	267.66b	502.6ab	356.64a	9536ab
p-value	*	**	**	**	**	**	**	**

Note: ns: ** Significant at 1% probability level, *Significant at 5% probability level, ns: Not significant. Means in each column with the same letters are not significantly different at P ≤ 0.05% level according to Duncan's Multiple New Range Test (DMNRT) (Mean ± SE, n=8).

CONCLUSION

The research finding revealed that application rate of T5= 200kg N + 40 kg P + 60kg K recommended for increasing in the T5 showed the highest parameter in total fruit (47.25), weight fruit without husk per treatment (9.75 kg), weight fruit per plant (413.78 g), number of grain per fruit (507.0), 1000-grain weight (360.03 g) and yield before drying (11,1169 kg/ha) compare the all treatment.

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061-072

RESPONSE OF APPLICATION OF GIBBERELLIC ACID AND PACLOBUTRAZOL ON FLOWERING IN LIBERICA COFFEE

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ABSTRACT

Simultaneous flowering in coffee plants is crucial for a synchronized harvest of coffee beans, ensuring high-quality coffee cherries. A study was conducted to assess the impact of growth regulators on flower synchronization and ripening in *Liberica* MKL 7 coffee clone trees. The trees were treated with gibberellic acid GA3 (GA) and paclobutrazol at concentrations of 100, 200, and 300 ppm, with varying spray frequencies. The study monitored the appearance of flowers from the first flower in May 2023 to the third flower in September 2023. The results showed that different spray frequencies significantly influenced all parameters ($P<0.05$),and there was a significant effect ($P<0.05$) between the hormones gibberellic acid GA3 (GA) and paclobutrazol at different concentrations on total flowering, bud fruit, and fruit development. The study found that spraying twice with GA3 at 200 ppm (T3) resulted in increased flower production compared to no hormone treatment. Additionally, the number of flower buds in the third flower stage decreased compared to the first and second stages on the same branch.

Keywords: Liberica MKL 7, Gibberellic Acid, Paclobutrazol, Total Flowering, Bud Fruit, and Fruit Development.

INTRODUCTION

The flowering process of coffee affects the yield and quality of coffee. The season of flower formation varies around the world, depending on the local climate. Significant rainy and dry seasons affect the process of coffee flower formation, mainly to obtain simultaneous flowering. However, at present coffee growers are finding it increasingly difficult to predict the flowering season because increasing temperatures, erratic droughts and unpredictable rains have affected the flowering process of coffee and its fruit yield (Shapiro, et al). Coffee trees of the Liberica species grown from seeds start flowering approximately one year after being planted in the field. Around 50% of the plant population will flower at the age of 15 months, and almost all of the population will flower by the age of 30 months. Clone plant material typically starts to flower about 6 to 9 months after being planted in the field (Muhammad Ghawas and Wan Rubiah 1991).

Simultaneous flowering in coffee plants results in a simultaneous harvest of coffee beans, which is essential for producing high-quality coffee cherries. However, this process requires consistent and predictable rainfall (Byrareddy et al, 2020). Unfortunately, the changing climate patterns in coffee growing regions are leading to more erratic rainfall, primarily due to climate change. This inconsistency in rainfall can disrupt the simultaneous flowering process, causing flowers on the same tree or branch to bloom at different times, resulting in uneven fruit maturity (DaMatta et al, 2018). Climate change also impacts the overall quality of the coffee crop, increases the risk of plant diseases, and may render certain areas unsuitable for coffee cultivation.

In the northern region of Peninsular Malaysia, coffee beans typically mature from October to January. Conversely, in the southern and central states of Peninsular Malaysia, particularly in regions with unpredictable or pronounced rainy and dry periods, coffee trees bloom multiple times annually. Nevertheless, there is a higher flower yield during specific periods due to the presence of a notable dry season at certain times of the year. For Liberica. coffee, there are typically two production seasons annually, occurring from April to June and from October to December in the southern region (Muhammad Ghawas and Wan Rubiah 1991).

Uniformity in the formation of coffee flowers on the same plant is crucial. When there is inconsistency, some coffee fruits may be ripe while others are unripe, leading to difficulties in harvesting. This can result in a mix of healthy and damaged fruits, making the harvesting process more challenging for coffee farmers who have to pick at different times. Entrepreneurs are left with no option but to manually handpick the coffee beans, as mechanized harvesting is not feasible. This manual process requires more manpower, prolongs the harvesting time, and increases the production costs. Ultimately, this inconsistency in coffee yield at the farm level can have repercussions on the coffee industry, especially considering the high global demand for coffee (Ramanathan and Ali 2021).

To address this issue, a study is being conducted to boost the yield of coffee fruits by utilizing hormone induction methods along with proper agronomic practices like irrigation and fertilization. The application of flowering induction involves hormonal variations that could potentially trigger flowering at the desired time. Initial research focuses on assessing flowering and fruit development. Insights on the optimal concentration and application of this technique are anticipated to assist local coffee growers in achieving successful simultaneous flowering and high-quality coffee fruit production.

MATERIALS AND METHODS

Experiments are currently underway at the coffee research plot in MARDI Pontian, Johor, utilizing a 15-year-old Liberica MKL 7 coffee clone tree. The planted trees cover an area of 80 m × 20 m and are situated in peat soil. The plant growth regulator treatments included gibberellic acid GA3 (GA), and paclobutrazol at concentrations of 100, 200, and 300 ppm, with variations in spray frequency. Control treatments were sprayed with distilled water. Each treatment was applied to tagged plagiotropic branches using a hand sprayer until runoff. Pruning activities were conducted to remove lateral branches. The treatment evaluation was divided into two phases: Phase I involved assessing the period from floral initiation (candle stage) to fruit set. The number of flower buds, flowers at anthesis, and fruit set were recorded. Phase II focused on the fruit harvesting period from the initiation of ripening to the end of the harvesting period. Ripe fruits were hand-picked at onemonth intervals. Tagged plagiotropic branches and total production were recorded during the period, with only ripened fruits being collected. The experimental plots were managed as a commercial plantation following recommended agronomic and cultural practices for coffee production. The experiment was analyzed using a split-plot design with three replicate trees per treatment and five branches per tree as subsamples. Treatment differences were compared using analysis of variance, and means were compared using the LSD Test.

RESULTS AND DISCUSSION

Evaluation of Period from Bud Formation to Fruit Set

In this study variations in spray frequency significantly affected all parameters ($P<0.05$), while between hormone gibberellic acid GA3 (GA), and paclobutrazol at concentrations of 100, 200, and 300 ppm also showed significant ($P<0.05$) affected total flowering, total bud fruit and total fruit development. The first flower tag (May 2023) showed significant interactions between the main effects were recorded in all parameters. The frequency of the hormone spray at the first time (1x), the GA3 hormone concentration of 200 ppm (T3) produced a high number of flowers (15.47) On the 2nd spray, also showed a concentration of GA3 200 ppm (T3) produced a high number of flowers (29.93). The 3rd spray, also showing a concentration of 200 ppm (T3) produced a high number of flowers (24.53) and the amount of flower formation without hormones showed the lowest number of flowers (Fig 1). The frequency of the hormone spray the first time (1xGA3 at a concentration of 200 ppm (T3) produced a high number of buds (15.46). The 2nd spray, also showed a concentration of Paclobutrazol 200 ppm (T6) produced a high number of fruit buds (29.66) While the 3rd spray, also showing a concentration of 200 ppm (T3) produced a high number of fruit buds (24.53) the amount of flower formation without hormones showed the lowest number of fruit buds (Fig 2). The frequency of the hormone spray the first time (1x), GA3 at a concentration of 200 ppm (T3) produced a high amount of fruit (13.00). The 2nd spray, also showed a concentration of GA3 at 200 ppm (T3) produced a high amount of fruit (25.86). While the 3rd spray, also showing a concentration of GA3 at 200 ppm (T3) produced a high number of fruits (20.87) the amount of flower formation without hormones showed the lowest number of flowers (Fig 3).

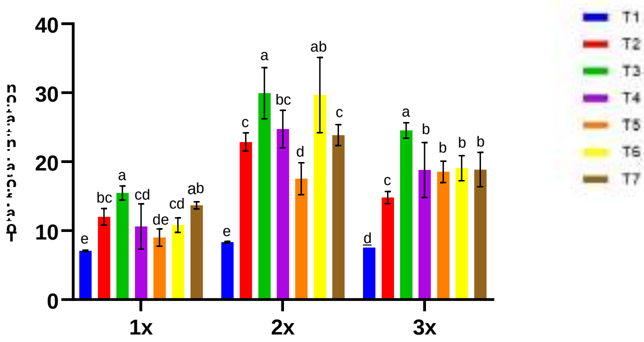

Figure 1: Effects of different levels of GA3 and Paclobutrazol hormones on the numbers of flowers at different hormone spray frequencies during the first flower stage for MKL 7 coffee clone. . Significant differences between means in each column with distinct letters for each factor were determined using the LSD test at a significance level of P≤0.05%.

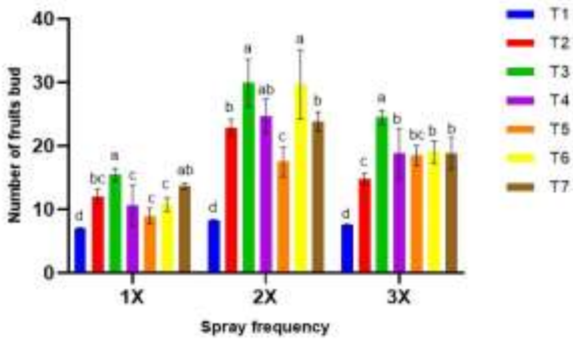

Figure 2: Effects of different levels of GA3 and Paclobutrazol hormones on the number of flower buds at different hormone spray frequencies during the first flower stage for MKL 7 coffee clone. Significant differences between means in each column with distinct letters for each factor were determined using the LSD test at a significance level of P≤0.05%.

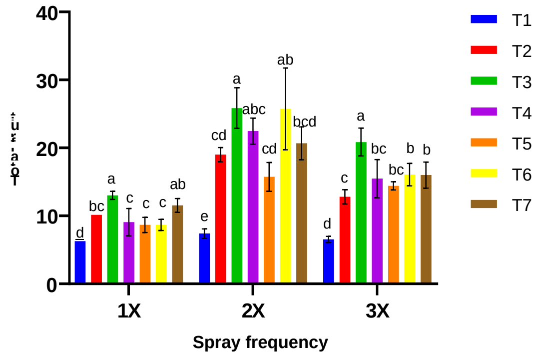

Figure 3: Effects of different levels of GA3 and Paclobutrazol hormones on the numbers of flower buds at different hormone spray frequencies during the first flower stage for MKL 7 coffee clone. Significant differences between means in each column with distinct letters for each factor were determined using the LSD test at a significance level of P≤0.05%.

The second flower tag (July 2023) showed significant interactions between the main effects were recorded in all parameters. The emergence of a new flower bud on the same branch is also documented as the second flower stage. The initial application of the hormone spray at a concentration of 300 ppm GA3 (T4) resulted in a significant number of flowers (25.53). Subsequent application of Paclobutrazol at a concentration of 100 ppm (T5) in the second spray led to a high flower count (34.27). The third spray, which involved a GA3 concentration of 200 ppm (T3), also resulted in a high number of flowers (28.26). The control group without any hormone treatment exhibited the lowest (Fig 4). The initial application of the hormone spray at a frequency of 1x with a GA3 concentration of 300 ppm (T4) resulted in a high fruit buds count of 23.47. After the 2nd spray, the application of Paclobutrazol at 100 ppm (T5) yielded a high fruit buds count of 30.33. The 3rd spray, with a GA3 concentration of 200 ppm (T3), also led to a high fruit buds count of 26.00. In contrast, the control group without any hormone treatment exhibited the lowest flower buds count (Fig 5).

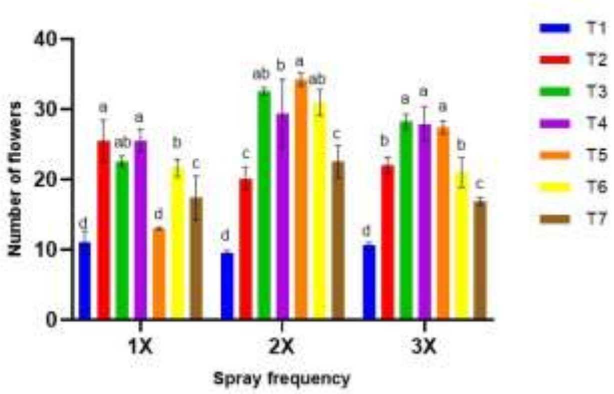

Figure 4: Effects of different levels of GA3 and Paclobutrazol hormones on the number of flowers at different hormone spray frequencies during the second flower stage for MKL 7 coffee clone. Significant differences between means in each column with distinct letters for each factor were determined using the LSD test at a significance level of P≤0.05%.

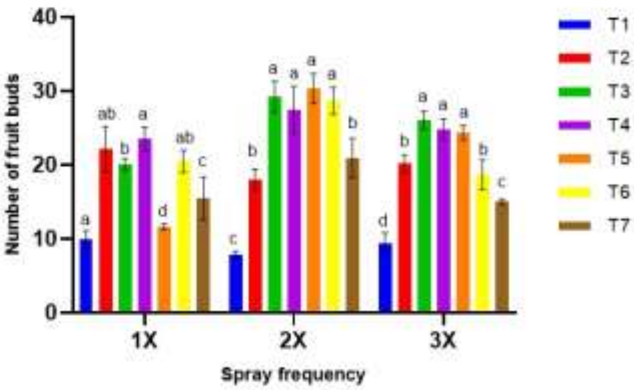

Figure 5: Effects of different levels of GA3 and Paclobutrazol hormones on the number of fruit buds at different hormone spray frequencies during the second flower stage for MKL 7 coffee clone. Significant differences between means in each column with distinct letters for each factor were determined using the LSD test at a significance level of P≤0.05%.

In the third tag (Sept 2023), there was no significant difference in the effect of the frequency of hormone spray across all treatments. Specifically, using the first spray resulted in a higher number of flowers compared to using the third spray. Among the hormone treatments, the GA3 treatment at a concentration of 300 ppm (T4) produced the highest number of flowers (5.64), while trees that were not sprayed with hormones had a lower number of flowers (Table 1).

Table 1: Effect of different concentrations of GA3 and Paclobutrazol hormones on flower production at different spray frequencies during the third flower stage on a single branch of the MKL 7 coffee clone.

	Total floral initiation
Spray frequencies	
1X	5.21a
2X	4.97a
3X	4.50b

Hormone treatment	
T1	2.93d
T2	4.84bc
T3	5.38ab
T4	5.64a
T5	5.60a
T6	5.20abc
T7	4.67c

Spray frequencies Hormone treatment	* **Spray frequencies x hormone** ns **treatment**

**Significant at a 1% probability level, *Significant at a 5% probability level, ns: not significant. The mean in each column with a different letter within each factor is significant at P≤0.05% based on the LSD test.

CONCLUSION

Throughout the study at MARDI Pontian until December 2023, it was observed that branches tagged for the first flower were still in the immature fruit stage. The branches tagged for the second and third flowers were at the fruit bud and flower stages, respectively. The study results indicate that spraying twice and using GA3 200 ppm hormone (T3) led to higher flower production compared to not using hormones. The number of flower buds in the third flower stage decreased in comparison to the first and second flower stages on the same branch.

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EXPLORING URBAN GARDENING IN MALAYSIA: INSIGHTS, CHALLENGES, AND FUTURE DIRECTIONS

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ABSTRACT

Urban gardening has become a trendy and notable means of improving food security, supporting sustainability, and strengthening community well-being. This review article explores the world of urban gardening in Malaysia by looking closely at current initiatives, the advantages and challenges involved, and the importance of sustainability. To investigate the motivations and experiences that drive urban gardening practices, several urban gardening projects were reviewed, including studies conducted in Kuala Lumpur and cities of other countries. It also looks at the impact of urban gardening on plant species diversity, community involvement and mankind's natural ties with nature. The review also discusses the importance of urban agriculture in maintaining food security, especially during the COVID-19 pandemic. In addition, it explores the possibilities of urban gardens as areas for socialising, leisure activities, and learning about the environment. Policy recommendations and outlook are provided to guide the development of urban gardening in Malaysia, with a focus on promoting sustainable practices and fostering community engagement. In general, this review provides valuable insights into the current state of urban gardening in Malaysia and presents a plan for promoting urban agriculture initiatives in the country.

Keywords: Urban Gardening, Sustainability, Food Security, Community Well-Being.

INTRODUCTION

Recently, urban gardening has been the global highlight to address numerous issues on food supply. It has been practised in the city environment or building structures such as rooftops, unused lands, and community spaces. Seen as having multifaceted roles, urban gardening is viewed as able to enhance food security as the communities harvest sustainable local produce. Accordingly, the gardening activities contribute to green areas in cities which also become shared spaces for social engagements among the communities. (Egerer et al., 2018; Rogge & Theesfeld, 2018; Russo & Cirella, 2019).

In Malaysia, urban gardening has also received similar attention to address food security that can sustain families in the urban environment. However, extensive study and exploration should be made regarding the challenges, further implementation and development of this method. Norziha et al. (2022) have documented issues faced by urban garden initiatives in Kuala Lumpur, whereas Milah and Rosmiza (2021) have highlighted the importance of empowering social assets in developing urban communities in Malaysia. Thus, this paper aims to explore the practices, issues and opportunities for future development through examining urban initiatives in Malaysia and other countries.

PROBLEM STATEMENTS

Accordingly, this paper seeks the view of writers on several questions as stated:

1. What are the current urban gardening initiatives in Malaysia?
2. What are the primary impacts of urban gardening initiatives in Malaysia?
3. What are the main challenges faced by urban gardening initiatives in Malaysia?
4. What are the future directions of urban gardening initiatives in Malaysia?

METHODOLOGY

Articles reviewed were obtained online, via Google Scholars search engine. The thematic search focuses on related keywords such as: urban garden, community garden, urban farm, community farm, sustainability, food security, rooftop garden, urban greenery system, environmental benefits, sustainable urban development, urban habitat, urban agriculture.

Consequently, 32 articles (2019 – 2024) were analysed to explore the initiatives of urban gardening, its impacts, challenges and directions, specifically in Malaysia setting.

URBAN GARDENING INITIATIVES IN MALAYSIA AND OTHER COUNTRIES

Several notable initiatives have emerged in Malaysia, which shows interest in urban farming is increasing. A study by Norziha et al. (2022) has listed some urban gardens or farming initiatives in Kuala Lumpur that utilize spaces in the city for agricultural purposes to enhance food security among the communities (Table 1). Implemented under Local Agenda 21 Kuala Lumpur (LA21KL) in 2005, the project aims to form a partnership between local authorities, the community and the private sector in working together to plan and manage the environment towards sustainable development and a higher quality of life.

Urban Gardens in Kuala Lumpur (2016-2020)
Laman Urban Agro Seri Semarak
Kebun Komuniti PPR Intan Baiduri
Laman Agro Muhibbah
Taman Herba LA21 KL
Kebun Kejiranan Sunway SPK Damansara
TTDI Edible Community Garden
Harmoni Agro Perumahan Awam Sri Sabah 3A
Kebun Kejiranan Kampung Malaysia Tambahan
Kebun Kejiranan Happy Garden 7 Continental Park
Kebun Komuniti KRT Taman Setapak Indah
Kebun Kejiranan Persatuan Penduduk Islam (PKPI) Setapak Indah
Kebun Kebun Bangsar
Grow X Dignity
Kebun Bandar Taman Sungai Bunus
Kebun Kejiranan SMK Seri Sentosa
Kebun dan Dusun SK Sentul 1

Table 1. List of urban gardens in Kuala Lumpur around 2016 to 2020.
Source: Secretariat of LA21KL, Dewan Bandaraya Kuala Lumpur, Malaysia.

Urban garden initiatives in Malaysia have been introduced since 1997 with the mobilization of community garden programs through the "*Semai Indah*" program by the Ministry of Housing and Local Government. Subsequently, the Edible Garden project was launched in 2008. The community garden program then evolved consistent with the current needs and requirements of the community.

In relation, the Selangor Neighborhood Garden (*Kebun Kejiranan Selangor*) is an initiative that encourages agricultural activities in the neighborhood whether cultivated individually or by the community. It is an effort that can promote fresh local food production and become a source for the supply of agricultural products for own use or to be sold to improve the community economy in the area (PLANMalaysia, 2023). In addition, this neighborhood or community garden is a step that can be implemented to green and beautify the surrounding area. The Selangor State Government, through the local authorities of the State of Selangor, has implemented community gardens under the Local Agenda 21 Action Plan (LA21) to encourage agricultural activities among urban residents.

On March 3, 2006, the Green Earth Program (Program Bumi Hijau) was launched at the national level. Consequently, on October 26, 2006, the first community garden were launched in Putrajaya. By October 2, 2009, 8 community gardens had been developed, involving more than 110 participants. The implementation of these urban gardening initiatives plays an important role in inculcating the culture of growing one's own food sources as a community. Programs like these allow citizens (i.e. participants) to be gradually equipped with knowledge and practical skills that can assist in easing the burden of household expenses. Also, these programs aim to empower the citizens with the strategies to survive events that may restrict the flow of food supplies such as natural disasters (PLANMalaysia, 2023).

Evidently, the Green Earth Program is developed as a platform to instil awareness and interest in agriculture, especially among residents of urban and rural housing estates. It is designed to promote the consumption of fresh, nutritious and safe food in addition to the beautification of the housing environment. By growing vegetables, herbs and fruit trees in the community garden, participants produce their own food for daily consumption and secure access and availability to food (Norziha et al., 2022). This will also aid in reducing the financial impact faced by the participants due to the rising price of food in Malaysia. Moreover, they provide a platform for people in the community to come together, cultivate plants, contribute to the aesthetics of the city areas and foster the spirit of environmental sustainability (Maantay & Maroko, 2018; Koay & Dillon, 2020; Lal, 2020; Mamun et al., 2023).

There are many similar initiatives around the world that agree with the vision of community gardens and urban gardening in Malaysia. To support this idea, research done by Huaiyun et al. (2019) on community garden initiatives in Shanghai, China, discusses the value of community engagement in ensuring healthy urban environments. The initiatives, which are supported by government departments and NGOs, are viewed as a strategic tool to foster community participation and unity. These are attained while working together towards sustainable urban development. Thus, it is relevant to claim that urban gardening does not only soften the hardness of the city through the creation of green spaces, as documented in Zurich and Lausanne (Jahrl et al., 2022). It also provides opportunities for social interaction and community well-being (Martínez-Núñez, 2024).

Moreover, in Cape Town, Africa, urban gardening has been shown to alleviate the heat island effect in the city areas due to urbanization. The introduction of green spaces improves thermal comfort, lowers the risk of flooding and saves the energy (Kanosvamhira, 2023). Meanwhile, in Singapore, urban gardening has been prominent in strategic urban planning, specifically to increase the level of liveability and resilience of the city. In this case, urban gardening is regarded as a tool to achieve the social, environmental, and ecological objectives of city greening (Ploessl, 2023).

During the COVID-19 pandemic, Malaysians have learned more about the importance of urban farming as many people attempt to grow their own food at home. This provides the government with an opportunity to encourage collaborations between citizens and relevant organizations to expand the practice of urban gardening (Rosmah et al., 2022). At this point, urban gardening has been recognized to enhance food and economic security in times of crisis (i.e. the COVID-19 pandemic), as observed in neighbouring country, Indonesia (Haryanti et al., 2023).

The pandemic has caused numerous disruptions including lockdowns, job losses, and economic instability. These troubles later snowballed into food insecurity issues among vulnerable populations (Wolfson & Leung, 2020). Such negative impacts inevitably hit Malaysia during the pandemic. Many would remember the difficulties to journey to the shops to purchase food and the paranoia of contracting the virus. The disruptions caused by the pandemic crisis have made it harder for Malaysians to have access to nutritious food, as observed by Regina et al. (2021) in household food security in Sarawak, Malaysia. To tackle the problem, resilient food systems and community-based solutions are required (Dombroski et al., 2020; Zurayk, 2020). Consequently, urban gardening or community farming are some feasible solutions to managing vulnerabilities and enhancing food security among Malaysian citizens.

The COVID-19 pandemic has also made social media a platform for netizens to share about their gardening experience. Some of the social media influencers have started to post informative contents about their urban garden and tips for fellow followers. These influencers serve as catalysts for promoting urban gardening as a rewarding and impactful activity. In Malaysia, there are some prominent online gardening gurus that are well-known in their local landscape. Batrisya (2023) from the Pokok website has written about Malaysian urban and organic gardeners such as Nuraishah Shamsuddin (Instagram: @nuraishahshamsuddin), Bone Wan (Instagram: @kebone.wan) and Safiuddin MS (Instagram: @dekamsan). They have promoted self-sustenance gardening, whether through innovative gardening methods, promoting native plant species, or creating aesthetically pleasing and sustainable garden designs. Through this scenario, social media plays an important role in fostering urban gardening awareness and sustainability (Park & Shin, 2021). These initiatives are made visible to the public thus making growing your own food becomes a trend to be followed. Consequently, it attracts more people to join the activities at home.

BENEFITS AND IMPACTS OF URBAN GARDENING

At this point, it has been mentioned countlessly that urban gardening initiatives contribute to ongoing work on sustainability attainment and the well-being of the community. In Taipei, for example, the “Garden City Initiative” supports community gardens, rooftop gardens and school garden projects. These projects are deemed to aid in creating resilient individuals, communities, and the environment. This includes dealing with climate change and fostering environmental awareness (Hou, 2018).

Subsequently, the review on urban food security in Malaysia during the COVID-19 pandemic stresses the necessity of urban farming in securing food sources for families that live in cities. Undeniably, this information strengthens the role of urban gardening as a tool for food sustainability and empowers its citizens with self-sufficiency knowledge (Rosmah et al., 2022). To support this, Mamum et al. (2023) study adds that a country can become less dependent on imported food as they increase their food security.

Apart from the benefits mentioned previously, urban gardening holds the potential to enhance the physical look of city areas. A study by Nazanin et al. (2020) on the effectiveness of an urban gardening program in Malaysia discusses the many benefits it can offer to the community. Interestingly, one of the gains of implementing the program is the decline of vandalism in the participating area. Vandalism in the city often occurs in vacant or underutilised spaces. By transforming these spaces into occupied gardening spaces, vandalism can sensibly be reduced. The increase of community engagements in the garden can also deter unwanted delinquent activities near the vicinities which leads to a safer community. This is supported by Jahrl et al. (2022) emphasizing the effectiveness of community gardens in deterring urban vandalism.

On the ecological aspect, special spaces for food production, like the urban garden, improve biodiversity and air quality. For instance, selecting plant species that mimic natural habitats can promote specific plant biodiversity (Ong et al., 2022), which later, hypothetically, may provide habitat for pollinators like bees (McDougall et al., 2018), support local bird species in urban ecosystem (Mayorga et al., 2020; Prihandi & Nurvianto, 2022) and grow shrubs with roots that help to stabilise the soil and reduce erosion (Nazanin et al., 2020).

Subsequently, several studies have shown that urban gardening benefits participants' well-being. Taking care of a garden or farm as a team in the urban setting improves both the physiological and psychological health of the citizens (Zutter & Stoltz, 2023). Working with plants offers the opportunities to perform healthy physical and mental activities (Janowska et al., 2022). To elaborate, participants do various activities, such as lifting bags of soil, pushing wheelbarrows, harvesting and watering, that enhance body coordination, muscle strength and cardiovascular health. Managing gardening projects as a team creates opportunities for them to search for strategies or solutions for certain issues, which leads to utilizing cognitive function and social engagement (Zhang et al., 2021). Moreover, natural components inside the garden are deemed to have positive effects on humans. For instance, cool breeze, colourful flowers, green plants and other tangible garden elements become pleasant experiences that encourage urban gardeners to unwind and ease mental stress (Nazanin et al., 2020). Consequently, the feelings of achievement, encouragement from other members and social discourse during collaborative work contribute to elevating confidence and building other social skills (Poulsen et al., 2017; Koay & Dillon, 2020; Wolf & Waitt, 2023).

SUSTAINABILITY AND FUTURE OUTLOOK

The United Nations announced the Sustainable Development Goals (SDG) in 2019 which encompass of 17 goals that focus on achieving sustainable development. Issues such as high food prices and the struggles to attain food security require effective solutions. Therefore, one of the goals, namely "Goal 2: Zero Hunger", is developed to guarantee food security, eliminate hunger, enhance nutrition, and promote sustainable agricultural practices globally. (United Nations, 2019).

Accordingly, urban gardening has become an effective strategy for tackling sustainability issues globally, including Malaysia. To start with, city dwellers face a lack of personal land to grow their own food (Norziha et al., 2023). In Malaysia's Green Earth Program, the local authorities play the role of identifying vacant lands, such as reserve lands, open spaces or leasehold areas to be cultivated as community gardens (PLANMalaysia, 2023). These spaces, when fully utilised as community gardens or farms, become local food production spots that can guarantee a variety of fresh crops, support local economic development and promote community-based agriculture. Thus, it is relevant to say that urban gardening ensures the sustenance of food supply from local sources, which also lowers citizens' dependency on food imports. Such a situation strengthens some scholars notes on how urban gardening helps in creating food security and resilient citizens that are competent to survive in changing global trends (Lal, 2020; Koay & Dillon, 2020; Zurayk, 2020; Norziha et al., 2022; Mamun et al., 2023). At least at a time of emergency or natural disaster, such as flooding or pandemic outbreaks, these community gardens will ensure the availability of food for several households.

However, urban agriculture initiatives, such as the programs and projects discussed earlier, are linked to more than providing food for citizens. The work of utilizing vacant and abandoned plots in urban areas is indeed a way to mitigate the negative impacts of urbanisation. As cities expand and the population increases, more challenges begin to seep into the urban areas. Problems such as vandalism at hidden locations, abandoned construction projects, deterioration of air quality and overwhelming heat are often linked with the bad side of urbanisation. Urban gardening can become the strategic solution to the matters aired previously. The utilisation of hidden locations for community gardens puts vandalism at a halt as more people will be present at said locations. Similarly, issues of waste of space can be decreased as abandoned spots are used for agricultural projects. As these community gardens and farms mature, they aid in the diversification of plant species and suitable habitats for animals. This does not only benefit in diversifying flora and fauna species but is also valuable in enhancing the physical outlook while adding more green spaces to the urban setting. Consequently, all these lead to a better environment (i.e. improved air quality) and cities that are safer to live in.

Accordingly, the previous discussions have placed urban gardening as a realistic and promising solution to numerous problems that often arrive in one package with urbanisation. Thus, based on this insight, it is appropriate to recognise urban gardening initiatives as part of a strategic tool in any urban planning. However, it cannot be denied that there are some challenges in urban gardening initiatives. A study by Norziha et al. (2022) on Malaysia's setting has underlined some important matters that need to be anticipated when implementing individual or community gardening in urban areas. Some factors related to technical expertise, resources, finance, social aspects and environment should be attended to ensure the success of urban gardening plans in the country. Hence, by leveraging these challenges and other successful urban agricultural programs, Malaysia can formulate a stronger plan and framework for future urban gardening initiatives.

POLICY AND RECOMMENDATION

In Malaysia, several community garden implementation guidelines have been issued by various agencies focusing on respective aspects. However, having different sets of guidelines from each of these agencies results in a lack of standardisation. In relation to that, the Ministry of Housing and Local Government has taken the initiative to formulate and coordinate guidelines for the implementation of community gardens that focus on urban areas. Hence, the Urban Community Garden Policy was implemented based on five cores that are in line with the 12th Malaysia Plan.

Firstly, planning, platforms and urban community garden development programs are provided to support the implementation of green development throughout the country. This includes the identification of locations that are appropriate for the development of urban community gardens. The second core ensures that urban community gardens are developed in an organised and systematic manner that supports environmental sustainability. This development should adhere to the guidelines of the local authorities to maintain the safety and cleanliness of the environment. Consequently, the third core encourages the cultivation of cooperation and social integration among the urban garden community. The next core is to facilitate the participants during the operation period of the garden and to assist in the coordination with any relevant agencies. Lastly, the fifth core encourages access to quality food sources to support daily needs. This is linked to the quality and safety of the crops produced (Ministry of Housing and Local Government, 2021).

The policy is well-written and provides complete guidance to urban communities to manage, plan, design, develop and implement urban community gardens according to the rules and existing procedures. However, the program requires active promotion nationwide to ensure that information about the program can be widely broadcast. Social media, for instance, is one of the most effective promotional platforms to ensure that information is delivered comprehensively throughout the country. Consequently, a lack of sufficient promotion will give the impression that the urban community garden program is not widely implemented throughout Malaysia.

DISCUSSION AND CONCLUSION

Long-term research is crucial for understanding the true effects of urban gardening on community well-being, biodiversity, and food security in Malaysia. By monitoring these initiatives over an extended period, we can observe their actual impact on communities and the environment, whether positive or negative. This comprehensive data enables us to make well-informed choices and establish strategies that guarantee the efficiency and longevity of urban gardening. This, in turn, leads to the improvement of public health, the conservation of biodiversity, and the enhancement of long-term food security.

Additionally, future study should investigate the economic advantages of urban gardening by examining the cost savings for households, the possibility for employment development, and the revenue produced from selling crops from these initiatives. For instance, it is beneficial to analyse the cost savings received by individuals on grocery expenses, the job opportunities generated by gardening initiatives, and the overall economic influence on communities. Furthermore, it is crucial to evaluate the expenses associated with establishing and upkeeping gardens in order to determine their financial feasibility and their impact on local economic development.

As urban gardening offers numerous potential benefits to education, there is a necessity to investigate how urban gardening can be used to engage young people in learning about the environment, science, and healthy eating. Further studies may include investigating how school and community gardening programs can instruct students about ecosystems, spark interest in science and technology (i.e., STEM subjects), encourage sustainability competency and promote better eating habits. Consequently, information on the long-term effects of these initiatives on young people's attitudes and behaviors towards the above matters may provide useful insights for educators and policymakers in the future.

In conclusion, urban community gardening or farming is a strong tool for Malaysia to attain food security, achieve sustainability and foster community well-being. The urban agriculture initiatives by Malaysia are consistent with the global trends particularly to meet the Sustainable Development Goals. Despite challenges faced during the implementation of urban gardening in the country, the program has many beneficial impacts on the physical, mind and social health of the citizens. The program is also useful in addressing problems that often occur in urban areas. To guarantee a continuous implementation of community gardens in urban areas, supporting policies and community participation are inevitably needed. Lastly, any future initiatives should incorporate urban gardening into any urban planning in our country.

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074-076

IMPACT OF LAYER AND PLANTING DISTANCE ON GROWTH OF KAILAN IN A MULTILAYER PLANTING SYSTEM

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ABSTRACT

The multilayer or vertical farming system is gaining popularity and receiving more attention in Malaysia due to their effectiveness of space optimization. Planting density also another focus to maximize the production capacity with compromise of growth and yield. However, uneven lighting and shading was an issue on multilayer planting system in a greenhouse. Therefore, a study on the effect of layer and different planting distance on growth of Kailan in a multilayer planting system was studied. This study was conducted in greenhouse at the Malaysian Agricultural Research and Development Institute (MARDI) using two factor factorial Randomized Complete Block Design (RCBD). The first factor is layer which consists of 3 levels, namely below, middle and top. The second factor is planting distance which consists of 3 distances arranged with 13cm, 18cm and 23cm in a single row. Parameters observed were plant height, number of leaves, leaf area, plant fresh weight, relative chlorophyll content, air temperature, relative humidity and light intensity. Planting distance of 13cm, 18cm and 23cm was significantly different for all parameters except for number of leaves and relative chlorophyll content. Meanwhile, top and middle layers show better results for all parameters compared from below layer. In summary, layer which is below, middle and top was significantly affect on growth of Kailan on a multilayer planting system.

Keywords: Multilayer, Planting Distance, Kailan, Green House.

INTRODUCTION

Brassica oleracea is a species of plant that includes various cultivars such as kailan (kale), cabbage, broccoli, and cauliflower. Generally, these cultivars can be grown in Malaysia, indicating a wide choice for production using vertical farming (Stephen & Gobilik, 2022). Multilayer or vertical farming system is getting popular and more attention in Malaysia, involve growing plants in a vertically oriented structure, typically against a wall or other vertical surface. These systems are popular in urban environments with limited space and can provide various benefits, including increased crop yield per unit area, improved air quality, and aesthetic enhancements. This method is commonly used in urban agriculture and indoor gardening. The logic of vertical farming is simple: produce more food on less land (Touliatos et al., 2016; Muller et al, 2017).

Research suggests that vertical planting systems can be used effectively for cultivating *Brassica oleracea* crops, including kailan. Some potential advantages of vertical cultivation for these plants include; i) space optimization: vertical systems allow for the efficient use of limited space, making them suitable for urban agriculture or areas with restricted land availability, ii) higher yield: vertical planting can provide increased crop yield per unit area by utilizing the vertical space effectively. This is especially beneficial when cultivating crops with compact growth habits like kailan, iii) protection and management: vertical systems can provide better protection against pests and diseases, as well as easier access for maintenance, harvesting, and pruning and iv) microclimate control: vertical farming can help regulate temperature, humidity, and light exposure, which may contribute to improved growth and yield of *Brassica oleracea* crops. It is important to note that the success of vertical planting systems for kailan or any other crop depends on various factors, including the specific requirements of the plant, the design and construction of the vertical system, appropriate irrigation and nutrient management, the numbers of layers adequate lighting conditions and the planting distance.

The number of layers in a multilayer planting system has a significant impact on the growth of vegetables. The more layers there are, the more plants can be grown in the same space, which can lead to higher yields (Eigenbrod & Gruda, 2014). However, too many layers can also lead to reduced light and air circulation, which can hinder plant growth.

Therefore, it is important to find the right balance between the number of layers and the amount of light and air each plant needs.

The distance between plants within each layer also affects their growth (Choy & Son, 2005; Wiangsamut & Koolpluksee, 2001). Plants that are too close together may compete for nutrients and light, leading to stunted growth and lower yields. On the other hand, plants that are too far apart may not make full use of the available space and light, which can also result in lower yields. Therefore, it is important to find the optimal planting distance for each type of vegetable. As previously stated, among many vertical designs, the column hydroponic system (CHS) is the most effective in increasing leafy vegetable yield per unit area. However, some CHS-features limit Kailan production in the system, especially factors associated with plant spacing (i.e., related to column spacing) and light intensity (Stephen & Gobilik, 2022). Accordingly, a study on the effect of layer and different planting distance on growth of Kailan in a multilayer planting system was studied.

MATERIALSAND METHODS

The study was conducted in greenhouse at the Malaysian Agricultural Research and Development Institute (MARDI) using two factor factorial Randomized Complete Block Design (RCBD). The first factor is layer (L) which consists of 3 levels, namely below (L1), middle (L2) and top (L3). The second factor is planting distance (S) which consists of 3 distances arranged with 13cm (S1), 18cm (S2), 23cm (S3) in a single row.

The recommended planting distance for Kailan is based on the plant's growth habit and the space it needs to grow and develop properly. Kailan is a leafy vegetable that grows in a compact rosette shape. It has shallow roots and does not grow very tall, usually reaching a height of about 15-20 cm. Spacing the plants about 13-25 cm apart in rows that are 30-45 cm apart provides enough room for each plant to develop a healthy root system and to grow a mature rosette of leaves without being crowded by neighbouring plants.

Parameters observed were plant height, number of leaves, leaf area, plant fresh weight, relative chlorophyll content (SPAD meter), chlorophyll fluorescence (Fluor Pen), total chlorophyll A and B (Spectrophotometer), light intensity (light meter), air temperature and relative humidity (thermometer and a hygrometer). Data was analyzed by analysis of variance (ANOVA) using SAS software version 9.4 and tested for significance using Duncan's Multiple Range Test at $P \leq 0.05$.

Multilayer system

A multilayer system consists of a 6 layer of growing container per rack, which is two layers from bottom is consider as below layer (L1), two layers in middle is consider as middle layer (L2) and two layers at the top is consider as top layer (L3). All container is fulfilled with mixture media such as perlite, vermiculite and peatmoss (1:1:1). Structure and layout of multilayer system showed in Figure 1.

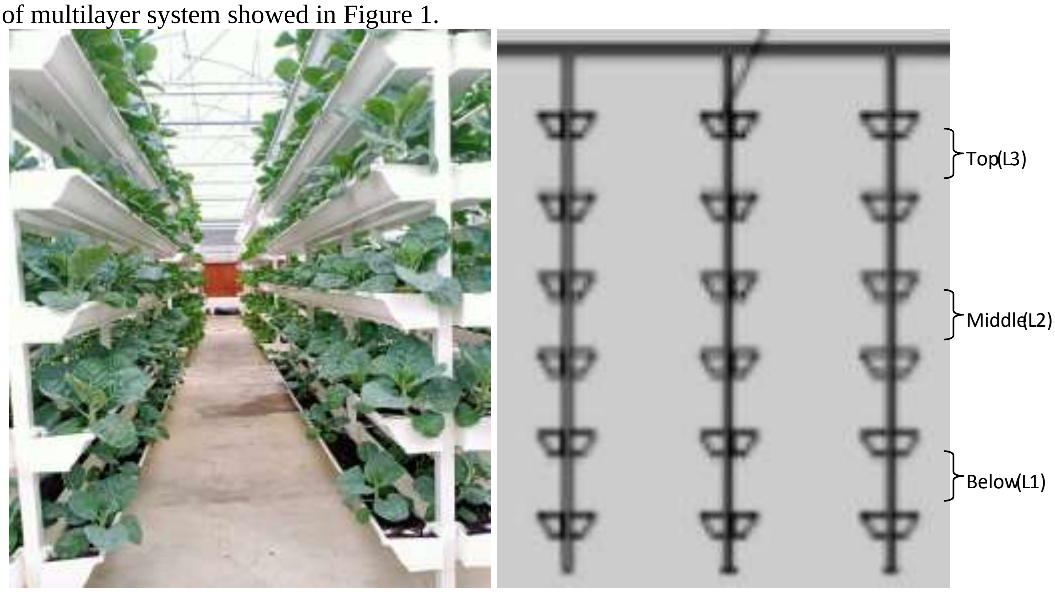
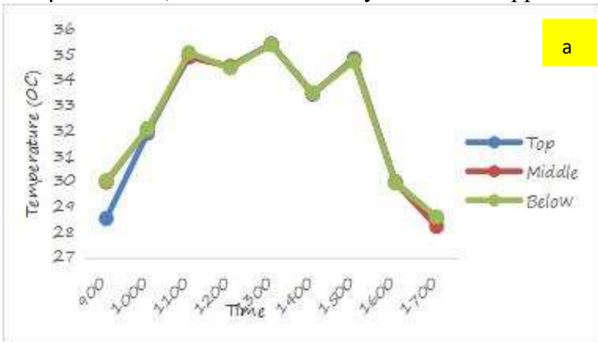


Figure 1. Structure and layout of multilayer which consist of 6 layers of growing container

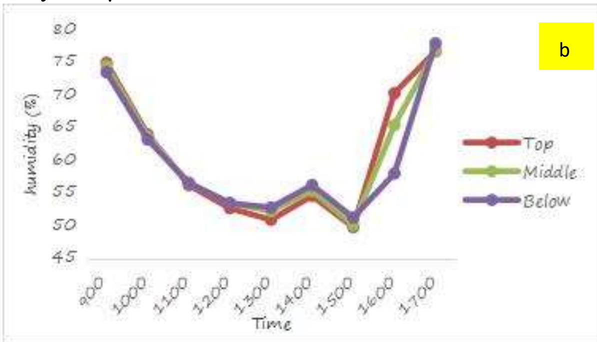
RESULTS AND DISCUSSION

Environment condition

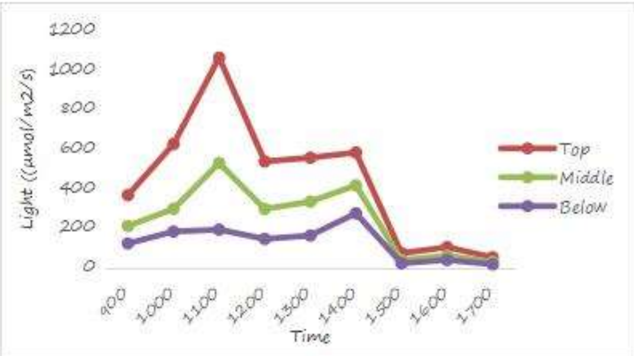
Greenhouses are designed to create a controlled environment that is optimal for plant growth. The air temperature, relative humidity and light intensity are some of the factors that are typically controlled in a greenhouse. By controlling these environmental factors, greenhouse growers can create an ideal growing environment that promotes healthy plant growth and maximizes crop yields. The environment in greenhouse in this study were showed in Figure 2. The temperature during 11:00 am to 3:00 pm for all layer's ranges from 33-35 ˚C. On sunny days, the relative humidity varies between 50-75%, while during rainy days, it increases beyond 75% for all layers. The top layers receive the highest amount of light, followed the middle and while bottom layers experience relatively lower light conditions. For instance, during the period of 10.00 am to 11.00 am, the top layer receives 1100 µmol m²/s¹, the middle layer receives 500 µmol m²/s¹, and the bottom layer receives approximately 200 µmol m²/s¹.



(a) air temperature



(b) relative humidity



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(c) light intensity

Figure 2: The environment in greenhouse (a) air temperature, (b) relative humidity and (c) light intensity

Effect of different layer conditions on growth characteristics

Table 1 showed the layer level of top, middle and below was significantly different for all parameters which is plant height, number of leaves, leaf area, plant fresh weight, plant dry weight and relative chlorophyll content of Kailan planting on multilayer system in a greenhouse. Top and middle level grew more vigorously than below level. The shortest plant (10.10cm) was found in the below condition. The tallest plant (11.24cm) was recorded under the middle condition which was at par to top level (11.02cm).

Result was shown for growth characters of Kailan at different layer levels, which is the increase of layer levels (bellow to top) will increase significantly growth for number of leaves, leaf area, plant fresh weight, plant dry weight and relative chlorophyll content (Table 1). Top and middle layers showed better results compared from below layer because in greenhouse natural light were limited for below layer. Overall, multilayer systems in greenhouse can offer several benefits for plant growth, including increased productivity, better nutrient absorption, reduced water usage, and pest and disease control. However, the effectiveness of multilayer systems will depend on the specific design, plant species and growing conditions.

Lighting plays a critical role in vertical farming since plants rely on light energy for photosynthesis and their growth and yield depend on the quantity and quality of light they receive (Wang & Folta, 2013). Inadequate lighting can result in slow plant growth and development. Vertical farming systems can face shading issues where plants at the bottom receive less light than those at the top, leading to slow growth and reduced marketability (Touliatos et al., 2016; Gobilik et al., 2021). In a multilayer system, light intensity is suspected to decrease from top to bottom and from outer to inner sides, affecting plant growth and yield (Touliatos et al., 2016; Gobilik et al., 2021). Artificial light is commonly used to supplement natural light in top vertical farming systems, although its cost-effectiveness for small-scale operations remains debatable (Shao et al., 2016). However, relying solely on artificial light increases the initial cost of vertical farming systems, which may be a challenge for smallholder farmers who make up the majority of vegetable farmers in Malaysia.

Table 1: Growth responses of Kailan in different levels and planting distance on multilayer planting system in a greenhouse.

Treatment	**Plant height (cm)**	**Number of leave**	**Leaf area (m^2)**	**Plant fresh weight (g)**	**Plant dry weight (g)**	**Relative chlorophyll content**
Level (L)						
Top (L3)	11.02±0.86ab	8.05±0.44a	96.58±12.15a	60.24±8.37a	44.92±7.40a	48.30±0.59a
Middle (L2)	11.24±0.81a	7.38±0.36b	81.50±9.37b	26.90±3.34b	18.32±3.03b	46.22±0.65b
Bellow (L1)	10.10±0.83b	6.91±0.30c	51.46±5.68c	13.71±1.76c	8.02±1.34c	44.26±0.59c
Planting Distance (PD)						
13cm (S1)	11.43±0.96a	7.54±0.38a	82.48±10.16a	36.64±6.03a	26.17±5.41a	46.10±0.66a
18cm (S2)	10.66±0.75ab	7.46±0.36a	76.33±9.51ab	34.79±5.81a	24.16±4.98ab	46.27±0.63a
23cm S3)	10.25±0.77b	7.33±0.38a	70.07±9.10b	28.64±5.08b	20.35±4.33b	46.35±0.62a
L	*	***	***	***	***	***
PD	*	NS	*	**	*	NS
L x PD	*	NS	NS	NS	NS	**

Data are means ± SE. Values in each column followed by a different lower-case letter indicate significant difference by the Duncan's Multiple Range Test ($P \leq 0.05$).

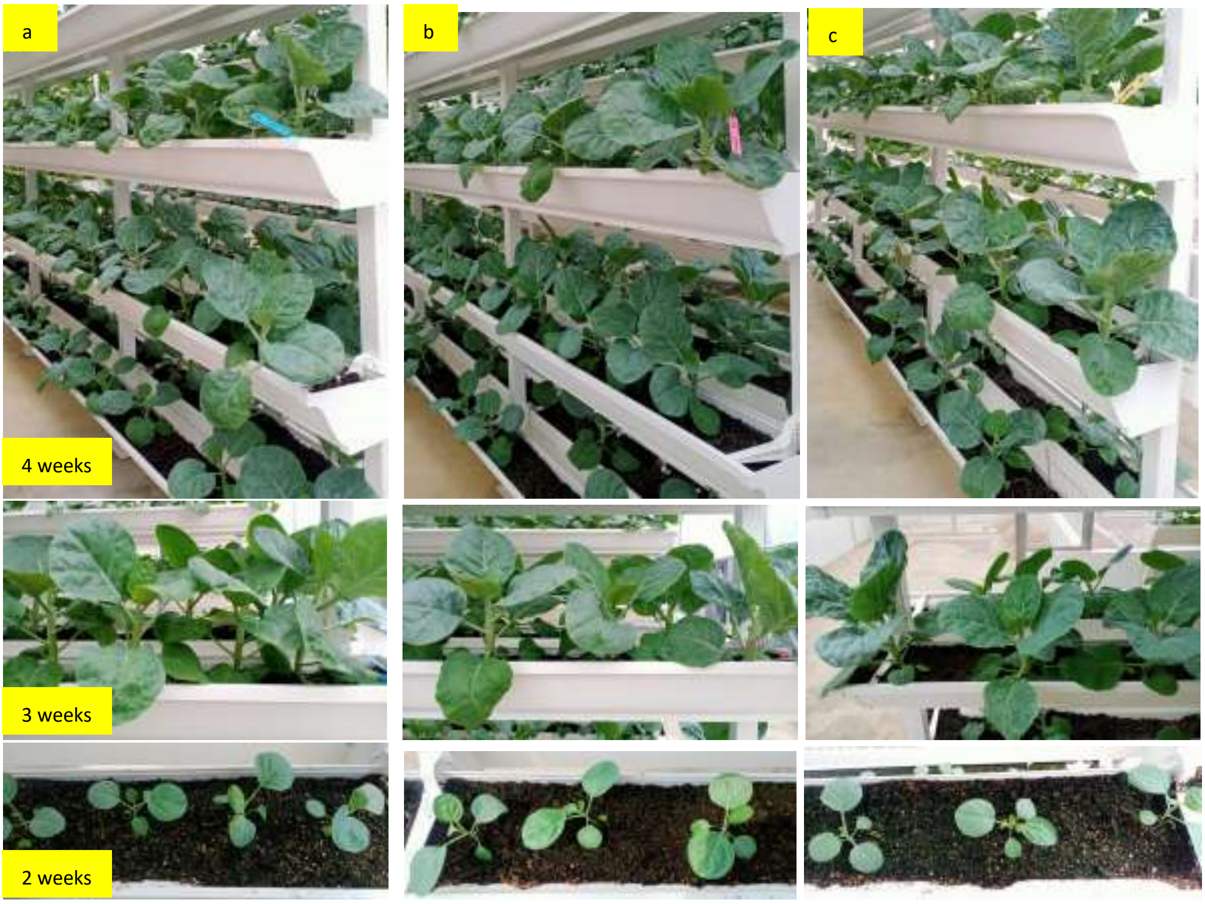
*= significant at 5% level of probability; **= significant at 1% level of probability; ***= significant at 0.1% level of probability; NS = not significant.

Effect of different planting distance on growth characteristics

Choosing the right distance for a specific crop is crucial to maximize resource utilization, minimize competition, and minimize the risks of pests, diseases, and weeds. Table 2 showed the results of this study that a planting distance of 13cm, 18cm and 23cm was significantly different for all parameters except for number of leaves and relative chlorophyll content.

Result was shown for number of leaves at different planting distance, which is the increase of distance (13cm to 23cm) will decrease for number of leaves, which is planting distance of 13cm (7.54) gives the best results followed by 18cm (7.46) and 23cm (7.33). While, result for relative chlorophyll content at different planting distance was shown, the increase of distance (13cm to 23cm) will increase for relative chlorophyll content, which is planting distance of 13cm (496.10) gives the lowest results followed by 18cm (46.27) and 23cm (46.35). Figure 3 displays pictures of varying planting distances, including 13cm, 18cm and 23cm at 2, 3 and 4 weeks after transplanting.

There have been several studies on the optimal planting distance for Kailan, but the specific recommendations may vary depending on the growing conditions, climate, and variety of Kailan. The difference in recommended spacing between these studies may be due to differences in the specific varieties of Kailan used, as well as differences in growing conditions and cultural practices. It's also possible that newer varieties or changes in growing practices may have led to different optimal planting distances compared to older studies. Overall, the recommended planting distance for Kailan is based on a combination of scientific research, practical experience, and observations of plant growth and development. It's always a good idea to experiment with different planting distances to find what works best in your specific growing conditions.



a) 13 cm of planting distance (b) 18 cm of planting distance (c) 23 cm of planting distance

Figure 3. Different planting distances of (a) 13cm, (b) 18cm and (c) 23cm at 2, 3 and 4 weeks after transplanting.

CONCLUSION

For optimal growth of Kailan using a multilayer planting system, it is advisable to space them 18cm apart to achieve a higher yield. It is crucial to effectively plan and manage the system, as some vegetables may perform better than others. The combination of the layer and planting distance significantly affects the yield of Kailan. However, the study's results were obtained from a single season trial, which may not be sufficient to determine the sustainability of the findings. Therefore, it is suggested to conduct similar experiments in at least one additional season to arrive at a conclusive outcome.

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076-077

THE IMPACT OF VARIED WATER DEPTHS ON GERMINATION AND EARLY SEEDLING GROWTH IN SELECTED MALAYSIAN RICE VARIETIES: A PRELIMINARY STUDY

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ABSTRACT

A preliminary investigation was undertaken in February 2020 to explore how varying water depths affect the germination and initial growth of three rice varieties: Mardi Siraj 297, Mardi Sempadan 303, and Mardi Sebernas 307, under controlled laboratory conditions. Water depths of 0, 5, 10, and 15 cm were employed with three replications using transparent containers measuring 20 cm in height and 15 cm in diameter. Data was collected on the fifth day after seeding. Findings revealed that both the depth of water and the specific rice variety significantly influenced the germination and early growth of rice seedlings. The highest germination rates and indices were observed in a water depth of 5 cm, surpassing those in 0 cm water depth by 8%. Additionally, variety MS 297 exhibited the highest vigor index, surpassing MS 303 by 40% and MS 307 by 47%. Furthermore, shoot height and root length of variety MR 297 were notably highest in 0 cm water depth and consistently higher across all water depth treatments compared to the other two varieties. Overall, MR 297 demonstrated superior early growth performance and vigor compared to the other varieties, suggesting its potential as a genotype tolerant to submerged conditions based on seedling observations.

Keywords: Mardi Siraj 297, Mardi Sempadan 303, Mardi Sebernas 307, Water Depths, Germination, Seedling Growth.

077-078

IMPROVING HARUMANIS MANGO PRODUCTION IN GREENHOUSE: A COMPARATIVE STUDY OF STINGLESS BEE (*Trigona* sp.) POLLINATION

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ABSTRACT

Harumanis mango has been recognized as a high-value crop in Malaysia. However, its cultivation in greenhouses often encounters challenges, particularly insufficient fruiting, attributed to the absence of effective pollinators. Therefore, finding potential pollinators adaptable to greenhouse conditions becomes imperative to address this issue. In this research, stingless bee species were utilized as pollinators to enhance Harumanis mango production. The objectives of this study were to investigate Harumanis production using stingless bees in a greenhouse. A randomized complete block design was employed in the greenhouse at the Horticultural Research Centre of the Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor, spanning two periods: without stingless bee pollination (November 2021 to June 2022) and with stingless bee pollination (November 2022 to June 2023). The results revealed a significant impact of stingless bee pollination on reproductive growth, with a notable increase in fruit number and fruiting percentage. However, there was no significant difference in panicle number or yield per fruit observed with stingless bee pollination. Additionally, fruit weight, length, diameter, and total soluble sugar remained unaffected. Notably, stingless bee pollination significantly increased yield per tree, amounting to 10.38, with an average fruit weight per tree of 5.63 kg. This emphasizes their potential as effective pollinators for Harumanis mango cultivation in greenhouse environments.

Keywords: Harumanis Mangoes, Fruit Production, Greenhouse, Pollinator, Stingless Bee.

INTRODUCTION

Harumanis mangoes stand out as one of Malaysia's premier mango varieties, celebrated for their exceptional flavor profile distinguished by a creamy texture and a captivating aroma (Siti Raihan, 2022). This unique combination of taste and fragrance has garnered widespread popularity among consumers, despite its relatively higher price point and the meticulous cultivation it requires (Uda et al., 2020). Year after year, Harumanis continues to experience consistent demand growth, a testament to its enduring appeal (Abdullah & Maamun, 2022; Sulino et al., 2023). Similar to other mango varieties, Harumanis typically bears fruit once a year during the main production season from April to June (Amirul et al., 2020; Zul Helmey et al., 2023). The heart of Harumanis cultivation lies in the northern states of Peninsular Malaysia, particularly Perlis and Kedah, where the majority of production is concentrated (Muhamad Hafiz et al., 2020).

However, without pollinator the production of fruit will decrease. Moving beyond Harumanis, mangoes are fascinating as andromonoecious plants, featuring both hermaphrodite and staminate flowers on the same inflorescence (Ramírez & Davenport, 2016). While hermaphrodite flowers possess the capability for self-pollination, challenges arise from instances of pollen-stigma incompatibility, leading to potential fruit set failures (Sutherland, 1986; Gehrke-Vélez et al., 2012; Dutta et al., 2013). Hence, the process of cross-pollination facilitated by a diverse array of insects, including wasps, ants, flies, butterflies, beetles, and bees, plays a crucial role in enhancing mango fruit set (Kleiman et al., 2021; Dhokane & Chavan, 2023).

Among these pollinators, stingless bees (*Trigona* sp.) have emerged as significant contributors to the pollination process in diverse plant species, spanning natural and agricultural environments (Eltz et al., 2003; Slaa et al., 2006) including Harumanis. There are more than 30 species of stingless bees inhabit in Malaysia agriculture site (Mohd Fahimee & Rosliza, 2016). This group comprises small to medium-sized bees with vestigial stings, commonly found in tropical and subtropical regions worldwide. They play a major role as pollinators for numerous flowering plants in tropical regions (Pushpalatha et al., 2022). Their significant role in pollinating fruits such as mangosteen, rambutan, and starfruit underscores their importance in ecosystem dynamics (Mohd Fahimee et al., 2022). Recognizing the substantial impact of stingless bees as pollinators for many fruit trees, this study delves into assessing the efficacy of *Trigona* sp. in pollination and its influence on Harumanis mango (*Mangifera indica* L.) yield within a controlled greenhouse environment.

MATERIALS AND METHODS

Experimental Location and Design

The research was carried out within a greenhouse facility located at the Horticultural Research Centre of the Malaysian Agricultural Research and Development Institute (MARDI) in Serdang, Selangor. The study spanned two periods: from November 2021 to June 2022 (without stingless bees as pollinators) and subsequently, from November 2022 to June 2023 (with stingless bees as pollinators). A randomized complete block design (RCBD) with three replications, each comprising six samples, was employed as the experimental design.

Management Practices

Seven-year-old *Mangifera indica* cv. Harumanis trees were cultivated in sedentary soil with a spacing of 4 m × 4 m between trees. Thirty-six healthy trees, exhibiting uniform shape and size, underwent regular pruning after each harvesting season. All trees received identical fertilization and other agricultural practices tailored specifically for the study.

Data Collections

Reproductive growth was meticulously monitored, starting from the flowering stage. Flower counts per plant were recorded, along with fruit yield. Data collection also involved noting the percentage of trees bearing fruit. To determine this percentage, the fruit yield was divided by the total number of inflorescence-bearing trees per tree. Additionally, precise measurements of fruit diameter, weight, and length were taken to evaluate overall fruit yield.

Statistical Analysis

Statistical analyses were performed using SAS software (Version 9.4, SAS Institute Inc., Cary, North Carolina, USA) via Analysis of Variance (ANOVA). Differences among treatments were assessed using the Least Significant Difference (LSD) method at a significance level of $P \leq 0.05$.

RESULT AND DISCUSSION

The impact of stingless bee pollination on reproductive growth was found to be significant result. Table 1 demonstrates a notable increase in the number of fruits (11.07) with a corresponding fruiting percentage of 55.70%. Cross-pollination has significantly boosted mango set (Nurul Huda et al., 2015), often facilitated by honey-sucking insects and bees seeking nectar from flowers (Usman et al., 2001). For instance, in Gadung Mango, fruit formation increased by 267.5% with Trigona sp. pollination (Kusuma & Windriyanti, 2022). Beside stingless bees, other bees such as *Apis mellifera*, *Apis cerana Braunapis hewitti*, and *Halictus* sp. has been identified as a mango pollinator (Sung et al., 2006). However, the number of panicles did not show any significant difference. Moreover, the emergence of flowers on the Harumanis mango is influenced by factors such as environmental climatic conditions. High temperatures, coupled with drought conditions, have been identified as the main triggers for flowering in mango (Farook et al., 2012; Herbert & Cavalcante, 2022).

Concerning yield per fruit, As indicated in Table 2, stingless bee pollination did not yield any significant differences. Despite the significant role of stingless bees in enhancing fruit production, no significant difference was observed in the number of fruit weight, length, diameter, and total soluble sugar produced upon the introduction of stingless bee pollination. This indicates that while stingless bees are effective in fruit set, the yield per fruit produced by the Harumanis mango is not influenced by their pollination. In addition to environmental factors that exert significant influence (Shaidatul Azdawiyah et al., 2020), fruit weight, fruit length, fruit diameter, and total soluble solid is primary determined by its cultivar (Mohd Asrul et al., 2018), agro-ecological conditions (Zul Helmey et al., 2019), age of the tree (Meena & Asrey, 2018), and cultivation practices (Azam et al., 2022; Singh et al., 2017).

Furthermore, the influence of stingless bee pollination on yield per tree was found to be significant, as shown in Table 3. Each tree produced an average of 10.38 fruits, with an average fruit weight of 5.63 kg per tree. Stingless bees have proven successful in increasing the yield of fruits per tree. This increase in fruit weight per tree can be attributed to the higher number of fruits produced. Stingless bees are recognized as highly effective pollinators due to their pollen-gathering behaviour, visiting numerous flowers and facilitating successful fertilization, seed development, and fruit production (Chuttong et al., 2022; Bueno et al., 2023).

Table 1. The effect of stingless bee pollination on the number of panicles, number of fruits, and fruiting percentage of *Mangifera indica* cv. Harumanis.

Treatment	Reproductive growth		
	Number of panicles	Number of fruits	Percentage of fruiting
Without stingless bees	19.1a	3.47b	22.59b
With stingless bees	20.6a	11.07a	55.70a

Means with different letters within each column are significantly different at P≤ 0.05 using LSD.

Table 2. The effect of stingless bee pollination on the fruit weight, fruit length, fruit diameter, and total soluble solid of *Mangifera indica* cv. Harumanis yield per fruit.

Treatment	Yield per fruit			
	Fruit weight (kg)	Fruit length (cm)	Fruit diameter (cm)	Total soluble solid (%)
Without stingless bees	434.10a	14.75a	9.39a	15.35a
With stingless bees	495.73a	15.88a	8.95a	15.43a

Means with different letters within each column are significantly different at P≤ 0.05 using LSD.

Table 3. The effect of stingless bee pollination on the number of fruits, and fruit weight of *Mangifera indica* cv. Harumanis yield per tree.

Treatment	Yield per tree	
	Number of fruits	Fruit weight (kg)
Without stingless bees	4.13b	1.795b
With stingless bees	10.38a	5.63a

Means with different letters within each column are significantly different at P≤ 0.05 using LSD.

CONCLUSION

In conclusion, the study underscores the significant impact of stingless bee pollination on the reproductive growth and yield of Harumanis mango within greenhouse environments. Despite the absence of significant differences in panicle number and yield per fruit, stingless bee pollination led to a notable increase in fruit number and fruiting percentage. This highlights the crucial role of stingless bees as effective pollinators for mango cultivation. Future research should explore strategies to further optimize stingless bee pollination and maximize mango production in greenhouse settings.

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CONFLICT OF INTEREST DECLARATION

Each author played a role in conceiving and designing the study, writing the article, critically revising it for essential intellectual content, and approving the final version. The paper has not been submitted to another journal or any other publishing outlet, and it is not currently under review elsewhere.

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079-080

THE PRODUCTION OF 'PITCHER PLANT', *NEPENTHES AMPULLARIA*, USING THE TISSUE CULTURE METHOD

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ABSTRACT

Nepenthes ampullaria, named after its flask-like modified leaves, can be found in secondary or swampy forests, as well as damp open regions ranging in altitudes from the lowlands to approximately 2,100 metres. This species has traditionally been used to regulate the menstrual cycle, facilitate delivery, alleviate asthma, treat eye inflammation, and improve strength. The components utilised were the liquid in an unopened pitcher and the root. Aside from its medical properties, N. *ampullaria* has grown in favour as a beautiful plant, and the pitchers were used as cooking pods for the Malays' famous food, "Lemang periuk kera." The existing pot-like pitcher sources come from the original ecosystem. To avoid *N. ampullaria* species loss due to demand and to lessen total reliance on the source from the original habitat, this species must be propagated. The tissue culture approach is one method for mass-propagating *N. ampullaria.*

Propagation of *N. ampullaria* was done using two different media types, semi-solid and liquid, to test for their proliferation rate. The propagation using semi-solids has resulted in a mean number of shoots produced in MS media supplemented with 2.5 mg/L BAP (2.8 shoots per explant). While in liquid media using Temporary Immersion Bioreactor Systems (TIBs), the culturing of *N. ampullaria* using the same media, MS, with the addition of 2.5 mg/L BAP, has resulted in a higher mean number of shoots (3.18 shoots per explant) compared to the semi-solid. A lower-salt-strength medium, ½ MS, with a supplementation of BAP 0.5 mg/L has shown a two-fold increment in the number of shoots produced compared to the semi-solid, MS + 2.5 mg/L BAP. The number of shoots produced was collected after 8 weeks of culture. In addition, no explant was found rooted in the semi-solid media, while nearly 80% of explants were rooted in the liquid media (½ MS) without the addition of a plant growth regulator. The parameter of *N. ampullaria* culture in TIBs, the frequency of immersion (3x, 4x, d, and 4x), was also tested. The frequency of three immersions per day has shown the highest mean number of shoots, 4 shoots per explant. However, the difference in shoot production between different immersion frequencies has shown no significant difference. The propagated tissue culture seedling was later acclimatised in the weaning chamber before being transplanted into the nursery.

Keywords: Periuk Kera, Microrpropagation, Plant Growth Regulator.

075-084

HEAVY METAL DETERMINATION AND HEALTH RISK ASSESSMENT OF EDIBLE MUSHROOMS COLLECTED FROM SELECTED MARKET IN KELANTAN, MALAYSIA USING ATOMIC ABSORPTION SPECTROMETER (AAS)

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ABSTRACT

Edible mushroom is a fungus used as food and medicine with high protein and are widely consumed by humans in daily life, especially in Asia. Despite high protein, edible mushrooms have the potential to accumulate heavy metals. Heavy metal concentration in edible mushrooms can negatively affect human health. Furthermore, edible mushrooms can be contaminated by heavy metals from the soil and environment. The aim of this study is to determine the heavy metal extraction (Zn, Pb, Cu, and Cd) in four different samples of edible mushroom species (*Pleurotus ostreatus*, *Lentinus edodes*, *Agaricus bisporus*, and *Flammulina velutipes*) that were purchased from selected markets in Kelantan, Malaysia. The heavy metal was determined by Atomic Absorption Spectrometer (AAS) and the result was compared to the permissible level by WHO/FAO. Besides, the human health risk was assessed through target hazard quotient (THQ), hazard index (HI), estimated weekly intake (EWI), and provisional tolerable weekly intake (PTWI). The average heavy metals concentrations in four edible mushroom species were found to be in the descending order where Zn > Cu > Pb > Cd. Furthermore, the average THQ for Zn, Pb, Cd, and Cu are 6.413×10^{-5}, 5.877×10^{-4}, 3.05×10^{-4} and 6.55×10^{-5}, respectively, whereas, EWI were found not to exceed the PTWI. There is no significant risk because HI values are less than 1 which is 0.001. Based on the results, it can be concluded that the analysed edible mushroom species do not pose a health risk to humans when consumed for a long term due to the relatively low present heavy metals concentration in the investigated mushroom.

Keywords: Edible Mushroom, Heavy Metal, Atomic Absorption Spectrometry, Health Risk, Concentrations.

1. INTRODUCTION

Mushrooms are special for their distinct taste, aroma, nutritional value, and medicinal properties. Mushrooms are known for their low energy content and high concentration of important biologically useful elements such as ß-glucans and antioxidants (A. Ihugba et al., 2018). Moreover, mushrooms have been identified as therapeutic foods with antibacterial, anti-mutagenic, and anti-tumoral properties. Most edible mushrooms have a full essential amino acid profile that makes them as good source of high-quality protein (González et al., 2020). Mushroom is also a component of natural forest ecosystems and plays an important role in element and organic matter cyclic pathways. Macro fungi research in Malaysia focuses on the ecology, cultivation and health benefits of edible mushrooms (Samsudin & Abdullah, 2019). Mushrooms contain 5–15% dry matter and have a well-balanced mineral and vitamin composition. Malaysia has a high demand for a mushroom which is expected to increase in line with per capita intake and population growth.

On the other hand, studies of toxic elements in macro fungi have revealed a connection between mushroom heavy metal concentration and metal contamination sources (Semreen & Aboul-Enein, 2011). In addition, environmental and fungal factors have been shown to influence heavy metal accumulation in macro fungi. It accumulates in varying concentrations of heavy metal in different plants which, some plants will obtain more metals than others. Even though certain heavy metals are needed for plant growth, but with excessive accumulation of these metals by plants grown on polluted land is a sign of increased pollution (Leblebici et al., 2020). Hence, it is crucial to lower the possibility of human toxic metal poisoning and regularly monitor the heavy metal load in mushroom species.

Heavy metal also comes out with any metallic chemical element and they are classified as essential and non-essential in general. Xenobiotics or foreign elements are non-essential metals (Al, Cd, Hg, Sn, Pb, Cr) that have no proven biological role, and their toxicity increases with growing concentrations. Essential metals (Cu, Zn, Ni, Co, Mo, Fe) are considered to play important biological roles and are toxic at high concentrations (Authman, 2015). In addition, free radicals are known to be generated by some heavy metals that can lead to oxidative stress and cell damage.

The heavy metal concentration of edible mushrooms has been found to have both direct and indirect effects on the environment and human health. The concentrations of heavy metals in certain edible mushroom components such as the stipe and cap were also evaluated and the results revealed that Cr, Zn, Fe, Cd, Pb, and Cu easily accumulate in greater amounts in the caps, whereas Ni and Mn appear to accumulate more in the stipes (Dowlati et al., 2021). Consumption of these heavy metals is extremely dangerous because their accumulation in the body is linked to a variety of diseases. Many researches have looked into the accumulation of heavy metals in the fruiting bodies of edible mushroom species (Nnorom et al., 2020). Therefore, information on the levels of heavy metals in food and the dietary intake of their consumers is essential.

Besides that, edible mushrooms have the potential to accumulate heavy metals. The accumulation of heavy metals by edible mushrooms is a complicated process influenced by environmental such as soil, metal content, amount of organic material, and physiological factors. However, heavy metal accumulation in the human body causes significant damage to various organs, especially the respiratory, mental, and reproductive systems, as well as the digestive systems. When heavy metals are consumed more than their prescribed dietary intake, they have a significant impact on human health. Even though these metals have no biological function, their toxic effects persist in some form that is harmful to human health and its proper functioning. It also exposes the health effect of humans such as irritation of the skin, ulcers and kidney problems.

There are several previous studies conducted to analyze heavy metal residue in edible mushroom species. According to Huang, Jia, Wan, and Jiang (2015), in China indicated that the 18 heavy metals contained in various types of edible mushrooms are high concentrations of some heavy metals such as Pb, Cd, and Hg that exceeded the permissible limits by the Chinese standard (MH 2012) (Huang et al., 2015). Besides, according to research conducted by Cocchi, Luigi, Vescovi, and Luciano (2006), in Italy, the majority of edible mushroom species contain very high amounts of mercury from the maximum acceptable that recommended by WHO which is not more than 0.3 mg. However, edible mushrooms particularly those found near heavily populated highways, can contain higher levels of heavy metals than plants.

This study was conducted to determine the heavy metal residue in edible mushroom using Atomic Absorption Spectrometry and its risk to human health.

2. MATERIALS AND METHODS

2.1 Sample collection

Four types of species of edible mushrooms were selected for this study are *Pleurotus ostreatus* (Oyster mushroom), *Lentinus edodes* (Shiitake mushroom), *Flammulina velutipes* (Enoki mushroom) and *Agaricus bisporus* which is the most highly consumed by residents in Kelantan, Malaysia.

2.2 Preparation of solutions

5 mL nitric acid (HNO_3), 5 mL sulphuric acid (H_2SO_4), and 1 mL hydrogen peroxide (H_2O_2) were used to prepare blank reagent solution and were marked up to 50 mL with a volumetric flask. Then, serial dilution of 10^{-1}, 10^{-2}, 10^{-3} and 10^{-4} were conducted (Cantwell, 2019).

A concentration of 1000 mg/L stock solution for each of the heavy metals Zn, Pb, Cu, and Cd was prepared. The standard calibration solution for each heavy metal was prepared by dissolving 1.5980g of lead nitrate ($Pb(NO_3)_2$), 2.1032g of cadmium nitrate ($Cd(NO_3)_2$), 3.798 g of copper nitrate ($Cu(NO_3)_2$) and 1.245 g of zinc nitrate ($Zn(NO_3)_2$), respectively with deionized water and then diluted to 1000 mg/L in a volumetric flask with deionized water (Ribeiro, 2014). Thus, at concentrations ranging from 5 µg/L to 100 µg/L, the normal calibration solution for each heavy metal was prepared.

2.3 Sample preparation and analysis

Each of the four samples of edible mushrooms were washed with deionized water and the sample was dried at 60˚C for 24 hours in a food dehydrator (Leblebici et al., 2020). Then, each of the samples was crushed and blended in a ceramic mortar into a very fine quality powder. Each sample was then homogenized with an agate pestle and stored in pre-cleaned polyethylene bottles at -20 °C. Atomic Absorption Spectrophotometer (AAS) was used and the concentrations of Pb, Cu, Zn, and Cd in the digests were determined (A. Ihugba et al., 2018).

Then, the digestion method that was used is dry ashing digestion that is particularly suitable for First of all, 1.00 g of dried sample of each mushrooms type was placed into a high porcelain crucible. The porcelain crucible will be heated and placed in a muffle furnace at 600°C for 6 hours and steadily increased to make the sample dry ashing (Tsegay et al., 2019). Then, the samples were ashed until they left a white or grey ash residue. The porcelain crucible is the best vessel for dry ashing because it is unaffected by all of the common acids except concentrated, high-temperature phosphoric acid and aqua regia (Hu & Qi, 2013).

After that, 10 mL of concentrated mixed acid (HCl: HNO_3=1:1) was used to digest the ashed samples. The digestion solution was heated to 150 °C on an electric hot plate for one hour or until it was nearly dry. Then, the residue was filtered using a PTFE filter and syringe filter. The sample was moved into a volumetric flask with added 3% HNO_3 to make up to 50 mL. Move 15 ml from the stock sample into falcon tube for sample analysis and serial dilution. Before the analytical phase, the digested solution was placed in a polyethylene bottle. However, the blank digestion tests were carried out in the same way. All the four heavy metals (Zn, Pb, Cu, and Cd) were analyzed by using the AAS. Furthermore, the data was displayed in μg/L (w/v) units and then, the conversion formula was used to convert (w/v) to dry weight (mg/kg).

2.4 Survey conducted

There was a survey conducted using Google Forms and the survey was blasted to the respondent in Kelantan. This survey was provided with three sections which are sections A, B, and C. This section consists of social demographic, intentions of Kelantan residents towards edible mushrooms as a source of food, and frequency of intake edible mushrooms in a day, week, and month among Kelantan, Malaysia residents.

2.5 Health Risk Assessment

2.6 Estimated Daily Intake (EDI)

The following equation was used to calculate the estimated daily consumption (EDI) (mg/kg body weight) of trace metals from eating four types of edible fungi (*P. ostreatus*, *F. velutipes*, *A. bisporus*, and *L. edodes*) (Chungu et al., 2019).

$$EDI = \frac{C_{metal} \times IR}{BW_{average}}$$

Where, Cmetal is average heavy metals content in edible mushroom (mg/kg), IR is ingestion rate daily intake of edible mushroom (gram/day person) and ABW is average body weight. Some of the standard default values were used in estimating and determining the aspect of body weight (60 kg/person). Next, the assumption value for the ingestion rate (0.3 kg/person) was used.

2.7 Estimated Weekly Intake (EWI)

The following equation was used to calculate the estimated weekly consumption (EWI, mg/kg body weight/week) of trace metals from eating four types of edible fungi (A. Ihugba et al., 2018):

$$EWI = EDI \times 7 \times \frac{x}{7}$$

EWI was calculated based on the questionnaire, the local people in the Kelantan consume edible mushroom species with a frequency of two times per week (*x*).

2.8 Target Hazard Quotient (THQ)

The equation below was used to calculate THQ (A. Ihugba et al., 2018).

$$THQ = \frac{EF \times ED \times IR \times C_{metal}}{RfD \times BW_{average} \times AT} \times 10^{-3}$$

The standard default values used in estimating and determining the aspect: body weight (60 kg/person) and exposure duration (70 years/person). Next, the assumption value also was used in this calculation, where: exposure frequency (365 days/year) and ingestion rate (0.3 kg/person). The standard value from USEPA of RfD (Cu (0.04), Zn (0.3), Cd (0.001), Pb (0.0035) were used.

2.9 Hazard Index (HI)

The sum of the hazard quotients is the hazard index (HI) derived from THQ (Dee et al., 2019). The following equation below was used to calculate the hazard index in Pb, Cu, Zn, and Cd:
$HI= THQ_{Cu} + THQ_{Zn} + THQ_{Cd} + THQ_{Pb}$

3. RESULTS AND DISCUSSION

The differences among the concentrations of Cd, Zn, Pb and Cu in four different edible mushrooms were significant in some cases, and this is likely due to its species and cultivated land. All of the metals analysed were found to be not above the detection limit of the FAO/WHO. From Table 1, clearly shown that heavy metal with highest concentration was zinc, Zn (1.393 mg/kg), whereas the lowest concentration recorded is cadmium, Cd (0.013 mg/kg). *P. ostreatus* had higher Zn, Pb, and Cd metal concentrations than *L. edodes*, *A. bisporus*, and *F. velutipes* while Cu is higher in *A. bisporus*.

In addition, the results were obtained by calculating the average amounts of means concentrations (Zn, Cu, Pb, and Cd) in the edible mushroom samples in the unit of mg/kg. The average concentrations of heavy metals obtained were; Cu: 0.131 mg/kg; Zn: 1.2953 mg/kg; Cd: 0.01525 mg/kg and Pb: 0.10275 mg/kg, respectively. The concentrations of heavy metals in edible mushroom samples were found to be in the following order where Zn > Cu > Pb > Cd. Moreover, the presence of heavy metals in each of the edible mushroom samples was compared to the authorized levels by World Health Organization/ Food and Agriculture Organization WHO/FAO (2011). According to Table 1, all heavy metals (Zn, Cu, Pb, and Cd) in edible mushroom samples did not exceed the WHO/FAO 2011 permitted level respectively.

The concentration of zinc in edible mushrooms, *P. ostreatus* had the highest mean concentration, with 1.393 ± 0.0190 mg/kg, followed by *L. edodes* (1.327 ± 0.0061 mg/kg), *A. bisporus* (1.259 ± 0.0084 mg/kg) and the lowest mean concentration of zinc in *F. velutipes* with a ranging concentration of 1.202 ± 0.0162 mg/kg. Zinc is a major aspect of a wide range of enzymes, where it serves as catalysis, architectural, and regulatory element. Furthermore, zinc insufficiency can be caused by a lack of zinc in the diet, poor absorption, excessive output, or hereditary zinc metabolism abnormalities (Zhu et al., 2011). Because of the maximum concentration of this element in the cap of the fruiting body for the 56 examined growing edible mushrooms species, zinc has a better characteristic in the analysed species of mushrooms.

Zinc was found to be the most abundant heavy metal in all of the edible mushrooms species samples in this study followed by Cu, Pb and Cd. However, zinc concentration reported in this study can be regarded as negligible in all samples and are not detrimental to human bodies because it does not exceed the permissible level by WHO/FAO. This result was matched with a previous study in Nigeria, in which samples of mushrooms were taken from a prominent supermarket in Imo State. According to A. Ihugba et al. (2018), zinc was found to be the most abundant ranged from 0.03 mg/kg to 3.25 mg/kg, followed by Cu (0.03 mg/kg to 0.83 mg/kg) and Pb (0.04 mg/kg to 0.36 mg/kg). The average concentration result acquired from this study was approximate to the previous study's observation. Since crop mineralisation and an environment polluted with zinc based chemical compounds, this observation predicts that zinc enters the mushrooms through the soil.

Copper is the human body’s natural third most abundant trace element, and it has a nutrient effect on living processes. Copper is essential for a range of metabolic activities, yet it can be harmful to people when concentrations are exceeded (Elements & In, 2019). the maximum concentration of cooper was found in *A. bisporus* with the concentration 0.193 ± 0.0013 mg/kg, whereas the lowest concentration was found in *L. edodes* at a concentration of 0.059 ± 0.0032 mg/kg. Then, 0.176 ± 0.0036 mg/kg for *P. ostreatus* and 0.096 ± 0.0029 mg/kg for *F. velutipes*. The results revealed that the mean concentration of every edible mushroom sample did not exceed the WHO/FAO permissible level of 40 mg/kg.

In addition, mushrooms have a greater copper concentration than vegetables in general. It is required for a wide variety of biological reactions. Some copper in the environment is less securely bonded to soil particles, and it may be soluble enough in the water for plants such as mushrooms to absorb (Dowlati et al., 2021). The mean Cu concentration in this study is lower than in a recent study in China, which used mushroom samples from Yunnan Province and found that the mean concentration was greater than in this research. According to the Fangkun Zhu et al. (2011), the mean Cu concentrations in the latest research ranged from 7.44 mg/kg to 31.9 mg/kg. These levels in mushrooms are still below the WHO/FAO acceptable guidelines for both research and safe for consumption by humans.

Among the four edible mushroom species, *P. ostreatus* had the highest lead concentrations, at 0.128 ± 0.014 mg/kg, and was followed by *A. bisporus*, which had 0.102 ± 0.0135 mg/kg. Then, the mean concentrations of lead followed with *L. edodes* and *F. velutipes*, where the concentrations in these samples are 0.100 ± 0.0095 mg/kg and 0.081± 0.006 mg/kg, respectively. The lead concentrations in all four edible mushroom samples did not exceed WHO/FAO (2011) permitted levels. After cadmium and mercury, lead is the third most dangerous trace metal found in mushrooms. Lead has no positive function in human metabolism and causes gradual poisoning (Zhu et al., 2011). In addition, lead slowly builds in the bones and can substitute for calcium. It is also can enter the human body through the atmosphere, water, and food. The digestive tract, consuming contaminated water and contaminated foods, inhalation, and the transdermal pathway are all ways for lead elements to enter the body (Muszyńska et al., 2018).

According to the previous study conducted by Semreen & Aboul-Enein (2011) on edible mushrooms collected from Jordon, where the mean concentrations of Pb range from 2.01 mg/kg to 4.81 mg/kg. In comparison to this study, the results of previous research show significant lead concentrations that exceed the WHO/FAO permissible level which is 0.3 mg/kg. Effective soil quality and assurance control are the improvement efforts that are critical components of growing safe edible mushrooms for consumers. This also can help to reduce lead from accumulating in edible mushrooms.

Among the four edible mushroom species, the highest concentrations of Cd were shown in *P. ostreatus*, with a value of mean concentration is 0.02 ±0.0011mg/kg. However, Cd concentrations were found to be lowest in *L. edodes* and *F. velutipes*, both of each edible mushroom had the same amounts of 0.013 ± 0.0006 mg/kg. All of the edible mushroom samples contain low levels of Cd and that the levels of Cd do not exceed the limitations set by WHO/FAO regulations. Then followed by *A. bisporus* which has the second highest mean concentration of 0.015 ± 0.0008 mg/kg. Most importantly, none of the four edible mushroom samples tested exceeded the permissible limits of Cd concentration which is 0.2 mg/kg that set by WHO/FAO. Since cadmium is a hazardous element for humans, acute poisoning is uncommon but chronic poisoning induced by the accumulation of small amounts of cadmium over time is more prevalent such as in food (Muszyńska et al., 2018). Cadmium also a chemical substance has referred to processes and carcinogenic properties, as well as disrupting liver processes, the reproductive organs, and bones. Moreover, Cd is commonly found in soil and is absorbed into the food chain by plants such as mushrooms (Karami et al., 2020).

Beside from that, research carried out by Liu et al. (2021) collected edible mushrooms from Jilin Province, China, also shown that the level of Cd concentrations was lower than the permissible level by regulations. The concentrations of Cd from previous research in mushrooms ranged from 0.007 mg/kg to 0.07 mg/kg (Liu et al., 2021). When the Cd concentrations in edible mushroom samples are compared to the previous studies, it can be concluded that the Cd concentrations in edible mushrooms for this study are significantly the same because it does not exceed the permissible limit by WHO/FAO.

The comparison of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) for adults in all four edible mushroom samples in this research is shown in Table 2. The amount of EDI is lower than the amount of EWI because their time is different. It is because of EDI is used to determine the metal requirements in mushrooms for adults in days, whereas EWI is used to determine the metal requirements in mushrooms for adults in weeks. The EWI in *P. ostreatus* were found to be high for the first metal, Zn which is 0.014 mg/kg/week, followed by Zn in *L. edodes* with 0.0132 mg/kg/week and Zn in *A. bisporus* with 0.0126 mg/kg/week. *F. velutipes* has been found is the lowest of EWI for Zn with 0.012 mg/kg/week. Then, Cu has the highest EWI in *P. ostreatus* and *A. bisporus*, both at 0.0018 mg/kg/week, and followed by Cu in *F. velutipes* at 0.001 mg/kg/week and *L. edodes* which is the lowest EWI for Cu with 0.0006 mg/kg/week. Next, EWI of Pb for adults in *P. ostreatus* has an EWI of 0.0012 mg/kg/week, *A. bisporus* has an EWI of 0.00102 mg/kg/week. Then, followed by *F. velutipes* has an EWI of 0.001 mg/kg/week, and *L. edodes* has an EWI of 0.0008 mg/kg/week. The most estimated daily intake of Cd for adults are in *P. ostreatus* with 0.0002 mg/kg/week and followed by *A. bisporus* with 0.00016 mg/kg/week. The lowest estimated weekly intake of Cd in edible mushrooms was 0.00014 mg/kg/week for both *L. edodes* and *F. velutipes*. However, EDI of heavy metals was determined using the average mean of each heavy metal in the mushrooms and the consumption rates associated with each heavy metal (Leblebici et al., 2020). Then, according to the body of adult requirements, the Tolerable daily intake (TDI) (mg/kg/day) of edible mushrooms is collected from WHO/FAO (2011) stated that for Zn is 60 mg/kg/day, for Cu are 4.3 mg/kg/day, for Pb are 0.03 mg/kg/day and lastly for Cd in edible mushrooms are 0.01 mg/kg/day.

In addition, provisional tolerable weekly intake (PTWI) is the maximum number of contaminants that a 60 kg consumer can consume in a week which is set by WHO/FAO (A. Ihugba et al., 2018). Whereas, PTWI (mg/kg/week) for adults in edible mushroom species is 360 mg/kg/week for Zn because Zn is essential in human bodies but not excessively, 30 mg/kg/week for Cu, 18 mg/kg/week for Pb, and 7 mg/kg/week for Cd. Furthermore, EDI and EWI for the edible mushroom samples in this research do not exceed the TDI and PTWI recommended by WHO/FAO (2011). This indicates that all of the edible mushroom samples were safe for human consumption, especially Kelantan residents.

The results of the Target Hazard Quotient (THQ) and the Hazard Index (HI) for adults in the four edible mushroom species samples were shown in Table 3. THQ indicator links metal concentrations in mushroom to their toxicity, as well as the quantity of mushrooms consumed and the body weight of consumer. The average value of THQ in Zn is 6.413×10^{-5}, while the average of THQ in Cu is 6.55×10^{-5}. Whereas, the average value of THQ for Pb in the four edible mushroom samples is 5.877 × 10-4 and followed by the last metal which is Cd that show the average value of THQ are 3.05×10^{-4}. The Hazard Index (HI) for adults in the edible mushroom species that was examined were discussed. However, to determine the HI value, sum all of the THQ in all of the metals together. In this research, the HI result came out to be 0.001. There is no significant risk in four edible mushroom species that purchased from a selected market in Kelantan because the Hazard Index (HI) values are less than 1, and the edible mushrooms are safe to consume.

4. CONCLUSION

This study provides an overview of heavy metal accumulation in four edible mushroom species which are *P. ostreatus* (Oyster mushroom), *L. edodes* (Shiitake mushroom), *A. bisporus* (Button mushroom), and *F. velutipes* (Enoki mushroom) purchased from a selected market in Kelantan, Malaysia. The mean concentrations of heavy metals identified in four edible mushroom species in this research sorted in increasing order, with Cd < Cu < Zn being the most prevalent. According to regulations guidelines, the accumulation of heavy metals in mushrooms is not in a critical level. The average heavy metal concentrations identified in each edible mushroom species were Zn (1.2953 mg/kg), Cu (0.131 mg/kg), Pb (0.1028 mg/kg) and Cd (0.0153 mg/kg). The levels of heavy metal concentration in all edible mushroom species in this research do not exceed the WHO/FAO (2011) permissible limits.

Furthermore, EDI for adults observed in all edible mushroom species was determined to be lower than the WHO/FAO recommended TDI for all heavy metals examined. Besides, EWI for an adult consuming 300 g of mushroom and weighing 60 kg was found to be lower than the PTWI for Zn, Cu, Pb, and Cd levels given by WHO/FAO. As a result, there is no risk to people in Kelantan who consume these four edible mushroom species. The Hazard Index (HI) was 0.001, which indicates that no significant risk exists in all edible mushroom species.
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Conflict of interest - Disclose any potential conflict of interest appropriately.

The authors declare no conflict of interest.

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Table 1. Level of heavy metal concentration (mg/kg) in edible mushroom species samples

Type of Edible mushroom species	Concentration of heavy metal; Mean ± SD (mg/kg)			
	Zn	Cu	Pb	Cd
Pleurotus ostreatus	1.393 ± 0.0190	0.176 ± 0.0036	0.128 ± 0.014	0.020 ± 0.0011
Lentinus edodes	1.327 ± 0.0061	0.059 ± 0.0032	0.081 ± 0.006	0.013 ± 0.0006
Agaricus bisporus	1.259 ± 0.0084	0.193 ± 0.0013	0.102 ± 0.0135	0.015 ± 0.0008
Flammulina velutipes	1.202 ± 0.0162	0.096 ± 0.0029	0.100 ± 0.0095	0.013 ± 0.0006
WHO/FAO	60	40	0.3	0.2

Table 2. Comparison of Estimated Daily Intake (EDI) and Estimated Weekly Intake (EWI) for adults.

Edible mushroom species	EDI (mg/kg/day)				EWI (mg/kg/week)			
	Zn	Cu	Pb	Cd	Zn	Cu	Pb	Cd
Pluerotus ostreatus	0.0070	0.0009	0.0006	0.0001	0.014	0.0018	0.0012	0.0002
Lentinus edodes	0.0066	0.0003	0.0004	0.00007	0.0132	0.0006	0.00014	0.0008
Agaricus bisporus	0.0063	0.0009	0.0005	0.00008	0.0126	0.0018	0.00102	0.00016
Flammulina velutipes	0.0060	0.0005	0.0005	0.00007	0.012	0.001	0.001	0.00014

TDI (mg/kg/day)	60	4.3	0.03	0.01	-	-	-	-
PTWI (mg/kg/week)	-	-	-	-	360	30	18	7

Table 3. Target Hazard Quotient (THQ) and Hazard Index (HI) for adults in edible mushroom samples

Type of Edible mushroom species	Target Hazard Quotient (THQ)				Hazard Index (HI)
	Zn	Cu	Pb	Cd	
Pleurotus ostreatus	$2.32 \text{ x } 10^{-5}$	$2.2\text{x}10^{-5}$	$1.83\text{x}10^{-4}$	$1\text{x}10^{-4}$	3.282×10^{-4}
Agaricus bisporus	$2.09\text{x}10^{-5}$	$2.41\text{x}10^{-5}$	$1.46\text{x}10^{-4}$	$7.5\text{x}10^{-5}$	2.66×10^{-4}
Lentinus edodes	$2.21\text{x}10^{-5}$	$7.38\text{x}10^{-6}$	$1.16\text{x}10^{-4}$	$6.5\text{x}10^{-5}$	2.105×10^{-4}
Flammulina velutipes	$2.003\text{x}10^{-5}$	$1.2\text{x}10^{-5}$	$1.43\text{x}10^{-4}$	$6.5\text{x}10^{-5}$	2.4003×10^{-4}
Average value	6.41×10^{-5}	6.55×10^{-5}	5.88×10^{-4}	3.05×10^{-4}	0.001

087-087

ORGANIC VERSUS CONVENTIONAL JACKFRUIT PLANTATION: INFLUENCE OF FERTILIZATION ON PLANT NUTRIENT STATUS

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ABSTRACT

Organic farming is an alternative method of food production that differs from conventional methods due to its non-use of synthetic chemicals (pesticides and fertilizers). This approach aims to prevent food contamination, soil pollution, and maintain soil fertility. Many people choose organic food, associating organic dietary models with a healthier and more environmentally friendly lifestyle. The aim of this study was to compare the impact of an organic cropping system (ORG) and a conventional one (CONV) on jackfruit plantations. Leaf nutrient analysis was conducted to measure levels of Al, B, Ca, Cd, Cu, Fe, K, Mg, Mn, Na, P, S, Zn, N, C, H, and the C/N ratio. The results showed that most micronutrients measured in both cropping systems did not differ significantly (Al, B, Ca, Cd, Cu, Fe, Na, S, Zn). However, there were notable differences for Mg and Mn. Mg levels were 31% higher in the CONV system compared to ORG, while Mn levels were 73% lower in CONV compared to ORG. K and carbon analysis showed no significant difference between CONV and ORG cropping systems. However, P and N levels, and the C/N ratio showed significant differences between the two systems. P and N contents were higher in CONV by 3.6% and 39.4%, respectively, whereas the C/N ratio was higher in ORG than in CONV. In conclusion, while the ORG cropping system did not differ significantly from CONV in terms of most micronutrients, including P and K, the differences observed in other nutrients suggest that organic jackfruit plantations may offer benefits for sustainable food production

Keywords: Leaf Analsis, Micronutrient, Macronutrient, J33, C/N Ratio.

INTRODUCTION

The use of chemical fertilizer has been a popular and widespread practice in the effort to address global food security challenge resulting from low fertile agricultural soil. The dependence on these chemical fertilizers, particularly nitrogen fertilizer has become necessary to replenish soil nutrients and invariably improve the quantity and quality of agricultural produce. By application of chemical fertilizers and the remaining percentage are lost to the environment (Norse, 2005; Mózner et al., 2012). Conversely, the use of organic manure, has been reported to improve biological, chemical and physical properties of the soil and invariably increase plant growth and yield because of its high organic matter content due to high microbial activity (Stephen et al., 2014; Mitran et al., 2017). Organic manure, as opposed to inorganic fertilizer has also been reported to increase the level of secondary metabolites like phenolic, flavonoid, and antioxidant activity in plant (Zeinab et al., 2013; Fließbach et al., 2007). For example, Wiebel et al. (2000) reported a 19% higher phenolic content for organically produced apples compared to the inorganically produced ones. Higher level of phenolic content was also recorded for organically grown strawberries compared to inorganically cultivated ones (Hakkinen and Tomonen, 2000). All these reported cases have led to growing preference by consumers for organic agricultural produce (Hughner et al., 2007; Mie, et al., 2016). All over the world, attention has now shifted towards the use of organic manures because of the perceived health benefits and higher nutritional contents believed to be derived from products from these types of farming (Worthington, 2001; Magkos et al., 2001; Tarozzi et al., 2006). This submission is supported by the studies which reported that the positive attitude of the consumers towards organic food is based on their perception of higher nutritional contents and better tasting of organic food compared to the conventional or inorganic foods (Hunter et al., 2011; Smith-Springer et al., 2012).

Recent systematic reviews and meta-analyses reported that organic production methods result in lower yields, but higher concentrations of phytochemical/antioxidants and higher antioxidant activity in crops, and this was primarily linked to a lower and/or more balanced supply of nitrogen when organic instead of mineral N-fertilisers are used

[11,12]. Jackfruit (Artocarpus heterophyllus Lam) is one of the most important minor fruit crops in tropical and sub-tropical regions. Every part of the tree and fruit is used for various purposes. The green ripe fruit is consumed as vegetable while ripe one is enjoyed as fresh fruit due to its nutritional value and delicious taste. Systematic jackfruit plantation in the country is very rare. Most of the cases it is found in homestead garden and in roadside plantation. Jackfruit is reported to be a suitable choice degraded lands in Asia and Africa. Generally, jackfruit trees are given fertilizers and thus little, is known about its organic fertilizer requirement. However, due to the complexity and variability of organic fertilizers available in the market, it is necessary to conduct a study that can serve as a recommendation to farmers producing organic jackfruit. In this experiment, the comparison between organic fertilizer and conventional fertilizer on the growth of J33 jackfruit plants were examined. We also analyse the comprehensive effects of both fertilizrs on the plant nutrient of jackfruit.

MATERIALS AND METHODS

Overview of the test area. The experiment was carried out at the Ladang Organik Bersepadu, Malaysian Agricultural Research and Development Institute (MARDI) from July 2001 to July 2022. This area with an average annual temperature of 25-28 °C, an average annual rainfall of 10.2 mm, and an average relative humidity 85%. The physical and chemical properties of the soil layer at 0–20 cm before fertilization are as follows:

Table 1. Soil nutrient content before planting.

Nutrient	pH	CEC	Total carbon (C)	Total nitrogen (N)	Available-P	Exchangeable cations		
						K	Ca	Mg
		($cmol_c$/ kg)	(%)	(mg/kg)	(mg/kg)	($cmol_c$/ kg)		
contents	5.12	5.21	1.30	0.46	7.00	0.03	1.01	0.19
Status	Low	Low	Low	Good	Low	Low	Low	Low

During the trial period, the drip irrigation quota was 135 m^3 /hm^2, and irrigation was carried through drip irrigation if there is no rain.

Planting and Treatment. We selected the 1-year-old jackfruit seedling from the nursery with a uniform tree body and good growth. We set up 3 fertilization treatment as listed below:

T1: Conventional (15:15:15) – 1kg/plant
T2: Organic 1 (Chicken manure and mushroom compost) at 2:3:3 – 300 g/plant

The experiment area was 1.3 acre, and the planting distance was 8 m x 8m. A standard agricultural practice such as fertilizer application, weed control, and irrigation were implemented accordingly.

Measurement indicators and methods. Organic fertilizer was applied every 8^{th} of the month from 2021 to 2022 starting July 2021. The data measurement was plant height, plant circumsentence and plant nutrient. Plant height was determined with tape measurement meanwhile plant diameter was measured by using vernier caliper. Plant canopy was measured by using by measuring east-west and north-south length.

Nutrient Analysis. Leaf tissue samples were wet-ashed using H_2O_2:H_2SO_4. A 0.25-g tissue sample was added to a 100 mL Kjeldahl flask. After 4 mL of H_2SO_4 were added,the flask was heated to boiling for 6 min, and then 10 mL of 30% H_2O_2 were added drop-wise with heating. The clear solution was cooled, diluted to 100 mL,and filtered. Cations were determined using inductively coupled plasmaspectrometry (model Atomscan16, Thermo Jarrell Ash, Franklin, MA) (27). Total reduced N and P were determined in the tissue digests by colorimetricprocedures (28)

Statistical Data Analysis
This experiment was designed as CRD as organic fertilizer and conventional as treatment. Each treatment consisted of 4 plants divided into 4 replications. The data obtained for each variable was analysed using Statistical Analysis Software (SAS Institute, Cary, NC, USA). Differences among treatments were determined by one way analysis of variance (ANOVA). Mean comparison were conducted using the T-test comparison test at $p < 0.05$.

RESULT AND DISCUSSION

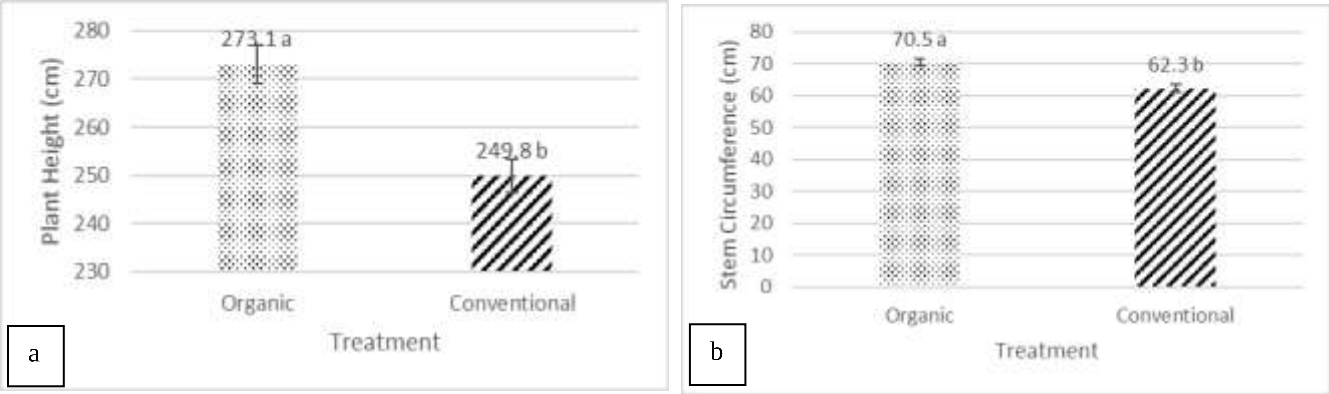

Figure 1. Effect of Organic and Conventional fertilizer on A) plant height, B) Stem circumference at vegetative stage of jackfruit.

By adopting organic and conventional fertilizer applications, a significant difference in plant height and stem circumference was observed in jackfruit at vegetative stage (Figure 1). Organic fertilizer was higher in plant height (9%) and stem circumference (13%) as compared to conventional fertilizer applications. The enhanced plant development in response to organic application is well known (Diacono, 2011), exhibiting a significant steady increase in plant growth due to improved nutrient conditions in the root rhizosphere. The nutrient content in organic fertilizer (2:3:3) probably was enough to meet the requirement of stabilizing growth as compared to excessive nutrient in conventional fertilizer (15:15:15). The quantity and quality of organic manure have tremendous effect on nutrient balance of the soil, while other abiotic and biotic factors need to be considered to maintain nutrient efficiency (Hati et al., 2008). Organic manure application improves soil physical-chemical properties by actively facilitating bacterial growth as well (Wang et al., 2016 & Weitz et al., 2001).

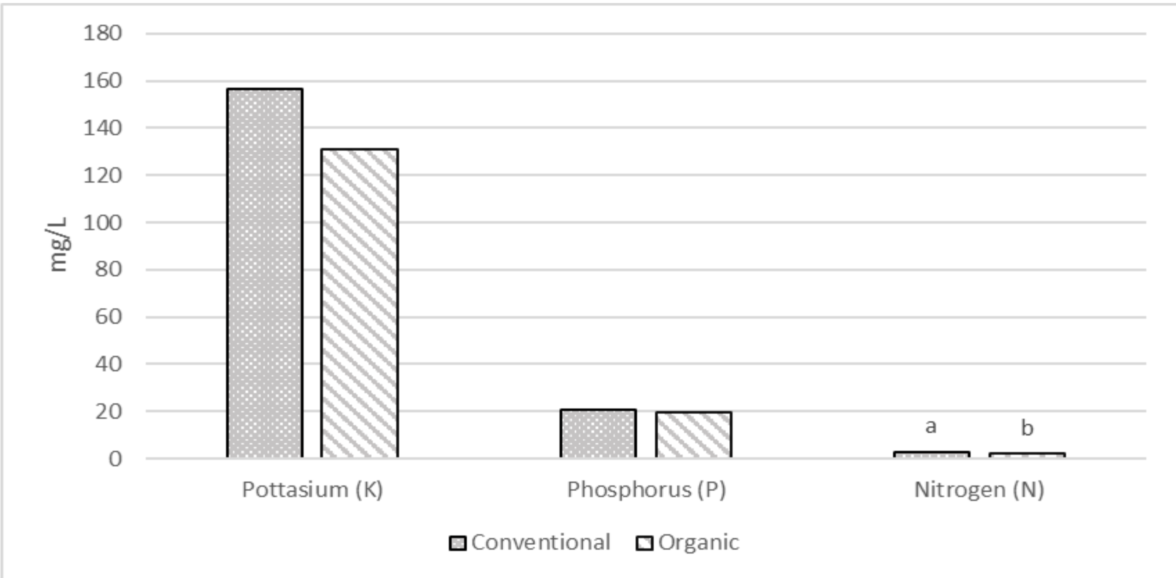

Figure 2. Effect of organic fertilizer and conventional fertilizer on Nitrogen (N), Phosphorus (P) and Pottasium (K) of leaf nutrient at vegetative stage of jackfruit. Soil pH at organic at 6.2 and conventional at 5.3.

Soil organic matter and fertilizers are crucial resources for agriculture. The soil pH in the organic plot was within the normal range, while it was slightly acidic in the conventional plot (Figure 2). Soil pH influences the plant's ability to absorb essential nutrients for growth and production. There was no significant difference between organic and conventional fertilizers regarding phosphorus and potassium levels (Figure 2). However, nitrogen levels were higher in conventional fertilizers compared to organic ones. The reliance on chemical fertilizers, particularly nitrogen, has become necessary to replenish soil nutrients and improve the quantity and quality of agricultural produce. High crop yield and plant biomass from nitrogen fertilizer application drive the increased dependence on these fertilizers (Camara et al., 2003; Guo et al., 2010).

However, intensive use of nitrogen fertilizers has been reported to cause nitrogen residue accumulation, nutrient toxicity, metal pollution, greenhouse gas emissions, groundwater contamination, and soil acidification (Han and Zhao, 2009; Sierra et al., 2015). Research indicates that increased nitrogen application leads to higher soil electrical conductivity, available nitrogen, a distorted C/N ratio, disruption of soil microbial communities, and a reduction in the nutritional quality of agricultural produce (Stuart et al., 2014; Sierra et al., 2015; Norman and Dazzo, 2016; Wei et al., 2018). It is established that crops only absorb 30-50% of the applied nitrogen.

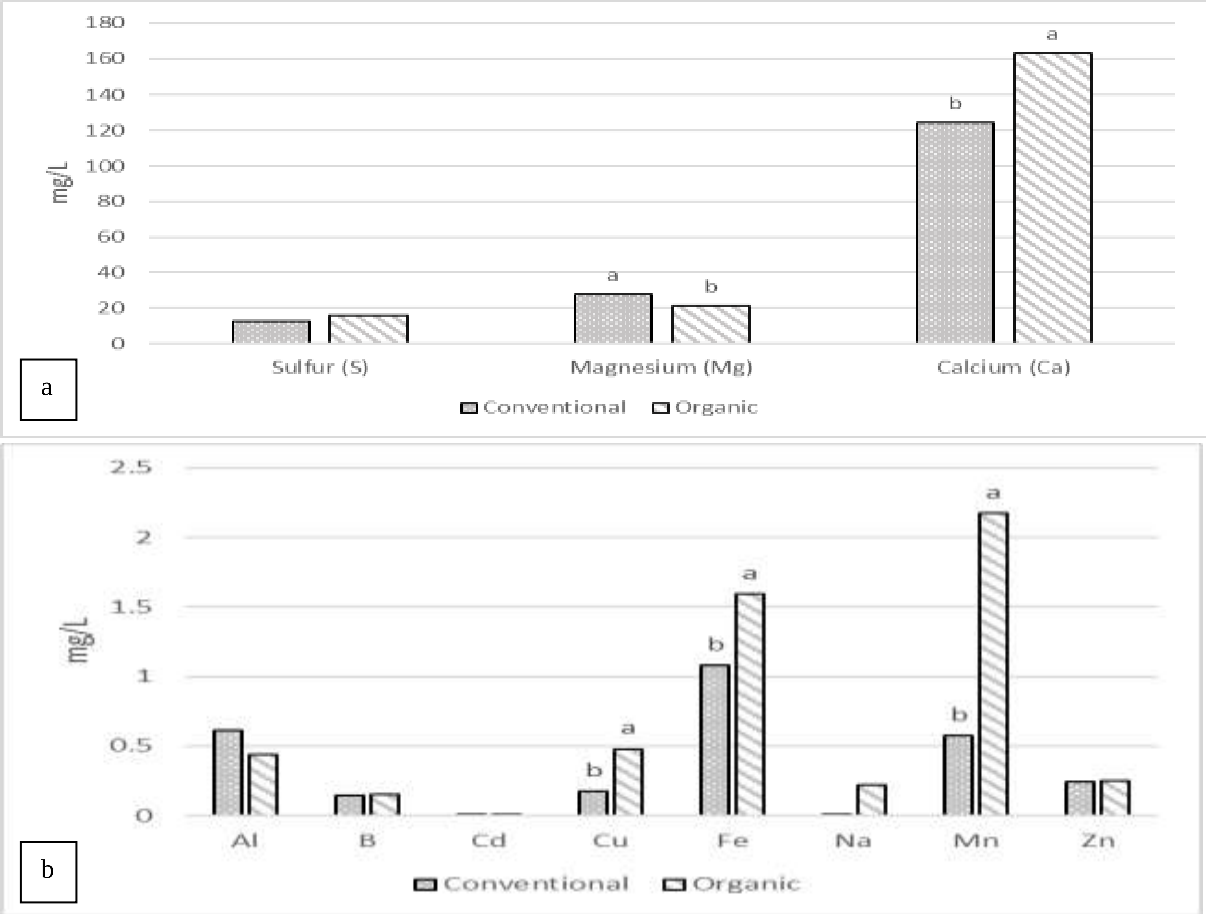

Figure 3. Effect of organic fertilizer and conventional fertilizer on **(a)** Sulfur (S), Magensium (Mg) and **(b)** Calcium (Ca), Aluminium (Al), Boron (B), Calcium (Ca), Cadmium (Cd), Copper (Cu), Ferum (Fe), Natrium (Na) and Zinc (Zn) of leaf nutrient at vegetative stage of jackfruit. Soil pH at organic at 6.2 and conventional at 5.3.

As shown in Figure 3 and Figure 4, there is no significant difference between organic and conventional fertilizers for aluminum (Al), boron (B), cadmium (Cd), sodium (Na), sulfur (S), and zinc (Zn). However, there are significant differences for magnesium (Mg), calcium (Ca), copper (Cu), iron (Fe), and manganese (Mn). Mg levels in organic fertilizer are 23% lower than in conventional fertilizer. In contrast, Ca, Cu, Fe, and Mn levels are higher in organic fertilizer compared to conventional fertilizer. This experiment utilized chicken manure obtained from waste disposal and spent mushroom substrate (SMS) left after harvesting mushrooms, which are widely recommended for beneficial recycling in organic fertilizer. This practice helps establish natural nutrient and organic carbon cycles, promoting long-term sustainability (Grimm & Wosten, 2018; Ksheem, 2014). Organic materials hold significant value as components of composts (Polat et al., 2009). Additionally, due to their alkaline nature, these materials can help reduce soil acidity (Malinska et al., 2018; Dikinya, 2010) and improve nutrient availability (Ukalska et al., 2020). Part of these yield responses can be explained by differences in the amount of optimum nutrients and the combination of soil physical and biological inputs received by the two systems.

CONCLUSION

With the rapid growth of Malaysia's industrial economy and the accelerated construction of energy and chemical bases, there is a need to further optimize the layout, production structure, and product structure of the agricultural industry. Utilizing the vast reserves of farmyard manure and farm waste can contribute to the development of characteristic organic agriculture, offering an effective means to increase farmers' income. The current research reports represent the initial step in exploring the effects of applying organic fertilizers on the plant growth of jackfruit during the vegetative stage. This research can provide a theoretical basis and technical support for the production of organic jackfruit. However, further studies are necessary to determine the appropriate application rate of organic fertilizer, assess the comprehensive effects of organic fertilizer application, explore the cumulative effects of long-term organic fertilizer use, and understand its impact on soil fertility.

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088-088

REFORMULATION OF COOKIE STICKS USING EDAMAME POD SHELL POWDER AS FUNCTIONAL INGREDIENT

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ABSTRACT

Most cookies in the market are high in calories and low in dietary fiber. Edamame (*Glycine max)* pod shells are rich in dietary fiber and health-beneficial bioactive compounds but are often discarded as waste. Hence, this project aimed to enhance the fiber content of cookie sticks using edamame pod shell powder (EPP) as a functional ingredient. The sample cookie sticks with 15% EPP (EPP-sample) and the control without EPP were prepared. The proximate composition, physicochemical properties and sensory quality of the cookie sticks were determined. EPP-sample contained significantly higher levels of crude fiber (7.54%) than that of the control (0.53%); but contained significantly lower carbohydrates (43.29%) than that of the control (54.75%), with almost 10% reduction in total calories. The EPP-sample also exhibited relatively high DPPH radical scavenging activity (42.44%), TPC (42.03 mg GAE/100g), TFC (26.99 mg QE/100g) compared to the control (DPPH radical scavenging activity 38.02%, TPC 37.50 mg GAE/100g, TFC 18.00 mg QE/100g). Sensory results showed that there was no significant difference between EPP-sample and the control in terms of their appearance, color, aroma, taste and texture, although more panellists preferred the control. The acceptance index of EPP-sample was 76%, indicating panellists' satisfaction towards the product. In conclusion, edamame pod shell powder could be used as a functional ingredient to reformulate cookie sticks for healthier products. This project aligns with the sustainable development goals, SDG 2 - promoting good health and SDG 12 – responsible consumption and production.

Keywords: Edamame Pod Shell, Fiber, Antioxidant, Sensory Quality.

FORESTRY

014-013

MANGROVE RESPONSE TO CLIMATE CHANGE: MACHINE LEARNING-BASED LONG-TERM MAPPING TO DETECT MANGROVE CHANGES IN MATANG MANGROVE FOREST RESERVE (MMFR), MALAYSIA

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ABSTRACT

Mangrove ecosystems, important for biodiversity, coastal protection, carbon sequestration, and supporting fisheries and livelihoods, also hold cultural and recreational importance, emphasizing the critical need for their conservation. This study investigates the response of mangrove ecosystems to climate change through the application of machine learning-based long-term mapping techniques in the Matang Mangrove Forest Reserve (MMFR) in Malaysia. Using Landsat time-series data, this study aims to analyze mangrove changes over the period of 40 years (1983-2023) in the MMFR through the Random Forest algorithm. Furthermore, the sea level rise data was added to understand the relationship between mangrove change and climate change. Analysis of remote sensing data revealed significant spatial and temporal patterns of mangrove extent and health changes over the study period. The correlation analysis between mangrove changes and sea level rise revealed a significant relationship, highlighting the impact of climate variability on mangrove ecosystems in the MMFR. The results illustrate varying levels of mangrove loss, gain and stability across different MMFR regions, with certain regions showing higher changes than others. Furthermore, temporal analysis reveals fluctuations in the level and health of mangroves over time, showing the dynamic response of these ecosystems to environmental drivers. These findings are expected to deepen our understanding of the effects of climate change on mangrove ecosystems and inform targeted conservation and management efforts.

Keywords*:* Machine Learning-Based Long-Term Mapping, Mangrove Ecosystems, Matang Mangrove Forest Reserve (MMFR), Random Forest Algorithm, Remote Sensing.

034-035

EXPLORING TREATMENT CYCLE EFFECTS ON CELLULOSE NANOFIBRILS FROM *GIGANTOCHLOA SCORTECHINII* (BULUH SEMANTAN) FIBER

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ABSTRACT

This research investigated the impact of treatments involving sodium hydroxide and benzoylation on the extraction of cellulose nanocrystals from Semantan bamboo (Gigantochloa scortechinii). The study demonstrated the effectiveness of isolation methods and chemical treatments in removing non-cellulosic constituents and obtaining bamboo cellulose nanocrystals (BCNs) through acid hydrolysis. X-ray diffraction analysis revealed that benzoylated BCN (B) had a crystalline cellulose II structure, while NaOH-treated BCN (S) had a crystalline cellulose I structure. BCN (S) exhibited a higher crystallinity index (80.55%) than BCN (B) (67.87%). BCN (B) had a higher yield of 23.68 ± 1.10%, compared to BCN (S) at 20.65 ± 2.21%. Transmission electron microscopy images showed that BCN (S) had a mean diameter of 7.95 ± 2.79 nm, while BCN (B) had a mean diameter of 9.22 ± 3.38 nm. Thermogravimetric analysis indicated that BCN (B) had lower thermal stability than BCN (S), with charcoal residues at 600°C of 31.06% and 22%, respectively. The zeta potential values were -41.60 ± 1.97 mV for BCN (S) and -21.80 ± 2.54 mV for BCN (B). These findings highlight the potential of Gigantochloa scortechinii for eco-friendly and sustainable applications in the construction, furniture, and renewable energy industries. BCNs derived from Gigantochloa scortechinii are versatile and hold promise for multiple applications.

Keywords: Bamboo, Cellulose Functionalization, Nanocellulose, Bioplastic, Reinforcement.

039-037

QUALITY OF ACACIA SAWN TIMBER FROM ACCELERATED KILN DRYING

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ABSTRACT

Generally, acacia timber species are prone to drying defects due to their inherent characteristics. The prolong processing time for acacia timber is associated directly to the drying issues of the timber. In this study, a dedicated drying regime was established for the acacia species, to ensure that proper treatment can be applied to minimise the occurrence of various drying defects in an efficient manner, whilst improving the quality kiln throughput of the timber, mainly cater for export market. Freshly sawn acacia lumber was dried from green condition in a 100 m^3 capacity accelerated drying system. Result showed that the drying time of acacia timber has been reduced down to about 2 weeks compared to the conventional processing time of 6-8 weeks for 30-mm thick timber. The moisture content (MC) dispersion within individual sawn timber and between sample boards after drying, ranged from 0.15 to 1.67% and were within general permissible range of less than 2% MC difference. In general, a uniformly dried sawn timber, with small MC variations along the length and across the thickness, is less stress and dimensionally more stable during service.

Keywords: Sawn Timber, Moisture Content, Sawn Dimension, Accelerated Drying

INTRODUCTION

Generally, *Acacia mangium* tress are renowned for their robustness and adaptability, which makes them good plantation species (Lim et al. 2003). The density of *A. mangium* ranges from 290 kg m^{-3} to 675 kg m^{-3}. *Acacia spp* is fast emerging as an important timber resource in the South East Asia region. It is a fast growing plantation species and is one of the major species in timber plantation sector in Sarawak and Sabah, covering more than 300,000 ha of planted acacia trees in both states.

The challenge to produce quality kiln dried acacia sawn timber on a consistent basis has always been one of the major concerns of the local acacia timber producer (Ramzul Iklas & Sik 2019). Acacia timber species are prone to drying defects due to their inherent characteristics. Wood defects such as checks, cup and crook (warp along the length of the edge of lumber) in Acacia board increases after drying, and it is higher than other plantation species. Besides, acacia lumber had high incidence of collapse and splits, further compounded by the occurrence of moisture/ wet-pockets in the lumber.

According to Tenorino and Moya (2011), one of the main factors influencing variability in final moisture content of *A. mangium* is the formation of wet pockets during drying, which tend to form in lumber from trees growing in very humid, tropical climates. Piao et al. (2000) also reported the drying problems related to *A. mangium*. Whereas, Haslett (1983) identified that the extreme variability of drying rate between the heartwood of a quartersawn compared to flat-sawn as the major problem encountered in the drying of *Acacia melanoxylon* R. Br.

The conventional steam drying method is conducted according to wood moisture content based recipe, which are derived from the moisture content (MC) changes of the timber during drying process. In previous studies, a moisture content based recipe was employed in drying of *Acacia mangium* sawn timber using a conventional steam-heated kiln, with the initial dry bulb temperature (DBT) limited to 50°C or lower and final DBT to be maintained below 70°C (Sik et al. 2018).

In recent years, drying timber at high temperatures and employing heat treatment has been regarded as having a competitive edge in drying efficiency and improved properties of heat-treated wood. Heat treatment was conducted on *Acacia mangium* wood by means of drying and heating with high temperature in air to improve the dimensional stability of the wood (Tran 2013). The results showed that the dimensional stability and the water-repellent effectiveness are increased by about 15-46% and 8-18%, respectively. Whereas, Gan et al. (2015) reported that hot water bath and microwave pretreatments with appropriate settings after air drying could expedite drying process and alleviate the occurrence of wet pockets in *A. mangium.*

Prolong processing time for acacia timber is associated directly to the drying issues of the timber. Consequently, the extended kiln resident time required for acacia timber has become inevitable in current drying mill practice, and is a detrimental factor for better kiln turnaround time and as well as the drying energy efficiency. To address these issues, a dedicated drying regime has been developed for this particular timber species, to ensure that proper treatment can be applied to minimise the occurrence of various drying defects in an efficient manner (Sik et al. 2019). The research further assessed the quality of the kiln dried acacia dimension stocks produced in a commercial kiln dryer using the optimised accelerated drying-cum-treatment protocol.

MATERIALS AND METHODS

Sample Preparation

The commercial scale drying trial was carried out in a timber processing plant located in the Kemena Light Industry Area in Bintulu, Sarawak, Malaysia. *Acacia mangium* logs of 2.3 m in length and 6-13 cm in diameter were converted into 30mm-thick dimension sawn of 100mm in width. The timber charge for the drying trial were made up of 14 stacks of sawn *A. mangium* with each box-stacked bundles measuring approximately 3m in height and 1.5m in width. The sawn timber in each bundles were stickered in layers with 25mm-thick wooden stickers properly aligned at a distance of 45cm apart.

Drying Method

In this drying study, timber charge consists of 100m^3 of boxed-stack sawn timber was dried in a high performance drying system with a heating capacity of up to 200ºC. The optimised accelerated drying-cum-heat treatment schedule conducted for this commercial scale drying study was based on relative humidity control with an initial dry bulb temperature (DBT) of 75°C and temperature reduction of not more than 5°C in all stages of drying (Table 1). Two (2) pairs of high heat resistant relative humidity (RH) sensors were placed inside the kiln throughout the drying process. A total of fifteen (15) sample boards were selected and used as control for the drying trial. Each of the sample boards were inserted with thermal couple wire at the core section of the board, as temperature sensors to obtain real-time temperature reading of the sample boards at set time interval during drying. The sample boards were placed in each of the timber stacks at different height, and in various stack-compartments in the kiln.

Table 1 Drying-cum-treatment schedule based on relative humidity (%) control

Relative Humidity Control (%)	Dry Bulb Temperature Setting (℃)	Corresponding Ambient Environment: Equilibrium Moisture Content, EMC (%)
95	75	11
80	75	10-11
70	73	9
60	70	8
55	68	7-8
50	66	7
45	64	6
35	62	5
20	60	<5
95	110	<5

Moisture Content Determination

Prior to drying, initial moisture content (MC) values of 15 pieces of randomly selected sample boards were determined by obtaining a 25mm-broad strips (MC.avg=A1 & B1; MC.sec=A2 & B2) at a distance of 50mm from both end of each sample boards. To obtain sectional moisture content (MC.sec), specimen A2 and B2 are further sliced into shell and core sections (Figure 1). The sample boards were weighed before commencement of drying and at the end of drying. After completion of the drying run, average MC of the dried sample boards were again determined. In addition, MC variation within individual sample board was determined by slicing another 25-mm-wide strip from each of the boards into shell and core sections. The moisture content of the timber was determined based on standard oven drying method and calculated based on the formula (1):

$$\text{Moisture content (\%)} = \frac{\text{Initial weight (g)} - \text{Oven} - \text{dry weight (g)}}{\text{Oven} - \text{dry weight (g)}} \text{ x } 100 \qquad (1)$$

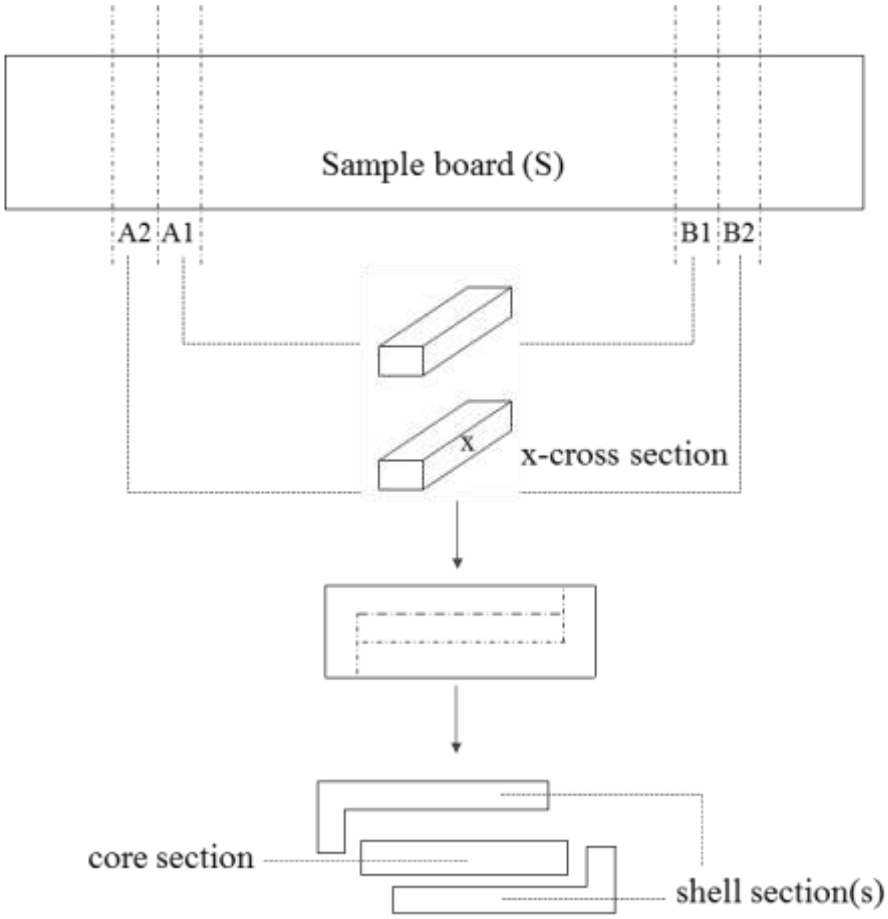


Figure 1 Specimen preparation for average moisture content (MC.avg) and sectional moisture content (MC.sec) for sample board (S): S1-S15

RESULTS AND DISCUSSION

Drying Time

The total kiln resident time including cooling down period for drying-cum-treatment up to 110°C for a kiln charge of 100m^3 acacia sawn lumber was about 15 days. Using the established accelerated drying system, the drying time of acacia timber has been reduced down to 2 weeks compared to the conventional processing time of about 8 weeks for a 30-mm thick sawn timber.

In this study, the acacia timber charge was kiln dried from green condition until the charge reached the targeted moisture content of 7-9%. Haslett (1983) reported that air drying of 25-mm *Acacia melanoxylon* was achieved in 12-20 weeks in Rotorua with final kiln drying taking 4-5 days. In addition, accelerated drying methods could increase the moisture content (MC) differences in timber and recommended that all material, particularly 50 mm and thicker, be air dried to about 30% moisture content before kiln drying.

Contrarily to other common conventional drying regime for acacia timber, the acacia timber for this drying trial has not undergone any air drying prior to drying in a steam-heated kiln. This study showed that by employing proper drying regime suitable to the inherent characteristic of acacia, the air dying step can be exempted and thus, saving processing time and, subsequently the overall production cost.

Physical properties

Average moisture content

The moisture content (MC) of *Acacia mangium* was determined at green condition and after drying. The green MC of *A. mangium* ranged from 62.49 to 108.73%, with a mean of 85.36% (Figure 2). In previous studies, Sik et al. (2018) also reported high initial MC variations in *Acacia mangium* which ranged from 48.89 to 138.22% in another industrial drying trial conducted in Sabah, Malaysia.

Table 2 showed the moisture content variations within the respective sample board before and after drying. The moisture content difference is determined from both ends of the sample board, and it reflects the extent of the occurrence of uneven moisture content along the length of the sawn timber. The initial MC difference within the individual sample board of the same kiln charge, ranged from 0.91% to 31.09% (Table 2). It has been reported that significant MC variation was found along the length of green Acacia wood. Generally, high MC variations between the sawn wood in a kiln charge and within the individual sawn is regarded as a challenge in drying in all wood species especially defects prone species such as acacia wood.

The MC of *A. mangium* after drying ranged from 6.90 to 9.13%, with a mean of 7.77% (Figure 3). After drying, the moisture content (MC) dispersion within individual sawn timber and between sample boards after drying, ranged from 0.15 to 1.67% and were within general permissible range of less than 2% MC difference (Table 2). This is an important indicator that the smaller MC differences within an individual kiln dried sawn and between different pieces of kiln dried sawn especially in the making panel products, will render the products more dimensionally stable during service. Cai and Hayashi (2007) mentioned that lack of MC uniformity has a significant impact on the manufacturing process and will affect the quality of products manufactured.

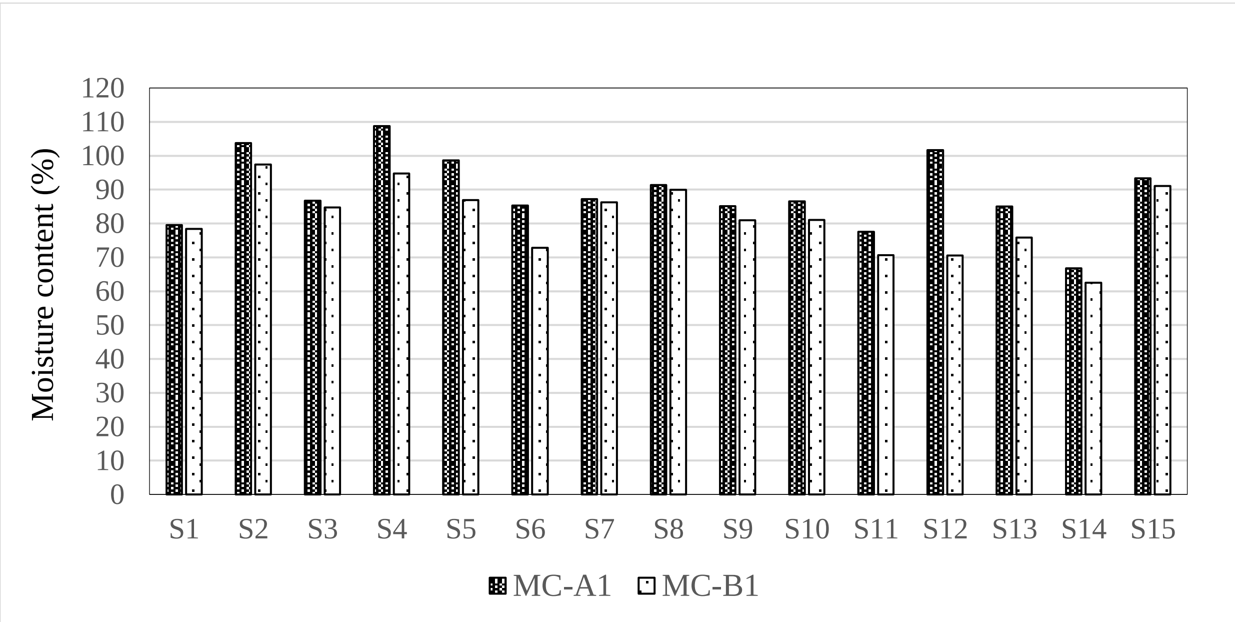


Figure 2 Initial moisture content variation between sample boards in a kiln charge

Table 2 Average MC (%) difference within the same sample board

Sample board	Moisture content difference (%)	
	Before drying	After drying
S1	1.22	0.17
S2	6.32	0.52
S3	2.00	1.06
S4	14.01	0.22
S5	11.66	0.15
S6	12.47	1.67
S7	0.91	1.27
S8	1.45	0.12
S9	4.17	0.63
S10	5.50	0.66
S11	6.90	0.22
S12	31.09	0.58
S13	9.25	1.29
S14	4.28	1.09
S15	2.26	0.27

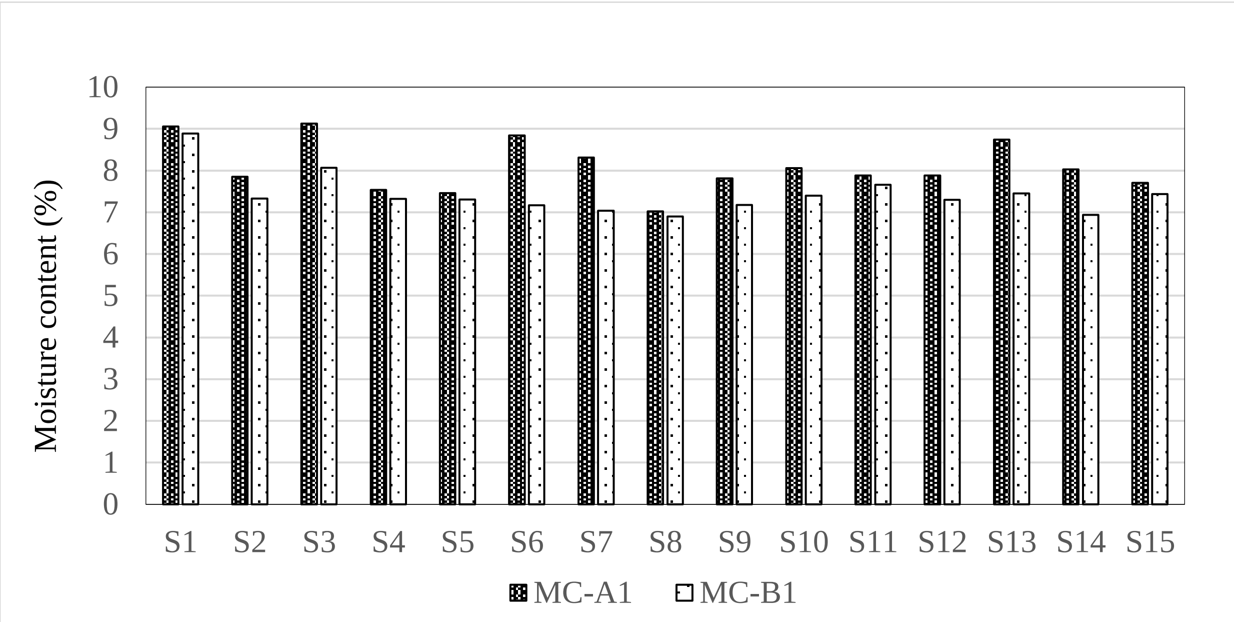


Figure 3 Moisture Content variation between sample boards in a kiln charge (after drying)

Sectional moisture content

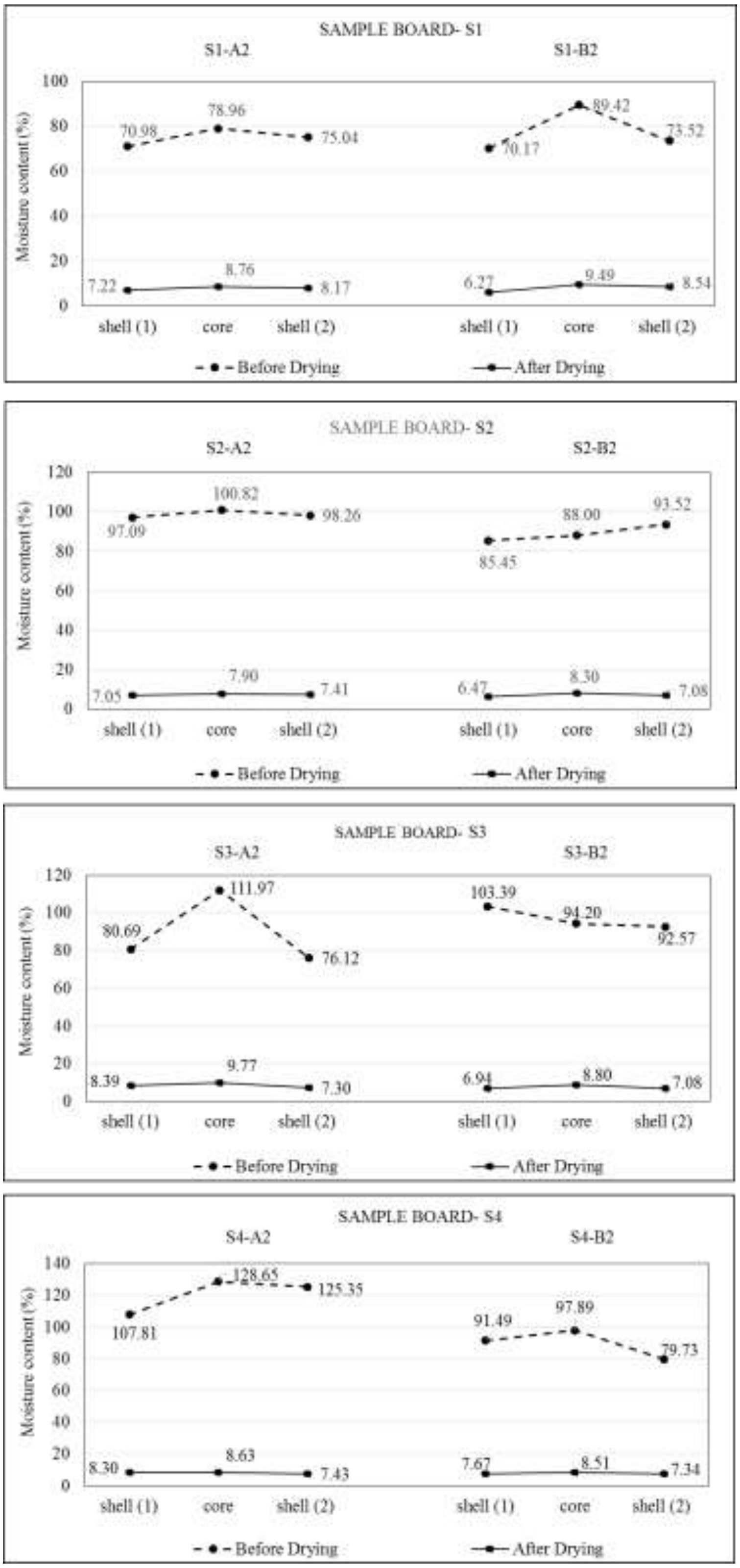
The variation of moisture content (MC) within individual dried *A. mangium* sample was determined in outer layers and inner layers (Figure 1) separately in order to check for presence of wet pockets.

It is important for dried sawn timber to have as minimum moisture gradient as possible, usually in the general permissible range of 2% MC. According to Tan (2014), high moisture content variation after drying of 7-year, 10-

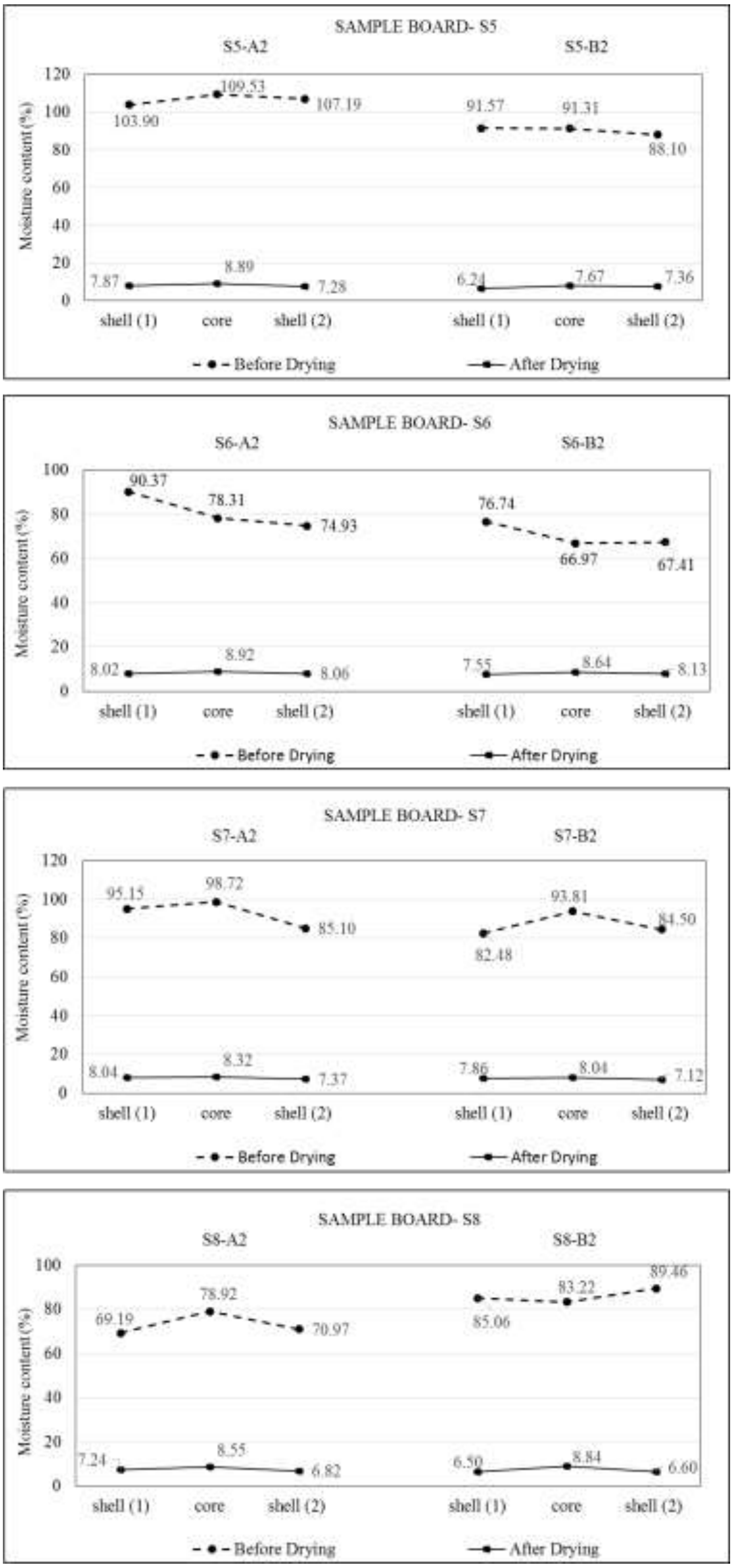
year and 13-year old *A. mangium*, whereby moisture gradient of more than 6% between the shell and core layers of *A. mangium* was found in 60%, 40% and 27% respectively in the order of these age-group.

Figure 4 illustrated the moisture content gradient (shell and core regions) within sample boards (S1-S15) before and after drying. Based on the total number of sample boards randomly selected for controls in the study, about 80% of *A. mangium* samples were able to dry uniformly throughout the timber and the variation of MC between the inner and outer layers was within 2.0% after conditioning treatment, which was carried out before the end of drying. Whereas, the remaining samples, albeit higher variation between the inner and outer layers, the MC difference between these samples recorded were below 3%. This is important as effective moisture movement from the core toward the surface during drying is crucial to minimise or prevent the occurrence of severe casehardening. A common problem faced when drying timber is casehardening. Case hardening occurs when timber has been dried too rapidly. Case-hardened timber may warp considerably and dangerously when the stress is released e.g. during cutting.

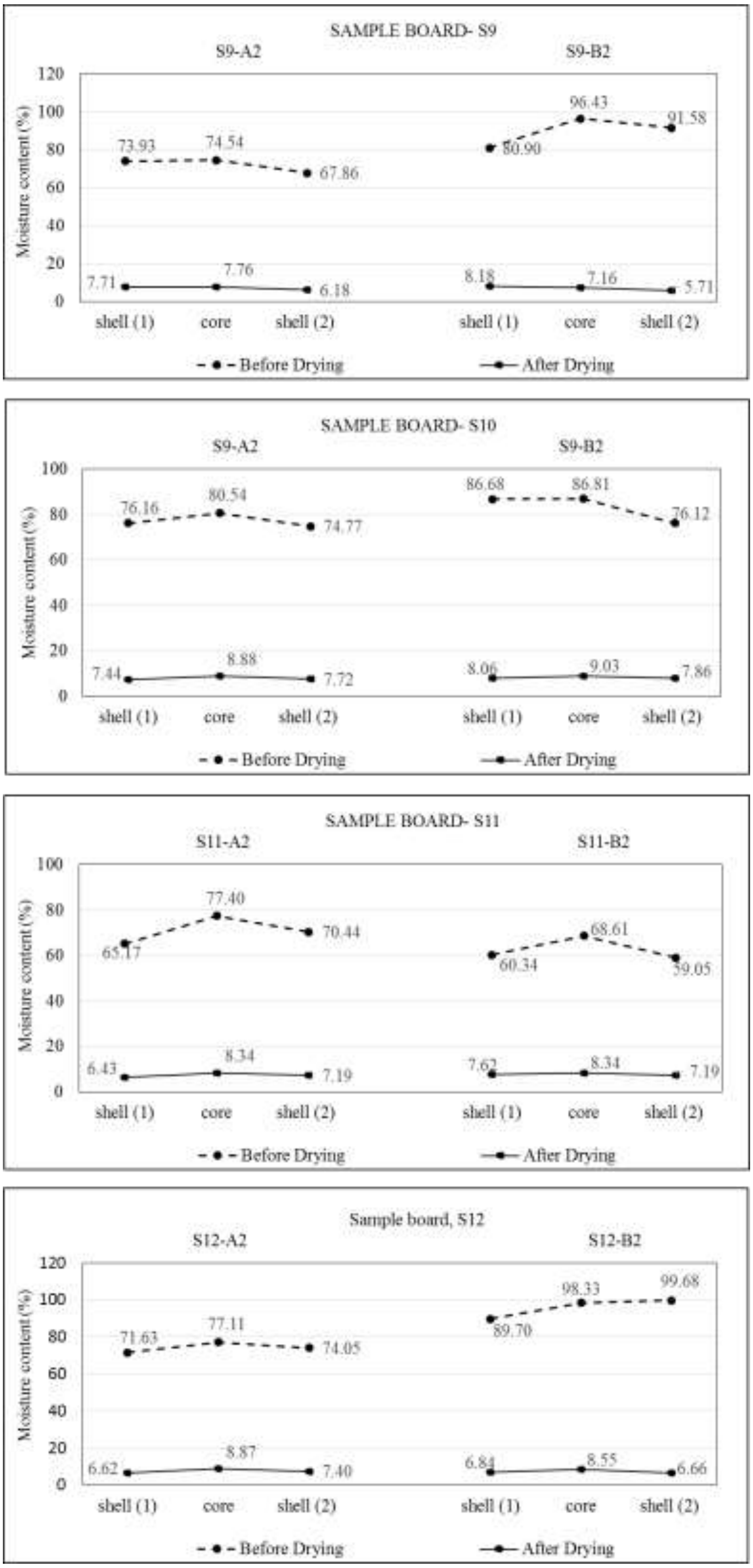
Relatively higher initial moisture content in shell layer compared to its core was detected in several sample boards; S2, S3, S6 and S8 (Figure 4). The moisture gradient between the outer and inner layers was more than 5% MC and this could indicate the presence of random moisture pockets in fresh sawn timber. Nonetheless, the moisture gradient of these sample boards was reduced down to less than 2.5% with slightly higher MC at the core regions. In general, a uniformly dried sawn timber, with small MC variations along the length and across the thickness, is less stress and dimensionally more stable during service.



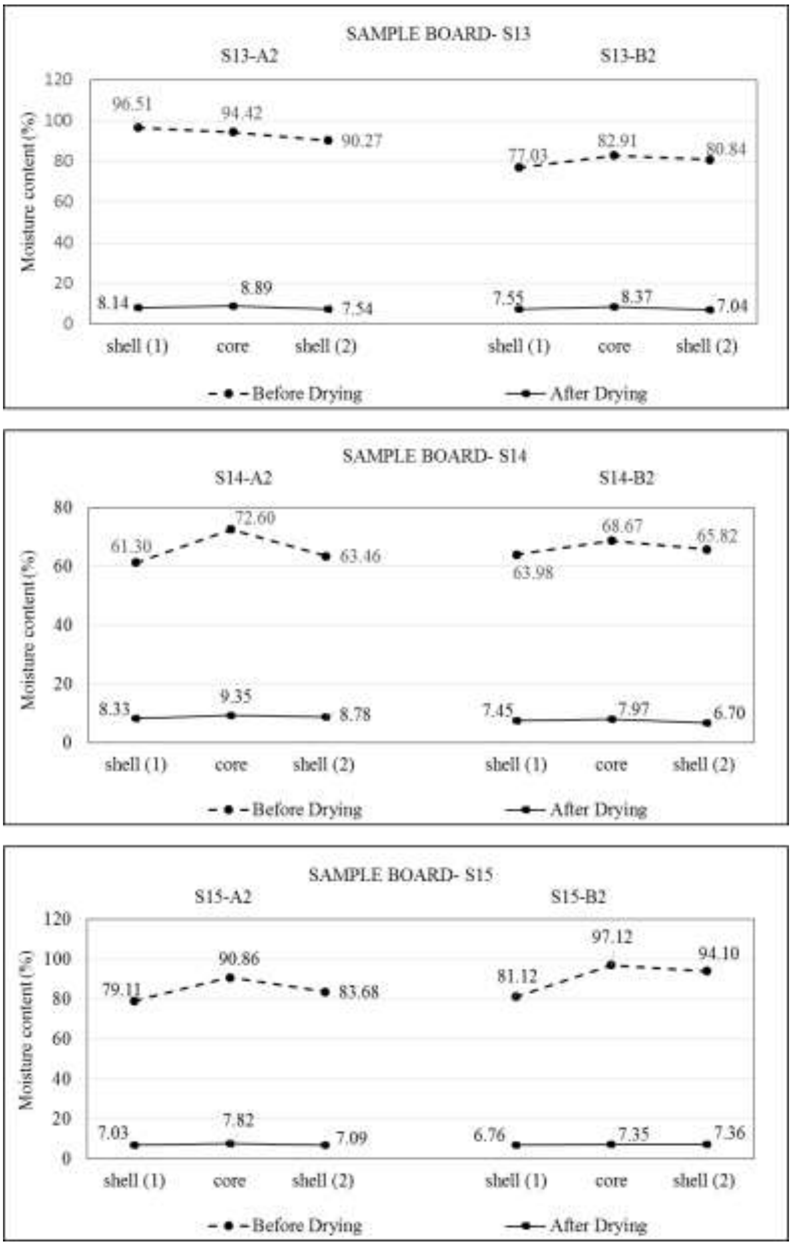
(a) Sample board S1-S4



(b) Sample board S5-S8



(c) Sample board S9-S12



(d) Sample board S13-S15

Figure 4 Moisture Content gradient (shell and core regions) within sample boards (S): S1-S15 (figure: a-d) before and after drying

CONCLUSIONS

This accelerated drying method is optimised specifically for lumber with drying issues such as acacia lumber with inherently presence of moisture wet pockets. An optimised drying-cum-treatment protocol to accelerate the drying of acacia timber was established, whilst improving the quality kiln throughput of the timber. Overall, the method of this invention has significantly reduced the drying period, minimised various drying defects, as well as improving the acacia timber properties. The dried acacia sawn timber produced in this study is suitable to be processed into semi-finished products such as manufacturing of truck flooring and other quality wooden joineries.

ACKNOWLEDGMENTS

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042-044

WATER STRESS, GROWTH, AND IOT APPLICATION IN NURSERY MANAGEMENT

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ABSTRACT

Nursery management is important in any agriculture, forestry or plantaon programme. However, it faces challenges especially in areas experiencing water stress. Fast growing species that typically characterised with high water demand such as Eucalyptus and Bamboo may be affected by the water availability, where both insufficient and excessive watering may influence plant health and overall performance. Knowledge on the plant interacons and growth performance according to different water availability can provide valuable insights in opmizing nursery operaons, predicng water requirements, and migang environmental risks in any plant culvaon program. Furthermore, in managing nurseries, water conservaon is an important economic factor to be considered as water usage can lead to water wastage and thus influence the operaonal costs. Inefficiencies in the usage of water resources highlight the need for advanced technologies for monitoring and management of the nursery. Using technology such as automaon and Internet of Things (IoT) in nursery management will beter assist the producon of good quality planng materials to address water stress issues and foster sustainable growth monitoring. This research explores the plant watering consideraons in plant growth performance, and the potenal of IoT technology applicaon in nursery management to gain beter control over seedling producon while maintaining high crop quality.

053-051

CO-OCCURRENCE NETWORK OF ARTHROPODS IN RIPARIAN HABITATS SIGNAL CONSERVATION NEED FOR PTEROPTYX FIREFLY (COLEOPTERA: LAMPYRIDAE) IN PENINSULAR MALAYSIA

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ABSTRACT

Riparian habitats are in imminent threat from anthropogenic impacts due to various land-uses. Of these impacts, the significant decline in riparian firefly populations is of major concern. While quantitative data on firefly population trends are often used in monitoring programs, the biotic integrity of each riparian habitat at a local scale remains unknown. We used the cooccurrence network approach and the traditional richness and compositional analyses to quantify the ecological stress represented by land use activities on the arthropod communities on firefly display trees in three firefly sanctuaries in Peninsular Malaysia: Sepetang River, Rembau River and Chukai River. Using 68 arthropod families consisting of 41,048 individuals, we generated three co-occurrence networks for each firefly sanctuary. In contrast to Sepetang River and Rembau River, Chukai River is characterized with smaller network size, lower interaction diversity and co-occurrence connectance with higher modularity co-occurrence network. All of these network attributes correspond to high land use intensification in Chukai River. These observations imply Chukai River as the most vulnerable firefly sanctuary, with lower biotic integrity. Our study showed the novelty and reliability of co-occurrence networks in the assessment of ecological stress upon arthropods in firefly riparian habitat for an informed conservation decision to be made.

Keywords: Lampyridae, Pairwise Co-Occurrence, Species Interaction, Habitat Disturbance.

060-058

ANTIOXIDANT STATUS BETWEEN SIRIH 'BERTEMU URAT' AND 'TIDAK BERTEMU URAT'

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ABSTRACT

In Malay tradition, "sirih bertemu urat" (converging veins) and "sirih tidak bertemu urat" (nonconverging veins) are terms used for betel leaves on the same plants but with different pattern. Morphologically, "bertemu urat" leaves exhibit the left and right veins (nerves lateral) meet at the central vein, while non-"bertemu urat" leaves lack this characteristic. Despite limited scientific evidence supporting their efficacy, Malay Traditional Medicine favors the use of *P. betle* "bertemu urat" leaves for medicinal purposes. Therefore, this study aims to investigate the antioxidant status of extracts from both "bertemu urat" and non-"bertemu urat" betel leaves by analyzing phenolic compounds and antioxidant activity. The leaves were subjected to a maceration process in an incubator shaker using 80% methanol to obtain the extract. The total phenolic content was measured using the Folin-Ciocalteu method, and antioxidant activity was evaluated through DPPH and FRAP assays. Statistical analysis revealed significant differences ($p < 0.05$) in the phenolic content between *P. betel* "bertemu urat" and *P. betel* non-"bertemu urat," indicating that *P. betel* "bertemu urat" has a higher phenolic content compared to *P. betel* non-"bertemu urat.". The results obtained from the DPPH and FRAP assays suggested that *P. betel* “bertemu urat" may have higher antioxidant activity compared to non-"bertemu urat". However, it is important to note that the results of this study could not be confirmed due to errors and limitations in the methodology. Nonetheless, it highlights how variations in leaf morphology within the same plant species can influence antioxidant properties. Considering these variations can assist researchers and practitioners in making informed decisions when selecting betel leaves with optimal antioxidant potential.

Keywords: *P.betle*, “Bertemu Urat”, Leaf Venation Patterns, Antioxidant Status.

069-074

APPLICATION OF GENOME-WIDE SNP MARKERS IN EVALUATING POPULATION STRUCTURE OF A NEAR THREATENED DIPTEROCARP, *RUBROSHOREA LEPROSULA* (MERANTI TEMBAGA)

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ABSTRACT

Rubroshorea leprosula is a well distributed species in Southeast Asia, mainly in Malay Peninsula, Borneo and Sumatra. However, this species is experiencing population decline due to over-exploitation of its valuable timber. This study aims to evaluate population structure of *R. leprosula* using high density single nucleotide polymorphism (SNP) markers obtained via whole genome resequencing. A total of 194 accessions of *R. leprosula* from 37 natural populations have been sampled and re-sequenced based on *R. leprosula* draft genome using Illumina Hiseq dan Novaseq platform. Initially, about 88,404,490 SNPs were obtained from the raw variant calling file (VCF). After a hard filtering process (genotype quality < 20, coverage depth < 5, minor allele frequency (MAF) < 0.01 missing rates < 20%, monomorphic and multiallelic SNPs), a set of 642,543 biallelic SNPs were retained for subsequent analysis. In order to avoid excessive linkage on population analysis, these SNPs were LD-pruned using the indep-pairwise option in PLINK software. Finally, a total of 373,367 LD-pruned SNPs with LD (r^2) ≤ 0.5 were employed to assess the population structure. ADMIXTURE analysis had classified the accessions into two distinct genetic clusters (K=2) of which 151 accessions (Malay Peninsula and Sumatra) were in Cluster A and the rest of the accessions from Borneo were in Cluster B. Interestingly, about six accessions from Malay Peninsula (Perak, Kelantan and Terengganu) were observed to exhibit genotype admixture with those in West Borneo, which might be related to Last Glacial Maximum (LGM) period affected Sundaland millions years ago. Furthermore, principal component analysis (PCA) and maximum likehood (ML) tree also confirmed the findings identified by the ADMIXTURE analysis. Overall, these genome-wide SNP markers revealed a clear genetic differentiation between populations in Borneo and those in the Malay Peninsula/Sumatra. This important finding provides new insight on the population structure of *R. leprosula* which will help in conservation strategy and management of the species.
(309 words)

Keywords: Admixture, Conservation, Linkage Disequilibrium, Sundaland, Whole Genome Resequencing.

073-081

ASSESSMENT OF THE PHYSICAL QUALITY OF *EUCALYPTUS PELLITA* SEEDLINGS

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ABSTRACT

Eucalyptus pellita is a popular species of plantation tree in many nations and regions because of its fast growth and excellent timber qualities. Moreover, *Eucalyptus* leaves are known as forest harvesting waste with the potential to generate essential oils. *Eucalyptus* is one of the plants utilized in the pulp and paper industry. This study aims to investigate the impact of two parameters which is types of fertilizer and polybags (black polybags and transparent polybags) on *Eucalyptus* growth performance in nursery. The present investigation was carried out at Main Nursery, Forestry Research Institute Malaysia under agro-climatic and irrigation conditions of the nursery. Twenty seedlings were prepared for this study consists of two treatments of eco-friendly soil conditioner and NPK (ratio of NPK 8:8:8). Survival and height measurements were collected accordingly. Seedlings without any treatment showed better growth than treatment with soil conditioner or NPK. Seedlings as in C1 shows consistently fastest growth compared to T1 (B) and T2 (SC), and the mortality rates were 0%, 15% and 5% respectively. The results demonstrated that fertilizer and soil conditioner applied at younger age of seedlings give less effect on growth performance.

Keywords: *Eucalyptus Pellita*, Potting Media, High Quality Planting Materials.

INTRODUCTION

In the past 200 years, plants have emerged as a significant source of innovative pharmacological chemicals. Throughout human history, natural plant products have formed the basis of phytomedicine. Seeking for biologically active chemicals, numerous researchers have turned to the kingdom of plants for assistance. Eucalyptus, a genus that can be found in Papua New Guinea, Australia, and Sulawesi Island, belongs to the Myrtaceae family which is well-known for producing aromatic compounds. Many studies on Eucalyptus bioactivities have demonstrated its effectiveness as an analgesic, antifungal, anti-inflammatory, bone resorption inhibitor, and natural repellent (Yelfi et al. 2022).

There are over 900 species in the genus Eucalyptus, of which over 300 have leaves that are known to produce essential oil, and roughly 20 of those species have been shown to possess a significant percentage of 1,8-cineole at more than 70% (Ben et al. 2011). Furthermore, Eucalyptus is an aromatic plant that can be used as an inhaler due to the high concentrations of methanol and ethyl acetate in their leaves. Murni et al, (2023) reported that with 1900 g of *E. pellita* leaves as a dry powder, up to 176.75 g of methanol, and 27.93 g of ethyl acetate were extracted, and phytochemical analysis of the extracts revealed that the methanol and ethyl acetate extracts contained flavonoids. In addition, according to Kartiko et al, (2021) study stated that *E. pellita* leaves could produce essential oils, which has a potential to develop in microbiology industry since it was a new natural source of antibacterial agents.

According to Orwa et al, (2009), *E. pellita* is a medium-sized to giant tree that may reach heights of 40 metres and breast heights of 1 metre in diameter. The bark is thick, dark to reddish-brown, coarse, and persistent to the little branches, and it is fibrous and shallowly to coarsely fissured. Eucalyptus is considered a rapid growth plant under plantation conditions. Therefore, South-East Asia has long produced *Eucalyptus pellita* as a source of fibre, mainly for pulp manufacture according to Hii (2017). It has also been noted as a species that might be used in conjunction with native hardwoods to produce veneer and solid wood. Hence, *E. pellita* and other species quickly took the place of *A. mangium* industrial tree plantations that had significantly declined in eastern Malaysia (Japarudin et al, 2022). Due to fewer mixed tropical hardwood species are being harvested from natural forests, and an increasing need for logs, *Eucalyptus pellita* planted forests offer an alternate source of wood to satisfy this demand.

In previous year, *E. pellita* had been planted in various nursery, such as in Kota Tinggi, Johore during the period from 2019 to 2020 where the height increment was 2.5 to 3.4 cm per year according to Zuhaidi et al, (2020). This study is to focus on growing Eucalyptus in nursery conditions to meet the high demand of this species for high quality planting materials. Suitable standard of procedure will be developedto fulfil the requirement of the production of quality planting materials for *Eucalyptus pellita* to support the forest plantation industry.

MATERIALS AND METHODS

a. Study location

The study was conducted at Main Nursery in Forestry Research Institute Malaysia (FRIM), Kepong, Selangor. The experiment was carried out in open space area with automated watering three times a day. The temperature was between 29 and 34°C.

b. Plant materials

The seeds were collected and germinated on a seed bed filled with sand medium. The seed bed was observed daily to check the watering system and availability of germinated seeds. The seedlings with four leaves (around 10 cm height) were transferred to the polybag (normal and transparent). The height of the seedlings was taken at 2 months old.

c. Treatments

Eucalyptus pellita seedlings were transferred from growing beds to polybags accordingly and nurtured to remove transfer shock. Seedlings were transferred to medium treated with either soil conditioner or fertilizer (NPK 8:8:8) after two months' transplant. Normal and transparent polybag were used to observe any effect on growth performance. The soil conditioner or fertilizer were applied to three months old seedlings of *E. pellita*.

Polybags without soil conditioner and fertilizer were the controls for this experiment (C1 – normal polybag and C2 – transparent polybag). Each treatment includes 10 sample with three replicates. The treatments for this experiment are summarized in Table 1:

Table 1 Different treatments for growth performance of Eucalyptus pellita in nursery

Tag	Treatment
T1(B)	NPK; normal polybag
T1(SC)	Soil conditioner; normal polybag
T2(B)	NPK; transparent polybag
T2(SC)	Soil conditioner; transparent polybag

d. Measurement on growth performance of *Eucalyptus pellita*

Height of the seedlings were collected at two months old, followed by each month after applications of fertilizer and soil conditioner to test their effect on plant growth. Survival of the seedlings was also observed and recorded to determine any effect of fertilizer or soil conditioner.

RESULTS AND DISCUSSION

The height of the seedlings at the nursery stage for *Eucalyptus pellita* are shown in Figure 1. In the first month of data collection, the height of each treatment did not show any difference. Eucalyptus seedlings (C1 - normal polybag) attained best growth rate. The height increment from 10 cm to almost 70 cm within 3 months of transplant. Seedlings in T1 (normal polybag) had a better growth performance than T2 in a transparent polybag. Hence, to compare the polybag types, all the polybag growth performance is relatively better than in a transparent polybag.

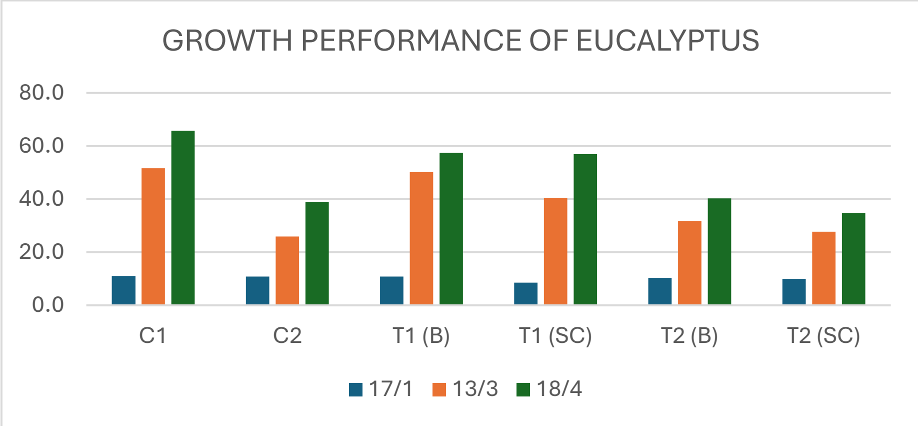


Figure 1. Growth performance of *Eucalyptus pellita* seedlings in nursery.

Eucalyptus pellita is a fast growing species. This factor might explain when there is low effect of fertilizer and soil conditioner on growth for seedlings as shown in figures 2 and 3. The lowest performance is for treatment T2 in transparent polybags.

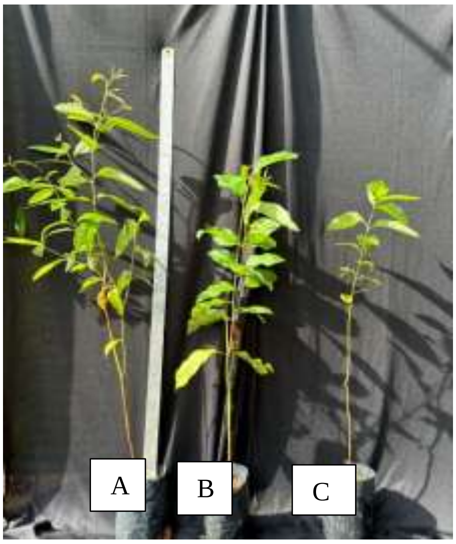
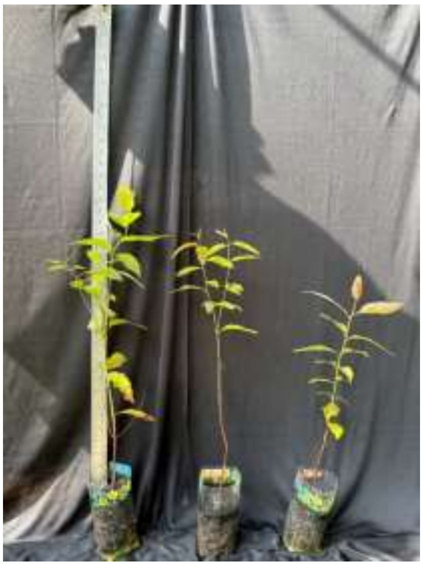


Figure 2. Heights of *E. pellita* seedlings grown in normal polybag. (A) is C1, (B) is T1 (B), and (C) is T1 (SC).



A B C

Figure 3. Heights of *E. pellita* seedlings grown in transparent polybag. A is T2 (B), B is C2, and C is T2 (SC).

A B

Figure 4. Heights of *E. pellita* where (A) is C1, and (B) is C2.

According to figure 4, C1 shows the highest *E. pellita* in normal polybag while C2 has the lowest height among those treatments. From observation made, the root was well developed for all treatments. Root growth is critical to the establishment of planted seedlings. Seedlings are subjected stress just after transplanting. The size and dispersion of the root system, the root-soil contact, and the root hydraulic conductivity of a recently planted seedling all influence its capacity to withstand planting stress. All seedlings survived in conditions without any fertilizer and soil conditioner (table 1). However, 5 to 15% death was observed for treatment T1(B), T1 (SC), T2 (B) and T2 (SC). More observation need to be done and chemical analysis of the potting medium for understood the factor causing the death of the seedlings.

Table 1. Percentage of *Eucalyptus* death for each treatment.

TREATMENT	DEATH %
C1	0
C2	0
T1 (B)	15
T1 (SC)	5
T2 (B)	10
T2 (SC)	15

The findings demonstrate that transparent polybags may stunt the growth of *E. pellita* seedlings. The normal polybag has a better growth rate without any fertilizer needed according to figure 4. As transparent polybag may still retain some heat and moisture, however it increased the exposure to direct sunlight, and temperature changes may have an impact on the plant development and well-being as observed the difference in figure 4. Figure 4 shows a difference between the tallest plant in a polybag and in a transparent polybag. Due to the extremely hot weather in Malaysia, the growth of *E. pellita* in transparent polybag might be affected. Furthermore, Omar et al, (2021) stated that white paper bag is not the best to grow BARI Aam 3 and BARI Aam 4 and brown paper bag proved to be extremely effective to improve the quality of the fruit. Compared to dark coloured containers, lighter coloured containers reflect more solar radiation away from them, resulting in less absorption of solar energy. In nursery production, heat stress is lessened by using lighter-coloured containers according to Seneviratne et al, (2019). In contrast, this study shows that transparent polybag stunted the growth of eucalyptussuggesting that transparent polybag is not suitable for *E. pellita* in nursery.

CONCLUSIONS

Results clearly show that using a transparent polybag will stunt the growth of *Eucalyptus* while adding a fertilizer and soil conditioner shows less effect on *Eucalyptus* growth. Furthermore, seedlings with fertilizer and soil conditioner shows higher mortality. The information from this study are preliminary data that require further investigation and discussion. Analyzing the chemical composition in the potting media will give more information in growing Eucalyptus seedlings in nursery. Further study should help in identifying the improved procedure to produce *Eucalyptus pellita* seedlings with high quality and faster growth to be planted in plantation/site.

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082-082

ESTIMATION OF BIOMASS IN JEMORENG, SARAWAK

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1.0 INTRODUCTION

Peat swamp forest is rich in carbon and store averagely 220Mg C ha^{-1} in the standing trees and 670Mg C ha^{-1} per meter depth of peat (Manuri *et al*., 2014.). This ecosystem is playing an important role in climate change because deforestation and land preparation for agriculture shall lead to substantial greenhouse gas emissions. Under the United Framework Convention on Climate Change (UNFCCC), Malaysia has committed to the REDD+ mitigation approach which target to reduce greenhouse gas emissions from deforestation and degradation, conserve forest carbon stocks, sustainably manage forest and enhance forest carbon stock. Since Malaysia will be paid by incentives based on quantified emission reduction, hence, accurate biomass and carbon estimation is essential.

Many of the aboveground and belowground biomass for peat swamp forest are determined via allometric equations instead of in-situ measurements. This could cause a serious error either over or underestimate of the true value. This systematic error possibly causes by using allometric from different geographic area or wood density.

There are several ways to assess the carbon stock namely via harvest method, carbon flux measurement, remote sensing, modelling and plot method. As for this Jemoreng study, simple plot measurement was chosen over other methods owing to the time and technical constraints.

2.0 METHODOLOGY

2.1 Study site

Figure 1. The expedition site located at Mukah Division, the central of Sarawak

This study was carried out during the expedition which was held from 14-31 Julai 2022 organized by Constitution and Conservation Division of Forest Department Sarawak Headquarters. The study site is at Kampung Jemoreng, a Matu subdistrict, Sarawak, within 2°40’0”N and 111°33’0”E under Mukah Division. The journey from Kuching to Matu took about six hours by road. Peatswamp forest with patches of wet heath forest are the main vegetation in Jemoreng. Sekiu trees (*Madhuca motleyana*) which produce highly price edible oil, name “*minyak sekiu*” are found in peatswamp forest in Jemoreng. Beside Jemoreng, Omar *et al*. (2022) reported that *M.motleyana* was widely distributed in nine states in Peninsular Malaysia and could be found also in Thailand, Sumatra (Riau, Belitung), Brunei and Kalimantan (Chai & Yii, 2022).

2.2 Experimental design

Randomized circular plot was applied during the expedition. A total of seven (7) circular plots were established and denoted as J1 to J7. Each of the circular plot contained three sub-circular of size 100m^2, 250m^2 and 1m^2.

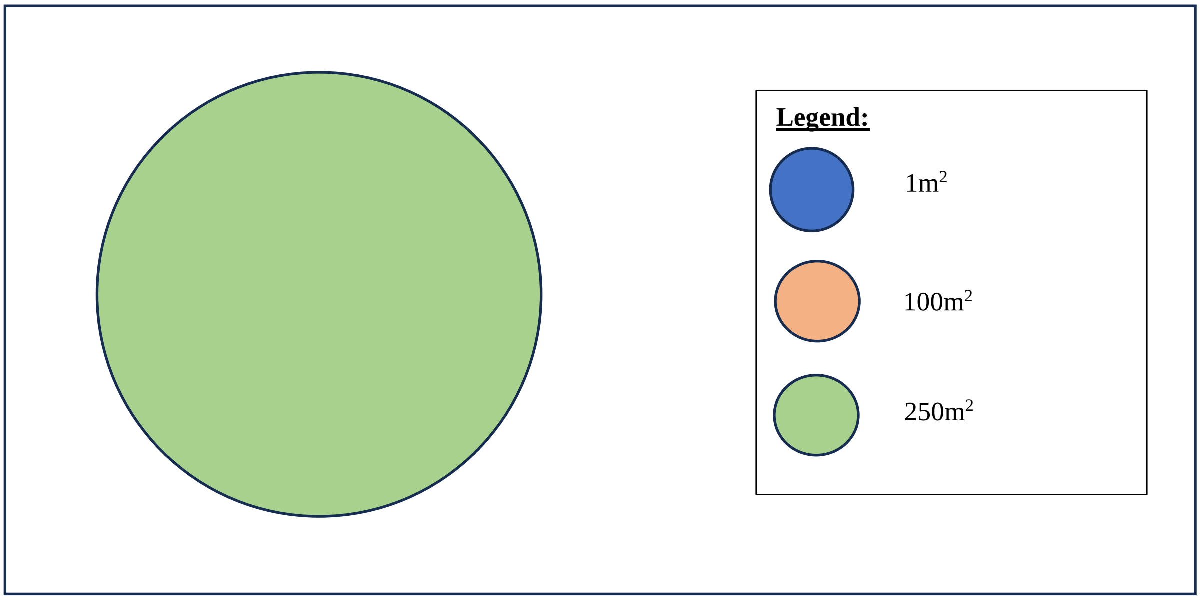

Figure 2. Experimental design for circular plot

2.3 Aboveground biomass (AGB)

Aboveground biomass comprised of three components which are the standing live trees, standing dead or lying dead wood and leaf litter. These three components were covered in this study. For standing live trees, only trees of diameter at breast height (DBH) of less than and equivalent of 5cm were measured in 100m^2 circular plot whereas trees of more than 5cm were recorded within 250m^2 circular plot. Standing dead or lying dead wood of more than 10cm diameter were measured within the 250m^2 circular plot. Litter such as leaves and twigs were being collected from the 1m^2 circular plot. The total weight of leaf litter was recorded and then sub-sampled to bring back to laboratory. The subsamples were oven dried at 70° C for 72 hours or until constant weight.

The equation used to calculate the biomass of Jemoreng Peat Swamp Forest was 0.136 x DBH ^2.51 based on Manuri *et al*., (2014) where this equation was developed for tropical peat swamp forest in Indonesia.

2.4 Belowground biomass (BGB)

There are two components of belowground biomass which are soils and roots. Biomass for soil in this study was determined via collection of core and auger samples. Core ring samples were used to determine bulk density whereas the auger samples (soil depth 0-40cm) were used to determine the organic matter content. The root component in this study was not carried out because root measurements involved digging out whole root systems and is very laborious (Niiyama *et al*., 2010).

The carbon in the soil was calculated using this equation:

Carbon in soil = Bulk density x depth x carbon content from ashing method

3.0 RESULTS AND DISCUSSION

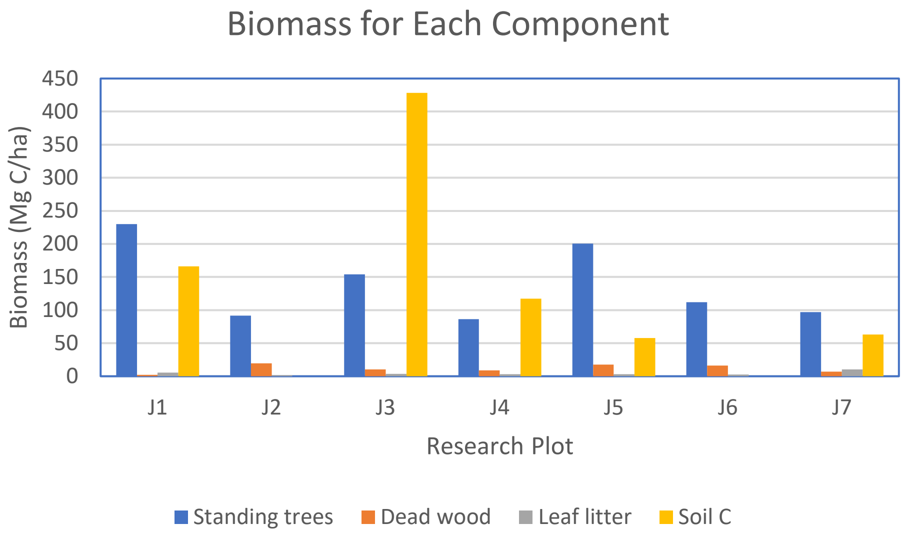

Figure 3. The biomass results of each component.

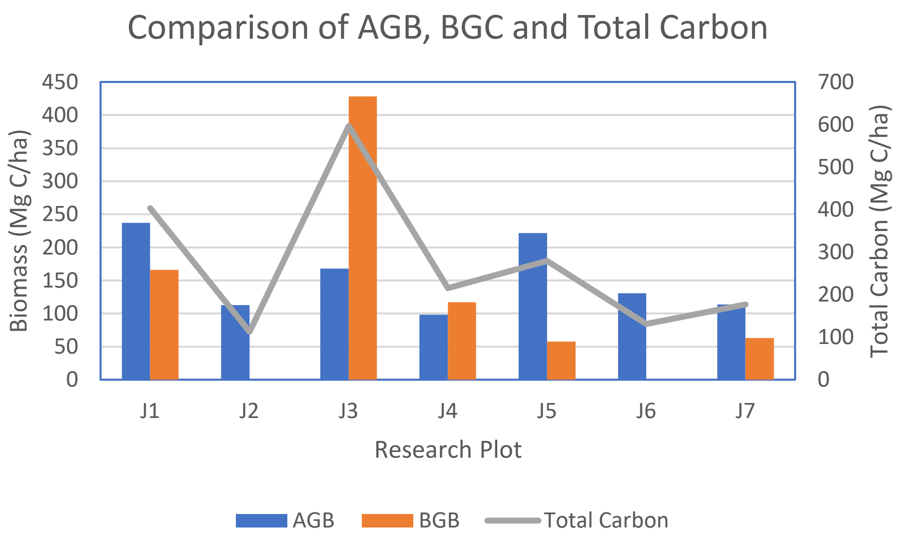

Figure 4. Comparison of aboveground biomass, belowground biomass and total carbon among study plots.

There was no soil sample collected for J2 and J6 plot due to the flooding condition on day of sampling (Figure 3). Nevertheless, the rest of the research plots showed that standing trees and soil components were the major contributors to the forest biomass. The standing trees biomass ranged from 86 to 230Mg C/ha. Whereas the below ground biomass ranged from 60 to 430Mg C/ha. According to Manuri *et al*., (2014), the pristine peat swamp forest stored about 220Mg C/ha in the standing trees and 670Mg C/ha per meter depth of peat. However, in Jemoreng, the peat swamp forests are partly disturbed thus some location had lower carbon storage. Another study done by Novita *et al* (2021)., at Central Kalimantan Peat Swamp Forest showed that average above ground carbon stock in primary forest was 168±31Mg C/ha and 140±16Mg C/ha for secondary forest. Thus our results in Jemoreng are similar to both studies conducted in Indonesia.

The downed wood was a significant C pool, whereby it was contributing 1-17% to the total aboveground C stocks in the study plot. According to Anderson *et al*. (2011), dead wood accounts for ~8% of total C pool in forest globally. The variability in dead wood C stocks may be attributed to the primary production, tree mortality, decomposition rates that are related to climate and species wood traits (Basuki *et al*., 2009).

During the expedition, several soil groups were observed in the research plots such as podzol, gley soil and organic soils. According to Sarawak Soil Classification (Teng, 2004), podzol is referring to leached soil with paled colour sand at the sub-layer of soil profile. Whereas gley soil occurred at the poorly drained soil. The high water-table and occasionally inundation are the main reason of gley floodplain. Organic soil or peat soils are locally defined as soils having 50cm or more of organic soil materials (>35% organic matter).

It was noticed that J3 had the highest belowground carbon (430 C tonne/ha) compared to the rest of the study plots. After close examination, it was noticed that soil in J3 consisted of organic material with 53% loss of ignition (Halipah *et al*., 2022). Whereas some plots having podzol layer denoted wet heath forest (Figure 6) with lower carbon content and higher sand content.

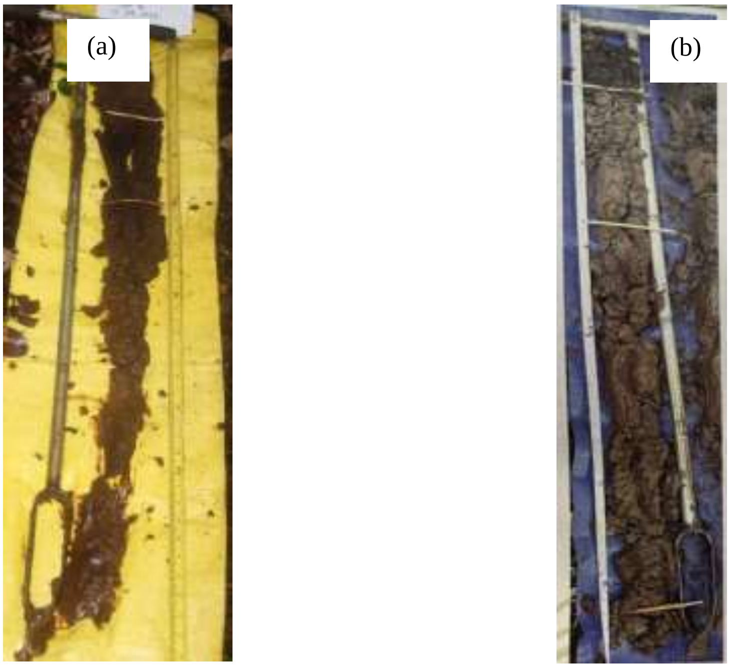

Figure 5. Soil profile for plot J3 (a) and plot J7 (b)

4.0 CONCLUSION

This study is the first biomass study in Jemoreng, Sarawak. During this study, only diameter at breast height (DBH) is used because DBH alone explains more than 95% of the variation in aboveground tropical forest carbon stocks (Brown, 2002). Although tree height could improve the accuracy of estimation for peat swamp forest, but this is not practical and necessary in this study because the standing trees are smaller in size. This study showed that Jemoreng forest consist of peat swamp forest and wet kerangas forest.

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081-089

EFFECTS OF DESICCATION AND LIQUID NITROGEN EXPOSURE TO EMBRYOS OF STERCULIA PARVIFLORA ROXB

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ABSTRACT

Sterculia parviflora is a species of tree in the family of Malvaceae. The fruits are often consumed by birds and storage of these seeds using a conventional method of seed storage is difficult, as they are easily damaged caused by fungal infection due to their high moisture content. This paper explored the possibility of long-term conservation of *Sterculia parviflora* germplasm using cryopreservation of excised embryos. Before immersion in liquid nitrogen, the moisture content of the embryos was reduced by desiccation in a sterile laminar airflow for up to 8 hours. The embryos that had been air-dried from 2 to 8 hours, withstand desiccation up to a moisture content of less than 6% where 100% of the embryos germinated. At this low moisture level too, the survival of cryopreserved embryos was in the range of 60 to 80%. The embryos did not survive cryopreservation at a moisture content of 10% or more. These findings demonstrated the feasibility of cryopreserving *Sterculia parviflora* embryos for long-term purposes.

Keywords: Storage, Cryopreservation, Desiccation, Embryos.

INTRODUCTION

Sterculia parviflora is a deciduous tree species in the Malvaceae family that grows in lowland and hill forests. It is called kelumpang burung because birds enjoy eating its fruit. In Roman mythology, Sterculia is named after "Sterculius" which refers to the disagreeable aroma of the flowers in this species. *S. parviflora* is regarded as one of the biggest Sterculia trees that can reach a height of 35 meters. Its' simple leaves are 10–27 cm long and 5–15 cm wide, having an oblong to elliptic-oblong form. Birds and squirrels are attracted to the enormous velvety orange or scarlet seedpods with their large black seeds. This tree is widely distributed in India, Indonesia, Malaysia, and other countries. It is a popular urban tree whose attractive form, flowers, and fruits make this tree suitable for roadside planting, gardens, and parks. Its white wood is usually used for indoor construction and plywood.

In our laboratory in Forest Research Institute Malaysia (FRIM) upon collection, *S. parviflora* seeds are kept in a 20ºC cold room at their initial moisture content. However, the seeds are only viable for a few weeks. In addition, the seed moisture content is high, around 42 to 54% on a fresh weight basis, and is prone to fungal infections and pest infestations. These can further reduce the seeds' viability when stored. As a result, preserving *S. parviflora* seeds in a conventional way for an extended time is very challenging. One option is available by using cryopreservation. Cryopreservation refers to the storage technique of germplasm at ultra-low temperatures in liquid nitrogen (LN) of -196°C or gaseous phase (-150°C), This technique is the most promising method for long-term storage of recalcitrant and intermediate seeds. Theoretically, at these temperatures, all metabolism activities were suspended and thus facilitated longer storage. It is also a safe and economical way of preservation. To survive these ultra-low temperatures, seeds must be dried before freezing.

Based on the *S. parviflora* seed characteristics, this species could be categorized as recalcitrant or intermediate/semi-recalcitrant (Ellis *et al.* 1990). These seeds can be kept in storage environments typically utilized for orthodox seeds, but with a shorter period. For this type of seed, desiccation and temperature reduction significantly lengthen the seeds' life. Roberts (1973) defines the second category of seeds that is orthodox. When mature, they have about 20% or lower moisture content on a fresh (FW) basis. The important characteristic of these seeds is that they survive longer as their moisture is reduced to 1-5% FW (King & Roberts, 1980). This means they can be safely dried to low moisture content and stored for a long time at sub-freezing temperatures. Another category is referred to as recalcitrant. At shedding, mature recalcitrant seeds contain a high moisture content between 40-60% (Tompsett, 1994). These seeds cannot be dried without causing damage. They are often sensitive to sub-freezing temperatures (Chin & Roberts, 1980). To stay viable, these seeds must maintain a high moisture content. Their life span is short thus long-term storage using a conventional method is difficult.

Until recently, no attempt has been made for long-term storage of *S. parviflora* whole seed. This study explores the prospect of cryopreservation as a method of preserving the *S. parviflora* genetic resources by assessing the effects of desiccation and freezing on embryo survival in liquid nitrogen.

MATERIALS AND METHODS

PLANT MATERIALS

Fruits of *S. parvifolia* were collected from mother trees in Pahang when they reached their maturity stage. They were transported back to the Seed Technology Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Selangor where the study was conducted.

SEED CHARACTERISTICS AND TESTING

Before experimenting, the freshly collected seeds were subjected to several seed and quality characteristics including the number of seeds per kilogram, the weight of 1000 seeds, seed moisture content, and germination tests. Five seeds with three replications were used for the seed moisture content test. The seeds were cut into four small pieces for uniform drying in the oven at 103±2°C for 17 hours. In a germination test, fifteen seeds with four replicates were used.

SURFACE STERILIZATION

Seeds were washed with a multi-purpose detergent (Teepol) and rinsed under tap water for 10 minutes. The seeds were further surface sterilized with 20% v/v hypochlorite solution for 10 minutes and then rinsed thrice in sterile water. The embryos were excised aseptically in a laminar airflow cabinet, surface sterilized with 70% (v/v) ethanol for 1 minute, and rinsed three times in sterile water.

EFFECTS OF EMBRYO DESICCATION

Embryos of *Sterculia parviflora* were extracted from the cleaned and surface sterilized seeds. The excised embryos were placed on an open petri dish (9 cm diameter) and desiccated in the air current of a laminar airflow cabinet for 2, 4, 6, and 8 hours at 26±2°C, 53±2% relative humidity. Batches of embryos were removed for embryo viability determination before and after direct plunge in liquid nitrogen. The moisture content of embryos was determined after each desiccation period (5x3 embryos).

CRYOPRESERVATION

After each desiccation period, 5 embryos were placed in 2 ml polypropylene sterile cryovials and directly immersed in liquid nitrogen where they were kept for 24 hours Five embryos with four replications were used in each treatment. Thawing was performed rapidly in a 40°C water bath for 10 minutes. After constant stirring, the cryovials were removed and placed on a sterile filter paper in the laminar flow to dry the excess water.

GERMINATION

Treated and non-treated desiccated and cryopreserved embryos were germinated in test tubes containing Murashige & Skoog (1962) medium (MS) supplemented with 30 gl^{-1} sucrose solidified with 8 gl^{-1} plant agar, and incubated in a germination room under white fluorescent light at 26±2°C with a photoperiod of 16 hours. Germination was assessed by the formation of shoot and root and was recorded as a percentage of the total number of plantlets after 4 weeks.

RESULTS AND DISCUSSIONS

CHARACTERISTICS OF S. PARVIFLORA SEEDS

The average size of *S. parviflora* seed is about 23 mm long and 14 mm wide, weighed 1.800 g each. Meanwhile, the embryo is small with 3x2 mm (width x length) and 0.7 g. The average moisture content of the seed is 48.8% and 57.8% for the embryos.

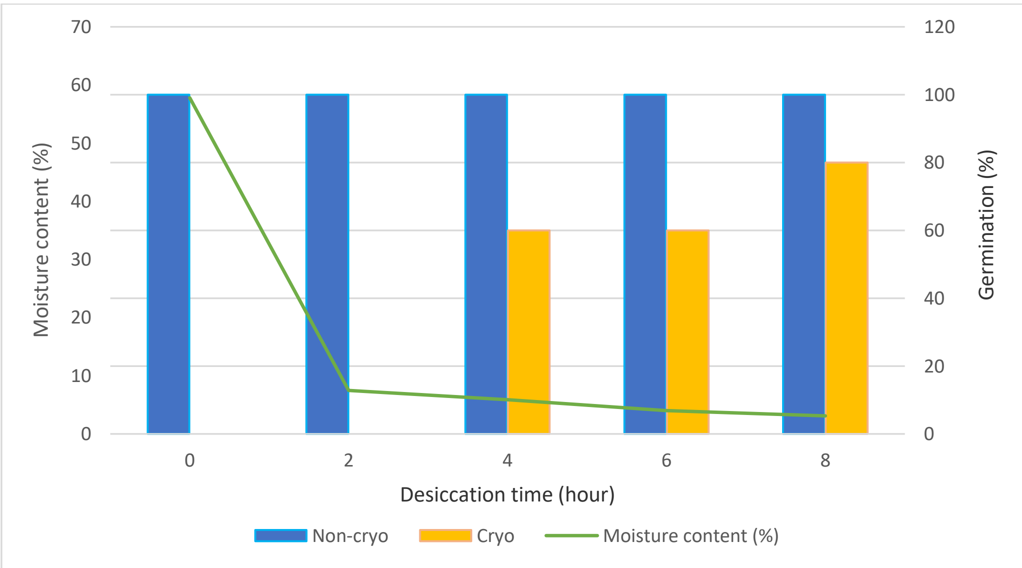
EMBRYO DESICCATION

Table 1. Moisture contents of *S. parviflora* embryos at shedding and after 2, 4, 6 and 8 hours of desiccation

Treatment	Mean
Shedding/0 hour	57.8
2 hours desiccation	7.5
4 hours desiccation	5.9
6 hours desiccation	4.0
8 hours desiccation	3.1

The moisture content of the fresh seeds was 48.8% and the embryos' moisture content was slightly higher, i.e. 57.8%. There was a decline in embryo moisture content as the desiccation time increased from 2 to 8 hours. The moisture content decreased drastically after 2 hours of drying in the laminar airflow cabinet (Figure 1). This was especially so in the first two hours where almost 50% of moisture was lost (57.8% to 7.5%). After that, there was a more gradual decrease to 5.9% and at the end of 8 hours of desiccation, the embryo moisture content reached 3.1%.

Figure 1. Effects of desiccation on moisture content and germination of treated (cryopreserved) and non-treated (non-cryopreserved) *S. parviflora* embryos.



GERMINATION OF NON-CRYOPRESERVED EMBRYOS

At shedding/initial moisture content, the germination of non-desiccated and non-cryopreserved embryos was 100%, respectively. Reduction in moisture content did not affect the germination percentage of the embryos at all desiccation levels, from 2 to 8 hours, where 100% of embryos germinated even at the lowest moisture content of 3.1% (Figure 1). These results show that *S. parviflora* embryos are not sensitive to desiccation, but tolerate low moisture content, from 7 to 3%.

GERMINATION OF CRYOPRESERVED EMBRYOS

Fresh embryos at a high moisture content of 57.8% and 7.5% did not survive exposure to liquid nitrogen but 60% to 80% of the embryos survived when they were dried to 5.9% moisture or less. In this case, survival increased with increased desiccation. After 4-8 hours of desiccation, the moisture content decreased to around 5.9 to 3.1% where survival rates were between 60-80%. In general, the desiccation of excised embryos for 8 hours to 3.1% moisture was optimal for preservation in liquid nitrogen.

Of greater significance is that many of these excised embryos were able to survive exposure to liquid nitrogen, indicating that excised desiccated embryos have the potential for cryopreservation of *S. parviflora* genetic resources. Excised embryos' survival in liquid nitrogen depended on their moisture content and freezing rate (Fig. 4). Generally, the best method for preserving excised embryos in liquid nitrogen was to desiccate them for eight hours until the moisture reached around 3.1%.

Air desiccation has been effectively used to cryopreserve embryos and embryonic axes of many intermediate and recalcitrant seeds, for instance, *Citrus indica*, *Elaeis guineensis*, *Citrus calaleriei*, and *Butia caarinensis* (25). This desiccation method is simpler and easier to apply than others that require cryoprotectants such as vitrification solutions. Drying embryos is also faster and in comparison to seed drying.

Seeds of tropical trees are usually large, and storing them through cryopreservation is difficult and will probably be damaged by freezing. However, by excising the embryos, survival is better, and preservation in liquid nitrogen is achievable. Hor *et al.* (1993) conducted a Differential thermal analysis of coffee seed tissues suggesting that the absence of freezable water is an important factor for successful cryopreservation.

CONCLUSIONS

The results of this study show that cryopreservation of excised embryos may be used to preserve some tropical plant species that fall into the intermediate to recalcitrant seed category. Hopefully, these findings will lead to a practical technique for cryopreservation and contribute to the development of conservation strategies for tropical plant diversity, in general.

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064-091

MANGROVE BLUE CARBON RESTORATION EFFORTS USING NATURE BASED SOLUTIONS (NBS) BREAKWATERS IN MALAYSIA

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ABSTRACT

Mangrove forests are a crucial component of blue carbon ecosystems, capturing and storing substantial amounts of carbon, with levels among the highest compared to other forest types. The destruction and disappearance of vegetated coastal habitats can jeopardize soil carbon to a depth of 1 meter, leading to its remineralization as CO_2. The total emissions resulting from mangrove loss are estimated to be around 14 million Mg CO_2, with an annual emission rate of approximately 0.5 million Mg CO_2 per year. Much efforts have been done to plant and restore mangroves. Nonetheless, numerous studies have demonstrated that conventional planting methods showed poor result, with survival rates of only 0 to 20% in replanted mangrove areas, particularly in high-risk zones. Study indicated that conventional planting techniques failed to effectively support and anchor the mangroves as early as 15 months, and in some cases, within the first 1 to 6 months. The coast of Sungai Haji Dorani, Sabak Bernam, Selangor are erosion prone and is recognized as a high-risk area, making conventional planting methods impractical. Planting mangrove species along its coast requires breakwater structure to reduce wave and current energy, prevent erosion, and promote planting. This study compared various coastal restoration efforts using different breakwater structures, including coastal blocks, L-blocks, geotubes, and mangrove poles. Mangrove pole breakwater structures, as a Nature-Based Solution (NbS), offer more advantages by being environmentally friendly, less expensive and less time-consuming. *Rhizophora* species are recommended for planting as they effectively mitigate both water depth and current velocity, have commercial value, and thrive in the muddy soil substrates typical via this region. Most degraded mangrove forests are unable to revive independently, thus necessitating human intervention to restore this degraded mangrove area such as in Sungai Haji Dorani, Sabak Bernam, Selangor.

Keywords: Mangrove Restoration, Blue Carbon, Nature Based Solutions (NbS), Breakwater Structure, Erosion.

090-092

TREE DISTRIBUTION AND FLORISTIC COMPOSITION OF PEAT SWAMP FOREST WITHIN RESAK TAMBAHAN FOREST RESERVE, PAHANG, MALAYSIA

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ABSTRACT

Studying the current status of peat swamp forests is important to provide information for future conservation and management planning. A study was conducted to assess the tree distribution and floristic composition of the peat swamp forest within the Resak Tambahan Forest Reserve in Rompin, Pahang. The study area was stratified into three categories based on Forest Canopy Density (FCD): low, medium, and high. Nine transect lines, each comprised of six circular sampling plots with a 10-meter radius, were established in seven forest compartments based on FCDs within the study area. All trees of equal and greater than 10 cm in diameter-at-breast-height (dbh) were measured and identified by species. The dominant families including Euphorbiaceae, Burseraceae, Clusiaceae, Sapotaceae, and Fabaceae. Important species in the study area include *Macaranga pruinosa* and *Syzygium* sp. Analysis of variance (ANOVA) indicated that there were no significant differences in the means tree density among the three FCDs ($p \geq 0.05$). The means basal area was found to be significantly different among the low, medium, and high FCDs ($p < 0.05$). The results of this study may provide valuable insights for conservation, management and ultimately safeguard this majestic ecosystem for future generations.

Keywords: Floristic Composition, Peat Swamp Forest, Resak Tambahan Forest Reserve, Tree Distribution.

INTRODUCTION

Information on tree distribution and species composition is essential for managing and conserving specific forest areas, especially in peat swamp forests. This information can be used to understand the population patterns or profiles of the area. According to 2022 Annual Report of Forestry Department Peninsular Malaysia stated that the peat swam forest covered 0.21 million hectares from total area of Permanent Reserved Forest throughout Peninsular Malaysia. Peat swamp forests provide significant services, including habitats for flora and fauna, food resources, carbon storage, economic benefits for the community, water catchments, and more (Astiani et al., 2021). However, this ecosystem are facing threats that affect their ecosystems, such as logging, land conversion, agricultural expansion, and canal developments (Atiningsih et al., 2024). A previous study in peat swamp forest located in the southern part of Peninsular Malaysia, reported that the forest is still rich in tree species despite being threatened by anthropogenic activities (Hyrul Izwan et al., 2022). Forests are capable resiliently recover from certain levels of disturbance, but in extreme conditions can ultimately impact their structure and integrity. Scientific studies are needed to explore more about this forest ecosystem that can be used as references by relevant stakeholders. Therefore, this study was assessed the tree distribution and floristic composition of the peat swamp forest within the Resak Tambahan Forest Reserve in Rompin, Pahang.

MATERIALS AND METHODS

Study area

This study was conducted in Resak Tambahan Forest Reserve, Rompin, Pahang. It is located at the southeast of Peninsular Malaysia and adjacent to the state of Johor. This area is basically administered by the Pahang Forestry Department. The overall condition of the Resak Tambahan Forest Reserve is good, with a healthy forest ecosystem supporting various species of flora and fauna. The study area was stratified into three categories based on forest canopy densities (FCD): low, medium and high. These FCDs were obtained from remote sensing analysis. The location of study area is shown as in Figure 1.

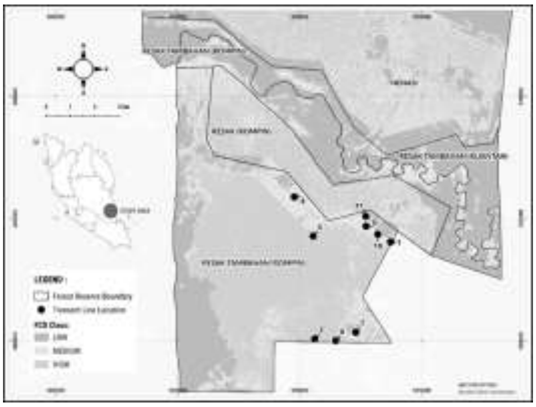


Figure 1: Location of study area

Data collection

Assessment on stand structure and species composition was conducted through survey transect method where each consisting of six circular sampling plots, were established in seven forest compartments based on Forest Canopy Density (FCD) within the study area. These circular plots were labelled as Plot A, B, C, D, E, and F, with a 10-meter radius between the midpoints of each circular plot. Figure 2 illustrates the layout of the transect lines used in this study.

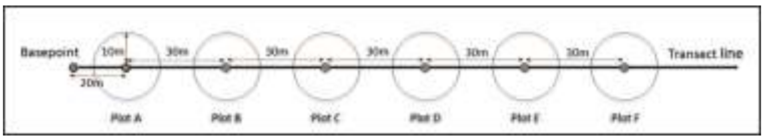


Figure 2: Layout of transect line

In addition, three transect lines were established for each FCD. The details regarding the location of the nine transects, based on FCDs in the Resak Tambahan Forest Reserve, are provided in Table 1.

Table 1: The location of transect lines established in Resak Tambahan Forest Reserve

No.	**Transect number**	**Forest compartment**	**Forest Canopy Density**
1.	1	28	Medium
2.	2	42	High
3.	3	25	High
4.	4	19	Medium
5.	5	27	Medium
6.	6	43	High
7.	7	44	Low
8.	10	28	Low
9.	11	27	Low

All trees with a diameter-at-breast-height (dbh) equal to or greater than 10 cm within the plots were measured, tagged, and recorded. Tree identification was made up to species level. The identification process was made possible with an aid of the Tree Flora of Malaya as the main reference for this study. Any unidentified trees were tagged and specimens were brought back to FRIM Herbarium for further process.

Data analysis

The information on stand structure in this study was assessed through a calculation of basal area and tree density. In term species composition, the dominant species in each FCD was identified on the basis of highest number of tree per hectare.

Basal area

Basal area is a measure of tree density that defines the area of a given section of land that is occupied by the cross-section of a tree. Equation (1) was used to calculate basal area that converts the dbh in cm to the basal area in m^2.

$$BA = \pi r^2 \qquad (1)$$
$$= 3.142 \times (dbh/200)^2$$

Where, BA is basal area in m^2, r is radius of tree in centimeter (cm) and dbh is diameter-at-breast-height.

Tree density

Tree density is the number of trees in a given area. To calculate tree density, the equation (2) was used. This information on tree density, especially species specific, is essential to facilitate efforts in management and conservation.

$$\text{Tree density} = \text{total number of tree/area in } m^2 \qquad (2)$$

Information calculated using equation (1) and (2) were converted to per hectare basis.

Floristic composition

Species composition refers to an assemblage of plant species that characterize the vegetation. There are various measurements for composition but the measurement is richness (the number of different species) and abundance (the number of individuals per species found in specified areas). In this study, the species composition was assessed through the measurement of relative density for each species. While, family composition was analysed through the information of tree density.

Important value index

Important value index or known as IVI analysis is a fundamental tool in ecological research, providing valuable insights into plant community dynamics (long term monitoring), biodiversity patterns, and ecosystem processes. A high percentage of IVI for a particular plant species within a community indicates that the species is relatively abundant, widespread, or both, compared to other species present in the same ecosystem. The IVI analysis is calculated as in equation (3) – (5).

Relative Frequency (RF) is the proportion of plots in which a species occurs.
RF = (Number of plots with the species / Total number of plots) * 100 (3)

Relative Density (RD) is the density of individuals of a species within the community.
RD = (Number of individuals of the species / Total number of individuals in the community) * 100 (4)

Relative Dominance (RDo) is the relative basal area occupied by a species within the community.
RDo = (Basal area of the species / Total basal area of all species) * 100 (5)

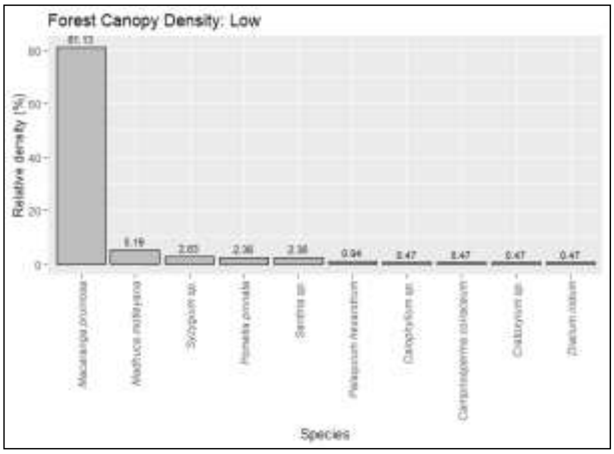
Total these three values were divided by three to determine the IVI for each tree species.

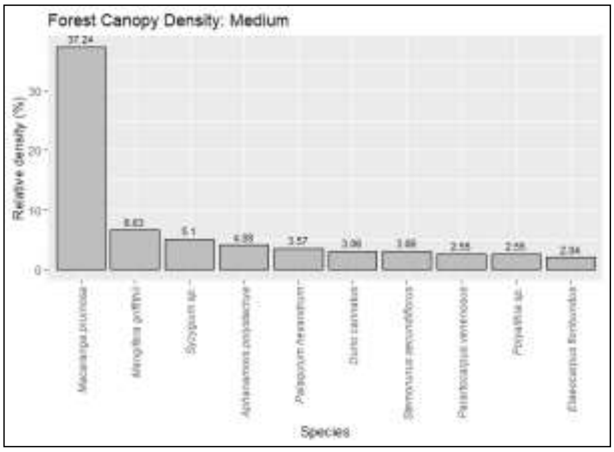
The analysis includes a comparison of calculated means between FCDs (i.e., low, medium, and high). A one-way Analysis of Variance (ANOVA) was performed to test the differences between the means of tree density and basal area. In the presence of significant differences in ANOVA, a multiple comparison Tukey test was used on the means by FCDs. All data analyses were conducted using R statistical software, including the 'RStudio', 'ggplot2', 'dplyr' and 'tidyr' packages.

RESULTS AND DISCUSSION

Floristic composition

A total of 686 trees were recorded in this study which comprise of 64 species and 28 families. The common species found in all FCDs were *Macaranga pruinosa* and *Syzygium* sp. Among tree families recorded in this forest reserve were Euphorbiaceae followed by Myrtaceae and Burseraceae. Figure 3 shows that the top ten tree species with the highest relative densities by FCDs. *M. pruinosa* exhibited abundance with high relative densities of 81.13% and 37.24% in low and medium FCDs, respectively. *M. pruinosa* is a pioneer species that easily grow under open areas or canopy gap within forest areas. A previous study in Central Kalimantan, Indonesia, found that pioneer species are common in open areas resulting from fire or other disturbances, such as illegal logging and land conversion (Qirom et al., 2022). In high FCD, *Syzygium* sp. reported the highest relative density, followed by *Calophyllum* sp. and *Santiria* sp. Species composition in peat swamp forests varies from one area to another depending on the forest conditions (e.g., soil, pH, depth, water level) and types of disturbances.





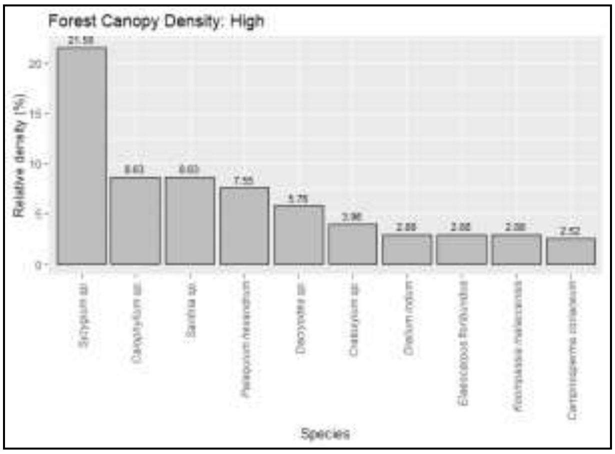


Figure 3: Top ten tree species with the highest relative densities by FCD

Table 2 shows the top five families with highest number of tree density for low, medium, and high FCDs. In terms of family distribution, Euphorbiaceae recorded higher tree densities in low and medium FCDs, with 304 trees/ha and 138 trees/ha, respectively. In high FCD category, the family of Myrtaceae, Burseraceae, and Clusiaceae had the largest number of trees within the study area, with densities of 110 trees/ha, 71 trees/ha, and 50 trees/ha, respectively. Compared to previous study found that the families Annonaceae, Myrtaceae, and Chrysobalanaceae reported a high number of individuals in Kuala Langat South Forest Reserve (Ruziman & Pardi, 2021). Information on family composition may be crucial for studying the forest profile, as it provides an overview of tree distribution that naturally grow within this peat swamp forest.

Table 2: Top five tree family with highest tree density by FCD

No.	**Family**	**Tree density (tree/ha)**
Forest canopy density: Low		
1.	Euphorbiaceae	304.12
2.	Sapotaceae	22.99
3.	Sapindaceae	12.38
4.	Myrtaceae	10.61
5.	Burseraceae	8.84
Forest canopy density: Medium		
1.	Euphorbiaceae	137.92
2.	Anacardiaceae	26.52
3.	Myrtaceae	19.45
4.	Meliaceae	15.91
5.	Sapotaceae	15.91
Forest canopy density: High		
1.	Myrtaceae	109.63
2.	Burseraceae	70.73
3.	Clusiaceae	49.51
4.	Sapotaceae	38.90
5.	Fabaceae	28.29

Important value index (IVI)

Table 3 displays the five species with highest IVI by FCDs. *M. pruinosa* recorded higher IVI in low (57.06%) and medium (21.08%) FCDs. This condition was influenced with the high number of *M. pruinosa* which one of pioneer species presented in both FCDs. Large gaps in tree canopies play a role in this ecological process, allowing pioneer species to germinate easily with the presence of sunlight on the forest floor. Apart from that, *Syzygium* sp., *Calophyllum* sp., and *Palaquium hexandrum* were among the three species with the highest IVI in high FCD. This study also showed that trees of the species *Syzygium* sp., locally known as 'Kelat', are important in this peat swamp forest, as this species was recorded among the top five with the highest IVI in all three FCDs. Hyrul Izwan et al.

(2022) reported that *Koompassia malaccensis*, *Syzygium inophyllum* and *P. hexandrum* were among trees with highest IVI in Ayer Hitam North Forest Reserve, Muar. Another genus, Syzygium, was found with high IVI in other peat swamp forests, indicating that trees from this genus are commonly found in peat swamp forests elsewhere.

Table 3: Top five species with highest IVI by FCD

No.	Species	RF (%)	RD (%)	RDo (%)	Total	IVI (%)
Forest canopy density: Low						
1.	*Macaranga pruinosa*	9.09	81.13	80.97	171.19	57.06
2.	*Madhuca motleyana*	9.09	5.19	7.77	22.05	7.35
3.	*Pometia pinnata*	9.09	2.36	2.59	14.04	4.68
4.	*Santiria* sp.	9.09	2.36	2.37	13.82	4.61
5.	*Syzygium* sp.	9.09	2.83	1.50	13.42	4.47
Forest canopy density: Medium						
1.	*Macaranga pruinosa*	4.29	37.24	21.70	63.23	21.08
2.	*Mangifera griffithii*	2.86	6.63	11.81	21.30	7.10
3.	*Palaquium hexandrum*	4.29	3.57	8.21	16.07	5.36
4.	*Syzygium* sp.	2.86	5.10	7.90	15.85	5.28
5.	*Parartocarpus venenosus*	4.29	2.55	5.29	12.13	4.04
Forest canopy density: High						
1.	*Syzygium* sp.	3.61	21.58	12.53	37.72	12.57
2.	*Calophyllum* sp.	3.61	7.91	14.95	26.48	8.83
3.	*Palaquium hexandrum*	2.41	7.55	11.73	21.70	7.23
4.	*Santiria* sp.	3.61	8.63	7.64	19.89	6.63
5.	*Koompassia malaccensis*	3.61	2.88	7.00	13.49	4.50

Stand structure

The means of tree density by FCD is shown in Figure 4. The mean of tree densities ranged from 346.56 trees/ha to 491.55 trees/ha. From the one-way analysis of variance (ANOVA), it was found that there were no significant differences in the means tree density across three FCDs ($p \geq 0.05$). This suggests that the tree densities had similar trend between FCDs. However, the tree density for high FCD was found to be slightly higher than that of low and medium FCDs.

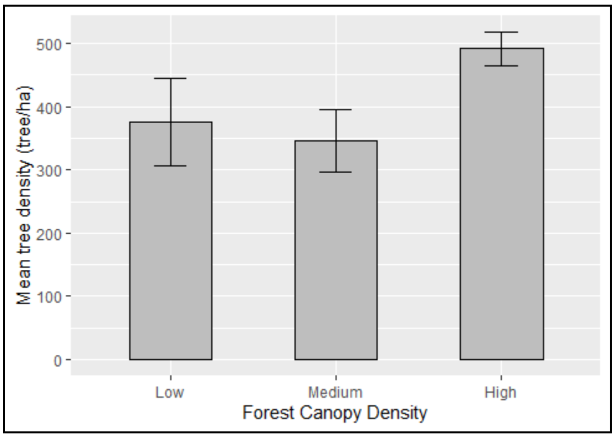


Figure 4: Means of tree density by FCD

The means of basal area obtained in the present study ranged from 8.52 m²/ha to 24.74 m²/ha (Figure 5). From the ANOVA analysis exhibited significant differences among the low, medium, and high FCDs ($p < 0.05$). The analysis of multiple comparison tests showed that the mean of basal area for the high FCD is significantly different from the low and medium FCDs. However, the mean of basal area for low FCD is not significantly different from the medium FCD ($p \geq 0.05$).

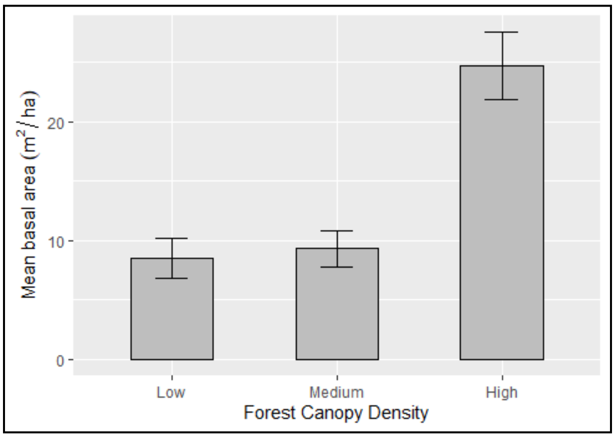


Figure 5: Means of basal area by FCD

CONCLUSION

This study shows that the Resak Tambahan Forest Reserve has a large number of pioneer species within low and medium FCDs. *Syzygium* sp. is found to be common in areas with high forest canopy density. In terms of family distribution, Euphorbiaceae was found to have a higher tree density in low and medium FCDs, while Myrtaceae was more dominant in high FCD. Similar to the trend in species composition, *Macaranga pruinosa* exhibits a higher Importance Value Index (IVI) in low and medium FCDs. Meanwhile, *Syzygium* sp. is an important species within all three FCDs of this forest reserve. The means of tree density was found to be similar across the three FCDs. Moreover, the mean basal area was found greater in high FCD over other FCDs. The findings from this study can provide useful insights and references for relevant stakeholders to take appropriate actions to conserve this majestic heritage for future generations.

ACKNOWLEDGEMENT

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091-093

THE LOCAL COMMUNITY PERCEPTION AND PARTICIPATION OF MANGROVE FOREST: THE CASE OF KUALA LINGGI FOREST RESERVE

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ABSTRACT

Mangrove forest is a unique forest located of extreme environment. The area not only serves as a natural habitat of various flora and fauna, but acts as natural protector against natural disaster such as big waves and strong winds. Mangrove area of Kuala Linggi Forest Reserves located at the border of Malacca dan Negeri Sembilan. The area is important area as mangrove coverage and also the breeding sites and the only crocodile sanctuary in Peninsular Malaysia. The forest management and restoration of mangrove in Kuala Linggi forest reserves should also benefited the local community. The involvement of the local community in mangrove forest conservation is a proactive step that has short-term and long-term effects. When a community is involved in the forest conservation program, the mangrove forest will be more assured of its preservation. Thus, this paper aims to assess the perception and participation of local community to the Kuala Linggi forest reserves. A total of 299 households were interviewed from four village's located surrounding Kuala Linggi forest reserve. Result show that the community is aware of the importance of Kuala Linggi mangrove area as a flora and fauna conservation habitat and also contributes to environmental research and education. Participation Index (PI) show that the status of community participation on management and conservation were below moderate at level 0.37. More awareness and conservation program should also be contributed by local community to enhance the participation towards the forest reserves.

Keywords: Mangrove, Perception, Participation, Local Community.

INTRODUCTION

Mangrove forest is a unique forest located of extreme environment. The area not only serves as a natural habitat of various flora and fauna, but acts as natural protector against natural disaster such as big waves and strong winds. Mangrove area of Kuala Linggi Forest Reserves located at the border of Malacca dan Negeri Sembilan. The area is important area as mangrove coverage and also the breeding sites and the only crocodile sanctuary in Peninsular Malaysia. The forest management and restoration of mangrove in Kuala Linggi forest reserves should also benefited the local community. The involvement of the local community in mangrove forest conservation is a proactive step that has short-term and long-term effects. When a community is involved in the forest conservation program, the mangrove forest will be more assured of its preservation.

In order to provide a reasonable justification, a basic economic evaluation study on the ecosystem needs to be conducted to provide information on the true value of mangrove forests, especially the contribution to the surrounding population. The existence of the HS Kuala Linggi Mangrove Forest is important as the economy of the local population such as marine life in addition to the role of the forest ecosystem as coastal protection against wave erosion, strong winds, waves and others. Directly, the people in this area benefit from aspects of livelihood resources and other aspects such as coastal protection.

Participation is a very broad concept that is often used by different people with very different meanings. Kelly (2001) states that involvement is a diverse process in which local communities are involved and play a role in issues that impact and affect them. Various methods have been applied and used in determining the community involvement level score. The level of community involvement can be measured using the Index of Participation (IP). Kamnap (2003); Fauziah et al. (2015) and Azreena AK et al. (2018) stated a formula to measure the Community Participation Index for five (5) Likert Scale points. In general, there are four (4) levels or levels of involvement which are planning, implementation, monitoring and benefit sharing. The index of involvement (IP) is used to measure the level of involvement of local communities in the conservation of protected areas. The classification of participation levels involving in planning, implementation, monitoring and benefit sharing. Hence the objective of this study is to assess the perception and participation of local community to the Kuala Linggi forest reserves.

STUDY AREA

The proposed area for the restoration project is located within the Kuala Linggi Forest Reserve. This area borders the Linggi River which is the border river between the states of Melaka and Negeri Sembilan.

Figure 1: Kuala Linggi Forest Reserves and surrounding villages

METHODOLOGY

The research methodology includes several approaches namely;

i) Rapid Rural Appraisal (RRA); The RRA technique is a tool that enables rapid assessment of the existing environment and the possible impact of the use of forest resources and other environmental services on local socio-economic life (Liswanti et.al, 2012). It provides information in terms of the importance of the area and mangrove/non-mangrove resources to local residents. The information collected includes the number of people, the economic activities of the people and the dependence of households on the resources in the mangrove area. The RRA was conducted in two series around February and March 2023.

ii) Focus group discussion; The method of group and individual discussion is a way to get the views of the parties involved on the importance of mangrove species restoration activities, the conservation of the area as well as the active involvement of the local community in the program.

iii) Questionnaire survey; The survey method is a method that will be used to interview households for the purpose of in-depth surveying the socio-economic status/impact of the local community as well as the level/status of community involvement in the restoration and conservation activities of the project area.

iv) The involvement index (IP); IP was used to measure the level of local community involvement in protected area conservation based on a five-point scale (Very often = 1.0, Often = 0.8, Sometimes = 0.6, rarely = 0.4 and Never = 0.2). Flow chart of methodology as Figure 2. The index of involvement for various levels in ecotourism activities and protected area conservation is obtained by using the formula below (modified from Kamnap, 2003):

Project Planning - rapid rural appraisal (RRA) and focus group

RRA was conducted prior to the survey and aims to gather some basic community information.

Questionnaire development

The formation of the questions in the questionnaire was based on the initial survey and the objectives of the study needs.

Sample size and pre-test questionnaire

The appropriate sample size is important in determining the accuracy of the estimated value of the results.

Household survey

Face-to face interview with the households in 4 villages located near Kuala Linggi FR. Survey was done in May 2023 and a total of 299 respondents were interviewed

Analysis

All the data from the survey been validating before the analysis was done using SPSS software. The analysis covering descriptive and statistical analysis. Participation index calculated by:

$$IP = [(X5* 1) + (X4* 0.8) +(X3 * 0.6) + (X2 * 0.4) + (X1 * 0.2)] / N$$

Where ; Where, IP = index of involvement/participation in activities related to ecotourism and conservation of protected areas, program level X5 = level of respondents very often participate in programs of a certain level, X4 = level of respondents often participate, X3 = level of respondents sometimes participate, X2 = the level of respondents who rarely participate, X1 = the level of respondents who never participate and N = the total number of respondents for each level of activity.

Figure 2: Flowchart of the methodology

RESULTS AND DISCUSSION

A total of 299 households were interviewed from four village's located surrounding Kuala Linggi forest reserve (FR), namely Kg. Kuala Linggi, Kg. Bukit Darat, Kg. Tengah and Kg. Tanjung Dahan as listed in Table 1.

Table 1:List of villages and households

No	Village	Households surveyed
1	Kg. Ayer Tawar	71
2	Kg. Bukit Kuda / Pasir Gembur	83
3	Kg. Merlimau Pantai	70
4	Kg. Merlimau Timur	75
	Total	**299**

Result show that the community is aware of the importance of the Kuala Linggi FR mangrove area as a flora and fauna conservation habitat (average scale: 4.3) and contributes to environmental research and education (average scale: 4.2).

The community view of this area is also important as a source of generating community income from mangrove products and generating income from river catches (average scale: 3.9). Table 2 show detail of the

Table 2: The importance of Kuala Linggi FR from community perspectives

The household's perception of the value of the HS Kuala Linggi mangrove area is important as...	**Scale**
Important areas as breeding habitat for fish seeds	3.6
Important areas generate income from river/lake catchment.	3.9
Important areas as flora & fauna conservation habitats	4.3
Important areas as a source of agricultural water supply	3.3
Important areas & contribute to environmental research and education	4.2
Important areas to generate income for the local community (eco-tourism/fish products/craft material wood & others)	3.9
Important areas as a source of drinking water supply and daily use	2.9
Important areas as daily food sources (leaves/shoots/fruits/grains/animals	3.3
Important areas as sources of traditional medicine	3.1
An important area as a tourist attraction and focus for recreation & leisure activities	4.0
Important & beneficial areas for climate and weather control	4.2
Contribution to heritage & cultural values	3.9

The view on the wise use of mangrove areas according to priority by the community shows that the first priority is for the preservation (left naturally) of the Kuala Linggi FR area. The second priority is the development of eco-tourism, followed by systematic logging and replanting and the fourth is open for agricultural development.

The level of participation index is to assess the local community's participation in activities related to ecotourism and local protected areas. There are four levels of evaluation of the level of engagement measured, namely the planning, implementation, monitoring and benefit sharing levels. Table 3 shows the index of the level of involvement for the level of the main programs where the local community participates in activities related to the local FR area. On average, the overall involvement index of the local community participating in activities/activities related to the FR area is (IP=0.37). The results of the study show that the level of local community involvement is generally still low at the three levels of the program studied (planning, implementation and monitoring), while the level of involvement at the level of benefit sharing index value is moderate. The level of community involvement for the planning and monitoring stage gave the lowest index (IP=0.29). This shows that the level of community involvement in channelling information to the authorities, in addition to involvement in patrol activities and monitoring of the FR area for any encroachment activities and other related activities is still low. In this study, it also shows that the engagement level index is relatively high in the benefit sharing stage (IP=0.57) compared to other engagement stages.

Table 3: Participation index results by stages

Participation	**Participation Index**
Planning	0.29
Implementation	0.34
Monitoring	0.29
Benefit Sharing	0.57
Average	**0.37**

CONCLUSION

Community’s perception towards mangrove around Kuala Linggi FR is valuable for habitat flora and fauna thus important to be conserve. More awareness and conservation program should also be contributed by local community to enhance the participation towards the forest reserves.

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092-094

EVALUATING CRITICAL SUCCESS FACTOR AND CHALLENGES IN NANOCELLULOSE PRODUCTS' COMMERCIALISATION

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ABSTRACT

Malaysia is blessed with abundant source of lignocellulosic biomass that could be converted to nanocellulose as new sustainable, renewable, biodegradable, high-performance value-added product. The presence of lignocellulosic biomass such as agriculture residue from oil palm e.g. empty fruit bunch, frond, trunk, rice straw; wood pulp, kenaf, forestry thinning waste and industry waste in the country are ample to create new cellulose-based industry that could lead to new revenue generation to the country and provide job to local society. Nanocellulose has shown strength enhancement in product manufacture. Having remarkable properties, nanocellulose is an attractive material for a wide range of applications in industries. The numbers show that the innovation on nanocellulose has been very progressive since the last decade however the commercialisation phase is quite slow in certain country. In Malaysia for instance, government support through Ministry of Science, Technology and Innovation (MOSTI) is available through various funding mechanism to embark on this area which offer up to pilot and commercialisation phase. Nevertheless, the commercial exploitation on nanocellulose in the local scenario has not been remarkably visible. It is imperative to acquire essential information on this industry as the availability of bioresource in the country is vast. This study through a face-to-face survey captures the view of chief executive officers (CEO) in the industry who have experience in commercializing nanocellulose in Malaysia. The qualitative data on critical success factors (CSF) and challenges were analysed using content analysis to extract meaningful insights and themes related to the commercialisation of nanocellulose. This study identifies the top five challenges and CSFs that include internal and external success factors in products commercialisation.

Keywords: Nanocellulose, Commercialisation, Critical Success Factors.

INTRODUCTION

Economy development around the globe is facing big challenge to reduce dependency on fossil resources as well as to address climate change issues in many areas like food, agriculture and energy. As a result of this scenario, lignocellulosic biomass has received growing attention for the new sustainable biomaterials and products since it could be converted to nanocellulose as new sustainable, renewable, biodegradable, high-performance value-added product. The increased interest in nanocellulose can be attributed to four mega trends which are; rooted from the increased growth of electronic communication and digital printing which leads to the low demand and consumption of paper, increased demand for green energy and bioenergy via biorefinery concept, due to the increased interest in sustainability, and last mega trend is the emergence of nanotechnology itself. According to recent statistics, the global production of natural lignocellulosic biomass is projected to be 181.5 billion tons per year, with only approximately 5% stated to be used. Of this 5%, majority biomass (85%) is available from forest, agriculture and grasses whilst the remaining comes from agricultural residues (Ashokkumar et al. 2022).

Nanocellulose has amazing qualities, making it an appealing material for a wide range of industrial applications. Looking at the industrial interest landscape, the number of nanocellulose-related patents filed worldwide has been increasing year after year. Between 2010 and 2017, the total number of documents related to nanocellulose was found to be more than 4000. (Charreau et al. 2020). Nanocellulose innovation has been quite progressive during the previous decade, however commercialization is slow in some countries. Malaysia, for example, is blessed with an abundance of lignocellulosic resources, and the government provides ongoing assistance through various financial mechanisms to enter this field, which offers up to the pilot and commercialization phases. Still, commercial utilization of nanocellulose in the local scenario has been quite low. It is imperative to acquire essential information on this area to assess the Malaysian nanocellulose scenario, given the country's richness of bioresources. Therefore, this paper will assess the critical success factors (CSF) and challenges in commercialization of nanocellulose in Malaysia.

MATERIALS AND METHODS

The data collection process consists of primary and secondary data collection to identify companies that produce nanocellulose and use nanocellulose in their production. The products listed under nanomaterial based products were collected from online internet searches included nanostat.com; a platform focused on nano science, technology, and industry (NSTI) information and from NanoVerify Sdn. Bhd. (NVSB), a wholly owned company of NanoMalaysia Berhad (NMB), an agency under the Ministry of Science, Technology and Innovation (MOSTI) which is mandated to monitor and facilitate nanotechnology development and commercialization in Malaysia. Identification of companies that produce nanocellulose and use nanocellulose in their production was made prior to a simple survey consisting of their basic information on type of nanomaterial they used in their production. A simple survey was conducted through email and phone calls to assess companies basic information on type of nano they used in their production. Only companies with nanocellulose production were selected for face-to-face survey. A questionnaire form was created for this purpose by the research team under the supervision of an industry specialist.

The critical success factor (CSF) were classified into internal and external success factors that were extracted from the strength and opportunities of products' commercialization through the viewpoint of the manufacturers. These CSFs and the challenges in nanocellulose commercialization will be analyzed through content analysis. Content analysis is one such method to interpret the qualitative data from interviews, open-ended questions, and is expressed through written language (Mariette Bengtsson, 2016). This paper employs summative content analysis, which identifies keywords before and throughout data analysis based on the study's interest. (Hsieh & Shannon, 2005).

RESULTS AND DISCUSSION

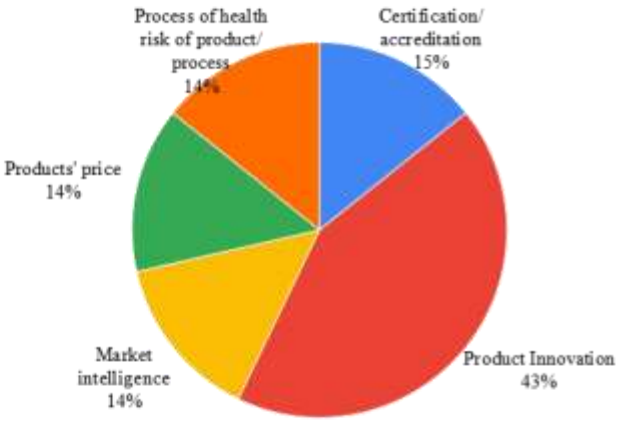
A total of 68 firms were discovered from 142 nanomaterial-based items listed in various sources. The study got 59% response rate from 41 companies. According to the results of the simple survey, four companies employ nanocellulose in their product development processes. This four companeies represents 6% share of nanocellulose based-products manufacturers in nanomaterial industry in Malaysia. Two out of four companies were visited by the research team for face-to-face interviews (Table 1).

Table 1. Nanomaterial based products and nanocellulose companies in Malaysia

Number of nanomaterial based products	142
Total companies identified	68
Total responses received	41
Number of companies use nanocellulose in product development	4
Total nanocellulose companies surveyed	2

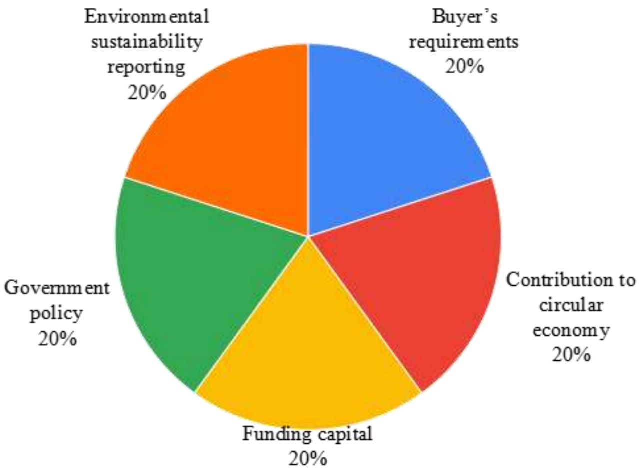
The top five internal success factor of products' commercialization are product innovation, the process or health risk of the product developed, certification or accreditation, market intelligence, and products' price. The main CSF is product innovation which includes the process of product innovation, type of products developed, and internal research, development, and innovation (R&D&I). The OSLO manual 2018 defined product innovation as goods and services include knowledge-capturing products and the product design characteristics.

Figure 1. Internal success factor of products' commercialization



As for the external factors that mostly affected the successful attempts are funding capital, government policy, buyer's requirements, contribution to circular economy, and environmental sustainability reporting. The external factors are are the aspects that constitute a significant opportunity for successful product commercialization.

Figure 2. External success factor of products' commercialization



The challenges in nanocellulose commercialization include a lack of client application development, a lack of industry interest, a lack of economic viability, a high level of technology expenditure, and an institute initiative.

CONCLUSIONS

Overall, the manufacturers recognize that utilizing their strengths in product innovation, the process or health risk of the product developed, certification or accreditation, market intelligence, and products' price, while capitalizing on funding capital, government policy, buyer's requirements, contribution to circular economy, and environmental sustainability reporting, is critical for their future success and growth.

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092-095

EXPLORING DRY LEAF POTENTIAL AS A GREEN RAW MATERIAL AT FOREST RESEARCH INSTITUTE MALAYSIA

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ABSTRACT

Increased awareness of green materials boosts the demand for sustainable products among Malaysians. In a survey conducted by Ipsos Malaysia, Malaysians claimed that 80% reduced the use of single-use plastics and 56% were concerned about the issue of single-use plastics. Malaysia's green technology agenda consists of a number of initiatives and rules designed to increase the use of eco-friendly practices in all industries and minimize reliance on fossil fuels and environmental impact. Currently there are 580 green industries in Malaysia consists of building, energy, transport, waste, and water industries. The previous analysis showed that Malaysia currently offered 10,613 different green products and services. In pulp making process, every biomass that contains cellulose can be processed into pulp/paper products. The product developed not only able to create income to the technology owners, but its' application is also currently on demand globally and has huge potential. Dry leaves have a potential for use as a new raw material for development of green products in Malaysia. These sustainable forest resources can be converted to useful value-added products besides other uses of dry leaves such as compost. Forest Research Institute Malaysia (FRIM) as a man-made forest resource with 92% of the forested area from the total area of 545 ha, produces dry leaves as forest and agricultural residue. Therefore, this study aimed to determine the volume of felling dry leaves that have potential to be a new source of green product development. The result of this study showed the availability of dry leaves available in the area.

Keywords: Dry Leaves, Waste To Wealth, Green Material, Green Products.

INTRODUCTION

Forest Research Institute Malaysia (FRIM) owns 92% of the wooded area on a total of 545 ha and produces dry leaves as forest and agricultural residue. Dry leaves have fallen from trees every day causing a large amount of leaf waste that increases transportation cost and requires more landfill space. These dry leaves have the potential to be used as a novel raw material in the creation of environmentally friendly and sustainable products. Since 1998, wood-based materials have dominated global pulp production. Many academics are exploring for non-wood pulp basic sources to help combat global warming (Razak & Anwar, 2020). Frechka (2023) stated that fallen leaves may be used as raw material instead of wood to reduce the number of trees chopped down. They said that in order to produce 20,000 sheets of A4 paper, 1,681 kg of leaves must be collected, saving up to ten trees from being cut down. Unlike other alternative raw materials that contain cellulose, dried leaves are considered the most relatively inexpensive in comparison to other green materials.

This research is being conducted as part of an effort to uncover a potential new green raw material, coinciding with rising awareness of green materials and demand for sustainable products among Malaysians. Instead of being washed away, these sustainable forest resources can be transformed into useful value-added products.

MATERIALS AND METHODS

The inventory data will be collected for 12 months started on April 2024 at FRIM campus area in Kepong. This is to quantify the availability of dry leaves that FRIM can supply as raw materials for potential new sources products. The information on dry leaves weight that fall from various types of tree species were collected at several locations along the main road and around the selected buildings at the FRIM Kepong campus. The locations involved are the centre of the FRIM campus along the main road, Foxworthy Road and Jelutong Road which houses the administration, human resources, visitor information center, auditorium, herbarium, and FRIM mosque.

In order to extract the information of dry leaves availability and supply, an inventory form were designed. The form includes information of;

i. Location
ii. Coordinates of dry leaves collection
iii. Area coverage of dry leaves collection
iv. Weight of dry leaves with category of dry, wet, green and wood/branches
v. Number of workers involved
vi. Types of tools
vii. Area of dry leave dumped; and
viii. Method of disposal

The form were distributed to each dry leaves collection location to be filled by the person in charge. The data collection of dry leaves follows the administrative department's work schedule, which is carried out on three days per week. The tools used in this exercise is a scraper, a canvas, and a 50-kilogram scale. Every data collection required an estimated two hours, from sweeping to weighing dry leaves before loading into the truck. A quantitative analysis was performed to assess the availability of dry leaves obtained at the study site over the first two months.

RESULTS AND DISCUSSION

The total dry leaves for the six areas involved in the data collection activities is about 1.61 ha. Every collection involved ten workers and a one-tonne lorry before being dumped at FRIM organic waste designated area. The dry leaves data collection usually took one to two hours for each location. The cleaning workers normally conducted the activity in the afternoon to allow the fallen leaves to dry, as dried leaves make the cleaning process easier. The tree stick or branch that has fallen from the tree is separated early during sweeping. Therefore, the ratio of dry leaves in this study to moist leaves, green leaves, sticks, or branches is 100 to zero. The study's early findings revealed that the total amount of dry leaves collected per month was anticipated to be 2,747 kg per ha for a month. More than 30,000 kg of dried leaves are predicted to be accessible in FRIM each year.

Using Frechka's (2023) formula, we can create approximately 356,930 sheets of A4 paper from the estimated potential 30 thousand kilogram availability of dry leaves on the FRIM campus. This is equivalent to 714 reams of 500 sheets of A4 paper each. There are more than 5,000 products made from paper and paper by-products. Today, there are so many useful things that are produced with pulp, paper and paper byproducts. Bandages, coffins, eyeglass frames, hairspray, chewing gum, brushes and comb, handles, fabric softener, liquid soap, lipstick, board games, dollhouses, sports helmets, and pictures are just a few of the unusual paper-based products available today. Some examples of paper byproducts include cellulose, oils, and resins produced during the paper making process.

CONCLUSION

FRIM as a tropical forest institute with the vision to be a leader in tropical forestry commercialization and application is constantly looking into sustainability studies that have a substantial impact and promote economic and social progress. This study is one of the first to investigate the possibilities of forest residues as a new alternative raw material. It has been demonstrated that these dry leaves are extensively available that could be transformed into a more valuable new alternative raw material for green product innovation.

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PLANTATION

004-002

ASSESSING LANDUSE CHANGES ON RUBBER PLANTATION IN KOTA SETAR, KEDAH FOR THE YEARS 2018-2022

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ABSTRACT

The rubber industry in Malaysia remains relevant in producing rubber-based products and contributing to the economic development of Malaysia since the early stages of independence. Changes in land use and land cover are important variables that impact the dynamics of the global environment. They frequently ignite heated discussions and reflect the patterns of growth in certain areas. This study aimed to identify changes on rubber plantation in Kota Setar, Kedah employing a combination of Geographical Information System (GIS) and remote sensing techniques. Specifically, a comparison of land use between 2018 and 2022 was conducted, utilizing Landsat 8/9 OLI-TIRS data along with the Thermal Infrared Sensor. Landsat satellite imagery underwent conversion from TIFF to *img*. format in Erdas Imagine software, preserving the original data integrity. Supervised classification was then applied to categorize the images into distinct classes, including water bodies, paddy field, palm oil, built-up areas, vegetation, forest, and rubber plantation. In the process of the research work using GIS, the land use changes for rubber plantations in the Kota Setar district in 2022 was 18.72% (12417.74367 hectares), compared to 10.63% (7048.867556 hectares) in 2018, representing an 8.09% change in the area. This district is among those experiencing the highest land use change for rubber plantations in the state of Kedah. The study arrived at the conclusion that there has been a significant land use change due to increase in rubber plantation interest in Kota Setar over the period of 5 years. Even though Kota Setar and Kedah mainly recognized for its significant paddy production and is sometimes called the Jelapang Padi State, it was also among the first states to introduce commercial commodities like rubber. This rubber plantation has expanded quickly and significantly boosted the nation's economy. Through the establishment of RISDA responsible for replanting and new planting of rubber trees, it can essentially raise yields more methodically and subsequently have an impact on the GDP growth of the state of Kedah.

Keywords: Landuse Changes, Rubber Plantation, GIS, Landsat, Kota Setar

1.0 INTRODUCTION

The scientific name of the rubber tree is Hevea brasiliensis. The origin of the rubber tree is from the Amazon rainforest in Brazil. The rubber tree is a perennial plant with a straight trunk and taproots. It can grow up to 18 meters tall and has a lifespan of up to 30 years. Rubber trees thrive in climates with temperatures ranging from 26 to 28 degrees Celsius (Hazir et al., 2020). They are required 2000 to 2500 mm of rainfall annually (Hazir et al., 2020). It is also can be grown in well-drained, non-waterlogged soil with adequate drainage and low acidity. Rubber trees can grow in areas up to 1000km north or south of the equator. Rubber trees are grown in countries including Brazil, India, Philippines, Malaysia, Indonesia, and the Philippines. These trees are grown primarily for latex production. Latex is a white fluid that is extracted from the rubber tree trunk when it is tapped. Natural rubber is consumed to produce vehicle tyres (Hirata et al., 2014).

Rubber trees were first planted in Malaysia in the early 1900s. It began with 9 rubber trees brought into Malaya and planted in Kuala Kangsar, Perak. As global demand for rubber increased, more rubber plantations were established in Malaya to meet this demand, starting in 1921. This led to the growth of the rubber industry in Malaysia, becoming a major contributor to the country's economy until the late 1990s. However, the production of natural rubber began to decline with the introduction of synthetic rubber, causing a decrease in the country's rubber output. Nevertheless, there is still demand for natural rubber, and Malaysia remains one of the countries that produce it, alongside Indonesia, Philippines and Thailand. In 2020, Malaysia produced 0.5 million metric tons (3.5%) of natural rubber, placing it seventh globally. (Department of Statistics Malaysia Official Portal, n.d.).

Although many industries that produce products from rubber prefer used a synthetic rubber, the production of natural rubber is still necessary for manufacturing various types of products. Furthermore, behind establishing plantation forests was to fulfil the increasing demand for timber products worldwide (Ratnasingam et al., 2021). Therefore, the rubber industry in Malaysia remains relevant in producing rubber-based products and contributes to the economic development of Malaysia. Malaysia has a wide variety of topographies and landforms. Flat land areas are formed from marine deposits, rivers, and low terraces, while hilly and sloping areas are created from high terraces and sedimentary soils, featuring undulating, rolling, hilly, and mountainous terrain. In the early stages of agricultural development in the country, farmers focused more on cultivating the flat lands due to lower operating costs. However, as most flat land areas became increasingly developed and scarce, the agricultural focus shifted to sloping areas near towns and villages. This shift was driven by better infrastructure development potential, easier access to agricultural inputs and marketing facilities. Land use changes in an area that demand exploitation of the environment have raised various land-use-related issues, such as forest areas being replaced by urban and agricultural areas. There are several factors cause the land use changes such as policy improvements, population expansion, transportation networks, economic issues, and urbanization (Nasir Ahmad & Mustafa, 2019).

In 2013, rubber is the second largest commodity produced in Malaysia after palm oil, contributing 4.7% to the gross domestic product (Sharib & Halog, 2017). Rubber industry has played a significant role in Malaysia’s economy since independence but it has changes because several factors such as the dynamic of global market, climate, policy changes and soil management. This situation raised concerns regarding the trend of changes in the area of rubber replanting from 2018 to 2022, which may reflect larger problems in the management and sustainability of the rubber industry. Firstly, it is necessary to determine how the area of rubber plantations has changed throughout this time, which reflects the effects of economic factors, such as rubber prices, and government support through initiatives such as RISDA. Secondly, it is critical to understand the history of the rubber growing area, especially how changes in land usage and terrain from flat to sloping places impacted the industry. Third, the factors contributing to changes in the area of rubber replanting need to be identified, including changes in policies, population growth, infrastructure development, and economic pressures.

This study is important because it is not only related to the economic sustainability of smallholders and the rubber industry as a whole, but also to broader socio-economic and ecological impacts. Changes in the rubber industry can have profound implications for rural community income, natural resource management, and environmental sustainability. Therefore, understanding these dynamics is crucial for planning effective strategies for the future of the rubber industry in Malaysia.

Ultimately, this study contributes to a broader understanding of how agricultural industries like rubber can adapt and thrive in the face of global and local challenges. This is crucial not only for Malaysia but also for other countries in the Southeast Asian region that rely on rubber cultivation as a primary source of income.

Therefore, this study is not only important in terms of economics and agriculture but also in the context of social development and environmental sustainability, making it relevant to various stakeholders from smallholders to national and international policymakers. The objective of this study is to determine the development of rubber plantation areas from 2018 to 2022.

2.0 STUDY AREA

The state capital of Malaysia's Kedah is Alor Setar. It is one of the most significant cities on Peninsular Malaysia's west coast and the second-largest in the state after Sungai Petani. Alor Setar has long since emerged as a city that is so fast and advanced in many respects. Starting with its role as a new state administrative centre in 1735, Alor setar continued to make stimulating progress until the city emerges as a fast-paced commercial centre with economic, agriculture and commercial activities (Yusof et. al 2021). In general, the research area's annual rainfall discharge for the Kota Setar District, Sik, and Padang Terap (south) is approximately 2400 mm, whereas the area's annual rainfall is lower in the north, near the District Kubang Pasu and Padang Terap of 2200 millimeters. However, this region frequently has exceptionally high rainfall, which can lead to flooding (Husain et. al 2015). Alor Setar is situated in the Copenhagen climate welding's tropical monsoon climatic zone which the rainy season is long in Alor Setar.

The Malaysian Peninsula's western region is home to Alor Setar. Setar City District, often known as Alor Setar Town, is home to more than 417,800 people in Kedah as of 2020. Additionally, Kubang Pasu, Sena Tree, and Pendang districts are bordered by it. The city of Alor Setar is situated on the shore of the Malacca Strait, which divides Indonesia and Malaysia, at 6°7′N and 100°22′E. The city's 666 km² overall territory is currently being reduced to 424 km² as Sena Trees is becoming a new district. Significant river systems, including the Kedah, Langgar, Tajar, Anak Bukit, Malai, and Red Alor, encircle Alor Setar.

Figure 1. Study Area of Kota Setar , Kedah

3.0 RESEARCH METHODOLOGY

To access information, data is required. Before beginning any GIS project, it needs to be ascertained. The project's conclusion will depend on the quality of the data. The source limit determines the precise data. Every kind of data are supposed to be predicated on appropriate structure, precise and dependable, up-to-date, high-quality and completely necessary.

There are two types of data which is primary and secondary data. The initial data source from which the researcher gathers data for a particular project or goal is known as primary data. Secondary data, on the other hand, is information that has been gathered in the past for different reasons and by parties unrelated to research projects. Primary data sources were used to obtain and compile all of the survey's data. Landsat 8/9 OLI-TIRS satellite image data for the research area's years 2018 and 2022 were used in this research. Landsat 8/9 satellite data has a 30m space resolution that is suitable for land-use change detection applications. The research process flow chart until the creation of the thematic map is displayed in Figure 2.

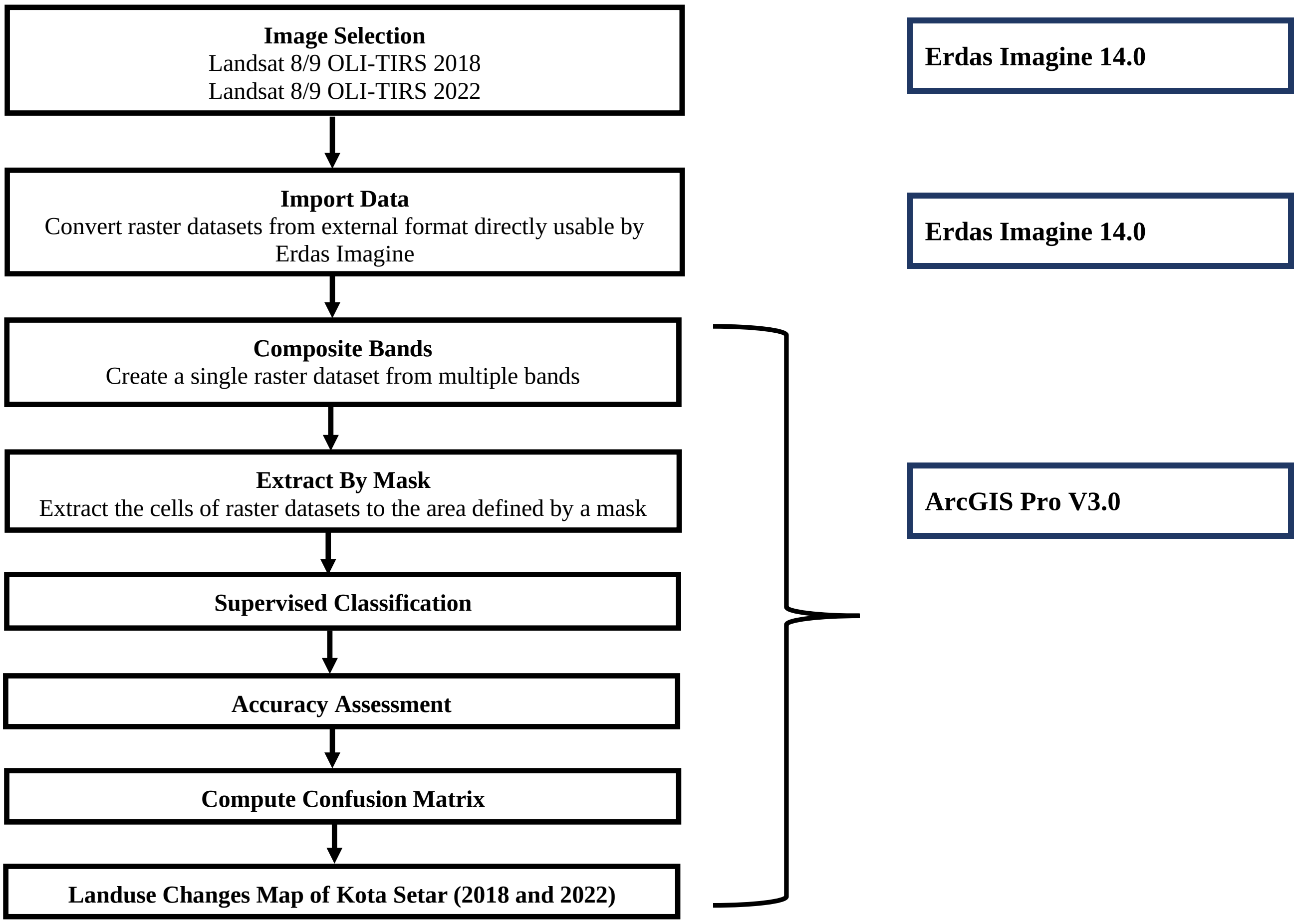

Figure 2. Satellite image processing and land use analysis flowchart

3.1 SATELLITE IMAGE DATA

This study uses satellite images that was acquired from the United States Geological Survey website (https://earthexplorer.usgs.gov/) to investigate changes in land use that occurred between 2018 and 2022. The main sources of information for the land cover classification were Landsat 8/9 OLI-TIRS for the years 2018 and 2022, which correspond to February 16, 2018, and December 9, 2022, respectively. The satellite image was selected because, in comparison to the other dates, it is the best observed image and has reduced cloud cover, especially in the research area.

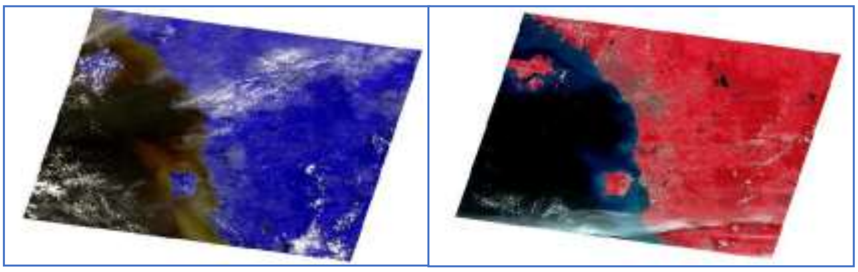

Figure 3. Landsat 8/9 OLI-TIRS satellite images for 2018 (left) and 2022 (right)

3.2 IMAGE PRE-PROCESSING

It is necessary to do various pre-processing steps on the satellite data that is obtained from the USGS website, such as converting digital pixel values to surface reflectance values and performing geometric correction to address any distortion that may be present. Once the pre-processing processes are completed, the composite bands and extract using mask tools are applied.

The images that were obtained from the USGS encompass a large area, including the sea and also parts of another district of Kedah. The environment of the research area is covered in photographs created by the Extract by Mask technique. By using the "Extract by Mask" tool, it can provide the input raster, the output raster's name and location, and the raster or object that will be used to determine the areas to be extracted (the input raster or feature mask data).

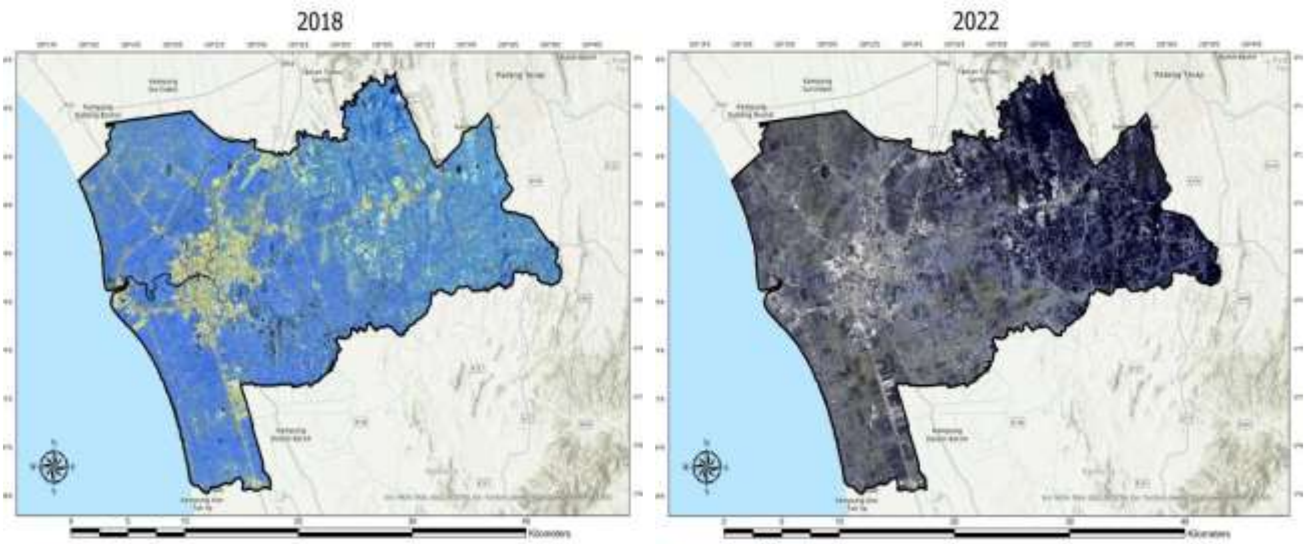

Figure 4. False color composite as display for Landsat 8/9 OLI-TIRS satellite images with combination bands of 5,4,3 RGB for 2018 (left) and 2022 (right)

3.3 IMAGE CLASSIFICATION

Supervised and unsupervised classification are two methods that can be applied to the classification of remote sensing data. A supervised classification method is used in this study's image classification procedure to classify images into many categories, including forests, built-up areas, agricultural, water bodies, open spaces, and vegetation.

In a supervised categorization, fieldwork, aerial picture interpretation, map analysis, and firsthand knowledge are used to determine the identification and location of some land-cover types (such as urban, agricultural, or wetland) a priori. In the remotely sensed data, the analyst looks for certain locations that are representative of these recognized land-cover types in a homogenous manner.

Because the spectral features of these well-known locations are utilized to train the classification algorithm for the ultimate land-cover mapping of the remaining portion of the picture, these areas are often referred to as training sites. For every training site, multivariate statistical parameters (such as means, standard deviations, covariance matrices, correlation matrices, etc.) are computed. After that, each pixel—both inside and outside of the training sites—is assessed and put into the class that it most likely belongs to.

3.4 MAXIMUM LIKELIHOOD CLASIFICATION ALGORITHM

Although the Maximum Likelihood Method (MLM) is one of the most widely used classification techniques in the field of remote sensing, there have been some drawbacks to it as well (J Susaki et. al 2000). A maximum likelihood (ML) classifier is a potent classification method that relies on the quality of training samples, which are often chosen using local knowledge and ground-trusted land cover maps. ML classifiers are based on the maximum likelihood decision rule. It is typically chosen by many remote sensing data consumers to categorize land covers globally due to its applicability, objectivity, and excellent discrimination between land covers (Asmala et. al 2012). Generally, depending on the model's assumptions and how it performs the classification, these techniques can be classified as either statistically based procedures or data mining (J Hogland et. al 2013).

3.5 ACCURACY ASSESSMENT OF CLASSIFIED IMAGE

The measurement of estimation to classification conditions using a remotely sensed dataset is called accuracy assessment. It is helpful in assessing the effectiveness of a classification strategy and in identifying potential errors (Z Abbas et. al 2020). It contrasts the categorized image with data from a different source that is regarded as reliable or ground truth. It is possible to get ground truth data in the field, but doing so requires money and time. Interpreting existing classified imagery, highresolution imagery, and GIS data layers can also yield ground truth data. The accuracy assessment in this research evaluates the categorized result's accuracy using a reference dataset. Creating a set of random points from the ground truth data and comparing it to the classified data in a confusion matrix is the most popular method for evaluating the accuracy of a classified image. Table 1 and 2 shows the accuracy assessment of Landsat 8/9 image year 2018 and 2022 respectively.

Classification	Water Bodies	Paddy Field	Palm Oil	Built-up Areas	Vegetation	Forest	Rubber	Total
Water Bodies	14	1	0	0	0	0	0	15
Paddy Field	0	20	0	0	0	0	0	20
Palm Oil	0	1	14	0	1	0	4	20
Built-up Areas	0	1	0	19	0	0	0	20
Vegetation	0	1	0	0	19	0	0	20
Forest	0	0	0	0	0	19	1	20
Rubber	0	0	0	0	0	1	19	20
Total	14	24	14	19	20	20	24	135

Table 2. Accuracy Assessment of Landsat 8/9 Image year 2022

Classification	Water Bodies	Paddy Field	Palm Oil	Built-up Areas	Vegetation	Forest	Rubber	Total
Water Bodies	15	0	0	0	0	0	0	15
Paddy Field	0	35	0	0	0	0	0	35
Palm Oil	0	0	17	0	0	0	3	20
Built-up Areas	0	3	0	20	2	0	0	25
Vegetation	0	14	0	0	6	0	0	20
Forest	0	0	0	0	0	17	3	20
Rubber	0	0	0	0	1	0	29	30
Total	15	52	17	20	9	17	35	165

Table 1. Accuracy Assessment of Landsat 8/9 Image year 2018

A set of 165 and 135 random sampling points was generated on the Landsat images of 2018 and 2022 using GIS application. The Landsat satellite image was classified into 7 categories such as water bodies, paddy field, palm oil, built-up areas, vegetation, forest and rubber.

For generating sample points of Landsat image 2018, there are 14 random samples of water bodies, 24 random samples of paddy field, 14 random samples of palm oil, 19 random samples of built-up areas, 29 random samples of vegetation, 20 random samples of forest and 24 random samples of rubber. Otherwise for Landsat image 2022, there are 15 random samples of water bodies, 52 random samples of paddy field, 17 random samples of palm oil, 20 random samples of built-up areas, 9 random samples of vegetation, 17 random samples of forest and 35 random samples of rubber.

The Kappa Coefficient is then generated from a statistical test to evaluate the accuracy of a classification. Kappa essentially evaluates how well the classification performed as compared to just randomly assigning values, i.e., did the classification do better than random. The formula for
Cohen's Kappa is calculated as:

where:
Po: Relative observed agreement among raters
Pe: Hypothetical probability of chance agreement
K : Kappa coefficient value

5.0 RESULTS AND DISCUSSION

5.1 CONFUSION MATRIX STATISTICS

According to Table 3, the results obtained from the comparison of random point sampling for Landsat images in 2022 are as many as 15 out of 15 random points for water bodies, 35 out of 52 random points for paddy field, 17 out of 17 random points for palm oil, 20 out of 20 points random for built-up areas, 6 out of 9 random points for vegetation, 17 out of 17 for forest and 29 out of 35 random points for rubber. The overall result shown that 139 out of 165 sampling points for the classification image of 2022 are accurate with the comparison that has been made with the reference image.

Table 3. Landuse changes matrix in 2022 (hectares)

Classification	Water Bodies	Paddy Field	Palm Oil	Built-up Areas	Vegetation	Forest	Rubber	Total	User Accuracy	Kappa
Water Bodies	15	0	0	0	0	0	0	15	100%	0
Paddy Field	0	35	0	0	0	0	0	35	100%	0
Palm Oil	0	0	17	0	0	0	3	20	85%	0
Built-up Areas	0	3	0	20	2	0	0	25	80%	0
Vegetation	0	14	0	0	6	0	0	20	30%	0
Forest	0	0	0	0	0	17	3	20	85%	0
Rubber	0	0	0	0	1	0	29	30	96.67%	0
Total	15	52	17	20	9	17	35	165	0	0
Producer Accuracy	100%	67.3%	100%	100%	66.67%	100%	82.85%	0	84.24%	0
Kappa	0	0	0	0	0	0	00	0	0	81.15%

$$\text{Overall Accuracy} = \frac{\text{Total Number of Correctly Classified Pixels}}{\text{Total Number of Reference Pixels}} \times 100\%$$

$$= \frac{(15 + 35 + 17 + 20 + 6 + 17 + 29)}{165} \times 100\%$$

$$= (268 / 308) \times 100$$

$$= 84.24\ \%$$

The kappa coefficient of agreement was introduced to the remote sensing community in the early 1980s as an index to express the accuracy of an image classification used to produce a thematic map (Congalton et al., 1983; Rosenfield and Fitzpatrick-Lins, 1986). The results of the calculations that have been obtained indicate the reliability index of the kappa coefficient as a whole is 81.15%. This also indicate that the reliability of the land use that has been interpreted and classified is high using the remote sensing approach.

5.2 LANDUSE IN KOTA SETAR

There are seven categories which have been classified using a supervised classification method such as water bodies, paddy field, palm oil, built-up areas, vegetation, forest and rubber. The thematic land use map that has been produced for 2018 and 2022 is shown in Figure 6. Meanwhile Figure 7 shows the rubber landuse changes map on Kota Setar district for the years 2018 and 2022.

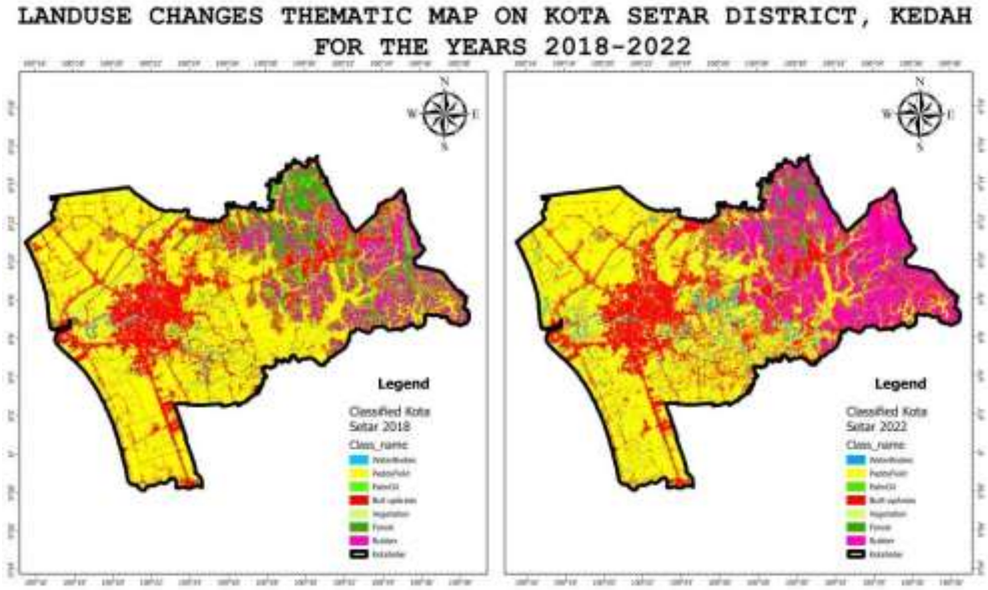

Figure 6. Kota Setar land use thematic map in 2018 (left) and 2022 (right) after the supervised classification

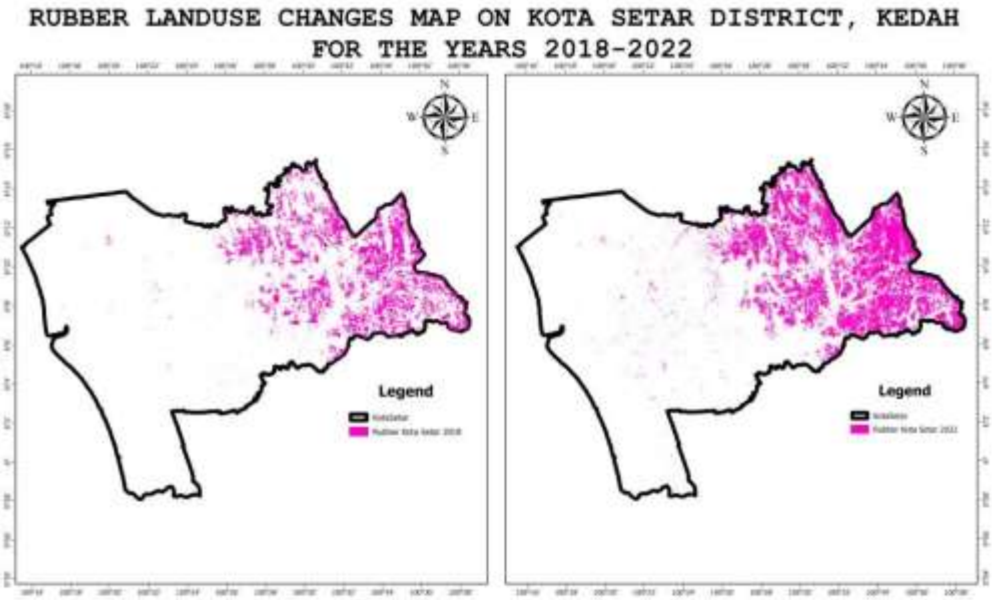

Figure 7. Rubber Landuse Changes map on Kota Setar district, Kedah for the years 2018 to 2022

5.3 CHANGE DETECTION OF RUBBER LAND USE IN KOTA SETAR

Table 5 shows the matrix of land use changes in the study area that took place between 2018 and 2022. Rubber land use changes have experienced an increase in 2022 was 18.72% (12417.74 hectares) compared to 2018 was 10.63% (7048.87 hectares). A total of 8.09% of the area changes. This is one of the highest rubber planting areas in the state of Kedah. The land use that contributed to the increase in rubber land use is built-up area (1079.55 hectares), forest cover area (1733.40 hectares), paddy field (567.27 hectares), paddy field area (2972.03 hectares), vegetation area (204.24 hectares) and water bodies (5.89 hectares).

Table 5. Landuse changes matrix in 2018 and 2022 (hectares)

		2018							
		Built-up Areas	Forest	Paddy Field	Palm Oil	Rubber	Vegetation	Water Bodies	**Total (2018)**
2022	Built-up Areas	9816.30	186.10	2494.77	774.08	575.70	609.73	79.11	14535.80
	Forest	77.71	1076.38	144.80	257.83	256.24	32.11	0.79	1845.87
	Paddy Field	3045.86	116.58	24861.71	959.38	197.59	1625.83	484.39	31291.35
	Palm Oil	71.33	299.37	110.31	631.11	123.95	19.91	0.59	1256.58
	Rubber	1079.55	1733.40	567.27	2972.03	5855.35	204.24	5.89	12417.74
	Vegetation	560.55	14.89	1141.84	154.54	34.93	316.01	17.68	2240.44
	Water Bodies	280.98	8.97	1896.39	64.56	5.09	103.63	394.06	2753.68
	Total (2022)	14932.30	3435.70	31217.09	5813.52	7048.87	2911.46	982.52	66341.46

The findings also indicate some very insignificant changes in land use categories, such as the conversion of built-up land to vegetation or forest. Errors in interpretation or classification—where one land cover is mistaken for another causing these kinds of changes. This can easily occur when open land is designated as built-up land or when land usage resembles vegetation cover, making it impossible to distinguish between different plants. This is mostly because there are only so many wavelength bands available in Landsat satellite photos, which limits the properties of surface reflection that can be distinguished.

6.0 CONCLUSION

Satellite image analysis carried out in eleven districts in the state of Kedah has shown an interesting pattern in the use of land for rubber crops between year 2018 and 2022. The Kota Setar district recorded a very high increase, which is 76.17%, indicating a successful effort in increasing rubber production in the area. This increase may be associated with soil conditions that are more suitable for rubber growth, or may be the result of development initiatives aimed at promoting rubber production. Although there are variations between other regions, the added land use area for rubber crops in Kedah shows a positive value with an increase of 5.04% between year 2018 and 2022. This reflects the ability of the rubber industry to grow and adapt in the face of challenges, as well as proving the importance of this sector in the agricultural economy of the state of Kedah. In conclusion, satellite image analysis provides a comprehensive view of the dynamics of the rubber industry in Kedah, revealing the diversity of the situation in each district. In general, there is an increasing trend, reflecting the potential for the development of the rubber industry in the state of Kedah. The results of this analysis are important in helping planning and implementation for stakeholders in the rubber industry, to ensure the sustainability and growth of this industry in the future.

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VARIABILITY OF OIL QUALITY TRAITS IN MPOB- OIL PALM (*Elaeis guineensis* Jacq.) GERMPLASM COLLECTION

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ABSTRACT

The oil palm germplasm collection has become the main source in MPOB's research to produce elite planting materials. A total of 11 germplasm collected from Nigeria, Cameroon, Zaire, Madagascar, Tanzania, Angola, Senegal, Sierra Leone, Guinea, Ghana and Gambia have been planted at MPOB Kluang Research Station and evaluated for fatty acid compositions, iodine value and carotene content using Gas Chromatography and UV-Vis Spectrophotometer. Analysis of variance (ANOVA) showed highly significant differences for all studied traits indicating presence of high genetic variability within the germplasm populations. For fatty acid composition, MPOB-Senegal, Guinea and Gambia had the lowest palmitic acid with the highest oleic acid. MPOB-Madagsacar germplasm showed unique characteristics with highest level of linolic acid which contribute to highest iodine value. Most of the germplasm evaluated had carotene content more than 1000 ppm, which is higher than current oil palm planting materials (500-700 ppm). The phenotypic correlations were negative between palmitic with stearic and oleic acid. Positive correlation was found between iodine with both unsaturated fatty acids. Carotene exhibited no association with IV and low correlation with fatty acid composition. The findings from this study are useful for developing planting materials with better oil quality.

Keywords: Oil Quality, Fatty Acid Composition, Germplasm, Oil Palm.

INTRODUCTION

Oil palm is one of the major drivers to the development of Malaysian economic. In 2023, the crude palm oil (CPO) production has increased by 0.5%, reaching approximately 18.55 million tonnes compared to 2022 despite a 0.4% reduction in planted area owing to replanting activities (MPOB, 2023). The oil palm in Malaysia begins with the seeds originated from the four Bogor palms and knowns as Deli *dura*. These seeds become one of the important commercial breeding lines that revolutionised Malaysia's agriculture sector. The narrowness of the oil palm gene pool in Malaysia has triggered MPOB to implement a genetic resources programme through collection, evaluation, utilization and conservation for future improvements of planting materials.

The first germplasm collection of oil palm (*Elaeis guineensis* Jacq.) was initiated in Nigeria in 1973 followed by Cameroon (1984), Zaire (1984), Tanzania (1986), Madagascar (1986), Angola (1991, 2010), Senegal (1993), Gambia (1993), Sierra Leone (1994), Guinea (1994) and Ghana (1996). Currently, Malaysia housed the world's largest oil palm germplasm collection which has been conserved in the form of a field genebank at MPOB Kluang Research Station, Johor. Concerted efforts must be conducted through rigorous characterisation and evaluation to identify the potential and actual value of these wild germplasm material before being incorporated into improved breeding lines (Kumar and Kaur, 2010).

Generally, palm oil contains an equal proportion of saturated and unsaturated fatty acid. Of these saturated fatty acids, approximately 44% is palmitic acid (C16:0), 5% is stearic acid (C18:0), and trace amount of myristic acid (C14:0). Meanwhile, the unsaturated fatty acid contents are approximately 40 % oleic acid (C18:1), and 10% polyunsaturated linoleic acid (C18:2) and linolenic acid (C18:3) (Montoya *et al*., 2014). One of the oil palm breeders' goals is to improve palm oil quality for a higher degree of unsaturated fatty acid. Therefore, this study is undertaken to assess fatty acid composition (FAC), iodine value (IV) and carotene content of all collections of *E. guineensis* germplasm in order to facilitate selection of breeding materials for future development of value-added oil palm planting materials in this country.

MATERIALS AND METHODS

Plant Materials

E. guineensis germplasms collected from 11 countries of Africa were planted in the form of 'open-pollinated' families in MPOB Kluang Research Station, Johor, Malaysia. The palms were planted either in a randomized complete block design (RCBD) or incomplete randomized design (ICRD). This study was carried out on 11701 *E.guineensis* palms from germplasm collection (*Table 1*). Both of the *dura* and *tenera* fruit forms from all germplasm were evaluated for FAC and IV. Out of 11 germplasm, only 9 germplasm were evaluated for carotene content including Nigeria, Madagascar, Angola, Senegal, Gambia, Sierra Leone, Guinea and Ghana.

Table 1: Number of samples representing the *E.guineensis* germplasm used in this study.

No	Germplasm	No. of samples (n)
1.	Nigeria	4098
2.	Cameroon	1504
3.	Zaire	2476
4.	Madagascar	18
5.	Tanzania	598
6.	Angola	1039
7.	Senegal	550
8.	Sierra Leone	498
9.	Guinea	511
10.	Ghana	258
11.	Gambia	151
	Total	11701

Sample collection and preparation

Ripe fruit bunches were harvested and chopped into three separate portions; apical, middle and basal. The samples spikelets were selected at random from each portion to represent a balanced composition of the fruit set. The oil from fruits was conventionally extracted using solvent extraction method. The spikelets were softened by autoclaving for 45 minutes and later fruit mesocarp was dried in the oven at 105ºC for 3 hours. The dried mesocarp was ground using a blender and mixed with hexane. The crude mixture of oil and hexane was then separated using a rotary evaporator, and the oil converted to methyl esters before the FAC was determined.

Oil analysis

i. Fatty acid composition (FAC)

Oil samples are homogenized after being melted at 60-70ºC. A weighted of 0.1 g of the oil samples was placed into a 7 ml vial. Using a pipette, 1.9 ml of hexane solvent was added into the vial. The vial was capped and shaken to dissolve the oil. 0.1 ml of sodium methoxide was added into the vial and the mixture was vortexed for 5 seconds. The clear upper layer was pipetted into a Gas Chromatography (GC) vial bottle and sealed with Teflon cap for GC-MS analysis.

Fatty acid methyl esters (FAMEs) from sample derivatization were injected into GC instrument and the GC analyses were performed on 7890B Gas Chromatography System (Agilent Technologies, California, US). Fatty acids components were displayed based on peaks on the chromatogram.

ii. Iodine Value (I.V)

IV was determined based on the official method introduced by America Oil Chemist's Society (AOCS) official methods (Kyriakidis and Katsiloulis, 2000). IV is calculated by combining the following unsaturated fatty acids components which were gathered from the data output based on GC:

IV = (Oleic acid (C18:1) x 0.8601) + (Linoleic acid (C18:2) x 1.732) + (Linolenic acid (C18:3) x 2.616) + (Palmitoleic acid (C16:1) x 0.9504)

iii. Carotene analysis

The carotene content was determined by using a spectrophotometer. A weighted of 0.1 g of the oil samples was placed into a 25 ml of volumetric flask. The oil samples were dissolved with a few milliliters of iso-ocatne and diluted up to a volume of 25 ml. The solution was transferred into cuvette and the absorbance at 446 nm was measured using a UV-Vis spectrophotometer. The beta carotene was calculated using this formula:

Carotene content (ppm) = $$\frac{25 \times 383 \times \text{absorption rate}}{100W}$$

where W is sample weight.

Statistical analysis

Data on FAC, IV and carotene were systematically extracted from the MPOB-Breeding Information System (MPOB-BIS) (Mohd Din *et al*., 2012). All the data were then analysed for simple analysis of variance (ANOVA) by using SAS 9.4, while the comparison between means was by Fisher's Least Significant Difference (LSD) at 5% level of probability. The relationship between the traits were computed and determined by the simple correlation method using the PROC CORR of the SAS programme.

RESULTS AND DISCUSSIONS

Germplasm performance

Mean square (MS) (*Table 2a and 2b*) showed a highly significant variation for all studied traits among the germplasms. Palmitic acid (C16:0), a saturated fatty acid is the major component of refined palm oil had the average of 39.75% (*Table 3*). The oil palm germplasm from Senegal, Guinea and Gambia had the lowest level C16:0 around 37%, whilst the highest levels were found in Madagascar, at 47%. Tan *et al*., (1981) found that commercial DxP material in Malaysia ranges from 41.8% to 46.8%. Meanwhile, Sujadi *et al.* (2016), who study the composition of fatty acid in various background of DxP commercial planting material in Indonesia reported the C16:0 ranged from 44.3% to 47.8%. As recommended by Tan *et al*. (1986), a lower content is preferred and the ideal C16:0 level is below than 25%. The lower value of C16:0 is more favourable to oil palm breeders as palmitic acid is the primary constituent of stearin which will raise the olein and influences the price fraction of palm oil. However, the richest sources of C16:0 in palm oil have several used in various industries such as surfactant, cosmetics, textile manufacturing and etc.

Table 2a: Mean square of fatty acid composition and iodine value in MPOB-*E. guineensis* oil palm germplasm.

Source of Variation	d.f	Mean squares				
		C16:0	**C18:0**	**C18:1**	**C18:2**	**IV**
Germplasm	10	1211.75**	215.46**	3331.64**	931.50**	2861.82**
Error	11690	9.32	1.74	9.89	2.47	7.64

Note: C16:0- Palmitic acid; C18:0- Stearic acid; C18:1- Oleic acid; C18:2-Linoelic acid; IV- Iodine value
** Significant at P<0.01
d.f -degree of freedom

Table 2b: Mean square of carotene value in MPOB-*E.guineensis* oil palm germplasm.

Source of Variation	d.f	Mean squares

		Carotene
Germplasm	8	118797296.00**
Error	4087	320840.00

Note: ** Significant at P<0.01
d.f = degree of freedom

Stearic acid (C18:0) is the second saturated fatty acids in palm oil. Madagascar germplasm which had higher C16:0 content, had the lowest C18:0 at 2.77%, whereas other *E.guineensis* accessions showed higher C18:0 level ranging from 4.78% to 6.02%. This result indicated that the germplasm had the diverse C18:0 content compared to commercial DxP materials, which ranged between 4.2% and 5.1% (Tan and Oh, 1981). There were no significant differences in the C18:0 content of crude palm oil from nine varieties of oil palm commercial materials as reported by Sujadi *et al.* (2016). Similar to C16:0 as saturated fatty acid, low C18:0 is more benefical for the palm oil industry in terms of improving palm oil quality and extending its application. High C18:0, nevertheless, could also be considerable since it is a quiet game-changer in oleochemical industry for the production of various products owing to its stabilising and high melting point properties.

Table 3: Performance of oil quality in MPOB-E.guineensis oil palm germplasm.

No.	Germplasm	N	C16:0 (%)	C18:0 (%)	C18:1 (%)	C18:2 (%)	IV	Carotene (ppm)
1	NGA	4098	39.92^{cd}	6.02^{a}	41.35^{d}	10.32^{d}	53.59^{g}	1166.75^{de}
2	CMR	1504	39.31^{de}	5.48^{b}	42.37^{c}	11.20^{c}	56.85^{d}	-
3	ZRE	2476	40.27^{c}	5.23^{bc}	41.29^{d}	11.63^{b}	56.51^{d}	-
4	MDG	18	47.09^{a}	2.77^{e}	27.96^{e}	20.65^{a}	60.30^{a}	1057.97^{e}
5	TZA	598	41.64^{b}	4.88^{d}	41.41^{d}	10.49^{d}	54.60^{f}	1178.95^{de}
6	AGO	1039	40.30^{c}	5.00^{cd}	41.49^{d}	11.74^{b}	56.89^{d}	737.62^{f}
7	SEN	550	37.37^{f}	5.53^{b}	45.24^{b}	10.46^{d}	58.28^{b}	2005.85^{b}
8	SLE	498	39.17^{e}	5.34^{b}	45.19^{b}	9.22^{f}	55.47^{e}	1310.30^{d}
9	GUI	511	37.14^{f}	5.99^{a}	46.73^{a}	9.07^{f}	56.52^{d}	1640.75^{c}
10	GHA	258	40.38^{c}	4.78^{d}	44.58^{b}	9.09^{f}	54.78^{f}	1990.12^{b}
11	GAM	151	37.50^{f}	5.35^{b}	46.05^{a}	9.81^{e}	57.54^{c}	2307.69^{a}
	Mean	11701	39.75	5.54	42.18	10.73	55.49	1341.50
	LSD		0.70	0.30	0.72	0.36	0.64	144.83

Note: NGA-Nigeria; CMR- Cameroon; ZRE- Zaire ; MDG- Madagascar; TZA- Tanzania; AGO- Angola; SEN- Senegal; SLE-Sierra Leone; GUI- Guinea; GHA - Ghana; GAM- Gambia; C16:0- Palmitic acid; C18:0- Stearic acid; C18:1- Oleic acid; C18:2- Linoelic acid; IV- Iodine value.

Means with the same letter are not significantly different at p≤0.05 based on Least Significant Difference (LSD)

Oleic acid (C18:l) is monounsatured, the abundant single fatty acid constituent of palm oil. All *E.guineensis* accessions recorded oleic acid content more than 40%, ranging from 41.29% to 46.73%, with the exception of Madagascar germplasm which had the lowest C18:1 at 27.96%. The C18:1 content of *E.guineensis* germplasm is slightly higher than in DxP materials which reported by Tan and Oh (1981) (37.3%-40.8%). Meanwhile, Sujadi *et al.* (2016) observed the C18:1 value in DxP commercial materials between 35.8% and 44.3%. Regardig the polyunsaturated fatty acid, the result showed that *E.guineensis* germplasm had the C18:2 ranged from 9.07% to 20.65%, with the mean of 10.73%. The mean of C18:2 in this study is comparable with the average DxP materials reported by Sambathanmurthi *et al*. (2000) and Sujadi *et al*. (2016). Is it interesting to note that unsaturated fatty acid in Madagascar is unique as it is high in C18:2 (20.65%) and lower level in C18:1. Currently, improvement of palm oil quality for a higher degree of unsaturation fatty acid (especially C18:1) is one of the goals of oil palm breeders as there is high demand for high oleic crops in food industry due to its resistance to oxidation (Suresh and Behera, 2020).

The IV, a multi-parameter measure of the global degree of unsaturation of fatty acids and reflects the liquidity of vegetable oil. In this study, the result showed that all the *E.guineensis* accessions had IV ranging from 53.39 to 60.30, higher than current commercial DxP (50-53). The best IV was found in Madagascar germplasm owing to its highest C18:2 content, followed by Senegal and Gambia germplasm. Rajanaidu *et al.* (1989) reported that the IV of individual performance in MPOB germplasm collection more than 60 and with the fractionation, the olein content should have attained IV close to 70. Compared with *E.guineensis*, the American oil palm collections known as *E.oleifera* has high level of IV up to 90 as it has higher level of C18:1 and C18:2 (Rajanaidu *et al.*, 2000). Breeding for a high IV planting materials is one of the main goals to cater the global trend in edible oil consumption towards the liquid oil. To achieve the objective for high unsaturated and liquid palm oil, one of the breeding stratergy is to take advantage of the traits present in *E. oleifera* palms using interspecific hybridisation and backcross without jeopardising the current yield (Montoya *et al*., 2014) .

High carotene content is desirable and useful in food and pharmaceutical industry. Based on the result, the *E.guineensis* accesions had carotene content between 700 and 2307 ppm, with the average of 1341 ppm. These result indicated that the *E. guineensis* germplasm contained high carotene content compared to current commercial DxP materials, 500-700 ppm. With high IV, Gambia germplasm had the highest carotene content, while the lowest was found from Angola. The wider carotene range is seen as an advantages to facilitate breeding for higher carotene palm oil. Mohd Din *et al.* (2006) found five individual palm from Tanzania germplasm which had high carotene content >2000 ppm with high fresh fruit bunch and oil yield. With interspecfic hybrid, the carotene content is some individual palms showed an oustanding value up to 3000 ppm (Alyasa *et al*., 2021).

Correlations

Simple correlation coefficients between the traits are given in *Table 4*. C16:0 showed a correlated negatively and significantly with C18:0 and C18:1, which is consistent with findings by Noh *et al.* (2002). This result indicated that the amount C18:1 decreased with the increasing of C18:0, and vice versa. Similar results were observed between unsaturated fatty acid. Negative correlation was observed between C18:1 and C18:2 indicated that an increase in one will cause a reduction in the other, which will affect the IV as both fatty acids are contributor to IV in palm oil. Meanwhile, IV is showed positive correlation with both unsaturated fatty acids, whilst negative to saturated acid as reported by Lamaisri *et al*. (2015). As expected, carotene exhibited no association with IV and low correlation with fatty acid composition.

Table 4: Simple correlation coefficient between oil quality traits on MPOB-*Elaeis guineensis* oil palm germplasm.

	C16:0	C18:0	C18:1	C18:2	IV
C18:0	-0.49**				
C18:1	-0.72**	0.04**			
C18:2	0.03**	-0.02*	-0.54**		
IV	-0.68**	-0.01ns	0.45**	0.50**	
Carotene	-0.24**	0.10**	0.30**	-0.24**	0.09**

Note: C16:0- Palmitic acid; C18:0- Stearic acid; C18:1- Oleic acid; C18:2- Linoelic acid.
*, **, and ns indicate significant at $p \leq 0.05$, $p \leq 0.01$ and not significant, respectively.

CONCLUSION

The MPOB-*Elaeis guineensis* germplasm showed wide variability in FAC, IV and carotene content. The uniqueness of Madagascar germplasm with highest C18:2 and IV has potential to be developed for improving oil palm qualities. The variations in FAC and IV in the germplasm collection could be exploited for breeding palms possessing higher unsaturated fatty acid. The negative correlation between C16:0 and C18:1 in the MPOB oil palm germplasm should be advantages in breeding. Selecting palm for high monounsaturated fatty acids, would likely be reducing the saturated fatty acid. It is in line with interest of palm oil industry to produce more liquid palm oil since it has the potential to open up markets for oil palm in the liquid oil sector and would compete more effectively with other oil crops. However, further studies should be carried out such as progeny testing to improve the genetic materials.

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PRODUCTIVITY OF RISDA RUBBER SMALLHOLDERS IN MALAYSIA

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ABSTRACT

Rubber Industry Smallholders Development Authority (RISDA) introduced the Mature Rubber Program under the Rubber Plantation Restructuring Program (PKG) during the 7th Malaysia Plan. Further improvements were made during the 8th and 9th Malaysia Plans by implementing the Rubber Productivity Improvement Programme (PPG). These efforts continued in the 10th and 11th Malaysia Plans with enhancements to three programs: the Rubber Productivity Improvement Programme (PPG), the Rubber Productivity Incentive Programme (SIPG), and the Marketing Infrastructure Programme (PIP). This study was conducted to identify the productivity of RISDA rubber smallholders who participated in productivity improvement programs under the guidance and supervision of RISDA to assess the extent of the impact of these programs in enhancing their productivity. This study was designed to determine the factors contributing to RISDA rubber smallholders' productivity in 13 states in Malaysia and it undertakes a descriptive research design using a quantitative approach as the methodology for the research. The key variables in the research framework are individual awareness, environment and situation, and management of rubber production. Data analysis is based on 428 respondents' feedback from the RISDA rubber smallholders in Malaysia and carried out using the SPSS 27.0 version. Linear regression was used to test the hypotheses. The results revealed that all three hypotheses were accepted. The programs implemented by RISDA have proven effective in helping RISDA smallholders improve productivity while increasing their income. Therefore, these programs should be continued, expanded, and improved for the overall welfare of smallholders.

Keywords: Rubber Productivity, RISDA Rubber Smallholders, Smallholder Income.

INTRODUCTION

Malaysia has historically been one of the largest producers of natural rubber globally. However, by 2014, its contribution to global production had declined, positioning Malaysia as the third-largest producer worldwide. Leading rubber-producing countries, including Thailand, Indonesia, Malaysia, Vietnam, India, and China, collectively contribute 90% of the world's rubber production. Rubber produced in Malaysia is exported to countries such as China, Germany, the United States, and Iran to be processed into rubber-based products like vehicle tyres and gloves (Abdullah, I. & Arshad, F.M., 2017).

Natural rubber is obtained through tapping, primarily conducted by smallholder farmers who rely on rubber cultivation as their main source of income. Unfortunately, there has been a consistent decline in rubber planting areas and productivity rates (Abdullah, I. & Arshad, F.M., 2017). This decline has resulted in a falling rubber process and increased poverty rates among many smallholders. Consequently, smallholders depending on latex production have sought alternative income sources to sustain their livelihoods (Siti Murni et al., 2018). Despite efforts by the Integrated Agricultural Development Project, poverty rates remain high, especially among smallholders. One proposed solution to this issue is the implementation of income-generating projects to create employment opportunities (Nor Diana et al., 2022). Furthermore, reliance on rubber productivity no longer guarantees a stable income for smallholders due to various factors.

To elevate the living standards of smallholders, improving efficiency and productivity is crucial. Efforts to enhance productivity and efficiency in the agricultural sector, particularly in rubber cultivation, have been entrusted to the Rubber Industry Smallholders Development Authority (RISDA). Rubber productivity directly impacts smallholder incomes. Consequently, RISDA has introduced various programs, that aimed at boosting rubber productivity, including the Rubber Productivity Improvement Programme (PPG), the Rubber Incentive Programme (SIPG), and the

Marketing Infrastructure Programme (PIP). The Ministry of Agriculture and Food Industries has also targeted a smallholder income of RM4,500 by 2025. To support smallholders, RISDA has devised strategies to increase rubber productivity to 1,800/kg/ha/year by 2025, thereby enhancing smallholder incomes by 30% through increased rubber sales.

This study aims to examine the productivity of rubber among smallholders and the technical efficiency of smallholders under the supervision of RISDA. Specifically, it seeks to determine the factors contributing to RISDA rubber smallholders' productivity in Malaysia. By addressing productivity and technical efficiency issues, the study aims to propose actionable strategies to improve the living standards of smallholders (Norhasikin Othman, 2008).

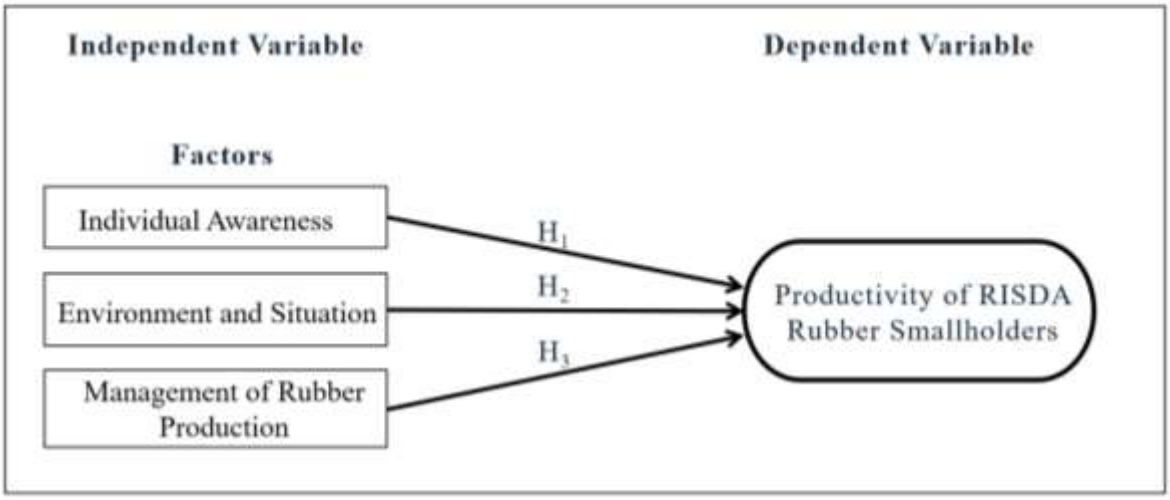

Figure 1: The research framework

The theoretical framework proposed for this study is adapted from three (3) components of factors that act as independent variables for this research.

METHODS

The study used the quantitative research approach because the researcher intends to determine the factors contributing to RISDA rubber smallholders' productivity in Malaysia. The independent variables in this study are factors in terms of individual awareness, environment and situation, and management of rubber production. While the dependent variable is the productivity of RISDA rubber smallholders. The study setting for this research is a non-contrived setting, and the time horizon for this study is a one-shot to answer the research question, which leads to a cross-sectional study. The current study adopted the cross-sectional design because the data were collected only once. Thus, the purpose of the study is correlational because it involves several variables to understand how they are related to one another.

The unit of analysis for this study is the individual, where respondents will answer the questionnaire independently. The study population is based on information from RISDA’s Productivity and Marketing (ProPem) for 2022 (Table 1). For this study, the sample respondents are RISDA rubber smallholders involving 13 states in Malaysia.

Table 1: Number of RISDA smallholders in Malaysia

STATE	NUMBER OF SMALLHOLDERS
PERLIS	262
KEDAH	4,032

PULAU PINANG	174
PERAK	3,000
SELANGOR	717
MELAKA	774
NEGERI SEMBILAN	3,251
JOHOR	2,352
PAHANG	5,157
TERENGGANU	2,609
KELANTAN	4,262
TOTAL	**26,590**

Source: Productivity and Marketing Unit (2022)

According to RISDA's Productivity and Marketing Unit (2022), there are a total of 26,590 smallholders in Malaysia and based on Krejcie and Morgan (1970), 379 samples were required when there is a population of 30,000. Therefore, this study was proposed to designate all RISDA rubber smallholders in Malaysia as the target population.

For data analysis, the collected data were examined by SPSS to determine the means and percentages for each of the questionnaires' items. Cronbach Alpha was used to check the reliability of the survey. Other than that, descriptive analysis was used for demographic and key variables. In addition, linear regression analysis was used for hypothesis testing to explain relationships between components of factors towards the productivity of RISDA rubber smallholders in terms of individual awareness, environment and situation, and management of rubber production.

RESULT AND DISCUSSION

The Cronbach's Alpha reliability analysis on all variables in this study, including individual awareness, environment and situation, management of rubber production, and productivity of RISDA rubber smallholders involving 13 states in Malaysia, resulted in values from 0.894 to 0.908. This finding indicates that the internal consistency obtained from all these instruments is very good and excellent. Table 2 shows a summary of the values of Cronbach's Alpha for each variable used in this study.

Table 2: Result of Reliability Analysis

No.	Variables	Cronbach's Alpha	Strength of Association
1.	Individual Awareness	0.908	Excellent
2.	Environment and Situation	0.894	Very Good
3.	Management of Rubber Production	0.903	Excellent
4.	Productivity	0.897	Very Good

N=428

Based on Cronbach's Alpha result in Table 2, the Cronbach's Alpha value regarding the factors towards the productivity of RISDA rubber smallholders in terms of individual awareness indicates an excellent Cronbach's Alpha value of 0.908, while the factors towards the productivity of RISDA rubber smallholders in terms of environment and situation shows Cronbach's Alpha value is 0.894. The component of factors towards the productivity of RISDA rubber smallholders in terms of management of rubber production recorded Cronbach's Alpha value of 0.903, while the productivity recorded Cronbach's Alpha value of 0.897, which depicts very good reliability of the instruments. As a result of the reliability test conducted, all dimensions in each variable exceeded Cronbach's Alpha minimum value of 0.7, so all instruments were reliable for further analysis.

As this was a correlational study, linear regression analysis was performed. Linear regression analysis can be used to analyse the relationship between an independent variable and a dependent variable.

Linear regression analysis was used to test if the tangible significantly predicted satisfaction. Based on Table 3, the R Square value for individual awareness is 0.385. The results of the regression for individual awareness indicated that the predictors explained 38.5% of the variance. Besides, the R square value for the environment and situation is 0.539. The value indicated that the environment and situation is able to explain 53.9% of the dependent variable, which is productivity. Additionally, the R Square of the management of rubber production is 0.595, which indicates that they can explain 59.5% of the dependent variable.

Table 3: Model Summary for Factors of Individual Awareness, Environment and Situation, and Management of Rubber Production

Model Summary

	R	R Square	Adjusted R Square	Std. Error of the Estimate
Individual Awareness	.620[a]	.385	.377	.53518
Environment and Situation	.734[a]	.539	.534	.46312
Management of Rubber Production	.771[a]	.595	.590	.43424

a. Predictors: (Constant), Individual Awareness, Environment and Situation, Management of Rubber Production

Table 4: ANOVA for Each Variable

ANOVA[a]

		Df	F	Sig.
Individual Awareness	Regression	1	274.944	<.001[b]
	Residual	426		
	Total	427		
Environment and Situation	Regression	1	166.249	<.001[b]
	Residual	426		
	Total	427		
Management of Rubber Production	Regression	1	195.064	<.001[b]
	Residual	426		
	Total	427		

a. Dependent Variable: Productivity of RISDA Rubber Smallholders in Malaysia
b. Predictors: (Constant), Individual Awareness, Environment and Situation, Management of Rubber Production
Based on Table 4, the F value of 274.944 is significant at $p<.001$ ($p<0.05$). Therefore, it shows that the independent variable individual awareness can reliably predict the dependent variable productivity of RISDA rubber smallholders, $F(427) = 274.944$, $p<0.05$. Thus, it can be deduced that the regression model is a good fit for the data.

In addition, the F value for environment and situation is 166.249, which is significant at $p<.001$ ($p<0.05$). Consequently, it shows that the independent variable's environment and situation can reliably predict the dependent variable, the productivity of RISDA rubber smallholders, $F(427) = 166.249$, $p<0.05$. Hence, the regression model is acceptable.

The table above also shows the ANOVA analysis for the management of rubber production with the dependent variable. Based on the result, the F value of 195.064 is significant at $p<.001$ ($p<0.05$). Besides, the independent variable, the management of rubber production, can reliably predict the dependent variable, the productivity of RISDA rubber smallholders, $F(427) = 195.064$, $p<0.05$. Therefore, the regression model shows a significant relationship.

Table 5: Coefficients for Each Variable

Coefficients [a]

		(β)	**t**	**Sig.**	**Result**
H1	KI	1.184	16.581	<.001	Accepted
H2	KPS	.936	12.894	<.001	Accepted
H3	PPG	1.077	13.967	<.001	Accepted

a. Dependent Variable: Productivity of RISDA Rubber Smallholders in Malaysia

According to Table 5, the coefficient for individual awareness is 1.184, which is statistically significant. Furthermore, the p-value is lower than 0.05 ($p<0.05$) it is considered that there is a significant relationship between the individual awareness and productivity of RISDA rubber smallholders. Moreover, the t-value is greater than 2, which is 16.581. Hence, from the table above, the researcher can conclude that individual awareness positively and significantly influences productivity. Therefore, the hypothesis can be accepted.

The coefficient for environment and situation is 0.936, which is statistically significant. Furthermore, the p-value is <.001, which is lower than 0.05 ($p<0.05$). Therefore, it is considered that there is a significant relationship between the environment and situation with productivity. In addition, the t-value is greater than 2, which is 12.894. Hence, from the table above, the researcher can conclude that the environment and situation positively and significantly influence the productivity of RISDA rubber smallholders. Therefore, the hypothesis was accepted.

The management of rubber production standardized coefficient beta value is statistically significant, with 1.077. Furthermore, the p-value is <.001, which is lower than 0.05 ($p<0.05$). Consequently, it is considered that there is a significant relationship between the management of rubber production and productivity. Additionally, the t-value is greater than 2, which is 13.967. Therefore, it can be concluded that the management of rubber production positively and significantly influences the productivity of RISDA rubber smallholders. The hypothesis is accepted.

Consequently, the hypotheses of the study are accepted:
H1: There is a positive relationship between individual awareness factors towards the productivity of RISDA rubber smallholders in Malaysia.
H2: There is a positive relationship between environment and situation factors towards the productivity of RISDA rubber smallholders in Malaysia
H3: There is a positive relationship between management of rubber production factors towards the productivity of RISDA rubber smallholders in Malaysia

The positive relationship between individual awareness and the productivity of RISDA rubber smallholders in Malaysia is supported by various studies and findings. Research has shown that factors such as awareness of farming techniques, access to information, and understanding of market dynamics significantly impact the productivity of smallholders. For instance, studies integrating theories like the Theory of Planned Behavior and Protection Motivation Theory reveal that higher awareness levels among farmers contribute to better decision-making and productivity outcomes (Suriansyah et al., 2024). By improving individual awareness through targeted interventions, RISDA's programs can significantly enhance the productivity and income levels of smallholders. This underscores the need for continuous education and support initiatives to sustain productivity growth in the sector.

Besides, several studies and articles highlight the positive relationship between environmental and situational factors and the productivity of RISDA rubber smallholders in Malaysia. initiatives like reforestation and intercropping with other crops have been recognized as effective strategies to enhance the productivity and sustainability of rubber farming. These practices not only improve the environmental footprint but also ensure consistent yields and resilience against climate change impacts (New Straits Times, 2023).

Other than that, to support the positive relationship between the management of rubber production and the productivity of RISDA rubber smallholders in Malaysia, several studies highlight crucial aspects. Implementing GAP significantly impacts productivity by ensuring sustainable farming methods. For example, studies on palm oil smallholders in Malaysia have shown that those who adopt GAP see improved productivity and well-being. The principles of GAP, which include efficient resource use and proper crop management, can similarly benefit rubber smallholders under RISDA by increasing yield and stability (Nurul Atiqah et al., 2023).

CONCLUSION

The study identified key factors determining the effectiveness of the Rubber Productivity Improvement Programme (PPG) among RISDA rubber smallholders, which are individual awareness, environment and situation, and management of rubber production. There is a positive correlation between these three (3) factors towards the productivity of RISDA rubber smallholders in Malaysia. The programs implemented by RISDA have proven effective in helping RISDA smallholders improve productivity and consequently increase their income. Therefore, these programs should be continued, expanded, and further enhanced for the overall well-being of smallholders. Thus, some recommendations can be made for future studies. Future researchers are advised to use moderator effects such as gender, age, and education level. This factor allows RISDA to get more detailed information about the effectiveness of the programme. If this recommendation can be taken into consideration by future researchers, it is believed that enhanced programme can be developed to help in futher improving the productivity of RISDA rubber smallholders in Malaysia.

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035-030

THE POTENTIAL USE OF RADIATION TO TREAT PHARMACEUTICAL-CONTAMINATED SEWAGE TREATMENT PLANT EFFLUENT FOR IRRIGATION PURPOSES

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ABSTRACT

A study was performed on the degradation of Diclofenac (DEC), Gliclazide (GLZ), Metformin (MET), and Amlodipine (AML), spiked in sewage treatment plant (STP) effluents by electron beam irradiation (EBI) technique. The degradation of DEC, GLZ, MET, and AML was investigated upon EBI at the energy of 1MeV, current of 3 mA and 9 mA, and irradiation doses ranging from 2 to 20 kGy. The degradation of DEC, GLZ, MET, and AML with the initial concentration of 100ug/l at the irradiation doses ranging from 2 to 20 kGy was quite significant. All the pharmaceutical compounds involved in the study are more easily degraded in acidic conditions while phosphate presence in the sample inhibits degradation. Meanwhile, COD, TOC, and NH_3-N decreased by 35.33%, 12.23%, and 8.33% at dose 2kGy. The present study showed radiation treatment over 100 ug/l of the DEC, AML, MET, and GLZ mixture in STP effluent is non-toxic to the growth of Amaritin, Caisin, Water Spinac, and Green bean seeds, as well as nauplii larvae.

Keywords: Degradation, Sewage Treatment Plant Effluent, Electron Beam Irradiation, Diclofenac.

030-034

CELLULOSE EXTRACTION FROM SUGARCANE BAGASSE ACROSS AGRICULTURAL, FORESTRY, AND PLANTATION SECTORS

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ABSTRACT

This study investigates the sustainable extraction of cellulose from sugarcane bagasse across agricultural, forestry, and plantation sectors. Sugarcane bagasse, a by-product of sugar production, emerges as a promising source of cellulose, offering avenues for value addition and waste reduction within these industries. Three extraction methods were evaluated: soxhlet extraction using ethanol, kraft pulping with $NaOH/Na_2S$, and iCEL machine which are producec cellulose, extractive, and lignin. Quantitative analysis reveals that soxhlet extraction yields the highest purity of cellulose (75.86±1.54%), followed by the CEL machine (50.20 ± 12.49%), while kraft pulping shows comparatively lower purity (48.42±3.95%) due to incomplete delignification. Morphological examination indicates that soxhlet extraction preserves cellulose's native properties, including its white color and fibrous morphology akin to raw cotton. In contrast, kraft pulping induces degradation, leading to yellowing and lignification of the cellulose. Fourier transform infrared spectroscopy confirms the presence of characteristic cellulose peaks in all samples, with additional peaks indicating residual non-cellulosic components, especially in kraft cellulose. Soxhlet extraction emerges as the optimal method due to its simplicity, higher purity, and non-destructive nature. Despite its industrial prevalence, kraft pulping exhibits lower efficiency in cellulose extraction. While the CEL method shows promise, further optimization is needed to enhance consistency. Future research directions may include adapting soxhlet parameters for nanocellulose production and characterizing materials properties for diverse applications. Economic feasibility and environmental impact assessments are crucial for the commercialization of lab protocols, thereby supporting sustainable cellulose production from abundant agricultural residues like sugarcane bagasse. In conclusion, this study underscores the importance of exploring efficient and sustainable methods for cellulose extraction from sugarcane bagasse, with implications for promoting circular economy principles and fostering sustainable development across agricultural, forestry, and plantation sectors.

Keywords: Sugarcane Bagasse, Soxhlet Extraction, Kraft Pulping, iCEL Machine.

1.0 INTRODUCTION

Sugarcane (*Saccharum officinarum*) is a well-known base material for producing white sugar. Sugarcane juice is a revitalising beverage due to its high vitamin, carbohydrate, and amino acid content. Due to variable amounts of hydrophilic components, sugarcane juice's status as a nutritional beverage is well-established. In addition, sugarcane juice's chemical profile reveals numerous phytochemicals with immense potential for pharmacological research. Sugarcane is among the top ten most-planted crops in the globe. Nearly one billion tones are harvested annually worldwide (Ahlfeld, 1996).

Cellulose extraction from sugarcane bagasse is gaining significant attention across the agricultural, forestry, and plantation sectors due to its potential for sustainable development and resource optimization. Sugarcane bagasse, a fibrous by-product of sugarcane processing, is traditionally used as a low-value fuel or disposed of as waste. However, recent advancements in biotechnology and materials science have highlighted its value as a rich source of cellulose, a versatile biopolymer with extensive applications.

The agricultural sector benefits from this process by converting waste into valuable products, thus enhancing the overall profitability of sugarcane cultivation. In forestry, cellulose extraction from sugarcane bagasse provides an alternative to wood-based cellulose, contributing to forest conservation and reducing deforestation pressures. Meanwhile, plantation sectors are exploring this process to diversify their product portfolios and create sustainable practices aligned with global environmental standards.
This comprehensive approach to cellulose extraction not only promotes a circular economy but also addresses critical environmental challenges such as waste management and carbon footprint reduction. By integrating these sectors in the cellulose extraction process, significant strides can be made towards achieving sustainable agricultural practices, preserving natural resources, and fostering innovation in bio-based materials.

2.0 MATERIALS AND METHODS

Sugarcane bagasse is the fibrous residue remaining after sugarcane juice extraction from stems. In this case, it is the primary raw material for producing cellulose. The Sugarcane are firstly separated from the tough skin and been dried in the oven for 24 hours or until completely dried before been grind into fine powder.

2.1 Extracting Cellulose with different methods

2.1.1 Extracting cellulose via Kraft pulping method

Kraft pulping is a widely employed method for cellulose extraction from sugarcane bagasse (SCB). In this process, SCB is treated with a mixture of sodium hydroxide (NaOH) and sodium sulfide (Na2S) under elevated temperature and pressure. The alkaline environment breaks down lignin and hemicellulose, leaving behind cellulose fibers. The resulting pulp is then subjected to a bleaching stage using agents like chlorine, chlorine dioxide, or hydrogen peroxide to remove residual lignin and enhance cellulose purity. The kraft pulping method is known for its efficiency in delignification and is a cornerstone in the paper and pulp industry due to its high cellulose yield.

2.1.2 Extracting cellulose via Soxhlet Extraction method

Soxhlet extraction is a solvent-based method for cellulose extraction from SCB. In this process, dried and finely ground SCB is placed in a Soxhlet extractor. Ethanol and water, is continuously cycled through the SCB in the extractor. The solvent selectively dissolves non-cellulosic components, such as extractives and some hemicellulose. The extracted solution is then filtered to separate the cellulose from the solvent and impurities. The cellulose is subsequently washed, dried, and weighed. Soxhlet extraction is known for its simplicity and effectiveness in removing impurities from cellulose samples.

2.1.3 Extracting cellulose via Integrated Cellulose, Extractive and Lignin (iCEL) method

The iCEL machine represents an integrated approach for comprehensive cellulose extraction from SCB. In this method, SCB undergoes a series of steps within the iCEL machine. Initially, the bagasse is fed into the machine, where solvent-based extraction removes extractives like waxes and resins. Following this, the remaining material undergoes a delignification process within the machine, selectively dissolving lignin and enriching the cellulose content. The final step involves meticulous separation to isolate the extracted cellulose. The iCEL machine is designed for efficiency, incorporating a closed-loop system to minimize solvent usage and often employing environmentally friendly solvents. This integrated approach streamlines the extraction process, making it a promising technology for obtaining high-purity cellulose from SCB in a sustainable and resource-efficient manner.

2.2 Characterization Cellulose from Sugarcane Bagasse

2.2.1 Fourier Transform Infrared Spectroscopy (FTIR) analysis of cellulose

Fourier Transform Infrared Spectroscopy (FTIR) analysis of cellulose involves preparing the sample by drying and grinding the cellulose into a fine powder, then mixing it with potassium bromide (KBr) to form a transparent pellet. This pellet is placed in an FTIR spectrometer, where its spectrum is recorded over the range of 4000-400 cm^{-1}. The resulting spectrum is analyzed to identify characteristic absorption bands, such as O-H stretching around 3400 cm^{-1} and C-H stretching around 2900 cm^{-1}, confirming the presence and purity of the cellulose. The process includes subtracting a background spectrum to account for any absorbance by KBr and atmospheric gases, ensuring accurate identification of cellulose-specific peaks.

2.2.2 Morphological Properties of Cellulose

Characterizing cellulose from sugarcane bagasse using stereo microscopy involves a detailed examination of the structural features and morphology of the extracted cellulose fibers. Stereo microscopy, also known as dissecting or low-magnification microscopy, offers a three-dimensional view of the cellulose structure. The cellulose sample is carefully mounted on a microscope stage, and a stereo microscope with dual optical pathways is utilized to observe the fibers. This method allows for the visualization of the cellulose's surface topography, fiber dimensions, and potential irregularities, providing insights into the quality and purity of the extracted cellulose. Through stereo microscopy, researchers can assess the integrity and characteristics of cellulose fibers derived from sugarcane bagasse, contributing valuable information for further applications in industries such as paper and textile.

3.0 RESULTS AND DISCUSSION

3.1 Cellulose Extraction of Sugarcane Bagasse with Different Methods

Table 1 directly compares the performance of three methods for extracting cellulose from biomass samples: Soxhlet extraction, kraft pulping, and iCEL pulping. Various metrics evaluate the efficiency and characteristics of cellulose obtained from each process. Soxhlet extraction, which uses solvents to remove extractives, achieved the highest removal rate of 6.68%, leaving behind holocellulose composed mainly of cellulose and hemicellulose. Kraft and CEL pulping processes more aggressively remove non-cellulose components, including extractives and lignin. The kraft process yielded the highest average cellulose content at 70.55%, with minimal hemicellulose content. In contrast, the iCEL process resulted in a cellulose content of 50.20% but retained a significant amount of hemicellulose at 27.98% on average.

Sample	Extractives (%)	Extractive Free (%)	Holocellulose (%)	Hemicellulose (%)	Lignin (%)	Cellulose (%)
SC	6.68 ± 0.42	10.07 ± 2.01	81.65 ± 1.61	5.79 ± 0.4	1.7 ± 0.44	75.86 ± 1.54
KC	0.00	10.79 ± 1.08	0.00	0.00	0.00	48.42 ± 3.95
CC	0.00	7.41 ± 4.13	87.03 ± 3.53	27.98 ± 3.73	0.00	50.20 ± 12.49

Notes: SC (Soxhlet Cellulose), KC (Kraft Cellulose), CC (CEL Cellulose)

Thus, kraft pulping outperformed CEL pulping in terms of cellulose specificity and purity. However, CEL pulping maintained higher utilization of the overall biomass by retaining hemicellulose sugars while liberating cellulose. The choice of process depends on the desired end use of the cellulose and the acceptability of hemicellulose contaminants. The consistency of results also differed between processes. Soxhlet extraction showed the lowest variability in cellulose yield, with a standard deviation of just 1.54%, which is expected for this mild chemical extraction method. In contrast, the harsher pulping processes exhibited wider variability in cellulose yields, with standard deviations of 3.95% and 12.49% for kraft and CEL, respectively. Further optimization for consistency is needed for these more complex pulping processes.

In summary, Soxhlet extraction reliably recovers cellulose while retaining hemicellulose and minimal lignin contaminants. Kraft pulping maximizes the purification and isolation of cellulose specifically but sacrifices hemicellulose yield and consistency. Finally, the CEL biochemical method recovers usable cellulose and hemicellulose as a combined renewable source stream, though purity and consistency need improvement. The choice of process ultimately depends on whether high-specificity cellulose or total utilization of biomass sugars is more desirable for the intended application.

3.2 Morphological Properties of Cellulose from Sugarcane Bagasse

As seen in Table 2, cellulose extracted via Soxhlet, iCEL, and kraft pulping techniques exhibit noticeable visual differences under low and high magnification stereo microscopy. Soxhlet-extracted cellulose appears white to slightly yellowish at 1.5x and 3.0x zoom, with a fluffy, cotton-like texture. This mild ethanol-based extraction preserves the native morphology of cellulose I crystals aggregated into microfibrils. Similarly, the iCEL method produces cellulose with an off-white color and fibrous texture by removing non-cellulosic binders like lignin and some hemicelluloses while minimizing cellulose degradation, resulting in a similar cotton-like appearance.

In contrast, kraft-derived cellulose exhibits a yellowish tinge at low magnification and appears golden-yellow at 3X zoom. The kraft fibers are more individualized and have a wood-like appearance due to residual lignin and lignin degradation products that resist alkaline extraction. The flat, ribbon-shaped fibres suggest partial fragmentation of the cell walls and coalescing of micro fibrils, as the harsh kraft process depolymerizes cellulose and alters its crystalline arrangement. This yellowish coloration and coarse texture indicate that kraft pulping extensively modifies the sugarcane bagasse's ultrastructure and cellulose assembly compared to the milder Soxhlet and CEL methods, which better preserve the innate cellulose properties.

Table 5. Cellulose was derived from three different methods under stereo microscopy

Sample	1.5x	3.0x
SC		
KC		
CC		

Notes: SC (Soxhlet Cellulose), KC (Kraft Cellulose), CC (CEL Cellulose)

3.3 Fourier Transform Infrared Spectroscopy (FTIR) of Cellulose from Sugarcane Bagasse

The Fourier Transform Infrared (FTIR) spectroscopy results characterize the functional groups present in Figure 1 which a cellulose extracted via three methods from sugarcane bagasse. The FTIR spectrum shows distinctive peaks corresponding to specific chemical bonds in the cellulose samples. A broad, intense peak from 3000-3500 cm^{-1} is visible in all samples, indicating O-H stretching vibration of hydroxyl groups, which signifies the presence of primary aliphatic alcohols integral to cellulose.

Specifically, at the wavenumber range of 3000-3500 cm^{-1}, SC1 shows a medium-intense peak at 3330.03 cm^{-1} corresponding to inorganic phosphate and primary aliphatic alcohol. KC1 also exhibits a medium-intense peak in this range, indicating primary aliphatic alcohol, alkynes monosubstituted, and aliphatic hydrocarbon. CC1 displays a similar medium-intense peak as SC1. This absorption range also suggests the presence of alkynes with monosubstituted groups or phosphates from residual extractives. The peak intensity suggests abundant hydrogen bonding in crystalline cellulose I.

All samples exhibit smaller peaks within 1000-1500 cm^{-1} associated with various functionalities. For instance, a medium peak at 1160 cm^{-1} in KC cellulose corresponds to the C-O stretch in aliphatic ethers within lignin or hemicelluloses, indicating surviving non-cellulosic entities. A defining cellulose peak at 895-896 cm^{-1} in SC and KC samples is due to β-glycosidic linkages between glucose monomers, verifying the cellulose structure. A comparable peak at 1018 cm^{-1} in CC cellulose also indicates the C-O stretch in cellulose.

Overall, strong broad hydroxyl peaks combined with smaller peaks indicating glycosidic bonds and ester linkages demonstrate the extraction of intact cellulose macromolecules using the three techniques. Medium peaks suggest the

Soxhlet method performed slightly better in removing non-cellulosic components. However, all samples confirm the presence of cellulose functionality alongside low quantities of residual biomass constituents.

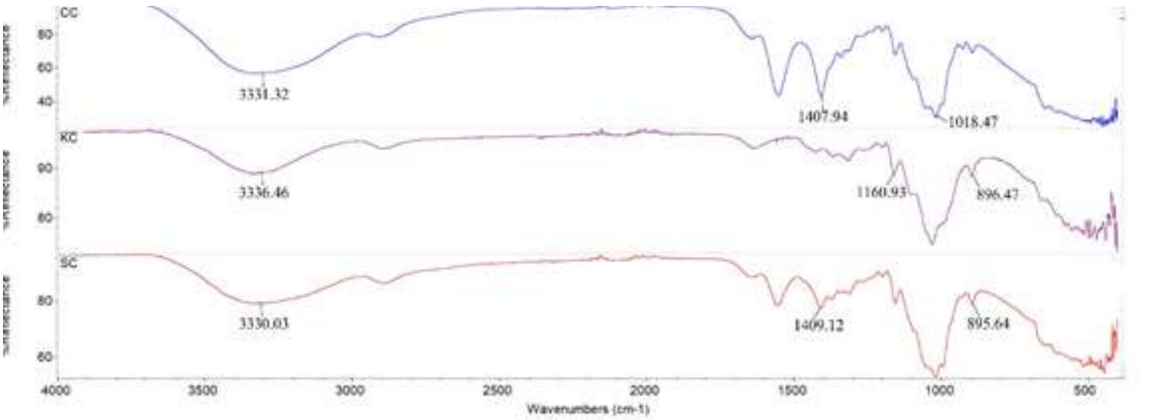

Figure 1. FTIR spectrum of cellulose from three different methods derived from sugarcane bagasse

4.0 CONCLUSIONS

The study compared three methods for extracting cellulose from sugarcane bagasse: Soxhlet extraction, kraft pulping, and the CEL machine. Quantitative analysis showed that Soxhlet extraction achieved the highest purity at 75.86 ± 1.54% and demonstrated superior consistency across samples. In contrast, kraft pulping yielded 48.42 ± 3.95% cellulose with higher variability, indicating incomplete delignification. The CEL machine performed between these values at 50.20 ± 12.49% cellulose. Stereo microscopy revealed distinct morphological differences: Soxhlet and CEL samples retained the native white color and fluffy, cotton-like texture of pure cellulose, while kraft pulping caused yellowing and lignification, suggesting more extensive alteration of the cell wall ultrastructure. FTIR spectroscopy confirmed characteristic cellulose peaks in all samples, with additional peaks indicating residual non-cellulosic components, particularly in kraft cellulose. Overall, the mild nature of Soxhlet extraction preserved sugarcane bagasse cellulose quantity and properties effectively. Its efficiency, reproducibility, and product purity make it optimal for extracting high-value cellulosic feedstocks for biorefineries, despite requiring more time compared to kraft pulping and the CEL machine, which produce cellulose faster but with lower purity and consistency.

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062-059

DETECTION OF A NEW ENDEMIC LEAF DISEASE IN MALAYSIAN RUBBER PLANTATIONS

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ABSTRACT

A rather new endemic leaf disease in rubber trees has affected many rubber-producing countries including Malaysia. The symptom was observed as a circular spot with a brown necrotic lesion on matured rubber leaf. The new leaf disease causes defoliation of rubber leaves and a reduction in latex yield. The identity of the causal pathogen is a matter of key importance to designing precise control measures in managing the disease. Thus, the objectives of this study were; (i) to isolate, characterize and identify the causal pathogen causing this disease in different localities using phenotypic and molecular characterization and (ii) to conduct in vitro pathogenicity test to fulfill Koch's postulates. Sampling was carried out at 7 different locations in 5 states in Peninsular Malaysia. Rubber leaves exhibiting symptoms of circular spots with brown necrotic lesions were collected and brought back to the Biological Control Laboratory, Faculty of Agriculture, Universiti Putra Malaysia for further analysis. Pure cultures of fungal isolates obtained were characterized based on cultural, morphological and molecular techniques. In addition, a pathogenicity test was conducted on three months old rubber seedlings clone PB 350. A detached matured rubber leaf was used in the in vitro pathogenicity test. Based on the isolation and identification results obtained, 24 isolates were identified as *Colletotrichum siamense*. Results from the in vitro pathogenicity test indicated that leaves inoculated with spore suspension or mycelial plug of *C. siamense* yielded a large lesion similar to the infected rubber leaf samples obtained from the plantations. Therefore, it can be concluded that *C. siamense* could be the primary causal pathogen of Colletotrichum Circular Leaf Spot (CCLS) disease in Malaysia.

Keywords: Rubber, Disease, Colletotrichum Circular Leaf Spot (CCLS), *Colletotrichum Siamense*.

068-066

VISION-BASED ROAD SIGNAGE RECOGNITION FOR AUTONOMOUS VEHICLE IN AGRICULTURAL PLANTATION

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ABSTRACT

The growth of self-driving vehicles demands dependable vision-based traffic sign recognition systems to maintain safety and efficiency in agricultural plantations. This research aims to create an enhanced traffic sign identification system based on the YOLOv3 algorithm, which will solve the limitations of standard human vision-based approaches, notably in low-light circumstances and with occlusions. The system leverages computer vision and machine learning techniques, requiring extensive training on diverse datasets to ensure robustness against environmental variations and regional signage differences. Implemented on platforms like Google Colab, the system was trained and tested using a comprehensive dataset, achieving a mean average precision (mAP) of 96.96%, precision of 94%, and recall of 95%. Despite its high accuracy and effective real-time processing capabilities, challenges like handling similar signs and occlusions persist. Future work will concentrate on increasing the dataset, refining the model, enhancing occlusion management approaches, and allowing real-time processing on edge devices like the Jetson Nano and Raspberry Pi, boosting system dependability and developing autonomous driving technologies.

Keywords: Vision-Based, Road Sign Recognition, Precision Agriculture, Autonomous Vehicle.

INTRODUCTION

The growth of self-driving vehicles demands dependable vision-based traffic sign recognition systems to maintain safe and efficient highway operations (K. Muhammad, A. Ullah, et al. 2021). The autonomous vehicle could also be beneficial for agricultural purposes for reducing the human burden of monitoring the plantation at all times, including pest monitoring (Kassim, A.M., Said, et al. 2022). Vision-based road sign recognition systems have received a lot of interest because they can potentially improve the safety and reliability of autonomous driving (M. I. Pavel, S. Y. Tan, and A. Abdullah 2022). These systems, which use powerful computer vision and machine learning techniques, are critical for autonomous cars' accurate navigation and decision-making capabilities (Kassim, A.M., *et. al.* 2011). They must withstand environmental variables such as weather, illumination, and occlusions and adapt to local and regional signage standards (D. Tabernik and D. Skocaj 2020). Creating such systems requires training on massive, diverse datasets, which is timeconsuming yet necessary for high-quality performance (Kassim, A.M., Yasuno, *et. al* 2015).

Traditional road sign identification methods relying on human vision are inadequate, especially under challenging conditions like nighttime or occlusions caused by environmental factors such as weather and physical obstructions (Bin Mohamed Kassim et al, 2021). These limitations necessitate an innovative and automated road sign recognition system for autonomous driving (Kassim, A.M., Termezai, *et. al* 2020). Current methods fail to accurately differentiate between various traffic signs and their meanings, posing safety risks due to visual similarities and obstructions (Kassim, A.M., Jaafar, *et. al* 2013). This project aims to develop a vision-based traffic sign recognition system using advanced computer vision techniques to identify, categorize, and interpret traffic signs reliably under diverse conditions, thereby enhancing traffic safety and supporting the advancement of autonomous driving technology (Kassim, A.M., *et. al* 2012).

Mashrukh Zayed, Al Amin, and Shohanur Rahman developed and implemented a real-time traffic sign detection and recognition system using the YOLOv3 algorithm to address challenges in traditional methods and improve road safety in Bangladesh. Huibing Zhan et al. (2020) study investigates the effectiveness of different components of MSA_YOLOv3, comparing its performance with other models and highlighting the computational complexity of certain aspects of the proposed algorithm. Some studies explore the implementation of traffic sign recognition using deep learning in autonomous vehicles, focusing on the YOLO method, dataset combination, training image implementation, and achieving robust and accurate real-time results (S. B. Wali *et. al.* 2019). There are also investigations on traffic sign recognition and distance estimation using deep learning models such as YOLOv3 and Disnet (G. S. R. Nath *et. al* 2021).

The study of control systems in autonomous vehicles focuses on designing and implementing algorithms and hardware that enable vehicles to operate without human intervention (C. Bila et al. 2017). This involves sensor fusion, where data from various sensors such as cameras, LIDAR, and radar are integrated to perceive the environment and decision-making algorithms that plan safe and efficient paths (Kassim, A.M., *et. al* 2021). The control system then translates these decisions into precise vehicle maneuvers using actuators for steering, acceleration, and braking (Kassim, A.B.M., Yasuno, *et. al* 2010). Advanced control techniques, such as Model Predictive Control (MPC) and Reinforcement Learning (RL), are often employed to handle the complexities and uncertainties of real-world driving scenarios (Hasim, N., *et. al* 2012). Research in this field aims to improve autonomous systems' reliability, safety, and efficiency, ultimately leading to the widespread adoption of self-driving technologies (Mohamed Kassim A. et al. 2016) along with battery performances (Azam, M.A. et al. 2021).

The Velibor Ilic focuses on developing classification and road sign detection algorithms for autonomous cars, the development of combining CNNs with YOLOv3 for real-time usage in ADAS development, traffic sign recognition with other sensor readings, and the academic nature of the research, leaving room for future advancements (B. Novak, V. Ilić, *et. al* 2020). Another study on traffic sign recognition with YOLOv3 focuses on building a solution, testing its performance on a specific collection of traffic signs, analyzing its real-time applicability, and evaluating its performance under various weather circumstances (E. Yurtsever, *et. al.* 2020). The project focuses on designing, developing, and testing a system for recognizing and categorizing traffic signs, emphasizing accuracy and reaction speed.

MATERIAL AND METHODS

Figure 1 shows a flowchart of training a YOLOv3 model for object detection. The process starts with collecting a dataset of images. Then, the images are labeled using Make Sense, a data labeling platform. Next, the images are divided into training and test datasets. The training dataset is used to train the YOLOv3 model, and the test dataset is used to evaluate the performance of the trained model. The YOLOv3 model is trained using the Google Colab platform. Once the model is trained, its accuracy is evaluated. The model is retrained with different parameters if the accuracy is not accepted. The process continues until the desired accuracy is achieved.

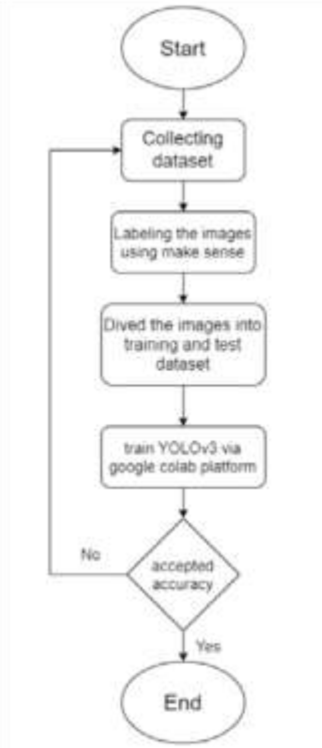

Figure 1 System flowchart

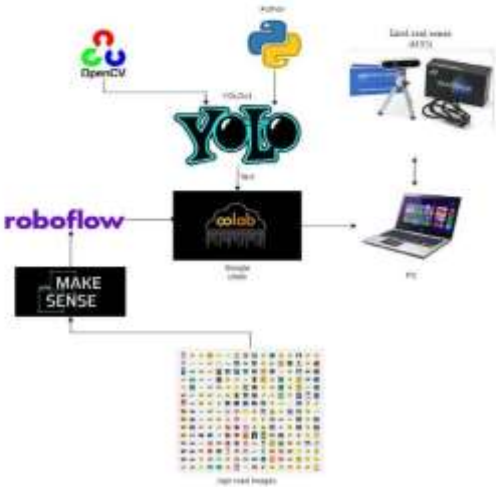

Figure 2 Components used

Figure 2 illustrates the system's item detection process. Initially, a camera or video clip captures the photograph. These images are then uploaded to MAKE SENSE, where they are annotated with bounding boxes around the objects. The annotated images are then used to train the YOLO model using the YOLO training script with Python and OpenCV. The trained model can detect real-time objects in any computing platform, such as Jetson Nano and Raspberry Pi. The dataset, comprising a collection of related data managed by a computer, is crucial for training the Convolutional Neural Network (CNN) on road sign properties (Aras, M.S.M., *et. al* 2013). Images used to train the algorithm are extracted from a video featuring various rotations and angles and divided into 80% training and 20% testing images. Each image undergoes pre-processing to adjust its size and file type.

Building an AI or deep learning model that functions like a human requires extensive training data. Accurate annotation of data is a critical step in training the model. Annotation involves marking data available in various forms, such as text, video, or images. "Make Sense," an online tool, allows users to name and annotate images based on desired classifications manually. The platform's UI and labeling process are illustrated in Figure 3.4. After labeling, users can export the dataset in the appropriate format. For this study, the dataset was exported in the YOLO format, with each image accompanied by a text file (.txt) containing labeling information and coordinates. The datasets were further processed using the RoboFlow web tool, which matches annotations with images and divides the dataset into training, testing, and validation subsets.

YOLOv3 represents an improvement over its predecessors, YOLOv1 and YOLOv2. Unlike exclusive labeling, YOLOv3 uses multi-label classification with a logistic classifier to determine the probability of an object fitting a label. It employs binary cross-entropy loss for classification instead of the mean square error used in previous versions. YOLOv3 makes multiple bounding box predictions and assigns an object value of 1 to the bounding box anchor that most overlaps with a ground truth object, ignoring other anchors with sufficient overlap. Utilizing the concept of feature pyramid networks, YOLOv3 predicts boxes at three scales, extracting features at each scale. The Darknet-53, a 53-layer CNN with skip connections inspired by ResNet, is the feature extractor. Darknet-53 starts with an ImageNet-trained network and adds 53 layers for detection, resulting in a 106-layer convolutional architecture. Convolution layers capture information from the input image, maintaining pixel relationships through mathematical operations. Pooling layers integrate feature points and resample feature maps to create new features. Before producing the classification output, the fully connected layer flattens the matrix into a vector.

The training procedure combines labels and images to teach the YOLOv3 module to detect and recognize traffic sign features. Google Colab facilitated the training process, utilizing Python. Out of 552 images, 414 were used for training, 83 for validation, and 55 for testing. The dataset and annotation files were uploaded to Google Drive and linked to Google Colab. Darknet was cloned to Google Drive using the command, (git clone https://github.com/AlexeyAB/darknet.git), and the YOLOv3 configuration file was adjusted to meet the desired specifications, setting the maximum number of patches to 6000 and steps to 4800 and 5400, representing 80% and 95% of the total patches, respectively. YOLOv3's core architecture is based on a 53-layer Darknet network, expanded to 106 layers for detection, and operates in Google Colab after downloading from GitHub and connecting to the cloud.

OPTIMIZATION OF RIS PLACEMENT AND CLUSTERING

This study will use the Intersection over Union (IoU) concept to identify objects. IoU computes the intersection of two bounding boxes: the red box represents the ground truth, while the green box represents the anticipated bounding box shown in Figure 3. To assess the validity of object detection, an IoU threshold value may be defined by confirming that the predicted and real bounding boxes are consistent. The picture of a car, with the predicted bounding box in red and the ground-truth bounding box in green shown in Figure 4.

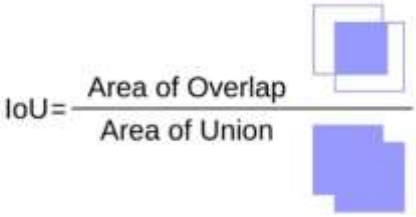

Figure 3 IoU concept.

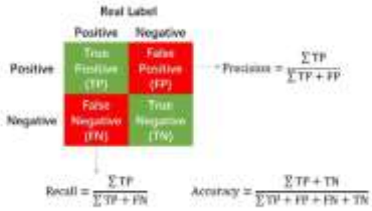

Figure 4 Predicted Label.

To evaluate performance, precision and recall metrics will be utilized, involving true positives (TP), false positives (FP), false negatives (FN), and true negatives (TN). The confidence score for each object detected by the model must be considered. Bounding boxes with a confidence score exceeding a certain threshold are regarded as positive, while those below the threshold are considered negative.

The YOLOv3 testing technique involves a systematic seven-step process. First, the image is uploaded and scaled to the correct dimensions. It is then divided into three grids of 13x13, 26x26, and 52x52 scales, where each grid unit predicts the object when its center point falls within it. Kmeans clustering is applied to each grid unit to examine bounding box priors, resulting in three clusters per grid unit, totaling nine clusters. A 13x13 small-scale feature map is constructed by feeding the image into the network for feature extraction. This feature map undergoes convolutional set and sampling twice, then combines with the 26x26 feature map to produce the prediction map response. Similarly, the 26x26 feature map is processed and combined with the 52x52 feature map. Finally, the three-scale predictive performance functions are combined, and a probability score threshold is used to filter out most low-scale anchors. YOLOv3 then employs Non-Maximum Suppression (NMS) for post-processing, resulting in more accurate bounding boxes and enabling real-time detection facilitated by the GPU's efficient computing capacity.

RESULTS AND DISCUSSION

The trained module was used to test a total of 600 photos (200 images for each class), and the average confidence rate of the results is remarkably accurate, with the Berhenti sign having a little higher average confidence rate than the other signs. However, the module has shown itself to be both extremely accurate and trustworthy. Since the study didn't explicitly state the average confidence rate for each class, there are no findings for the confidence rate of the research algorithm from the previous year.

The graphic in Figure 5 depicts the training results for a YOLOv3 model on a traffic sign detection challenge. The red line represents the model's mean average accuracy (mAP), which measures how effectively it can recognize traffic signs. The blue line represents the model's loss, which is a measure of how effectively the model can learn the job.

The mAP is initially low, but it increases as the model trains. This indicates that the model is learning to detect traffic signs more accurately. The loss is initially high, but it decreases as the model trains. This indicates that the model is learning to make better predictions. The model's performance reaches a peak after about 6000 iterations. This indicates that the model has learned to detect traffic signs very well.

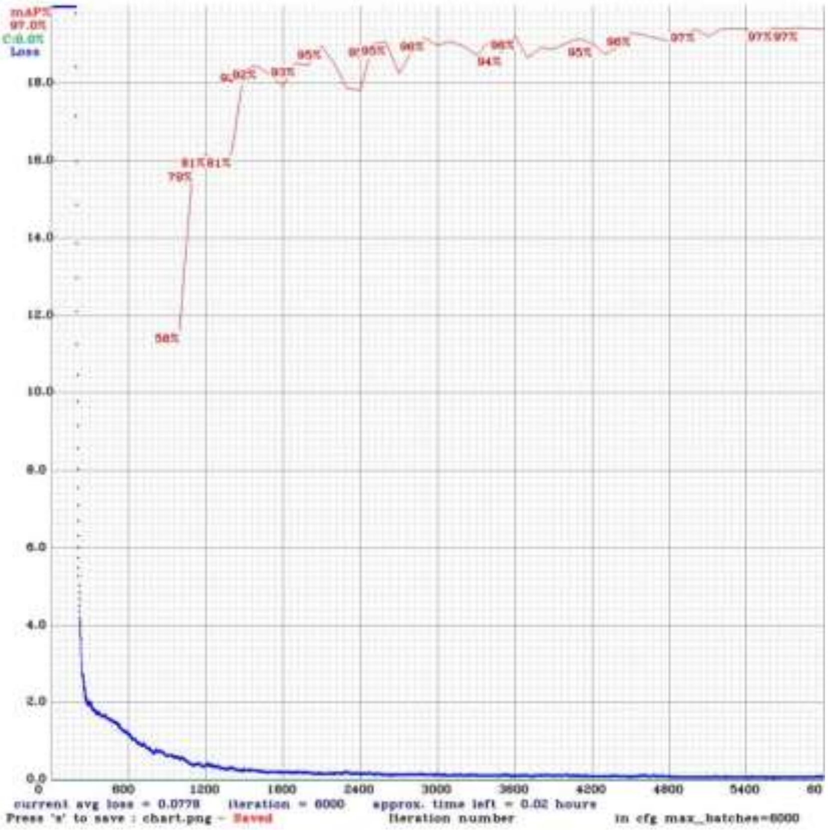

Figure 5 Output chart

High-performance deep learning programs frequently make use of high-level graphics processing units (GPU). Computers have grown into hardware that can work nearly endlessly as technology has advanced. Small, portable, low-cost, highp erformance computers with relatively high features are preferred over huge, expensive computers. In these cases, single-board computers are ideal. Single-board computers are made up of a single circuit board including input/output, memory, microprocessors, and other essential parts. FPS (Frames Per Second) is the measure of the number of full-screen pictures that are displayed consecutively in a second.

As shown in Figure 6 the outcomes are obtained by using (!./darknet detector map cfg/traffic_light.data cfg/yolov3x-traffic.cfg backup/yolov3x traffic_best.weights -map -dont_show 2>&1 > map_accuracy.txt) this code will produce the output of the evaluation of a deep-learning model for object detection. The model was trained on 545 images and was evaluated on 55 images. Figure 7 indicates that the model has a mean average accuracy (mAP) of 96.96%. The mAP measures the model's overall performance and takes into consideration both accuracy and recall. Precision is defined as the proportion of accurate detections, whereas recall is the fraction of ground truth objects discovered. The model attained a 94% accuracy and a 95% recall rate. The F1 score is a weighted average of accuracy and recall, and it measures the model's overall performance. The model obtained an F1 score of 95%.

```
seen 64, trained: 360 K-images (5 Kilo-batches_64)

calculation mAP (mean average precision)...
Detection layer: 82 - type = 28
Detection layer: 94 - type = 28
Detection layer: 106 - type = 28

detections_count = 140, unique_truth_count = 108
rank = 0 of ranks = 140
rank = 100 of ranks = 140
class_id = 0, name = 1-SPEED BUMB, ap = 95.34%          (TP = 28, FP = 2)
class_id = 1, name = 2-CROSSWALK, ap = 95.74%           (TP = 27, FP = 4)
class_id = 2, name = 3-BERHENTI, ap = 99.81%     (TP = 48, FP = 0)

for conf_thresh = 0.25, precision = 0.94, recall = 0.95, F1-score = 0.95
for conf_thresh = 0.25, TP = 103, FP = 6, FN = 5, average IoU = 79.76 %

IoU threshold = 50 %, used Area-Under-Curve for each unique Recall
mean average precision (mAP@0.50) = 0.969625, or 96.96 %
```

Figure 1 Coding in YOLOv3

<table>
<tr><td></td><td colspan="2">True Positive</td><td colspan="2">False Positive</td><td>Precision %</td></tr>
<tr><td>Speed bump</td><td colspan="2">28</td><td colspan="2">2</td><td>95.34%</td></tr>
<tr><td>Crosswalk</td><td colspan="2">27</td><td colspan="2">4</td><td>95.74%</td></tr>
<tr><td>Berhenti</td><td colspan="2">48</td><td colspan="2">0</td><td>100%</td></tr>
<tr><td colspan="5">Avrage Precision</td><td>97%</td></tr>
<tr><td colspan="2">Total Precision %</td><td colspan="2">Recall %</td><td colspan="2">F1 score %</td></tr>
<tr><td colspan="2">94</td><td colspan="2">95</td><td colspan="2">95</td></tr>
<tr><td colspan="2">IoU %</td><td colspan="2">Map %</td><td colspan="2">Iteration</td></tr>
<tr><td colspan="2">79.76</td><td colspan="2">97</td><td colspan="2">6000</td></tr>
</table>

Figure 2 Achieved model

Figure 3 Image recognition results

CONCLUSION

The use of the YOLOv3 algorithm to create a vision-based traffic sign recognition system improves the safety and reliability of autonomous driving by reaching a mean average precision (mAP) of 96.96%, precision of 94%, and recall of 95%. While the system demonstrates high accuracy and effective real-time processing, challenges like handling similar signs and occlusions remain. Future work should focus on expanding and diversifying the dataset, optimizing the model with advanced algorithms, improving techniques for handling occlusions and similar signs, and implementing real-time processing on edge devices like Jetson Nano and Raspberry Pi.

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DESIGN OPTIMIZATION AND NAVIGATION FOR AUTONOMOUS GUIDED VEHICLE (AGV) IN AGRICULTURE PLANTATION

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ABSTRACT

The Industrial Revolution (IR) 4.0 is altering the way we communicate, work, and live. However, automation and artificial intelligence (AI) have increasingly the potential to be applied in agricultural plantations. Nowadays, most plantations need humans to collect soil data and require high labor and time consumption. To solve this problem, designing and implementing an automated guided vehicle (AGV) system in the agriculture plantation has high potential. The AGV is based on plantation monitoring, and sending the data readings to the cloud server without humans is desired. In this project, the LIDAR is used as the main sensor for navigational purposes for travel around the experimental area. For obstacle avoidance, the same LIDAR is applied with the obstacle detection algorithm to detect the human or object around the AGV to prevent any collision. The multi-sensing system, such as the rotary encoder, is the supported sensor to accurately measure the wheel rotation and position. All signal data from the sensor fusion will be processed by the Robot Operating System (ROS) to optimize robot navigation, such as robot movement and data analysis for obstacle avoidance. Some simulations and experiments have been successfully performed in a computer-based simulation and farm.

Keywords: Autonomous Guided Vehicle, LIDAR, Sensor Fusion, Robot Operating System.

INTRODUCTION

Autonomous robotics in agriculture can offer several benefits for farmers, consumers, and society at large. Autonomous robots can reduce the dependence on human labor, which is often scarce, expensive, or unsafe in agricultural settings. Autonomous robots can also work around the clock, in harsh weather conditions, and in remote locations (Aras, M.S.M., *et. al* 2013). Productivity improvement: Autonomous robots can increase the yield and quality of crops by performing tasks more accurately, consistently, and efficiently than humans. Autonomous robots can also collect and analyze data from sensors to optimize crop management and decision-making (C. Zhang and N. Noguchi, 2017). Autonomous robots can reduce the environmental impact of agriculture by minimizing the use of inputs such as water, fertilizer, pesticides, and fuel (Kassim, A.M., Termezai, *et. al* 2020). Autonomous robots can also monitor and detect environmental conditions such as soil moisture, temperature, humidity, and pests. Autonomous robots can enhance the social welfare of farmers by improving their income, health, safety, and well-being. Autonomous robots can also contribute to food security by increasing the availability and affordability of food (LowenbergDeBoer J., *et. al* 2020).

However, autonomous robotics in agriculture also faces several challenges that need to be addressed before widespread adoption. Autonomous robots require sophisticated hardware and software components that are often costly, difficult to maintain, or prone to failure. Autonomous robots also need to cope with the variability and uncertainty of agricultural environments, such as terrain features, crop characteristics, weather conditions, and human interference (Gaus H., *et. al* 2017). Autonomous robots raise ethical questions about the impact of automation on human dignity, employment, responsibility, trust, and privacy. Autonomous robots also pose ethical dilemmas about the moral status of animals, plants, and ecosystems that are affected by their actions (Rose D.C., *et. al* 2018). Autonomous robots create legal issues about the ownership, liability, and regulation of robotic systems and their outputs. Autonomous robots also challenge existing legal frameworks that are based on human agency, intent, and control (Rose D.C., *et. al* 2021). Autonomous robots influence social issues such as public perception, acceptance, and adoption of robotic technologies and their implications for rural communities, culture, and identity. Autonomous robots also affect social issues such as power relations, equity, and justice among different stakeholders involved in agricultural production and consumption (Kassim, A.M., Said, *et. al* 2022).

Autonomous robots in agriculture can be classified into different types based on their mobility, functionality, or morphology. Unmanned aerial vehicles (UAVs): UAVs are flying robots that can carry cameras, sensors, or payloads to perform tasks such as aerial imaging, crop scouting, precision spraying, or pollination (Hunt E.R.Jr., *et. al* 2019). Unmanned ground vehicles (UGVs): UGVs are wheeled or tracked robots that can move on land to perform tasks such as soil sampling, weed control, harvesting, or transportation. Unmanned surface vehicles (USVs): USVs are floating or submerged robots that can operate on water to perform tasks such as water quality monitoring, fish farming, or irrigation management. Unmanned underwater vehicles (UUVs): UUVs are diving or swimming robots that can explore underwater environments to perform tasks such as aquatic weed removal, sediment analysis, or aquaculture inspection (Xu Z., *et. al* 2018)]. Stationary robots are fixed or modular robots that can perform tasks such as milking, packing, or sorting in indoor or outdoor settings (Bogue R. *et. al* 2018).

Autonomous robots in agriculture can be applied to various crops and livestock, such as cereals, fruits, vegetables, flowers, dairy, poultry, or fish. Crop monitoring involves collecting and analyzing data from sensors or images to assess crops' health, growth, and quality. Autonomous robots can perform crop monitoring by using techniques such as remote sensing, machine vision, or machine learning (Kise M., *et. al* 2019). Crop management involves applying inputs such as water, fertilizer, pesticide, or seeds to optimize the yield and quality of crops. Autonomous robots can perform crop management by using techniques such as precision agriculture, variable rate application, or site-specific management (Zhang C., *et. Al* 2020). Crop harvesting involves picking or cutting crops from the field or greenhouse. Autonomous robots can perform crop harvesting by using techniques such as mechanical manipulation, optical recognition, or force feedback. Livestock monitoring involves collecting and analyzing data from sensors or tags to assess the health, behavior, and productivity of animals. Autonomous robots can perform livestock monitoring by using techniques such as radio frequency identification (RFID), global positioning systems (GPS), or wireless sensor networks (WSNs) (Halachmi I., *et. al* 2019). Livestock management involves applying inputs such as feed, water, medicine, or hormones to optimize the welfare and performance of animals. Autonomous robots can perform livestock management by using techniques such as automatic feeding systems (AFS), automatic milking systems (AMS), or automatic weighing systems (AWS) (Bechar A., Vigneault C. *et. al* 2016).

The design and development of autonomous robots in agriculture involves several steps: problem definition, conceptual design, detailed design, prototyping, testing, and evaluation. The design and development process can be guided by various principles, such as user-centered design, participatory design, or responsible innovation (Mohamed

Kassim, A., *et. al* 2016). Problem definition involves identifying and analyzing the needs, requirements, and constraints of the intended application's users, stakeholders, and context. Problem definition can be done using interviews, surveys, observations, or focus groups (Kassim, A.M., Jamri, *et. al* 2012). Conceptual design involves generating and selecting ideas for the robot's structure, function, and behavior. Conceptual design can be done by using methods such as brainstorming, sketching, modeling, or simulation. Detailed design involves specifying and refining the robot's components, parameters, and algorithms (Bin Mohamed Kassim, A., *et. al* 2021). Detailed design can be done by using methods such as CAD, MATLAB, ROS, or Python (Kassim, A.B.M., Yasuno, *et. al* 2010). Prototyping involves building and testing physical or virtual models of the robot. Prototyping can be done by using methods such as 3D printing, Arduino, Raspberry Pi, or Gazebo (Ribeiro A.F., *et. al* 2018). Testing involves evaluating the robot's performance, reliability, and usability under different conditions and scenarios. Testing can be done by using methods such as experiments, trials, or benchmarks (Stahl B.C., *et. al* 2016). Evaluation involves assessing the robot's impact, value, and satisfaction for the users, stakeholders, and society. Evaluation can be done by using methods such as feedback, analysis, or indicators (Kassim, A.M., Jamri, *et. al* 2012).

This project explains the design and development of a multi-sensing system by ROS of autonomous guided vehicles (AGV) implemented using an Agriculture Mobile Robot. The multi-sensing system includes LIDAR scanning to avoid obstacles and detect the location, of other support sensors such as a motor encoder to most accurately know the turning of the Agriculture Mobile Robot wheel and position, this all-data signal of the sensor will be processed by Robot Operating System (ROS) and for the next action, like the movement of robot and data analysis on obstacles avoidance. The aim of this study is to implement a variable mission from user-given and varying terrain by the system the Agriculture Mobile Robot will base on missing given to execute it.

MATERIALS AND METHODS System Configuration

The system configuration of the agricultural machine is the core of the system's framework, as it must command and join countless components shown in Figure 1. The link between electronic sensors, connectors like LIDAR, encoder activators sensing the items, and extra detectors like wheel control units including the microcontroller (Kassim, A.M., Jaafar, *et. al* 2013). The concurrent connection of these detectors in the AGV is a tremendous stride towards building the machine's aptitude to journey across the region of agricultural areas, which led the agricultural robot to become more powerful and adaptive (Bogue R. *et. al* 2018). The appendage or chapter of inset in the chip and a regulator for data processing and machine learning operates the ROS panorama with the control AGV of expansion of the entire hurdle of the agricultural scene work. (Kassim, A.M., *et. al.* 2011)

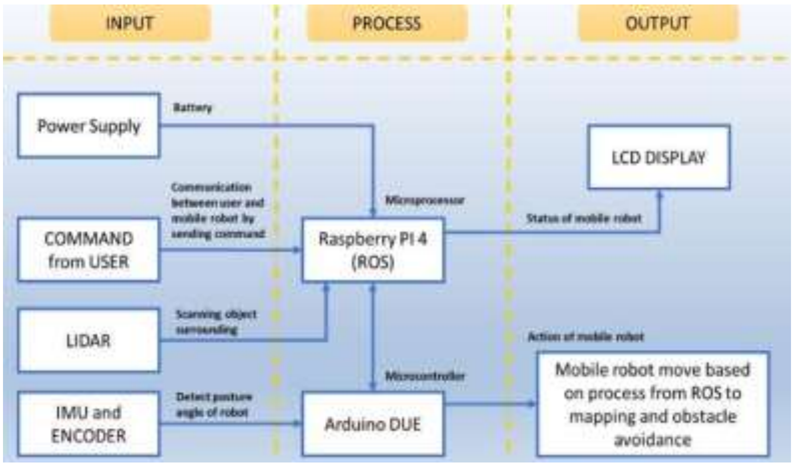

Figure 1 System configuration LIDAR Sensors for Agriculture Mobile Robot

A LIDAR sensor is selected to be built into the AGV as a sensing device for scanning any object surrounding that will be suitable for field use. LIDAR sensors are often used for AGV applications such as detection, mapping, localization, navigation, and position tracking (Kassim, A.B.M., Yasuno, *et. al* 2010). The function of LIDAR sensors are conversion devices that transform microwave echo signals into electrical signals. They use wireless sensing technology to detect motion by figuring out the object's position, shape, motion characteristics, and motion trajectory (Kassim, A.M., *et. al.* 2011). AGV uses the stage of the actions, the real-time facts of motion use the laser mechanism,

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and then the collected data of the surroundings of the AGV is used to make the latter able to roam in a variety of land patterns, and there is no collision of relay hurdles (Hasim, N., *et. al* 2012).

Figure 2 System configuration

Encoder Actuator: Transforming Motion into Signals

The other sensor used is the encoder actuator, which converts a shaft or axle's angular position or motion to an analog or digital signal shown in Figure 3. The function of it is to convert the angular position of a shaft to an electrical signal that can be read by a controller. The controller can use this signal to determine the shaft's speed, direction, or position (E. Yurtsever, *et. al.* 2020). Encoders are useful for providing feedback and control in many applications. The shaft-things are switching the shaft area in electrical signals that the controller can grasp and act on by controlling the device. For any part of the device, this gives real-time data about the area and direction of the device giving the order on the motion of the device (Kassim, A.M., *et. al* 2012). This is the way; the motion is given to take the form of the people. Or the device should be used for the varied tasks in farming. The place data from the shaft-things is used to follow the path of the farmer in the field. By putting sensors such as shaft-things in the devices, it is possible to be used to back the performance of such a control algorithm. This is the reason the reference path forms the manual control is matched with the present path of the device, and thus, command action is produced (Kassim, A.M., *et. al* 2021). It means that the enrichment is assured in the device that would follow the same path as of the people.

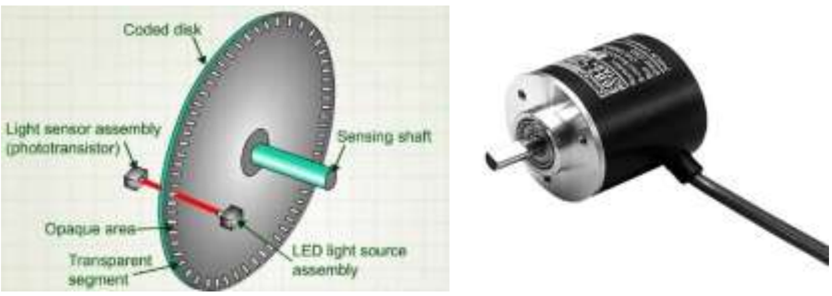


Figure 3: Encoder working principle and encoder example Inertial Measurement Unit (IMU) Sensor

IMU sensor is the sensor across the majority of gadgets and is intended to give data on motion and orientation of technical gizmos, accelerometers, and gyros are the stuff to implement a device to fix the speed and acceleration across any number of axes shown in Figure 4. Therefore, while also knowing where the device goes and what happens to it, it works on the motion in space (Kassim, A.M., Yasuno, *et. al* 2015). The significance of the sensors in many gadgets is tremendous so motion tracking is one of the major and significant things. Apparently, operating airplanes, drones, agricultural robots, and phones need the thingy indeed, moreover, all the technical gizmos need motion tracking parts to pass the control and operation. Followed by the possibility of mounting multiple sensors, Pecka and Osadčuks implemented the module of robots in agriculture, providing control at a time. It would be impossible to go on with their activity without a module providing realtime data on motion and speed.

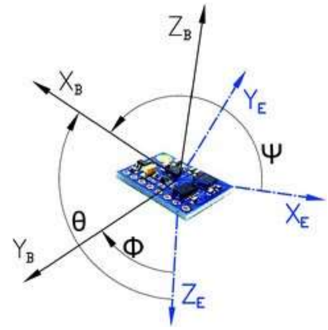


Figure 4 IMU sensor and orientation

Robot construction

The robot's base structure is made of aluminium profiles, and it looks sleek and sturdy. It has four wheels that glide smoothly on the ground. The AGV is powered by a DC motor on the rear wheels, which gives it speed and agility while the front wheels are controlled for the turning mechanism Azam, M.A., *et. al* (2021). The robot's size is optimal for moving between the rows of the fertigation farm without harming the crops.

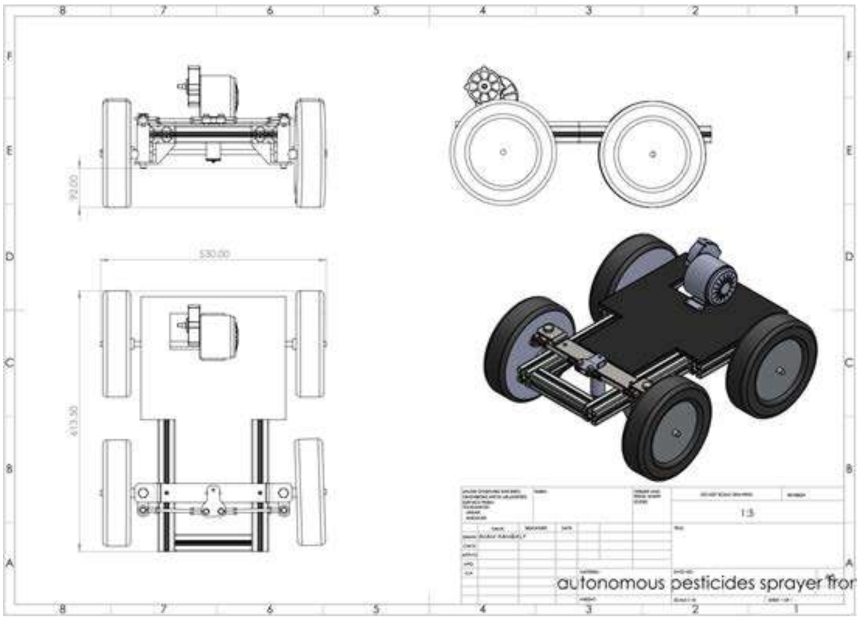
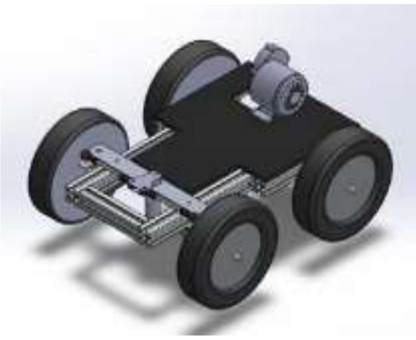


Figure 5 Mechanical structure 2D drawing using AUTOCAD



Mechanical structure 3D drawing drawing **Fabrication of agriculture robot Figure 6 Mechanical structure 3D**

RESULTS AND DISCUSSION A. Analysis of steering mechanism component by using Solidworks

We performed a static force analysis on the robot's chassis and steering mechanism using Solidworks software. The simulation results reveal the following insights.

i) **Material properties including force on the steering spindle**

The arrow in the simulation Figure 7 is the force push angle; when the part receives a large force from the bending force, the simulation diagram will show the strength force from blue to red; if the part is presented as red, it means that the part receives the most strength compared with the other side of the object.

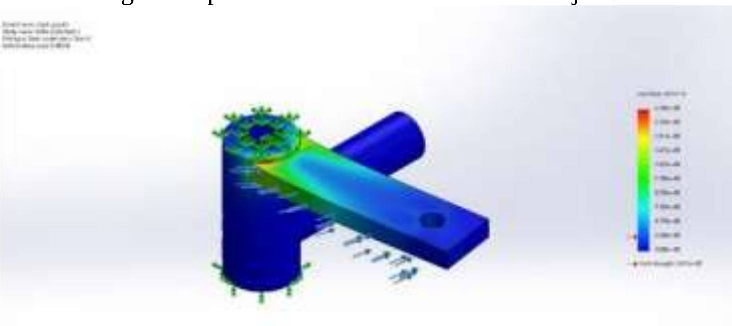


Figure 7: Spindle static stress test

ii) **Simulation of steering wheel angle and curvature radius**

After designing and simulating part of the agriculture mobile robot with the software Solidworks, run the simulation of the steering wheel angle and curvature radius and see the output of the design in the Figure 8, if it can turn preferred, if not change the parameters of the design.

Figure 8: Simulation of steering wheel angle and curvature radius B. Determining the turning radius of the AGV

To ascertain the turning radius of the multipurpose agriculture robot, a systematic experiment was conducted on a flat surface shown in Figure 9. This experiment aimed to determine the robot's turning capabilities, providing valuable insights for its navigation and operational efficiency in agricultural contexts. The robot, equipped with a wheel-base of 420mm, underwent the following steps:

1. Placement: The robot was positioned on the field, ensuring a stable starting point.
2. Steering angle setting: Manually, the steering angle was adjusted to +12 degrees and securely locked in place.
3. U-Turn motion: With gentle force, the robot was pushed forward, executing a U-turn.
4. Distance measurement: The distance covered from the initial position to the last point of the U-turn was meticulously measured.
5. Data recording: The obtained data was recorded in a tabular format.
6. Iterative process: Steps 2 to 5 were repeated for steering angles of +16, +20, and +24 degrees.

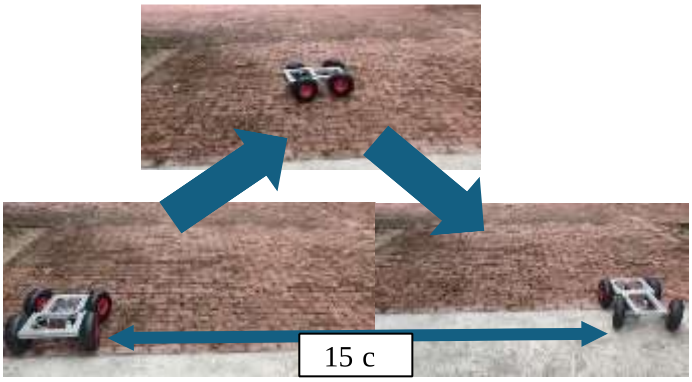


Figure 9: Real-field measurement of steering wheel angle and curvature radius

C. Experimental results of steering wheel angle & radius of curvature i. Simulation steering wheel angle & radius of curvature in Solidworks

The experimental results from the simulation and real field test can be compared, and the closeness of their output results can be seen. The output data analysis from the Simulation is shown in Table 1. The collected data are turn angle (°), wheelbase (mm), turn radius (mm), and turn distance (ft). The data analysis results are shown in the graph shown in Figure 10.

Table 1: Data analysis for simulation of steering wheel angle & radius of curvature

Turn angle (°)	Wheelbase, (mm)	Turn radius, (mm)	Turn distance, (ft)
4	420	6006.28	39.38544
8	420	2988.455	19.59643
12	420	1975.945	12.95701
16	420	1464.714	9.604682
20	420	1153.941	7.566823
24	420	943.3354	6.185806

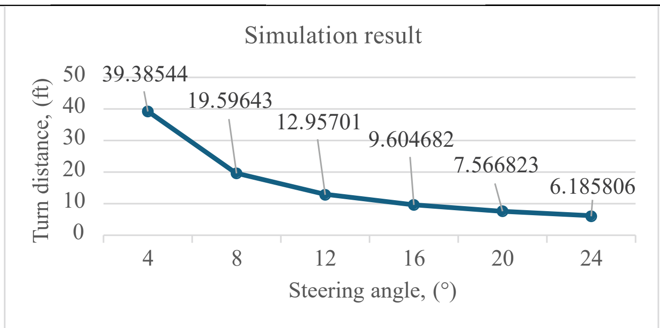


Figure 10: Data analysis for simulation of steering wheel angle & radius of curvature

ii. Data collection steering wheel angle & radius of curvature in real fields

The output data testing and collection from the real field test are shown in Table 2. The data include turn angle (°), wheelbase (mm), turn radius (mm), and turn distance (ft). The data analysis results in the graph shown in Figure 11.

Table 2: Data collection steering wheel angle & radius of curvature in real fields

Turn angle (°)	Wheelbase, (mm)	Turn radius, (mm)	Turn distance, (ft)
12	420	2012	13.19
16	420	1492	9.78
20	420	1175	7.71
24	420	961	6.29

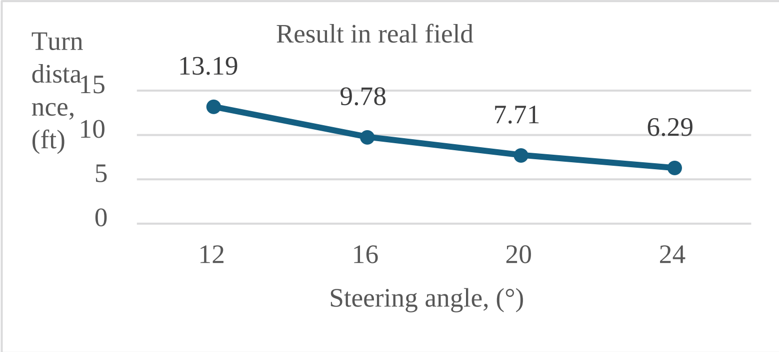


Figure 11: Data collection steering wheel angle & radius of curvature in real fields

iii. Comparison between simulation and real field test result

Figure 12 compares simulation and real field test results for steering angle versus turn distance. The blue line is data from the simulation, and for red line presents a real field test,

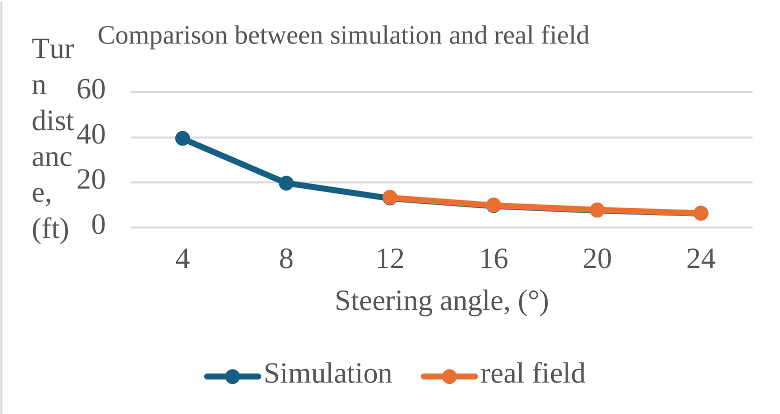
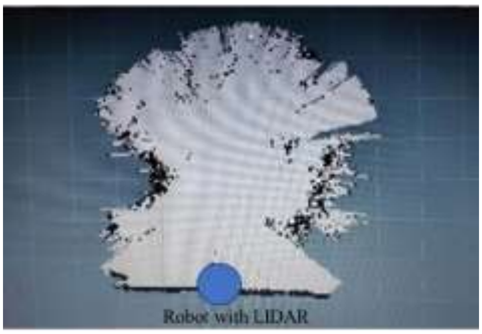


Figure 12: Comparison between simulation and real field test result

iv. LIDAR sensor outdoor field mapping by ROS

The robot's LIDAR sensor was used for the initial LIDAR sensor test. When the LIDAR was on, the robot connected to the system through ROS and showed the detection value at Windows RVIZ. Figure 13 shows the software's detection output (red-colored dot). The robot was used for outdoor mapping, and the scanning mapping was plotted at windows, as shown in Figure 14.

Figure 13: LIDAR sensor outdoor mapping



LIDAR sensor field detection using ROS **LIDAR sensor output mapping**

Figure 14: Output mapping from LIDAR sensor

CONCLUSION AND FUTURE WORKS

The design and development of an autonomous guided vehicle (AGV) system in an agricultural plantation is the subject of this article. The primary sensor for navigation while traveling within the experimental region was the LIDAR sensor. In order to avoid collisions, the AGV uses the same LIDAR in conjunction with an obstacle identification algorithm to identify any objects or people in the vicinity of the AGV. Besides, the rotary encoder is a multi-sensing device that may be used to precisely measure the location and rotation of the wheel. The Robot Operating System (ROS) processed all of the signal data from the sensor fusion to optimize robot navigation, including robot movement and data analysis for obstacle avoidance. Some experiments and simulations have been successfully conducted.

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071-073

GROWTH PERFORMANCE OF TISSUE CULTURE-DERIVED CLONAL MATERIAL OF SELECTED *EUCALYPTUS* HYBRID PLUS TREES IN THE NURSERY FOR THE ESTABLISHMENT OF CLONAL TRIAL PLOTS

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ABSTRACT

Eucalyptus hybrid has been planted widely due to its rapid growth, desirable wood characteristics, and being more disease tolerant compared to its parent species. These hybrid clones have been deployed commercially as monoclonal plantations for timber and pulp production. Systematic genetic improvement programs for *Eucalyptus* species have been initiated at Forest Research Institute Malaysia (FRIM) and selective breeding programs have been carried out for mass production through clonal propagation. Improvement was done through the evaluation based on tree selection, identification of candidate plus tree (CPT), and establishment of clonal trials. This enables rigorous conduct of the genetic improvement program on *Eucalyptus* hybrid to secure long-term response to selection in providing valuable stock for future selection. As a result, three clones have been selected based on their morphological traits and clonally propagated for mass production. Explants were collected from selected clones of *Eucalyptus* hybrid (*Eucalyptus grandis* x *Eucalyptus urophylla*) namely, EBH 501, EBH 507, and EUS 02 from Bukit Hari and Selandar research stations and brought back to be sterilized and multiplied through the tissue culture technique. Tissue culture plantlets were then hardened under nursery conditions after being acclimatized in jiffy peat pellets for one month. The plantlets were transferred to root trainers consisting of a mixture of topsoil, coco peat, slow-release fertilizer, and

Christmas Island Rock Phosphate (CIRP). The growth performance and survival rate of clones during the acclimatization period were continuously monitored and recorded. Pre hardening technique using jiffy peat pellets enhanced the survival rates of tissue culture plantlets during acclimatization in the nursery. It was found that there was a statistically significant difference between clones, and clone EBH 507 had the highest values in terms of height growth and survival rate. No significant differences were detected in diameter growth among the clones. These hybrid clones were found to be promising for the establishment of clonal trials. These clones will be evaluated more comprehensively in clone-by-site interaction trials with a standard silvicultural treatment. Performance and the value of a species and individual tree can be improved by identifying the best sources and selecting individuals within these to develop clones that can perform better for future plantation programs.

Keywords: Clonal Trial, *Eucalyptus*, Clonal Propagation, Tree Selection, Superior Genotypes, Tissue Culture.

078-079

HISTORY, PRESENCE, AND PERSPECTIVE OF USING SUN PROTECTOR FOR MANAGING SUNBURN AND FRUIT DISEASES IN FRUIT PRODUCTION: A REVIEW

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Sunburn injury due to high solar radiation levels and air temperatures, low relative humidity, and high elevations is common on fruits and can cause huge fruit production losses. Severe sunburn alters the cuticle and damages both the epidermal and sub-epidermal tissues. The injured fruits then can be easily attacked by insect pests and plant pathogens making them worse and unmarketable. Various economically important fruits such as apples, grapes, pineapples, pomegranates, and mangoes have been reported to be facing heavy losses due to sunburn and fruit rot diseases caused by fungal pathogens. To overcome this problem, several strategic managements of sunburn have been explored and used by growers including fruit bagging, shade netting, tree sprinkler cooling systems, and spraying with sun protectors. In this review, the use of sun protectors in managing sunburn and fruit rot diseases in various fruit productions is highlighted. Previous, current, and new perspectives on sun protector product developments such as suppressants, particle film, kaolin-based products, Calcium carbonate-based products, talc-based products, sunscreen, chemical protectants, and anti-transpirants will be critically reviewed and discussed.

Keywords: Crop Protection, Economically Important Fruits, Sunburn, Fruit Rot, Sun Protection.

083-083

EXPLORING THE POTENTIAL OF OIL PALM TRUNKS AT EARLY REPLANTING AGES AS ALTERNATIVE FEEDSTOCK FOR FUEL

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ABSTRACT

The utilisation of oil palm biomass as an alternative feedstock for fuel has been garnering increasing research interest in recent years due to the global demand for sustainable and environmentally friendly energy sources. The accessibility to an abundant supply of residual oil palm biomass during replanting activities, along with the prospect for early replanting to support plantation management, has led to the need for investigating the potential of younger OPT as a sustainably viable fuel source for green energy generation. This study explored the characteristics of OPT biomass at different replanting ages for their suitability as biofuel. Proximate, ultimate, calorific value and energy dispersive X-ray spectroscopy analyses were performed on OPT biomass collected at 19, 21, 23 and 32 years. Results from proximate by thermogravimetric analysis showed low moisture (<10%) and ash (<3%) contents in the OPT across all age groups, with relatively high volatile matter and fixed carbon contents which fell within the range of other biomass fuels. The ultimate analysis of all OPT exhibited appreciably high C (~40%) and O contents (>50%), whereas calorific values ranged from 15-16 MJ/kg, which were comparable to that of coal and other agricultural biomasses. Elemental content analysis revealed that the OPT contained trace amounts of Na, Mg, Al, Fe and Si, among others, indicating their suitability for thermochemical conversion. Based on the findings from this study, younger OPT have shown promising characteristics for their utilisation as a sustainable fuel source, further supporting their valorisation at early replanting ages.

Keywords: Oil Palm Biomass, Early Replanting, Fuel Properties, Sustainable Energy, Value Addition.

072-085

EVALUATING THE POTENTIAL OF *ENDOSPERMUM DIADENUM* (SESENDUK) FOR SUSTAINABLE FOREST PLANTATION IN PENINSULAR MALAYSIA

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ABSTRACT

The timber industry and wood-based products have long been pivotal to Malaysia's economy, a trend that is set to continue with increasing global demand. However, reliance on natural forests alone is unsustainable, leading to degradation and biodiversity loss. To address this issue and support the Forest Plantation Development Program (FPDP), the Forest Research Institute Malaysia (FRIM) has conducted extensive research and development (R&D) on several indigenous species, including *Endospermum diadenum* (Sesenduk). This paper presents the findings of FRIM's research on Sesenduk, highlighting its potential as a future forest plantation species. A notable achievement is the development of the FRIMsrp001 clone. Protocols for producing planting material through macropropagation (cutting technique) and micropropagation (tissue culture) have been established. Comparative field studies on tree growth rates from seed sources versus tissue culture have been conducted, leading to the identification of five new genotypes for further clonal testing. R&D results demonstrate that Sesenduk exhibits a fast growth rate, with a mean annual increment in diameter at breast height (dbh) of 2 cm/year. These findings support the use of Sesenduk in forest plantations, offering a sustainable alternative to natural forest dependency. FRIM is committed to supplying high-quality plant materials to assist the Forestry Department of Peninsular Malaysia in ensuring the sustainable use of forest resources

Keywords: Selection Of Plus Trees, Tree Breeding, Germplasm, Clonal Trial.

086-086

BIOREFINING OF AGROINDUSTRIAL BY-PRODUCTS FOR SUCCINIC ACID PRODUCTION

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ABSTRACT

The development of a biobased economy is highly dependent on efficient and sustainable refining of agroindustrial by-products, particularly in several areas suah as biofuels, biochemicals, and/or biomaterials. Succinic acid is an immensely valuable platform compound with diverse applications, and its market demand is continually growing. Sugar polymers from agroindustrial by-products or biomass such as oil palm trunk (OPT) and empty fruit bunch (EFB) serve as a promising low-cost feedstock for succinic acid production via biological transformation. In this study, an efficient process for producing succinic acid was developed using dilute ferric chloride ($FeCl_3$) as catalyst in the pretreatment step. Sequential enzymatic saccharification of the pretreated biomass was able to produce high sugar concentration. Fermentation of OPT and EFB hydrolysate with *Actinobacillus succinogenes* resulted in succinic acid yield up to 0.56 ± 0.06 g/g within 24 hours of fermentation. After purification, high purity of 99.93 ± 0.88% was achieved via evaporation and crystallization. Furthermore, the high product recovery rate of >90% could favorably increase provide profits, which encourages future industrial succinic acid bioprocessing and development.

Keywords: Oil Palm Biomass, Enzymatic Saccharification, Fermentation, Succinic Acid.

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096-097

ECONOMIC ANALYSIS OF KENAF PRODUCTION IN MALAYSIA

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ABSTRACT

This study provides an in-depth economic analysis of kenaf production among farmers in Perlis, Malaysia, aiming to investigate profitability, challenges, and key factors affecting its production. Kenaf (*Hibiscus cannabinus*) is a versatile crop utilized in textiles, paper, biofuels, and construction materials. In light of Malaysia's strategy to diversify its agricultural sector and decrease dependence on traditional crops, kenaf production has gained government's attention. However, the sustainability of Malaysia's kenaf industry is hindered by the imbalance between the supply and demand of raw kenaf resources and lack of established markets for Kenaf products. Therefore, understanding the economic feasibility and practical challenges faced by kenaf producers is crucial for making informed decisions and developing effective policies. The research utilizes multiple regression analysis to evaluate the influence of various socio-economic and environmental factors on kenaf production. These factors encompass age, education level, family size, farming experience, knowledge of kenaf cultivation, access to training programs, financial literacy, production costs, and environmental conditions. Data were gathered through surveys and interviews with kenaf producers, resulting in a robust dataset for analysis. Key findings indicate that education and training, contrary to expectations, have a statistically significant negative impact on kenaf production. This suggests a disconnect between educational content and the practical needs of farmers. Other variables, such as age, family size, years of experience, knowledge, financial literacy, production costs, and environmental conditions, did not show statistically significant impacts on production, although they exhibited the expected directional effects. Notably, experience and financial literacy were positively correlated with production, highlighting their potential importance, but these results were not statistically significant within the sample. The study emphasizes the need to re-evaluate current educational and training programs to better align them with the practical realities and requirements of kenaf producers. Furthermore, it highlights the necessity for additional research into the roles of experience and financial literacy in improving production outcomes. By offering these insights, the study aims to inform policymakers, stakeholders, and practitioners about the critical factors affecting kenaf production, ultimately contributing to the creation of more effective support programs and policies that can promote a sustainable and profitable kenaf industry in Malaysia.

Keywords: Perlis, Malaysia, Kenaf production, Multiple Regression Analysis, Cost Benefit Ratio.

INTRODUCTION

Kenaf (Hibiscus cannabinus) is a tropical plant noted for its diverse use in textiles, paper, biofuels, and building materials. It is a desirable agricultural crop because of rapid growth, minimal input needs, and tolerance to a wide range of climates. Malaysia, a country with a strong agricultural industry and a dedication to sustainable development, has identified kenaf's potential as a crop that corresponds with its economic and environmental aims. Malaysia's government has encouraged kenaf production as part of its aim to diversify the agricultural sector and minimize the country's reliance on conventional crops (Unfairtobacco, 2018).

Figure 1 Kenaf Cultivation Area by Region
Sources: NKTB,2019

In 2020, the planted area of kenaf had decreased to 1,626 ha, 32% of the projected planted area.
The total production of dried stems was 6,450 tons, with 45 tonnes of seeds produced by 1,061 smallholders. Between 2010 and 2020, dried stem kenaf output increased by 10%. In 2015, dried stem output peaked at 11,602 tons, but fell to 6,450 tonnes by 2020.

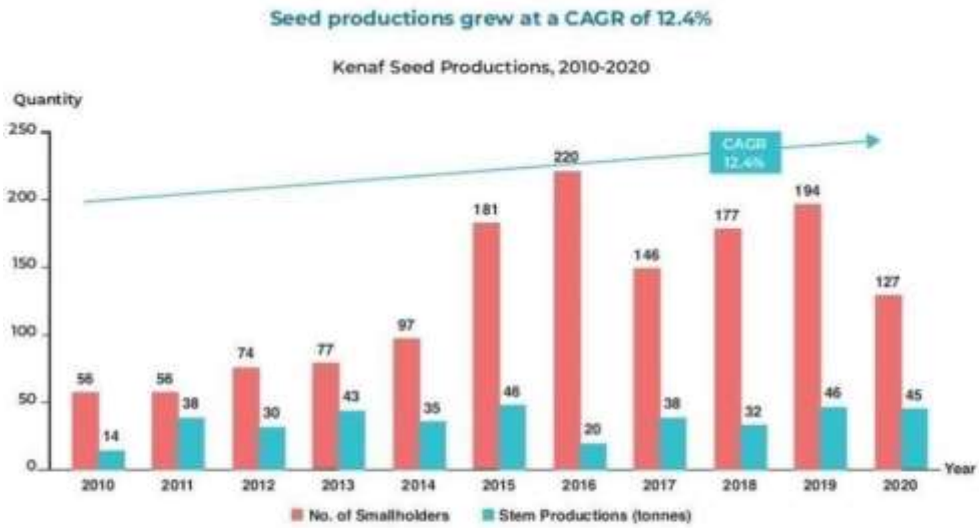

Figure 2 Kenaf Seed Productions,2010-2020
Source: MPIC, NKTB Statistics (2013)

On the other hand, kenaf seed output increased at a CAGR of 12.4% between 2010 and 2020.
CAGR stands for compound annual growth rate. It is a metric used to quantify the geometric progression ratio, which guarantees a consistent rate of return over a given time span. It's typically used to analyze investment returns or

business success. However, production fell in 2017 due to a protracted dry period, which reduced yield. As a result of the harsh weather, yields in succeeding seasons were impacted (National Agricommodity Policy, 2019).

The government subsidises kenaf production at an average of 61%-63% of the overall production cost through subsidies paid to participating smallholders. In 2020, the predicted value of kenaf production was RM28 million, based on a mean production cost of RM3,885 per tonne of dried stem (output 6,450 tons of stem) and RM4,655 per hectare of seed (45 tonnes of seed) (National Agricommodity Policy, 2019).

The economic research on Kenaf production in Malaysia contains significant gaps, preventing a complete knowledge of its feasibility and impact. For starters, there is a significant absence of indepth economic research of Kenaf farming. The current study lacks a full investigation of economic criteria such as cost- effectiveness, profitability in compared to other crops, and potential contribution to the agricultural industry. This lack of extensive economic study prevents lawmakers, farmers, and businesses interested in Kenaf from making informed judgments (Basri et al., 2014). The lack of established markets for Kenaf products is a significant barrier. Farmers are discouraged from cultivating Kenaf due to sales volatility and a lack of guaranteed markets. Furthermore, ineffective efforts to generate awareness and promote Kenaf products contribute to market barriers. In terms of marketing and promotion, it is difficult to establish a favorable market perception and achieve widespread adoption of Kenaf products (Vayabari et al., 2023).

Furthermore, farmers' lack of technical knowledge regarding optimal agricultural techniques for Kenaf cultivation poses an obstacle. Farmers may struggle to fulfill Kenaf's potential benefits without adequate training in harvesting operations, processing processes, and ecologically friendly ways, hurting the overall sustainability and economic feasibility (NAP2.0, 2021). Furthermore, farmers' lack of technical knowledge regarding optimal agricultural techniques for Kenaf cultivation poses an obstacle. Farmers may struggle to fulfill Kenaf's potential benefits without adequate training in harvesting operations, processing processes, and ecologically friendly ways, hurting the overall sustainability and economic feasibility (NAP 2.0, 2021).

Kenaf's current profitability condition presents issues. Kenaf's apparent lack of profitability is aggravated by high initial investment expenses, slow return on investment, and poor demand for its products. This financial element prevents potential farmers from seeking higher yields or more profitable solutions, limiting Kenaf output in Malaysia's agricultural economy (NAP 2.0, 2021).

Policy challenges in the Kenaf industry demand major reform to address issues such as a lack of collaboration among Ministries, Agencies, Research and Development Institutes, and Industries. Bridging these gaps and tackling these issues is crucial to the Kenaf industry's long-term success and development. Industry support and trade facilitation confront obstacles such as a lack of financial assistance and plantation loans. The little involvement of SMEs and social communities in the Kenaf sector complicates problems. The industry climate is influenced by challenges in creating Public-Private Partnerships (PPPs), reliance on private efforts, and contract farming constraint. Low investment in R&D, technology, design, and standards are among the issues confronting R&D and innovation. Inadequate research on pests, automation, and seed development impedes progress. Furthermore, the industry's potential is limited by a lack of innovation in areas like as construction, infrastructure, noise barriers, and environmentally friendly Kenaf products (Amanda,2023).

This study aims to explore the profitability of kenaf production and pinpoint the factors and constraints that impact profitability among kenaf producers. The research centers on three key objectives: identifying the costs and profits associated with kenaf producers in Perlis, Malaysia; investigating the challenges these producers encounter; and determining the factors influencing kenaf production in the area. By addressing these objectives, the study intends to offer a thorough insight into the economic feasibility and operational challenges faced by kenaf producers in Perlis.

METHODOLOGY

The study will be conducted in Perlis. The research will take place at Kenaf Plantation, which is characterized by extensive fields dedicated to kenaf cultivation. While the primary focus is on the outdoor plantation, the study may involve the field visits to the processing facilities for survey and gathering information for further analysis. This location has been chosen due to its status as a prominent kenaf plantation, providing access to diverse kenaf cultivars and real-world agricultural conditions. Besides, there were lack of information or research conducted in Perlis regarding economic analysis of kenaf production. Perlis is one of the major states that produce seed of kenaf, andtherefore conducting the research in this particular area is of important significant in filling in the research gap.

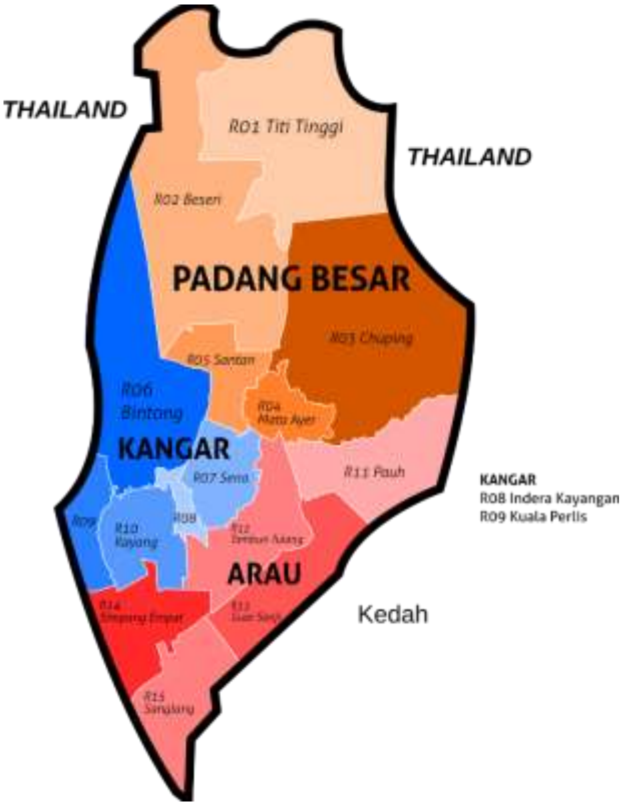

Figure 4: Map of Perlis
Source: NAP 2.0,2021

This is the map of Perlis including the district of Perlis. The pilot project for the development of the Kenaf Integrated Park (KIP) in the Chuping Valley and the Village Growth Center (PPD) in Batu Bertangkup, Chuping, Perlis aims to place the components of the kenaf production chain in an integrated manner under one roof. Based on the concept of From Farm to Market (seeds to market), KIP is a focal point linking all kenaf production activities starting from kenaf cultivation (upstream sector), crop processing (intermediate sector) to the production and marketing of kenafbased products (downstream sector).KIP's workforce includes kenaf growers for fiber and seed production, entrepreneurs and kenaf processing workers of the Kenaf Scheme for the People (KUR), premium fiber production site workers, kenaf pulp mill workers, entrepreneurs and small and medium enterprise (SME) workers of kenaf products and private companies for the marketing of kenaf-based products (LTKN,2023)

In 2016, the development of the Kenaf Plant Management System known as MyKenaf was implemented to meet the requirements of the National Kenaf and Tobacco Board (LKTN) in planning and managing the country's kenaf plants. MyKenaf was developed using the internal expertise of MYSA and LKTN. The operation of this system began in February 2019 in 4 pilot areas in the State of Pahang, namely Merchong, Tanjung Putus, Rompin and Cherating. Next, the operation of the system was gradually expanded to the states of Kedah, Perlis, Kelantan, Terengganu, Melaka and Johor (Malaysian Space Agency, 2016).

This study focused on Perlis namely Lembah Chuping, in which the number of growers 95 while the number of companies or organizations are 2 which comprise of Lembaga Kenaf and Tembakau Negara and Kenaf Global Ventures Sdn Bhd. Crop heritage is 25.00ha, damaged crop is 0, yielding crop is 25.00, harvested crop are 25.00, non-harvested crop is 0, production of Kenaf dried stems are 10.20 and lastly, and yield per hectare of Kenaf dried stems was 0.41.

Population and Sample Size

The population of this study refers to the group of individuals who will be subjected to research, which focuses on Kenaf's producers in Perlis which are smallholders. By using the formula from Raosoft Software, the population for this survey is estimated to be 57 individuals who plant kenaf and the sample size will be 50 individuals who will contribute to answering the questionnaire. According to Singh et al. (2014), as noted in Kish (1965) and Roberts

(2004), sampling is associated with population selection and overall population characteristics. Sampling offers two advantages: low cost and faster data collecting. Furthermore, random sampling is commonly used in corporate, medical, and agricultural studies to collect demographic data.
According to Tahardoost (2007), there are various statistical formulas for calculating sample size.
The statistical formula is expressed as:

$$n = \frac{p\,(100-p)z^2}{E^2}$$

Where,

n=the required sample size the percentage occurrence of a state or condition the percentage maximum error
P=required the value corresponding to level of confidence required

This formula contains two critical components. First, there are concerns for estimating the level of precision and risk that the researcher is ready to accept. E stands for margin of error, or the amount at which the researcher is willing to take the risk. The smaller the value of E, the larger the sample size needed (Baryman and Bell, 2003). The level of confidence is measured, which may represent the correctness of the population characteristics estimated using the sample size.

Sampling Method

Random method

A sampling technique in research is classified into two types: probability sampling and nonprobability sampling. Probability sampling is also known as random sampling. Probability sampling is classified into five techniques: basic random, stratified random, cluster random, systematic random, and multistage random. Probability sampling, often known as random sampling, implies that every member of the population has an equal probability of becoming a sample. Furthermore, it is free of bias; nonetheless, a researcher must devote more time and effort. In contrast, non-probability sampling can be employed in case studies and qualitative research (Taherdoost, 2016).

In this study, a simple random sampling has been chosen in order to select the number of respondents. The simple random sampling has two possible ways which are with or without replacement. An example of a simple random sampling with replacement is when a person has been selected as a sample, a person has to be possibly re-sampled because that person will be placed back in the population. Meanwhile, a simple random sampling without replacement is defined as once a person has been selected as a sample, he or she will not return to the population (Frerichs, R., 2008). Simple random sampling has the advantage of being easier to implement and requiring minimal knowledge about the population. Furthermore, the results may be accessible in a shorter amount of time, making this a cost-effective form of data collection. The disadvantage of this technique is that it takes time, especially for large populations, because it is impossible to reach all smallholders during the survey. Aside from that, the technique has disadvantages when respondents are far apart and difficult to contact due to higher travel costs and time constraints. In the context of a 50- person sample size, this technique requires assigning each individual a unique identity and then selecting research participants using a random mechanism, such as random number generators or tables. The main premise is to reduce biases and create a sample that is typical of the total population, hence increasing the generalizability of the study's results. The objective is to draw meaningful conclusions about the wider population based on the features found in a randomly selected group. While a precise source for this argument may not be available, it is based on fundamental ideas in research technique and statistics.

Measuring Instrument

Pre-Test
Pre-test is the instrument that will be conducted before the actual survey. The purpose is to ensure the respondents understand and to avoid any misinterpretation of the questionnaires. Pre-Test questionnaire will be distributed to the expert, to academician and to student. The strength and weakness able to be identified through pre-test regarding the question format for the survey. Through this pre-test, it conducted to examine the reliability and validity of the questions to be used in survey. The time needed and any obstacles able to be assessed through this test. The questions can be assured whether it provide understanding and comprehension to the issue that are going to be study.
Validity Test

Validity test purpose is to determine whether the questionnaire is valid or invalid. The test must be both reliable and valid. This tool ensures accurate measurements for the intended purpose.
Respondents provided feedback on the questions.

Pilot Test
Pilot Tests is an instrument used prior to conducting an actual survey. This pilot study aims to discover an unpredictable problem: questionnaire misunderstandings. This pilot survey includes 7% of the total responses. The pilot study aims to clarify statements for each question. This pilot study can identify weaknesses and change questionnaires if the alpha coefficient is < 0.70 (Nunnaly, 1994).

Reliability Test
Reliability Test is used to determine the reliability of the data obtained and will Use a pilot test to evaluate each variable in this study. Generally, the dependability test is It is critical to measure the aptitude and capability of the goods on the instrument used. Hair et al. (2010) defined dependability as the precision and stability of value or mark. of a measurement scale. Nunnally (1994) says that the instruments employed in the study is regarded to have low dependability levels if the value of alpha or the coefficient is less than 0.70. While reliability values above 0.7 indicate that the data collection process can be proceeded and are considered reliable. The questionnaire for this study were adapted from earlier research with various adjustments. Previous questionnaires lacked necessary questions about the factors influencing the system's implementation (48). The questionnaires were cited from Perera J. et al. (2007).

Data Analysis
Cost-Benefit-Analysis Benefit-cost ratio (BCR)

A cost-benefit analysis (CBA) is a methodology for determining the feasibility of a project or activity by comparing the costs incurred with the benefits obtained. Applying CBA to kenaf production entails weighing the costs of cultivation, processing, and marketing against the economic benefits of various kenaf end- uses, such as textiles, paper, and biodegradable products (Eval, 2023).

This idea emphasizes the importance of balancing economic growth, social progress, and environmental protection. When used to kenaf production, it entails determining how kenaf cultivation and utilization contribute to Malaysia's long- term economic development while taking into account social repercussions on local populations and the environmental sustainability of kenaf agricultural practices (Syafiqah, 2023).
It is is defined as:
$= \sum \{Rn/(1 + r)^n\} / \sum \{ Cn/(1 + r)^n \}$
Where: R = revenue

C = costs

n = number of years

r = interest rate

Thus, the overall formula of BCR is by dividing the sum revenues generated during production life over the sum of costs incurred.
The formula to calculate Benefit Cost Ratio (BCR) is shown as below:

Average Total Output (kg) Average Price **(A1 x A2) = A3**	A1 A2
Gross Margin (Revenue)	**A3**

Variable Cost + Fixed Cost	B B2
Total Cost	**B3**
Net Revenue **A3-B3** (Total Profit/ Loss)	C
Benefit Cost Ratio (BCR) **C / B3**	D

Table 1: Formula of Benefit Cost Ratio (BCR)

Thus, Benefit Cost Ratio will be calculated as the Net Revenue divided by the Total Cost.
BCR = Net Revenue / Total Cost

Cost-Benefit Analysis (CBA) is a systematic procedure for evaluating the prospective benefits and costs of a project or decision, commonly stated in monetary terms. It aids decision-makers in making informed decisions by assessing if the benefits outweigh the drawbacks.

Multiple Regression

$Yi = \beta0 + \beta1\chi1 + \beta2\chi2 + \beta3\chi3 \ldots\ldots\ldots \beta n\chi n + \varepsilon$
Y= Kenaf Production/ Yield $\beta0$ = Constant

$\beta1$- βn= Coefficient to be estimated $\chi1\chi3$
= Independent variables ε = the model's error term

This statistical technique examines the relationship between a dependent variable and two or more independent variables. It extends beyond simple regression by allowing for the investigation of how numerous factors influence the dependent variable concurrently. It provides insights into the strength and direction of these relationships.

RESULT AND DISCUSSION

This chapter presented the results of the data analysis. The data were analysed using the SPSS (Version 26) and Microsoft Excel.

Table 2: Pilot Test

Cronbach's Alpha	No. of Questions	No. of Respondent
0.853	51	7

The table presents the results of a reliability analysis using Cronbach's Alpha. The Cronbach's Alpha value is 0.853, indicating a high level of internal consistency among the items. This suggests that the items are reliably measuring the same underlying construct. The table also shows that 51 items were included in the analysis. A Cronbach's Alpha value above 0.7 is generally considered acceptable, while a value above 0.8 is considered good, and above 0.9 is excellent. Therefore, a value of 0.853 indicates that the set of 51 items has good reliability.

Reliability Test

Table 3 displays the results of a reliability test for a questionnaire using Cronbach's Alpha. The test assessed the internal consistency of the questionnaire, which consists of 51 questions and was administered to 50 respondents. The resulting Cronbach's Alpha value is 0.782, indicating a good level of reliability. This suggests that the questions on the questionnaire have a reasonable degree of internal consistency, meaning that they are measuring a cohesive underlying construct.

Generally, a Cronbach's Alpha value above 0.7 is considered acceptable (Hair, 2006), thus a value of 0.782 demonstrates that the questionnaire is reliable for the given sample.

Table 3: Reliability Test

Cronbach's Alpha	No. of Questions	No. of Respondent
0.782	51	50

Descriptive Analysis

The study shows that kenaf producers in Perlis, Malaysia, are predominantly middle-aged (31-50 years, 38%), male (76%), and married (74%), with small families (58% having 1-4 members). Educationally, 50% have high school education, and 44% have university degrees. Most producers (32%) have 7-9 years of experience and earn between RM2501-4000 monthly (38%). The majority are full-time workers (90%) and non-cooperative members (90%). Extension visits are frequent (46% received 3+ visits), with training and workshops being common services. Producers unanimously agree on the importance of high-quality planting materials and fertilizers, with 98% regularly applying fertilizer and 78% doing so themselves. Pesticide use is widespread (98%), and all can afford pesticides and follow safety procedures. Labor is crucial (94%), with 88% hiring additional help, and all respondents are aware of environmental hazards. Most have good knowledge of costs, revenue, and kenaf varieties, with all having formal training and a high selfrated ability in financial management. Key expenses are maintenance and labor, and 90% find kenaf cultivation profitable due to factors like easy management and fast returns. All respondents engage in workshops and primarily use WhatsApp for information, facing challenges like weather and itchiness. Essential skills include cultivation techniques and pest management.

Table 6: Descriptive Analysis of Smallholders Demographic

Variables	Categories	Number of Respondents	Percentage
Age	20-30 31-40 41-50 51-60 61-70 71-80	3 20 15 7 3 2	6 40 30 14 6 4

Gender	Male Female	41 9	82 18
Marital Status	Married Single	35 15	70 30
Family Members	1— 4 5-8 9-12	31 17 2	62 34 4
Educational Background	No education High School Primary School University	1 25 2 22	2 50 4 44
Year of Experience	0-3 4-6 7-9 10-15 More than 15 years	12 12 16 7 2	24 24 32 14 6
Income per month	2500 and below 2501-4000 4001-6000 8001-10000	18 19 12 1	36 38 24 2
Job Status	Part time Full time	5 45	10 90
Join Any Farmers Cooperation	Yes No	45 5	90 10

Extension Visit	1-2 Times 3-4 Times 5-6 Times Many Times	18 18 9 5	36 36 18 10
Serviced Received	Training Workshop Training and Workshop Training and Advice Training,workshop and Advice	2 10 14 11 13	4 20 28 22 26

Table 4 summarizes multiple regression analyses of impact of age, number of family members, education background, years of experience, knowledge, training, financial literacy, cost, and environmental factor on kenaf production among producers in Perlis, Malaysia. The result showed that a combination of all factors contributed 46% (R^2 = 0.460) in impacting Kenaf in Malaysia

Table 4: Model Summary of Multiple Regression

Model	**R**	**R Square**	**Adjusted R Square**	**Std. Error of the Estimate**
1	0.679	0.460	0.339	718.187

While Table 5 provides estimates of multiple regression model that shows the coefficients for each independent variable.

Table 5 : Summary of multiple regression analysis impact of age, number of family members, education background, years of experience, knowledge, training, financial literacy, cost, and environmental factor in affecting kenaf production among producers in Perlis, Malaysia.

Variable	**Unstandardized B**	**Standardized Beta**	***t***	***p***
Age	-186.764	-0.196	-1.003	0.322

Number of family members	-213.223	-0.130	-0.748	0.459
Education background*	-686.931	-0.515	-2.652	0.011
Years of experience**	264.734	0.354	1.669	0.103
Knowledge	-27.702	-0.086	-0.419	0.677
Training**	-411.229	-0.367	-2.474	0.018
Financial Literacy*	586.560	0.390	1.746	0.089
Cost	-276.789	-0.308	-1.158	0.254
Environmental Factor	235.895	0.129	0.780	0.440

**p < .05 (significant at the 0.01 level, 2-tailed): **p < .10 (significant at the 0.01 level, 2-tailed)* Firstly, the unstandardized coefficient for education background is B = -686.931, with a standardized Beta of β = -0.515. This result is statistically significant (p = 0.011), indicating a strong negative impact on kenaf production. Higher levels of education background are associated with lower kenaf production, suggesting that those with more education might be less engaged or efficient in kenaf production activities. A study by Rahman et al. (2004) on kenaf production in Malaysia discovered that farmers with higher education levels were less likely to engage in kenaf cultivation. The research indicated that individuals with more education often pursue employment in other sectors, thereby decreasing their participation in agricultural activities such as kenaf farming. A broader analysis by the Food and Agriculture Organization (FAO) found that higher educational attainment often results in a preference for white-collar jobs over farming, especially in developing countries. This trend aligns with the observation that individuals with higher education levels are less likely to be directly involved in agricultural production.

Moreover, the unstandardized coefficient for training is B = -411.229, with a standardized Beta of β = -0.367. This variable has a significant negative impact on kenaf production (p = 0.018), indicating that more training is associated with decreased production. This counterintuitive result suggests that perhaps the type or quality of training provided may not be effectively enhancing production capabilities. A review by Davis et al. (2010) highlighted that many agricultural training programs fall short in significantly improving productivity because they do not sufficiently address the specific needs and contexts of farmers. The study stressed the importance of context-specific training that considers local conditions and practical applications. A study by Ragasa et al. (2013) found that the quality of training is essential for its success. When training programs were poorly designed or delivered, farmers saw little benefit in terms of productivity improvements. The study emphasized that factors such as the relevance of the content, the delivery methods, and the followup support provided to farmers were crucial in determining the effectiveness of training program.

The unstandardized coefficient for age is B = -186.764, with a standardized Beta of β =

-0.196. This indicates that as age increases, kenaf production tends to decrease slightly. However, this relationship is not statistically significant, as evidenced by the p-value of p =
0.322. Thus, while there is a negative association, age does not have a significant impact on kenaf production among the producers in Perlis, Malaysia. For the number of family members, the unstandardized coefficient is B = -213.223, and the standardized Beta is β = -0.130. This suggests a negative relationship, meaning that having more family members slightly decreases kenaf production. However, similar to age, this effect is not statistically significant (p = 0.459), indicating that the number of family members does not substantially influence kenaf production. A study by Asadullah and Rahman (2009) found that family size had an insignificant impact on agricultural productivity in Bangladesh. The study suggested that beyond a certain point, additional family members did not significantly enhance productivity.

Years of experience has an unstandardized coefficient of B = 264.734 and a standardized Beta of β = 0.354. Although this indicates a positive relationship, suggesting that more years of experience lead to higher kenaf production, the p-value of p = 0.103 shows that this result is not statistically significant. Hence, while experience appears beneficial, it does not have a strong, significant impact on production in this context. Knowledge has an unstandardized coefficient of B = -27.702 and a standardized Beta of β = -0.086. This very slight negative impact on kenaf production is not statistically significant (p = 0.677). Therefore, the level of knowledge does not significantly influence kenaf production among the producers studied. In the study from Ali et al (2020), the researchers observed that factors such as resource availability, environmental conditions, and market access had a greater influence on kenaf production than the producers' level of knowledge. Their analysis showed that while having knowledge is beneficial, it is not the primary factor in successful kenaf cultivation. Practical elements like having the necessary farming inputs, suitable weather, and access to markets for selling the produce were more significant. This indicates that improving these external conditions could enhance kenaf production more effectively than solely increasing the producers' knowledge.

Financial literacy shows a positive impact on kenaf production, with an unstandardized coefficient of B = 586.560 and a standardized Beta of β = 0.390. However, the relationship is not statistically significant (p = 0.089). While better financial literacy tends to increase production, the evidence is not strong enough to confirm a significant effect. Cost has an unstandardized coefficient of B = -276.789 and a standardized Beta of β = -0.308. This negative relationship suggests that higher costs are associated with lower kenaf production, but the effect is not statistically significant (p = 0.254). Thus, while costs impact production, the influence is not strong enough to be considered significant in this analysis. Costs, including those for seeds, fertilizers, pesticides, labor, and machinery, are crucial factors that determine agricultural productivity. High costs can significantly reduce profitability, influencing farmers' decisions about crop management practices and the inputs they use. A study by Anriquez et al. (2011) emphasized the significant role these cost factors play in agricultural production decisions, affecting both yields and profitability.

When the costs of essential inputs are high, farmers may struggle to maintain profitability, leading them to make difficult choices about how to manage their crops. For instance, they might reduce the amount of fertilizer or pesticides they use, which can negatively impact crop yields. Similarly, high labor costs might force farmers to cut back on the workforce, potentially affecting of their operations.

Anriquez et al. (2011) highlighted that adopting cost-efficient farming practices is essential for achieving sustainable agricultural production. By finding ways to reduce expenses without compromising on the quality or quantity of their produce, farmers can improve their profitability and ensure the long-term viability of their farming operations. The study underscores the importance of supporting farmers in accessing affordable inputs and adopting efficient practices to enhance productivity and sustainability in agriculture.

The unstandardized coefficient for environmental factor is B = 235.895, with a standardized Beta of β = 0.129. This indicates a positive relationship with kenaf production, meaning better environmental conditions lead to higher production. However, this result is not statistically significant (p = 0.440), suggesting that environmental factors do not have a substantial or significant impact on kenaf production in this study. In the research Smith et al(2018), the authors discovered that environmental factors did not significantly influence kenaf production. Instead, their findings revealed that other variables were far more crucial in determining the yield and overall success of kenaf crops. Specifically, they noted that technological inputs and effective management practices had a more pronounced impact. The study suggested that advancements in farming technology, along with improved management techniques, were key drivers of productivity. This implies that even in varied environmental conditions, producers who adopt better technology and sound management strategies can achieve higher yields. Therefore, the research underscores the importance of focusing on these aspects rather than relying solely on environmental factors to enhance kenaf production.

In conclusion, the multiple regression analysis evaluating the impact of various factors on kenaf production among producers in Perlis, Malaysia, reveals several key insights. Education background and training are the only factors with statistically significant effects, both showing negative impacts. This suggests that higher education levels and more training may not be conducive to increased kenaf production, possibly indicating a misalignment between the education and training provided and the practical needs of kenaf producers. Other factors, including age, number of family members, knowledge, years of experience, financial literacy, cost, and environmental factor, do not show statistically significant impacts. While some of these factors have directional effects (positive or negative), their influence is not strong enough to be considered significant in this analysis. Notably, years of experience and financial literacy have positive coefficients, suggesting they could potentially enhance production, but their effects are not significant in this sample. Research suggests that both years of experience and financial literacy have the potential to enhance agricultural productivity, although their impacts may not consistently achieve statistical significance. For instance, a study conducted in Pakistan revealed that while these factors can exert a positive influence on farming outcomes, their effectiveness often hinges on various contextual variables such as the accessibility of financial services and the practical application of farming techniques (Adrian Wheeler ,2018).

Similarly, according to a World Bank report on agricultural finance, the combination of access to financial tools designed for farmers and financial literacy can contribute to improved agricultural productivity. However, the extent of this impact varies and is significantly shaped by the financial infrastructure and the successful integration of these tools within the local agricultural landscape (Alexander Lotsch, 2010).

These findings underscore a nuanced relationship where experience and financial knowledge offer advantages, yet their effects are intricately intertwined with other elements within the agricultural ecosystem. The interplay between these factors highlights the complexity of enhancing productivity in farming contexts, necessitating a holistic approach that considers local conditions, infrastructure, and the practical application of financial knowledge.

Overall, the study highlights that while certain educational and training aspects may need reevaluation to better support kenaf production, other factors like experience and financial literacy might benefit from further investigation with larger or more targeted samples to better understand their potential impacts. The findings suggest the need for tailored strategies that align more closely with the practical realities and needs of kenaf producers. In agricultural research, there is a clear emphasis on the critical need to customize strategies to suit the unique requirements, circumstances, and obstacles encountered by farmers. This tailored approach ensures that interventions are not only relevant but also practical, effectively addressing the constraints and leveraging the opportunities specific to diverse agricultural settings. For instance, findings from a study conducted by Davis et al. (2010) on farmer field schools underscore the importance of adapting agricultural interventions to local conditions. The study highlighted that interventions are most successful when they integrate indigenous farmer knowledge and practices, directly confront practical challenges, and are designed to resonate with the specific agricultural contexts they aim to improve.

This approach recognizes that agriculture is inherently contextual, shaped by varying ecological, economic, and social factors across different regions. By tailoring strategies to fit local realities, interventions stand a better chance of being embraced and effectively implemented by farmers. Moreover, this tailored approach enhances the sustainability of agricultural improvements, as solutions are rooted in the day-to-day experiences and needs of those directly engaged in farming activities. Therefore, the call for customization in agricultural strategies reflects a commitment to fostering resilience and productivity in farming communities through methods that are not only informed by research but also grounded in the practical wisdom of local farmers.

COST BENEFIT ANALYSIS

Table 6: Cost Benefit Analysis

	Kenaf farmer	
List	**R M**	**Percenta ge**

Production Cost		
A. Fixed Cost		
Plowing (Disc)	45 0	6.60%
Plowing (Turn 1)	40 0	5.87%
Plowing (Turn 2)	40 0	5.87%
Harvesting(Combine Havester)	45 0	6.60%
Total Fix Cost	17 00	24.96%
B.Variable Cost		
Planting + Basic Fertilization	30 0	4.40%
Pre-Emergence Herbicide Application	25 0	3.67%
Seeds	40 0	5.87%
NPK Green Ferrtilization (15:15:15)	16 56	24.30%
NPK Blue Fertilizer (12:12:17)	22 95	33.68%
Pre-Emergence Herbicide	13 6	2.00%
Insecticide	78	1.14%

Total Variable Cost	51 15	75.04%
C. Total Cost	68 15	100%

The cost-benefit analysis table offers a detailed breakdown of expenses for kenaf production in Perlis, Malaysia, categorized into fixed and variable costs. Fixed costs include essential expenses that remain constant regardless of output levels. The initial plowing with a disc plow costs RM 450, which constitutes 6.60% of the total production cost. This is followed by the first and second turns of plowing, each costing RM 400 and accounting for 5.87% of the total cost, ensuring the soil is adequately prepared for planting. Harvesting with a combine harvester also incurs a cost of RM 450, comprising another 6.60% of the total cost. Combined, these fixed costs amount to RM 1700, representing 24.96% of the total production cost.

Variable costs, which change with the level of production, cover inputs directly related to cultivation. Planting and basic fertilization cost RM 300, representing 4.40% of the total production cost, and provide essential nutrients at the beginning of the crop cycle. Pre- emergence herbicide application costs RM 250, accounting for 3.67% of the total cost, and helps control weeds before they emerge. Seeds cost RM 400, making up 5.87% of the total cost, underscoring the importance of quality seeds for good germination rates. NPK green fertilization (15:15:15) costs RM 1656, constituting 24.30% of the total cost, while NPK blue fertilization (12:12:17) costs RM 2295, the largest single expense at 33.68%. These fertilizers provide essential nutrients for healthy growth. An additional pre-emergence herbicide costs RM 136, representing 2.00% of the total cost, ensuring comprehensive weed control. Insecticides, costing RM 78 and accounting for 1.14% of the total cost, protect the crop from pest infestations.

Overall, the total variable costs amount to RM 5115, comprising 75.04% of the total production cost. When combined with fixed costs, the total production cost of kenaf reaches RM 6815. This detailed breakdown is essential for understanding the cost structure of kenaf production, highlighting areas where costs can be managed or reduced to enhance profitability. Fixed costs establish the necessary foundation for initiating and maintaining production, while variable costs represent ongoing investments that directly affect yield quality and production levels.

COST BENEFIT RATIO

Table 7: Cost Benefit Ratio

Parameter	**Cost/ha(RM)**
Plowing (Disc)	450
Plowing (Turn 1)	400
Plowing (Turn 2)	400
Harvesting (Combine Harvester)	450
Planting + Basic Fertilization	300
Pre-Emergence Herbicide Application	250
Seeds	400

NPK Green Fertilization (15:15:15)	1656
NPK Blue Fertilizer (12:12:17)	2295
Pre-Emergence Herbicide	136
Insecticide	78
Total Cost	**6815**
Yield (kg/ha)	**617**
Total Profit/loss (RM)	**5525**
Net Revenue (RM)	**5525**
Income (RM)	**12340**
Cost Benefit Ratio (CBR)	**2.23**

The table 12 provides a detailed overview of the costs and benefits associated with kenaf production per hectare (ha) in Perlis, Malaysia, leading to the calculation of the Cost Benefit Ratio (CBR). The costs are categorized, starting with plowing expenses: RM 450 for disc plowing, RM 400 for the first plowing, RM 400 for the second plowing, and RM 450 for harvesting with a combine harvester. These initial costs are essential for land preparation and maintenance.

The variable costs include RM 300 for planting and basic fertilization, RM 250 for pre- emergence herbicide application, and RM 400 for seeds. Fertilization costs are significant, with RM 1656 for NPK green fertilization (15:15:15) and RM 2295 for NPK blue fertilizer (12:12:17). Additionally, RM 136 is spent on pre-emergence herbicide and RM 78 on insecticide. The total cost per hectare for kenaf production is RM 6815.

On the benefits side, the yield is 617 kg/ha. The total profit or loss from this yield is RM 5525, which also represents the net revenue. The total income generated from kenaf production is RM 12340, providing a clear picture of the economic returns.

The Cost Benefit Ratio (CBR) is a crucial measure of investment and profitability, calculated by dividing total revenue by total cost. Using the table data, the CBR is obtained by dividing the total income (RM 12340) by the total cost (RM 6815), theoretically resulting in a CBR of approximately 1.81. However, the table reports a CBR of 2.23, suggesting there may be additional context or specific calculations influencing this figure.

A CBR greater than 1 indicates profitability. With a CBR of 2.23, it means that for every RM 1 spent on kenaf production, the return is RM 2.23. This notable return highlights the profitability of kenaf farming in Perlis. The detailed breakdown of costs and benefits, along with the CBR calculation, provides valuable insights for farmers and stakeholders, enabling them to make informed decisions about resource allocation and production strategies. The analysis emphasizes the economic viability of kenaf production, indicating it is a profitable agricultural venture in the region.

CONCLUSION

The study concludes that among the factors examined, education and training have a significant negative impact on kenaf production in Perlis, Malaysia. This suggests that higher education levels and existing training programs may not be well-aligned with the practical needs of kenaf farming, potentially leading to inefficiencies or misunderstandings in applying agricultural techniques specific to this crop. In contrast, age and household size do not significantly affect kenaf production, indicating that these demographic variables do not play a crucial role in influencing the productivity of kenaf farmers in the region.

Interestingly, experience and financial literacy showed positive correlations with production, indicating that these factors could potentially improve kenaf production if effectively utilized. However, their lack of statistical significance in this study underscores the necessity for further research with larger or more specific samples to validate these findings and to investigate the mechanisms through which experience and financial literacy impact production.

The study highlights the importance of reassessing and reforming existing educational and training initiatives to better meet the practical requirements and challenges faced by kenaf producers. This might entail incorporating more experiential, field-oriented training and emphasizing practical knowledge and skills that directly benefit kenaf production.

Furthermore, the results emphasize the significance of understanding the wider socio-economic and environmental conditions that influence kenaf farmers. Policymakers and stakeholders should consider these factors when developing support programs and interventions aimed at enhancing kenaf production. The study's findings offer a deeper look into the critical factors that affect kenaf production.

This understanding is vital for creating better support programs and policies that truly meet the needs of kenaf farmers. By using these insights, policymakers and stakeholders can help build a sustainable and economically successful kenaf industry in Malaysia. This aligns with the country's goals of diversifying agriculture and promoting overall economic growth. Ultimately, these efforts aim to strengthen the agricultural sector, ensuring that kenaf producers and the nation as a whole can thrive despite various challenges.

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CHALLENGES OF IMPLEMENTATION OF MALAYSIA SUSTAINABLE PALM OIL CERTIFICATION AMONG INDEPENDENT SMALLHOLDERS IN SELANGOR, MALAYSIA

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ABSTRACT

This research aims to identify and analyze the key challenges faced by palm oil independent smallholders in Kuala Langat, Selangor, regarding the implementation of Malaysia Sustainable Palm Oil (MSPO) certification. The study examines the factors influencing these challenges and evaluates the effectiveness of government support in overcoming them in order to help independent smallholders to get fully certified. Utilizing a quantitative method, the study collected data from local smallholders to understand their experiences and the hurdles they encounter. The findings reveal that the primary challenge for independent smallholders is financial constraints. Despite these obstacles, successful government initiatives appear to play a significant role in alleviating financial burdens, thereby facilitating the adoption of sustainable practices such as MSPO certification. The study highlights the critical role of government support in disseminating information and providing education to smallholders, which enhances their understanding and potential adoption of the certification. However, the study also suggests that while government support is beneficial, it often involves time-consuming processes, including compliance and training requirements, which add to the smallholders' time burdens. Based on these findings, several recommendations are proposed to maximize the benefits of government support. These include streamlining compliance and training processes to reduce the time burden on smallholders, enhancing financial assistance programs, and improving the dissemination of information related to MSPO certification. The research underscores the importance of continued government support in addressing financial and informational challenges faced by independent smallholders, ultimately promoting the adoption of sustainable palm oil practices in Malaysia.

Keywords: Malaysia Sustainable Palm Oil Certification, Financial Constraints, Government Support.

INTRODUCTION

Oil palm in Malaysia has turned into a huge industry as it contributes to gross domestic product (GDP), unlimited economic sectors, and emerging economic sectors in Malaysia. Hence, Malaysia plays a vital role in achieving the global needs for palm oil. In addition, as of 2019, Malaysia produced a massive crude palm oil which is 19.86 million metric tonnes from 5.9 million hectares (MPOB, 2020). Compared to 2018, there is a significant increase in production of crude palm oil by 0.34 million metric tonnes. However, due to low export prices for most part of the year 2019, there was a notable decline in export revenue as compared to 2018 by 4% from 67.52 billion to 64.84 billion (Parveez, 2020).

The distribution of oil palm production in Malaysia varies significantly across different states. Malaysia is one of the largest producers and exporters of palm oil globally, and the planted areas for oil palm are strategically located to maximize production and efficiency. In general, the states of Sabah and Sarawak in East Malaysia have the largest planted areas, contributing more than half of the total oil palm cultivation in the country. In Peninsular Malaysia, the states of Johor and Pahang also play a crucial role, with substantial areas dedicated to oil palm cultivation. The strategic distribution of oil palm plantations across Malaysia ensures a steady supply of palm oil for both domestic use and export, supporting the country's economy. The table below presents the distribution of oil palm planted areas by state in Malaysia, along with their respective percentages of the total planted area. This data illustrates the geographical spread and the significant role each state plays in the palm oil industry.

Table 1:Distribution of production of oil palm

State	Planted Area (ha)	Percentage (%)
Sabah	1,551,669	26.8
Sarawak	1,588,556	27.5
Johor	740,640	12.8
Pahang	757,882	13.1
Perak	354,193	6.1
Kelantan	139,984	2.4
Terengganu	161,846	2.8
Selangor	93,394	1.6
Kedah	69,204	1.2
Negeri Sembilan	169,107	2.9
Melaka	63,563	1.1
Perlis	7,683	0.1
Penang	13,160	0.2
Total	5,724,445	100

Source: MPOB, 2023

In supplying the global palm oil demand, there are several mandatory and voluntary certification schemes and its standard that exist in order to certify and validate sustainable palm oil production for all palm oil grower. One of the certifications that exist Malaysian sustainable Palm Oil (MSPO). The Malaysian Sustainable Palm Oil (MSPO) certification scheme transitioned from being voluntary on January 1, 2015, to becoming mandatory nationwide on January 1, 2020, covering all smallholders, plantations, and mills. In response to the ongoing drive for sustainable palm oil production, the Malaysian Standard (MS) on MSPO was developed to provide guidelines aligning with global demands for sustainable oil palm products. Compliance with MSPO requirements is monitored through audits conducted by third-party auditors, ensuring adherence to sustainability principles and criteria, as outlined by the Malaysian Palm Oil Board (MPOB, 2015). In addition, pressure on certification schemes has led to uses of technologies and increased research and development in the oil palm industry in order to produce yield with zero waste. The MSPO certification, customized to the specific conditions of the region, assists small-scale farmers in implementing sustainable farming methods. This might be used as a reply to counter the accusations about the environmental and social consequences associated with the Malaysian palm oil sector (Norhidayu et al., 2021).

Table 2 Malaysian Sustainable Palm Oil Certification

	Certified Planted Area(ha)
Independent Smallholders Certified Under MSPO Part 2	647,691,24
Organized Smallholder Certified Under MSPO PART 3	574,607,48
Plantation Certified Under MSPO Part 3	3,983,872.94
Total	5,206,171,66
	Unit Certified
Certified Mills Under MSPO Part 4	425

Source: Parveez, 2020

To successfully implement Malaysia Sustainable Palm Oil (MSPO) certification among independent smallholders in Selangor, it's crucial to address various challenges and strategic interventions. These include identifying obstacles such as financial limitations, technological gaps, and regulatory complexities (Chew F.T, 2019), analyzing how factors like educational background, agricultural experience, and current sustainable practices impact smallholders' ability to achieve MSPO certification (Ibrahim, M. H., & Jaafar, S. 2018), and evaluating the effectiveness of government initiatives, including financial assistance, capacity-building workshops, and regulatory simplification (Ministry of Plantation Industries and Commodities Malaysia, 2020). Addressing these challenges comprehensively will foster sustainable practices and enhance the long-term viability of Malaysia's palm oil industry.

Identifying challenges in sustainable palm oil certification for independent smallholders is crucial as it helps authorities develop measures to support certification efforts and evaluate government initiatives. For instance, an independent smallholder reported poor information dissemination about MSPO despite agreeing to certification (Rahmat et al., 2021). Addressing these challenges can increase the number of certified smallholders, boosting Malaysia's GDP, as the oil palm industry contributed 37.7% to the agricultural GDP in 2019 (Rahman, 2020). Certification also improves market access, meets legal requirements, and promotes environmentally friendly practices, enhancing consumer acceptance and supporting a shift towards sustainable and ethical consumption.

LITERATURE REVIEW

In Malaysia, oil palm smallholders are categorized into independent and organized smallholders. Independent smallholders manage or hire labor for holdings of 40.46 hectares or less, with 255,615 such smallholders as of December 2018 (MPOB 2019). Organized smallholders, managed by government agencies like FELDA, number 315,293 and manage 682,269 hectares. Under the

Malaysian Palm Oil Board (MPOB), independent smallholders are grouped into Sustainable Palm Oil Clusters (SPOC), which are overseen to improve the quality and quantity of Fresh Fruit Bunches (FFB) through the Sustainable Palm Oil Growers Cooperative (KPSM) (P Yap et al, 2021; Mail, 2022).

The Malaysia Sustainable Palm Oil (MSPO) certification, established in 2014 and managed by the Malaysian Palm Oil Certification Council (MPOCC), aims to improve agricultural practices and ensure sustainability. The MSPO covers independent and organized smallholders, plantations, and mills, each with specific criteria. It includes seven principles: management's dedication, transparency, legal compliance, social responsibility, environmental stewardship, best practices, and new plantation development (MPOCC 2019a, 2019b, 2019c; Palm Oil Health, 2017). This framework ensures sustainable palm oil production and worker rights in Malaysia.

The Malaysian Sustainable Palm Oil (MSPO) certification, established by the Malaysian government in 2013 and mandatory since January 2020, aims to ensure the sustainability of the palm oil sector. Despite this, applications from smallholders remain below 2% (Kumaran, 2019). The Malaysian government has urged the EU to recognize MSPO as a voluntary certification program, and international bodies like the Japanese Olympic Council and China Green Food Development Centre have already acknowledged it, boosting Malaysia's palm oil market (Bernama, 2019; Ismail, 2019).

Federal agencies such as FELCRA, FELDA, and the MPOB play crucial roles in promoting MSPO certification. FELCRA oversees Organised Smallholders (OSH), while FELDA provides technical, processing, and financial support. The MPOB is responsible for aiding independent smallholders in achieving certification, with approximately 600,000 hectares of smallholder palm oil farms certified as of May 2022 (Amran, 2023).

Smallholders in Malaysia face significant challenges in implementing the Malaysia Sustainable Palm Oil (MSPO) certification, including financial constraints, limited access to information, and time constraints. Financially, the high costs of sustainable practices, certification, and necessary infrastructure upgrades are often unaffordable, compounded by limited access to affordable credit (Smith, 2022; Lee et al., 2023; Tan et al., 2022). Additionally, a lack of critical information about the certification process leads to misunderstandings and noncompliance, hindering smallholders from making necessary adjustments (Johnson, 2021; Lee et al., 2023). Time constraints further complicate the situation, as smallholders struggle to balance daily farm operations with the demands of learning and implementing sustainable practices, often prioritizing short-term production over long-term sustainability (Tan et al., 2022; Lee et al., 2023). Addressing these challenges is essential for supporting smallholders in achieving MSPO certification.

The implementation of Malaysia Sustainable Palm Oil (MSPO) certification among smallholders is influenced by various factors, including education level, experience, and farm practices. Educated smallholders are better equipped to understand and meet certification requirements, recognizing benefits such as market access and sustainability (Smith, 2022; Lee et al., 2023). Experienced farmers may resist certification due to their reliance on traditional methods, while less experienced farmers are more open to adopting new practices for market benefits (Tan et al., 2022; Smith, 2022; Williams, 2023). Sustainable farming practices like integrated pest control and soil preservation align well with MSPO standards, facilitating certification, whereas traditional practices pose more challenges (Parthiban, 2021; Johnson, 2021; Mohd Suib, 2023). Encouraging sustainable methods and providing targeted support can enhance certification adoption among smallholders.

The Diffusion of Innovations Theory can aid in understanding the challenges smallholders face in adopting MSPO certification by focusing on perceived qualities such as complexity, trialability, and observability. Policymakers can enhance adoption rates by simplifying the process, offering trial projects, and showcasing success stories. Addressing compatibility and relative advantage by aligning certification with existing practices and highlighting financial benefits can further ease adoption, promoting sustainable palm oil practices in Malaysia.

The Theory of Planned Behaviour (TPB), developed by Icek Ajzen in 1991, helps predict and understand behaviour based on attitude towards the behaviour, subjective norms, and perceived behavioural control. In the context of MSPO certification, smallholders' attitudes, perceived control over the certification process, and the influence of education and experience can significantly impact their willingness to pursue certification. TPB provides a framework to analyze how these factors affect smallholders' intentions and behaviours, aiding in identifying obstacles and creating strategies to support certification adoption.

MATERIALS AND METHODS

The study contained information regarding characteristics or socio-demographics of respondents as well as other variables and methods for analysing the results. This investigation will be utilising a quantitative method. Quantitative research takes sample data and generalises its findings by analysing the complete sample. In quantitative research, data can be collected by the survey method or field observation strategy. The focus of the discussion on the research design adopted in this study is on how the sample and sampling will be selected, the instrument used in this study, the data collecting technique, and how the data will be analysed. Research will take place on several oil palm plantations among independent smallholder. The offered research framework (**Figure 1**) illustrates the thorough methodology utilized to carry out the investigation.

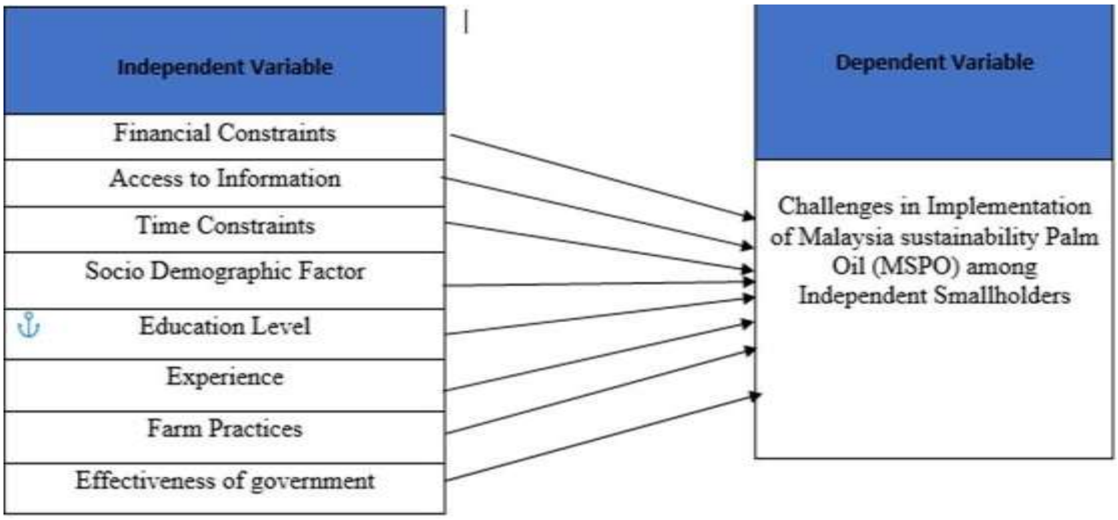

Figure 1: Research Framework

Location of study

The survey of this study has been focusing on oil palm independent smallholders located in MPOB branch Sustainable Palm Oil Cluster B2 Kelanang area Kuala Langat, districts of Selangor state of Malaysia. The study will employ a snowball sampling technique to select 245 independent smallholders from a population of 671 listed by the Malaysian Palm Oil Board (MPOB) under the Smallholders Palm Oil Cluster (SPOC) data. This sample size, determined using the Rao soft sample size calculator, ensures adequacy for statistical analysis using IBM SPSS Statistics. The study will gather both socio-demographic characteristics and other variables of the respondents, employing a quantitative method through surveys and field observations. The survey, conducted from April 10 to May 20, 2024, in Kuala Langat, Malaysia, will use a standardized questionnaire during site visits. Data analysis will be performed using SPSS to code and analyze the collected data, facilitating the interpretation of complex data sets and supporting meaningful conclusions.

Data Analysis

Data analysis is referred as the process of applying statistical or logical techniques in a methodical manner in order to describe and illustrate, condense and recap, and evaluate data is referred to as. It is essential for interpreting complex data sets and drawing meaningful conclusions, which ultimately support or refute hypotheses within a study (Shamoo Resnik, 2003). Therefore, the Statistical Package for Social Science, also known as SPSS, will be utilized in order to code and analyse the data that was collected through the questionnaire. Primary function of this application is to perform an analysis on scientific data in connection with the social sciences. This information can be put to use in a variety of research endeavours, including data mining, surveys, and market analysis. In its most basic form, SPSS works by first storing and organizing the data that is given to it, and then it compiles the data set in order to produce appropriate output. SPSS was developed in such a way that it is capable of managing a diverse range of data formats and coming to sound conclusions regardless of the circumstances. SPSS is utilized in order to conduct the analysis required for these research findings. In this study, the data were analysed using four different approaches, a reliability test, descriptive analysis, correlation analysis, and multiple linear regression analysis.

Descriptive statistics refers as the process of analysing, summarizing, and presenting findings in relation to a data set that is derived from either a sample or the entire population (Trinidad, 2020). In order to gain a general understanding of the respondents' profiles, a descriptive analysis was conducted. The mean and standard deviation of the independent variables as well as the dependent variable will be analysed with the help of the descriptive analysis. In terms of the demographic data, the computation will make use of both the frequency and the percentage. Researcher will have an easier time interpreting or drawing conclusions from the data that has been collected if they choose to use descriptive analysis.

The statistical method known as correlation analysis is applied to the task of determining whether or if there is a relationship between two variables or datasets, as well as the potential strength of such a connection. This means that correlation analysis is used in market research to analyse quantitative data obtained from research methods such as surveys and polls, with the goal of determining whether or not there are any significant links, patterns, or trends between the two variables. Main objective of correlation analysis is to identify design that can be found within datasets. A positive correlation means that both variables increase or that there is a strong correlation between them, whereas a negative correlation indicates that either one of the variables drops or that there is just a weak link between each other.

A Multiple regression analysis is used to find out if the constructs were positive predictors that were statistically significant. In the first analysis, a standard multiple regression will be estimated with all five construct inputs as independent variables and implementation of MSPO as the dependent variable. Result will be performed to test the idea of a cause-and-effect relationship that is built up during this research. Multiple regression is a good choice for this study because it lets you figure out how well you can predict an outcome when you know all the things that can affect it (Vogt, 2007). The method also can predict an outcome variable when all the predictors are known (Vogt, 2007).

RESULTS AND DISCUSSION Description of socio-demographic

The data provided represents the frequency and relative frequency of participants' ages grouped into different categories. The Gender table indicates that the majority of the participants are male, with 185 individuals representing 75.2% of the sample. In contrast, there are only 61 females, making up 24.8% of the respondents. This significant gender disparity suggests that the field of oil palm might be male-dominated, or that males are more likely to participate in this type of survey. Understanding the gender distribution is crucial for recognizing potential gender biases and tailoring interventions or support programs accordingly. Next is age socio-demographic. The age distribution shows a diverse range of ages among the participants. The largest group falls within the 41-50 years old category, with 104 individuals (42.3%). This is followed by participants aged 51 years and above, comprising 75 individuals (30.4%). The 31-40 years old group consists of 56 individuals (22.8%), while the youngest group, aged 20-30 years old, includes only 11 individuals (4.5%). This distribution indicates a relatively older demographic engaged in the oil palm sector, which could have implications for workforce planning and the introduction of new technologies or practices. The race is another important demographic variable captured in the table. There are 116 Malay participants, accounting for 47.1% of the sample, while Chinese participants number 130, making up 52.8%. This nearly even split highlights the racial diversity within the surveyed population. Such diversity can influence cultural practices, language preferences, and community dynamics within the oil palm industry, and understanding it is vital for ensuring inclusive and effective communication and policy implementation. Educational attainment among the participants varies significantly. Those with primary school education constitute 56 individuals (22.8%), while those with secondary school education represent the largest group, with 115 individuals (46.7%). Participants with tertiary education make up 75 individuals (30.5%). This distribution suggests that while a substantial portion of the workforce has a basic or intermediate level of education, a notable percentage has advanced education. This variation in education levels can impact the adoption of new techniques and technologies, as well as the overall productivity and efficiency within the industry.

Experience in the oil palm sector is split between two categories. Those with 11-20 years of experience form the majority, with 129 individuals (52.4%), while those with 21-30 years of experience number 117 (47.6%). This indicates that the respondents are quite experienced, with a considerable amount of time spent in the industry. Such extensive experience suggests a deep understanding of the sector, but it also highlights the potential need for succession planning and knowledge transfer to newer, less experienced workers. The types of land ownership among participants are fairly evenly distributed. Those who own their land number 117, representing 47.6% of the sample. Participants using rented land are much fewer, with only 11 individuals (4.5%), and those sharing land are 118 (48%). This balance between owned and shared land types suggests diverse land tenure arrangements in the oil palm sector, which can influence farming practices, investment decisions, and long-term sustainability strategies. Lastly, licensing status is another critical variable. The data shows that 129 participants (52.4%) are certified, while 117 (47.2%) are not certified. The near-even split between certified and non-certified participants underscores the importance of understanding the barriers to certification and the potential benefits it might bring. Certification could relate to sustainability practices, market access, and compliance with regulations, making it a significant factor in the industry's overall functioning.

Table 3: Descriptive Analysis of Smallholders Socio-Demographic

Profile		Frequency	Percentage (%)
Gender	Male	185	75.2
	Female	61	24.8
Age	20-30 years old	11	4.5
	31-40 years old	56	22.8
	41-50 years old	104	42.3
	51 years old and above	75	30.4
Race	Malay	116	47.1
	Chinese	130	52.8
Education Level	Primary School	56	22.8
	Secondary School	115	46.7
	Tertiary School	75	30.5
Experience in Oil Palm	11-20 Years	129	52.4
	21-30 Years	117	47.6
Land Type	Own	117	47.6
	Rental	11	4.5
	Share	118	48.0
License	Certified	129	52.4
	Not Certified	117	47.2

Reliability and internal consistency

The reliability test is utilized to indicates the extent to which it is without bias and thus to ensures consistent measurement across time and across the various items in the instruments. According to Sekaran (2009) the reliability also a measure of stability and consistency with which the instrument measure the concept and helps to assess the "goodness" of a measure. Cronbach alpha reliability coefficient that shows how the items in the set positively with each other. Cronbach Alpha is close to the 1. is higher the internal consistency reliability. In general, the reliability of less than 0.60 was considered poor, 0.6 to 0.69 are considered questionable reliability, they are in 0.70 are considered acceptable and those over 0.80 is considered good and lastly Cronbach Alpha values above 0.9 is considered. (Glien, JA, & Gliem, R. R, 2003).

The study employs a structured approach to identify and assess key challenges faced by independent smallholders in implementing Malaysia Sustainable Palm Oil (MSPO) certification. Three primary variables—access to information, financial constraints, and time constraints—are central to this investigation.in table 4 each variable is meticulously examined using a comprehensive questionnaire developed with high reliability, as indicated by Cronbach's alpha values: 0.983 for access to information, 0.971 for financial constraints, and 0.980 for time constraints. These high alpha values suggest strong internal consistency among the items within each variable, ensuring that the data collected accurately reflects the challenges experienced by smallholders.

Specifically, the variable of access to information scrutinizes the adequacy and clarity of information available to smallholders regarding MSPO certification processes and requirements. Financial constraints encompass the affordability of adopting sustainable practices, meeting certification costs, and accessing necessary financing. Meanwhile, time constraints gauge the difficulties smallholders face in managing the additional time demands imposed by MSPO compliance alongside daily farm operations. These variables collectively provide a detailed portrait of the obstacles hindering smallholders from fully embracing MSPO certification, thereby informing targeted strategies to enhance support and effectiveness of government interventions in promoting sustainable palm oil practices

Table 4: Croanbach Alpha Analysis

Variables (Challenges)	**No. Item**	**Croanbach's Alpha**	**Remarks**
Access to Information	5	0.983	Excellent
Financial Constraints	5	0.971	Excellent
Time Constraints	5	0.980	Excellent
Effectiveness	6	0.72	Good

Result of Descriptive Analysis

Descriptive analysis using SPSS is a straightforward process that involves entering data, selecting the appropriate descriptive statistics function, running the analysis, and interpreting the results. It provides essential insights into the dataset, helping researchers to understand the distribution and central tendencies. Financial constraints relate to financial challenges or limits that smallholders face. The mean score of 4.1535 shows that financial restrictions are a considerable obstacle, which is rather high on a scale of 1 to 5.8. The standard deviation of 0.78404 indicates low heterogeneity in answers, indicating that most smallholders face comparable degrees of financial limitations. Access to information assesses how aware or informed smallholders are about certain topics like as best practices, accessible resources, and legislation. The average score of 3.5200 indicates that access to information is a moderate issue. The greater standard deviation of 1.25925 suggests a more diverse range of replies, indicating that some smallholders have strong access to knowledge while others are much less educated. Time constraints are limits on smallholders' time that may affect their capacity to run their farms efficiently. The mean score of 3.5380 suggests that time restrictions provide a significant issue. The standard deviation of 0.93452 indicates a substantial dispersion in the replies, suggesting some diversity in how smallholders perceive their time restrictions.

Table 5: Descriptive statistics analysis

Descriptive Statistics

	N	Minim um	Maxim um	Sum	Mean	Std. Deviati on
Financial constraints	245	1.80	5.80	1017.6	4.1535	0.7840
Awareness	245	1.80	5.40	862.40	3.5200	1.2593
Time Constraints	245	1.40	5.40	866.80	3.5380	0.93452

The main challenge for independent smallholders is financial constraints, as evidenced by the greatest mean score of 4.1535. This implies that their operations are most significantly affected by financial challenges. Smallholders encounter substantial financial challenges, which may be linked to a variety of factors, including the high prices of resources, the absence of affordable credit, and the insufficient income earned from their agricultural operations. In order to improve the productivity and sustainability of smallholder operations and for influence the smallholder to involve in Malaysian Sustainable Palm Oil (MSPO) certification, it is crucial that we prioritize the solution of financial constraints.

Access to information and time constraints are also significant, although they are slightly less critical. While time constraints are a challenge, they are not as critical as financial constraints, as evidenced by their mean score of 3.5380. Smallholders frequently manage many responsibilities, including family obligations, community activities, and fieldwork. This burden can be reduced by offering assistance in enhancing time management and efficiency. This may involve instruction on the utilization of time-saving technologies, delegation strategies, and effective time management. The mean score of 3.5200 for Access to Information suggests that while it is imperative to enhance the spread of knowledge and information among smallholders, it may not be as vital as addressing financial issues.

In conclusion, financial constraints are the primary issue that must be addressed in order to effectively support smallholders, despite the fact that all three factors are challenges. The sustainability and productivity of smallholder farming in Selangor can be substantially influenced by the prioritization of financial support mechanisms. In addition, the improvement of time management and access to information are essential measures that can facilitate the attainment of MSPO certification, thus encouraging more sustainable and profitable palm oil production.

Results of Multiple Regression

In multiple linear regression analysis, the coefficients for farm practices level provide valuable insights into its impact on the financial constraint among smallholders in implementing MSPO certification. Based on Table 6 it has 3 independents variables which are education level, experience and farm practices. The table shows the significant values for education level and experience are

0.042 and 0.013 respectively while farm practices is 0. The farm practice is less than the rule of thumb of p-value of 0.05 and it can be said that farm practices has a positive influence on the financial aspects among smallholders. Moreover, the table 6.0 show the result standardize coefficient beta, the education level and experience get 0.228 and 0.258 and also the farm practices get 0.425. It can be concluded that. among those 3 independent variables, farm practices show the biggest influence as compared to education level and experience. Good agricultural practices, for example, may help smallholders to apply nutrient or fertilizers in a timely manner and reduce cost and gain higher profits, thus put a low constraint on their finance. According to Ahearn, M. C., & Newton, D. (2009), farming practices and the adoption of new technologies influence the financial challenges faced by beginning farmers to implement the Malaysian Sustainable Palm Oil (MSPO) certification.

Table 6: Multiple linear regressions that influences finance in implementing MSPO Certification

Model		Standardized Coefficients	Unstandardized Coefficients		t	Sig.
		Beta	**B**	**Std. Error**		
Level	Education	.228	-.247	.121	-2.048	.042
	Experience	.258	.404	.162	2.492	.013
Practices	Farm	.425	.413	.075	5.511	<.001

Based on Table 7, there are 3 independents variables which are education level, experience and farm practices that may influence access to information while implementing MSPO certification. The table shows the significant values for education level and experience are less than 0.001 as compared to farm practices which is at p-value of 0.06. The education level and experience are less than the commonly used threshold of 0.05 and it can be said that education level and experience were more significant to the access of information challenges. Moreover, Table 4-5 show the result of standardize coefficient beta, in which the coefficient levels for both education level and experience 0.228 and 0.258 respectively while the farm practices is 0.06. It can be concluded that among those

3 independent variables, experience shows a bigger influence as compared to education level and farm practices. Braunstein and Welch (2002) in his study asserted that there was a significant causal effect of experience on knowledge, suggesting that smallholders with more experiences will actually lead to greater implementation of Malaysian Sustainable Palm Oil (MSPO) certification.

Table 7: Multiple Linear Regressions that influence access to information in implementing MSPO Certification

Model		Standardized Coefficients	Unstandardized Coefficients		t	Sig.
		Beta	**B**	**Std. Error**		
Level	Education	.228	-.276	-.159	-3.967	<.001
	Experience	.258	-2.685	-1.067	-28.716	<.001
Practices	Farm	.006	.043	.004	.129	.060

Based on Table 8, there are 3 independents variables which are education level, experience and farm practices. The table shows the significant values for education level, experience and farm practices are 0. The education level and experience are less than the commonly used threshold of 0.05 and it can be said that education level, experience and farm practices were statistically significant to the time constraints challenge. Moreover, the table 4-6 shows the result of standardize coefficient beta for the education level and experience are 0.382 and 1.228 respectively while the farm practices is 0.327. It can be concluded that among those 3 independent variables, experience shows the biggest influence as compared to education level and farm practices. According to Rose, D. C., (2020) experienced farmers adapt quickly to new technology and techniques in smart farming, and their expertise have allowed them to better handle time limitations than less experienced smallholders.

Table 8: Multiple Linear Regressions that influences time or duration in implementing MSPO Certification

Model Coefficients	**Standardized Coefficients Beta**	**Unstandardized B**	**Std. Error**	**t**	**Sig.**
Education Level	.382	.683	.033	-20.760	<.001
Experience	1.288	3.342	.044	-75.530	<.001
Farm Practices	.327	.526	.020	25.726	<.001

Pearson Correlation Analysis

In research or data analysis, Pearson correlation analysis helps to understand how changes in one variable are associated with changes in another variable. It is widely used in various fields, including economics, social sciences, and natural sciences, to explore patterns and relationships in data.

Table 9: Pearson Correlation Analysis

Variable	Effectiveness	Financial Constraints	Awareness	Time Constraints
Effectiveness	1	-0.492**	0.920**	0.902**
Significant value = p		<0.001	<0.001	<0.001
Number of Sample = n		245	245	245
Financial Constraints	-0.492**	1	-0.460**	-0.432**
Significant value = p	<0.001		<0.001	<0.001
Number of Sample = n	245		245	245
Awareness	0.920**	-0.460**	1	0.920**

Significant value = p	<0.001	<0.001		<0.001
Number of Sample = n	245	245		245
Time Constraints	0.902**	-0.432**	0.920**	1
Significant value = p	<0.001	<0.001	<0.001	
Number of Sample = n	245	245	245	

In Table 9, financial restrictions and the perceived effectiveness of government assistance have a somewhat negative connection ($r = -0.492$, $p < 0.001$). This statistically significant link suggests that smallholders' financial restrictions tend to reduce as the perceived efficacy of government help rises. This implies that successful government initiatives are probably assisting in removing financial obstacles, which facilitates smallholders' adoption of sustainable practices such as MSPO certification.

Next, a strong positive correlation ($r = 0.920$, $p < 0.001$) exists between the perceived effectiveness of government support and awareness of the MSPO certification. This highly statistically significant relationship implies that as smallholders perceive government support as more effective, their awareness about the MSPO certification also increases. This underscores the critical role of government initiatives in disseminating information and educating smallholders, which in turn enhances their understanding and potentially their adoption of the certification.

Lastly, there is a strong positive correlation ($r = 0.902$, $p < 0.001$) between the perceived effectiveness of government support and time constraints faced by smallholders. This highly statistically significant relationship indicates that as smallholders perceive government support to be more effective, the time constraints they experience also tend to increase. This might suggest that while government support is seen as beneficial, it could also involve time-consuming processes, such as compliance and training requirements, which add to the smallholders' time burdens. The Factors Influencing the challenges in implementation MSPO Certification among Independent Smallholders.

The analysis achieves its second objective by exploring how education level, experience, and farm practices affect financial constraints, access to information, and time constraints for independent smallholders. It reveals that farm practices significantly influence financial challenges, indicated by a significant impact and a higher standardized coefficient compared to education level and experience. This finding supports earlier research emphasizing the importance of adopting new technologies in managing financial constraints in agriculture. For access to information challenges, education level and experience play more crucial roles, aligning with studies highlighting the role of knowledge and experience in facilitating certification processes. In terms of time constraints, all three factors education level, experience, and farm practices show statistical significance, with experience exerting the greatest influence, reflecting its role in enhancing efficiency and technology adoption among smallholders. Thus, the study effectively addresses how these variables impact different challenges faced by smallholders, meeting its intended research objective.

CONCLUSION AND RECOMMENDATION

The primary objective of identifying the main challenges faced by independent smallholders in Selangor has been achieved, with financial constraints emerging as the most significant obstacle, as indicated by the highest mean score of 4.1535. High resource prices, lack of affordable credit, and insufficient income from agricultural activities contribute to these financial challenges. While time constraints and access to information are also important, their lower mean scores of 3.5380 and 3.5200, respectively, make them secondary concerns. Addressing financial constraints is crucial for improving the productivity and sustainability of smallholder operations and encouraging participation in the Malaysian Sustainable Palm Oil (MSPO) certification. Prioritizing financial support mechanisms will significantly impact the sustainability and productivity of smallholder farming. Additionally, enhancing time management and access to information are essential measures that support the attainment of MSPO certification, promoting more sustainable and profitable palm oil production.

The multiple regression analysis reveals that farm practices significantly influence financial challenges, indicated by a significant impact and a higher standardized coefficient compared to education level and experience. This finding supports earlier research emphasizing the importance of adopting new technologies in managing financial constraints in agriculture. For access to information challenges, education level and experience play more crucial roles, aligning with studies highlighting the role of knowledge and experience in facilitating certification processes. In terms of time constraints, all three factors education level, experience, and farm practices show statistical significance, with experience exerting the greatest influence, reflecting its role in enhancing efficiency and technology adoption among smallholders. Thus, the study effectively addresses how these variables impact different challenges faced by smallholders, meeting its intended research objective.

Several recommendations may be made to maximize these benefits based on the study's findings about the influence of government support on financial restraints and smallholders' understanding of MSPO certification in Malaysia. First and foremost, government organizations must simplify procedures and lower administrative barriers to enable programs access. Smallholders may find it easier to participate in government programs if application processes are made simpler and there are clear instructions. Second, increasing outreach initiatives and communication channels can raise public knowledge of MSPO certification and its advantages. Digital media and neighbourhood networks may be used to distribute information to smallholders in a variety of geographic locations. Finally, establishing collaborations among governmental entities, farming groups, and private industry participants can enable all-encompassing assistance programs that cater to the monetary and instructional requirements of smallholders. By minimizing possible time-related obstacles and optimizing the effectiveness of government funding, these ideas seek to more successfully advance sustainable practices in Malaysian agriculture plantation landscape. It is also suggested that more data from across the states or areas in which implementation of MSPO is still a challenge, to be included in the future study, while considering it as a preliminary investigation aimed at establishing the foundation for a more comprehensive research study in the future

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