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## Foreword

It is a great privilege for us to present the authors and delegates of the conference with the proceedings of the inaugural International Conference on Food and Industrial Crops (ICFIC2022). We hope that you will find it informative, exciting and motivating. This version of e-proceeding contains a total of 28 papers submitted to the ICFIC2022 delivering the sustainable development of food and industrial crops in Malaysia and around the globe, concerning both crop-oriented and bio-based materials, which was held at The Waterfront Hotel, Kuching, Sarawak, Malaysia on 21<sup>st</sup> – 24<sup>th</sup> September 2022. This conference was one of the three concurrent international conferences under the umbrella of Congress on Sustainable Agriculture and Food Security (COSAFS2022). The conference was jointly organised by the Faculty of Agricultural and Forestry Sciences, Institute of Ecosystem Science Borneo, Universiti Putra Malaysia Bintulu Sarawak Campus and Ministry of Food Industry, Commodity, and Regional Development Sarawak (M-FICORD).

The conference under the COSAFS2022 aims to provide a platform to discuss and exchange knowledge between participants, promote and strengthen international and national collaborations in sustainable agriculture and food security as well as identifying and promoting smart agriculture solutions for the development of sustainable agriculture and food security. Hopefully, this event will also spark innovative ideas, and foster research relations or partnerships between the various institutions, corporate bodies, and communities. The conferences featured more than 300 participants including academicians, research scientists, policymakers, and officials from public and private sectors with thought-provoking keynote lectures, oral and poster presentations in the field of sustainable agriculture and food security.

The editors hope that this publication of e-proceeding will provide the reader with a broad overview of the latest research, development, and innovations and that it will be a valuable reference source for further research all over the world. The editors would like to express their sincere appreciation and thanks to all the authors for their contributions to this publication. We would like to express our utmost gratitude and appreciation to all the reviewers for their constructive comments on the papers. Nonetheless, we would also like to extend our special thanks to the organising team of COSAFS2022 who have work hard to ensure the success of the congress.

Thank you.

**Dr. Mugunthan Perumal**

Head, Scientific Programme Committee

ICFIC2022

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# Establishment of *in vitro* *Asparagus officinalis* Cultures

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## INTRODUCTION

*Asparagus* (*Asparagus officinalis*) is a long-lasting herbaceous perennial producing edible spears harvested every spring. The palatability of the species is highly dependent on its sugar content (Techavuthiporn and Boonyaritthongchai, 2016), with the agronomic and morphobiochemical traits influenced by the genotype, agronomics and especially the environment (Robb, 1984). The spears are known to be highly nutritious, containing high amounts of amino acids (Hurst and Clark, 1993), minerals (Lopez et al., 1996), and vitamins (Takahashi et al., 2019).

Seed-propagated asparagus are known to produce considerable differences in yield between each plant. Besides, the plants are highly susceptible to crown and root rot (*Fusarium*) (Pontaroli and Camadro, 2005). *In vitro* culture allows the selection of uniform materials with increased disease resistance (Pontaroli and Camadro, 2005; Jin et al., 1996). However, the biggest barrier in establishing *in vitro* asparagus cultures is the difficulty in initiating roots as the plants require robust and well-developed long fibrous root systems for successful acclimatisation (Conner et al., 1992). As such, the objectives of this study were to develop an efficient micropropagation protocol for *A. officinalis* and establish robust rooting systems in *in vitro* *A. officinalis* for local cultivation.

## MATERIALS AND METHODS

The study was conducted at the Postgraduate and Plant Tissue Culture Laboratories in QIU. Parameters assessed included the source of the asparagus spears as well as the surface-sterilisation protocols (Table 1) and culture media (Table 2) employed. The spears were sourced fresh from the QIU Sustainable AgriResearch (QSAR) centre or as packaged asparagus (EDSMA) from Aeon Kinta City Mall and surface-sterilised immediately. All surface-sterilised asparagus spears were subcultured in culture tubes containing 15 mL semi-solid medium supplied with 30

Table 1. Differences between Treatments A to H in the surface sterilisation of *A. officinalis* spears.

Step	Detail	Treatment							
		A	B	C	D	E	F	G	H
1	Spears agitated with a few drops of detergent for 5 minutes	✓	✓	✓	✓	✓	✓	✓	✓
2	Spears agitated with 5% Dettol for 5 minutes	×	×	×	×	×	×	✓	×
3	Spears left under running tap water (hours)	3	3	3	3	3	3	2	2
4	Spears rinsed with ethanol (%) for a few seconds	95%	95%	95%	95%	95%	70%	70%	70%
5	Spears rinsed with sterile distilled water	✓	✓	✓	✓	✓	✓	✓	✓
6	Type of agitation in Clorox® (%) + 3 drops of Tween 20 for 15 minutes.	10%, VS	20%, VS	25%, VS	30%, VS	MS	25%, HS	25%, HS	25%, HS
7	Spears rinsed with sterile distilled water	✓	✓	✓	✓	✓	✓	✓	✓
8	Spears excised into smaller explants	✓	✓	✓	✓	✓	✓	✓	✓
9	Explants swirled in ethanol (%) for a few seconds	95%	95%	95%	95%	95%	70%	70%	70%
10	Explants rinsed twice with sterile distilled water	✓	✓	✓	✓	✓	✓	✓	✓
11	Explants rinsed in antioxidant solution	✓	✓	✓	✓	✓	✓	×	×

✓ = step performed; × = step not performed; VS = vigorous stirring, MS = magnetic stirring, HS = hand swirling

g/L sucrose and 2.8 g/L gelrite. All cultures were subcultured monthly under standard plant tissue culture room conditions. Each treatment was conducted with a minimum of three replications and repeated twice. Observations included asepticity rates, shoot and root production as well as the influence of medium composition on recovery rate during subcultures. Means obtained were analysed using one-way ANOVA and differentiated via Tukey's HSD test using SPSS version 22 (IBM).

Table 2. Media composition used to culture *A. officinalis* spears *in vitro*.

Medium	Components (mg/L unless stated otherwise)						
	MS (strength)	NAA	Kin	BAP	IAA	IBA	ANC
Shoot Initiation 1 (SI 1)	1	0.2	0.2	0.2	-	-	-
Shoot Initiation 2 (SI 2)	1	0.1	-	2.0	-	-	-
½ SI 1	½	0.2	-	0.2	-	-	-
½ SI 2	½	0.1	-	2.0	-	-	-
½ Shoot Multiplication 1 (SM 1)	½	0.2	0.2	0.1	-	-	-
½ Shoot Multiplication 2 (SM 2)	½	0.1	-	1.0	-	-	-
½ NAA 1.0	½	1.0	-	-	-	-	-
½ NAA 2.0	½	2.0	-	-	-	-	-
½ NAA 3.0	½	3.0	-	-	-	-	-
½ IAA	½	-	-	-	1.0	-	-
½ IBA	½	-	-	-	-	1.0	-
ANC	1	0.2	-	-	-	-	1.3

MS = Murashige and Skoog medium (1962), NAA = naphthaleneacetic acid, Kin = kinetin, BAP = 6-benzylaminopurine, IAA = indoleacetic acid, IBA = indolebutyric acid, ANC = ancymidol.

## RESULTS AND DISCUSSION

A major consideration in this study was the source of explants. It was observed that spears harvested from the QSAR produced better results in terms of asepticity (Table 3). Bacterial and fungal contaminations were mostly observed in store-bought spears. The cultures were contaminated within days of the surface-sterilisation treatment despite displaying shoot proliferation. The antioxidant rinse step was also observed to have contributed to the contamination rates in the cultures, hence the removal of the step in subsequent repetitions of the experiment. The store-bought spears also displayed severe bleaching during the surface-sterilisation treatment.

Longer and stronger surface sterilisation treatments did not equate to better results in terms of asepticity for the asparagus spears (Table 3). Vigorous agitation was observed to be very damaging to the spear segments, while the use of harsh sterilisation agents such as Dettol bleached the explants. Gentle swirling proved to be the safest method of agitation during the disinfection process. However, no significant differences were found in the surface-sterilisation treatments. Treatments G and H were selected to proceed to the next stage of the study in the interest of time economy: the tap water rinse was reduced to two hours and the antioxidant rinse step eliminated for both treatments. Treatment H was further pared down by eliminating the Dettol wash step to reduce bleaching of the explants during the surface-sterilisation process.

Asparagus plants require robust root system and rootstocks for successful acclimatisation. Low survival was observed in acclimatised plants with poor root systems (Conner et al., 1992). Cultured asparagus contain high levels of endogenous gibberellic acid, causing inhibition of both shoot and root growth (Štajner et al., 2002) which may or may not be improved through the use of auxins and cytokinins (Khunachak et al., 1987). Ancymidol, a gibberellic acid suppressor, is known to improve both shoot and root development when used alone (Chin, 1982) or with increasing sucrose concentrations (Desjardins et al., 1987; Štajner et al., 2002). As such, further



investigation is required to optimise the use of ancymidol in *in vitro* asparagus plantlets to boost root production rates.

Table 3. Effect of surface sterilisation treatments and media composition on purchased and freshly-harvested *A. officinalis* explants.

Source of Spears	Treatment	% of Growth	
		SI 1	SI 2
Commercial	A	0.0±0.0	0.0±0.0
	B	0.0±0.0	0.0±0.0
	C	0.0±0.0	8.7±6.0
	D	0.0±0.0	0.0±0.0
	E	0.0±0.0	0.0±0.0
	F	3.2±3.3	16.1±6.7
	G	0.0±0.0	0.0±0.0
	H	4.8±2.4	4.8±2.4
QSAR	G	6.2±3.0	7.8±3.4
	H	38.4±3.5	36.7±3.5

Note. Values indicates mean ± standard error of mean.

A majority of the aseptic asparagus cultures displayed profuse shooting and callusing (Table 4 and Figures 1A-C), while roots were produced from shoots cultured in media supplemented with NAA (Table 4). The second subculture produced 100% recovery rate for all media used except for medium containing IAA. Some cultures displayed abnormal shoot production as a result of the PGR treatment (Figure 1D). The roots were observed to be small and thin and not suitable for acclimatisation (Figures 2A and 2B). Spears cultured in IAA displayed slow growth, with callus produced turning brown after a few weeks of culture.

Table 4. Effect of subsequent subcultures of surface-sterilised *A. officinalis* explants sourced from EDSMA and the QIU Sustainable AgriResearch centre.

Media	Recovery Rate (%)	
	Subculture 1	Subculture 2
½ SI 1	81.8±3.5 (shooting, callus)	100.0±0.0 (shooting, callus)
½ SI 2	76.9±3.5 (shooting, callus)	100.0±0.0 (shooting, callus)
½ SM 1	80.9±5.8 (shooting, callus)	100.0±0.0 (shooting, callus)
½ SM 2	78.4±6.9 (shooting, callus)	100.0±0.0 (shooting, callus)
½ NAA 1.0	63.6±15.2 (rooting)	100.0±0.0 (shooting, callus)
½ NAA 2.0	63.6±15.2 (rooting)	-
½ NAA 3.0	54.6±15.7 (rooting)	-
½ IAA	-	25.0±25.0 (slow growth, browning of callus)
½ IBA	-	100.0±0.0 (shooting, callus)

Note: Values indicates mean ± standard error of mean. Subcultures in ½ NAA 2.0 and ½ NAA 3.0 were discontinued as subculture in ½ NAA 1.0 produced similar results and was deemed economical. Spears subcultured in these two media were subcultured in ½ IAA and ½ IBA.

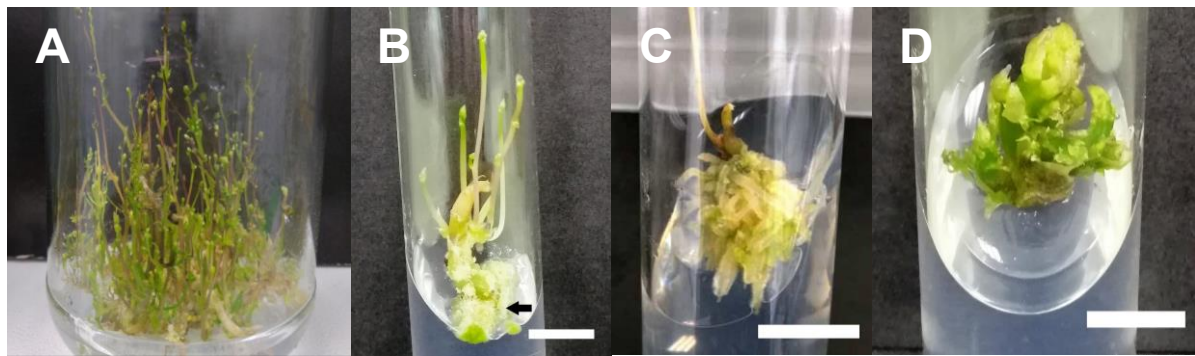


Figure 1. (A) Shooting and *in vitro* flowering, (B, arrow) callusing, (C) rooting and (D) abnormal shooting observed among the *in vitro* asparagus cultures. Bar = 1 cm.

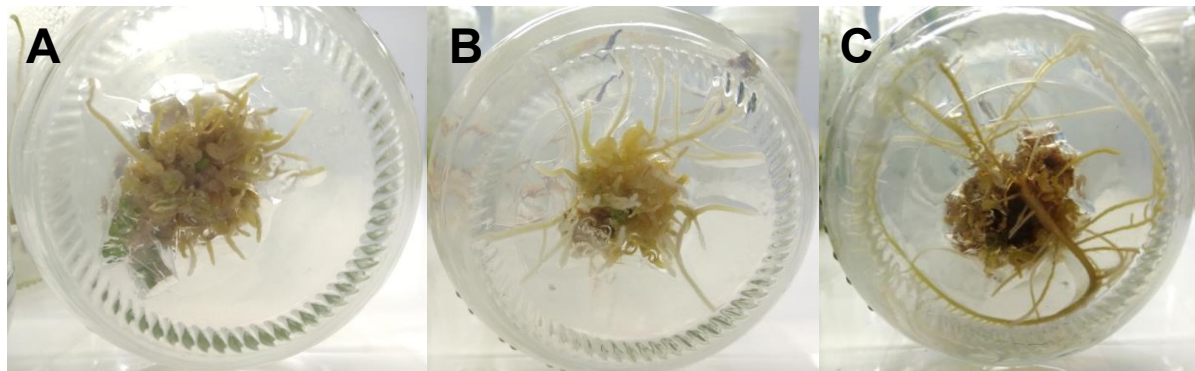


Figure 2. (A and B) Thin and fibrous roots produced by asparagus plants *in vitro* subjected to the ancymidol treatment; (C) a well-rooted *in vitro* asparagus plantlet ready for acclimatisation.

## CONCLUSION

Freshly-harvested *A. officinalis* spears produced the best results in terms of asepticity and subsequent growth when surface sterilised under Treatment H. Explant source plays a major role in the success of establishing *in vitro* asparagus cultures. Shooting and callusing were obtained in almost all treatments. Roots were produced from all asparagus cultures, regardless of source and tissue type, when cultured in medium containing ancymidol.

## ACKNOWLEDGEMENT

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# Nitrate in Leafy Vegetables Grown using Hydroponic System

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## INTRODUCTION

Planting of leafy vegetables using a hydroponic system is increasingly popular due to the limited arable land and is considered economically viable. However, cultivation method using hydroponic system may affect nitrate ( $\text{NO}_3^{2-}$ ) content in most leafy vegetables (Bian et al., 2018). Nitrate accumulation in most leafy vegetables remain a major issue and its reaction with amine in the body system can lead to the formation of nitrosamine, a carcinogenic compound (Walters, 1991).

Lettuce is among the popular leafy vegetables grown using hydroponic system because of its high yield, and contains minerals, vitamins and beneficial biologically active compounds. However, a high  $\text{NO}_3^{2-}$  content in lettuce has been reported (Novaes et al., 2009). Therefore, the objective of this study was to quantify the concentrations of  $\text{NO}_3^{2-}$  and other elemental nutrients in lettuce grown using the hydroponic system and fed with the recommended and a modified Sonneveld's fertiliser solutions.

## MATERIALS AND METHODS

### *Planting materials and conditions*

Green coral lettuce 'Lollo Bionda' (*Lactuca sativa* var. *crispa*) seeds were sown in a rockwool blocks and 7-day old healthy seedlings were transferred onto the hydroponic system. The lettuce plants were fertilised using the recommended and the modified Sonneveld's solutions. The fertiliser solutions were described as containing all the essential nutrients at various concentrations for healthy plant uptake (Table 1). The fertiliser concentrations in both solutions were controlled indirectly by measuring their electrical conductivity (EC). The EC was maintained at 1.5 mS/cm and pH 5.8 throughout the lettuce growing period for both fertiliser solutions. The plants were exposed to 24-hour lighting, average room temperature of 28°C and relative humidity of 50%. The plants were harvested at Day 35 and dried at 70°C for 3 days, grind and store prior to analyses. Twenty mL of ultrapure water was added to 2.0 g of each dried samples and then placed in 80°C water bath for 30 minutes. The filtrates were analysed for  $\text{NO}_3^{2-}$  using the ion chromatography (IC) (Metrohm, Herisau, Switzerland). Nitrogen was determined by Dumas combustion method using Nitrogen Analyzer (LECO FP528, St. Joseph, MI, USA). Other nutrients were determined by wet digestion method and measured using the inductively coupled plasma-optical emission spectroscopy (ICP-OES) (Perkin Elmer Optima 7300 DV, Waltham, MA, USA). The experiment was carried out in two rounds.

Table 1. The concentrations and fertiliser sources for nutrient solutions used in the experiment.

Stock Solution	Source (Chemical and formula)	Concentration (g/L)	
		Sonneveld's Solution	Modified Sonneveld's
A	Calcium nitrate, $\text{Ca}(\text{NO}_3)_2$	165.00	230.77
	Ammonium nitrate, $(\text{NH}_4)\text{NO}_3$	0.00	0.00
	Potassium nitrate, $\text{KNO}_3$	183.00	59.72
	Iron EDTA, $\text{C}_{10}\text{H}_{12}\text{FeN}_2\text{O}_8$	3.80	0.98
B	Monopotassium phosphate, $\text{KH}_2\text{PO}_4$	57.00	15.21
	Magnesium sulfate, $\text{MgSO}_4$	93.00	41.99
	Manganese EDTA, $\text{C}_{10}\text{H}_{14}\text{MnN}_2\text{O}_8$	0.29	0.0769
	Sodium borate, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$	0.35	0.51
	Ammonium molybdate, $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24}$	0.02	0.0056
	Zinc EDTA, $\text{C}_{10}\text{H}_{12}\text{N}_2\text{Na}_2\text{O}_8\text{Zn}$	0.20	0.05
	Copper EDTA, $\text{C}_{10}\text{H}_{14}\text{CuN}_2\text{O}_8$	0.03	0.0071

### Statistical analysis

Statistical analyses were performed using Graphpad Prism 7.05. The data were subjected to two-way ANOVA. Tukey's multiple comparisons test was used to determine the level of significant difference at  $P < 0.05$ .

## RESULTS AND DISCUSSION

The concentrations of nutrient in the modified Sonneveld's solution were formulated to reduce  $\text{NO}_3^{2-}$  content in lettuce. Nevertheless,  $\text{NO}_3^{2-}$  concentrations in lettuce grown using both recommended and modified Sonneveld's solutions are higher than the maximum permitted level of 4,500 mg  $\text{NO}_3^{2-}$ /kg for fresh vegetables (European Commission, 2011). In both Rounds, the concentration of  $\text{NO}_3^{2-}$  in lettuce grown using the recommended Sonneveld's solution was significantly higher ( $\pm 37\%$ ) than  $\text{NO}_3^{2-}$  concentration in lettuce fed with the modified Sonneveld's solution at  $P < 0.05$  (Figure 1). The  $\text{NO}_3^{2-}$  concentration in lettuce fed with the recommended Sonneveld's solution in Round 1 was not significantly different than in Round 2 at  $P < 0.05$  (Figure 1). Similar trend was observed for the  $\text{NO}_3^{2-}$  concentration in lettuce fed with the modified Sonneveld's solution in Round 1 and Round 2 at  $P < 0.05$  (Figure 1). The light intensity and duration, temperature, humidity, plant maturity, harvesting time, storage and N source were among the many factors contributed to the high  $\text{NO}_3^{2-}$  content in lettuce (Correia et al., 2010).

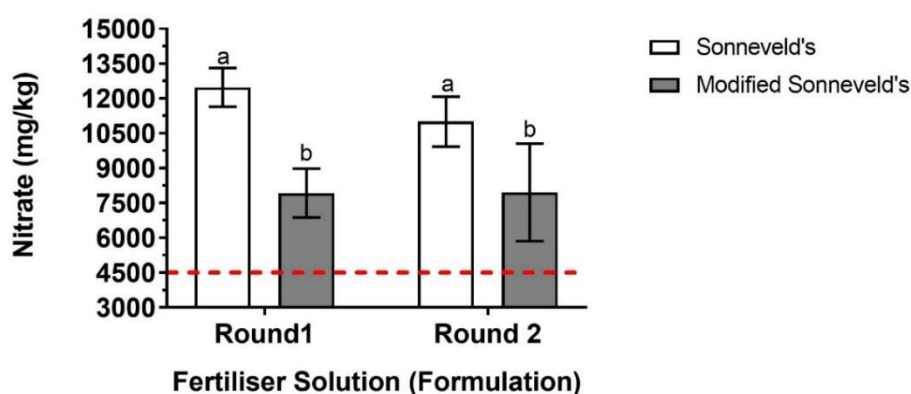


Figure 1. Nitrate concentrations in lettuce fed with the recommended and the modified Sonneveld's solutions. The dotted red line indicates the maximum permitted limit for nitrate in freshest vegetables (European Commission, 2011).

Macronutrients such as phosphorus (P) and potassium (K) were detected at higher concentration than what was considered sufficient or normal for most crops while calcium (Ca), magnesium (Mg) and boron (B) were at a deficiency level (Table 2). The nutrient imbalance may have adverse effects on the plant growth and is likely to affect the yield (Kumar and Mohapatra, 2021). Micronutrients such as iron (Fe), manganese (Mn), copper (Cu) and zinc (Zn) are adequate for growing most leafy vegetables (Table 2).

Table 2. Nutrient concentrations in lettuce grown using hydroponic system fed with the recommended and modified Sonneveld's solutions.

Element	Round 1		Round 2		Deficient*	Sufficient*	Toxic*
	Sonneveld	Modified	Sonneveld	Modified			
N (%)	4.53 ± 0.02	4.92 ± 0.02	4.78±0.08	4.56 ± 0.17	<2.5	2.5 – 4.5	>6
P (%)	0.82 ± 0.12	1.03 ± 0.09	0.70 ± 0.02	0.67 ± 0.02	<0.15	0.2– 0.75	>1
K (%)	7.36 ± 0.76	6.17 ± 4.45	5.35 ± 0.41	5.14 ± 0.16	<1	1.5 - 5.5	>6
Ca (%)	1.29 ± 0.21	2.24 ± 0.18	0.97 ± 0.03	2.30 ± 0.20	<1	1 - 4	>5
Mg (%)	0.32 ± 0.03	0.37 ± 0.03	0.30 ± 0.01	0.28 ± 0.01	<0.2	0.25 - 1	>1.5
Fe (mg/kg)	189 ± 45.39	426 ± 134.55	191 ± 43.86	186 ± 3.80	<50	100-500	>500
Mn (mg/kg)	54 ± 40.08	69 ± 2.52	47 ± 10.85	50 ± 4.10	15 - 25	20 - 300	300-500
Cu (mg/kg)	9 ± 1.73	6 ± 0.58	9 ± 0.25	8 ± 0.17	2 - 5	5 - 20	20 – 100
Zn (mg/kg)	51 ± 21.78	66 ± 8.62	52 ± 1.06	50 ± 0.94	10 - 20	27 - 100	100- 400
B (mg/kg)	15 ± 2.08	25 ± 1.53	9 ± 1.46	28 ± 3.80	5 - 10	10 - 50	50 - 200

Values are means ± standard deviation (SD). \*Average concentrations of elements in plants are based on Munson (1998).

## CONCLUSION

The  $\text{NO}_3^{2-}$  concentration in lettuce fed with the recommended and the modified Sonneveld's solutions is above the maximum permitted level. However, the use of the modified Sonneveld's solution reduced the  $\text{NO}_3^{2-}$  concentration to about 37% compared with the recommended formulation. Both fertiliser formulations have caused nutrient imbalance in lettuce. Further study is necessary to formulate a suitable fertiliser solution for growing lettuce using the hydroponic system. This is to ensure a healthy plant growth and the produce is safe for consumption.

## ACKNOWLEDGEMENT

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# **A Case Study of Technology Adoption through Asset-Based Community-Led Development (ABCD) in a Rice Farming Rural Community – Bario, Sarawak**

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## **INTRODUCTION**

Malaysia is a net importer of rice, with our rice self-sufficiency level (SSL) between 60% - 70%, and a growth rate of 0.03% (Omar et al., 2019). For Malaysia's aspirations to move towards a growing, equitable, and sustainable rice industry, one of the concerns is the empowerment of farmers by using eco-friendly paddy and rice production, technologies to promote sustainability (Arshad et al., 2021). Technology can be a disruption to the current food systems to feed growing world populations (Lee, 2019). Traditionally, technology adoption and technology diffusion are implemented with a top-down approach. The decision, strategies, and planning of the technology adoption and diffusion processes are driven by the agencies. The top actors introduced the technology, and the strategies of how the technology adoption and diffusion should be implemented, which often has a questionable success rate for the user groups, largely depends on how the whole process is being deployed by the top actors. It is also such technologies are not easily accessible by the user groups largely because of the cost incurred, and the scale of the technologies (Eneh, 2010). This study aims to explore the phenomenon of technology adoption within a community of rice farmers based in Bario, Sarawak through the lens of Asset-Based Community-led Development (ABCD) as an alternative to needs-based development, it is to map the available resources, and assets in the community, what the community can do with the peer support and help of outside agencies for achieving long-term ownerships of the development (McKnight et al., 2018). The study also discussed the role of agencies and their efforts to form partnerships, and peer collaborations based on those assets identified and community strength. A few technologies intensity with various technology classifications defined, namely low, low-med, med-high, and high technology with different process complexity, product complexity, and product development rate (Steenhuis et al., 2006). Technologies in any classifications are included in this study to be inclusive.

## **MATERIALS AND METHODS**

The study proposes a qualitative methodology based on participant observation and interviews, with open and semi-structured questions to capture a wider scope of relevant information. A total of 10 face-to-face first-round interviews and 5 second-round interviews were conducted in Bario, consisting of rice farmers, and opinion leaders. Informed consent interview sessions were conducted to stay neutral during the whole process, oblige to the true values, and respect of the interviewees, and be consistent in all the interview sessions. Themes were pre-selected to conduct data collection based on ABCD elements.

The intentions are to explore how all the elements interact and create and what is current activities happening based on those, by capturing the following ABCD resources and assets (McKnight et al., 2018).

- Local residents gift, skills, knowledge, passion.

- Associations, a club, farmers group, cultural group.
- Local Institutions, non-profit society.
- Local places, villages, town, shared places.
- Exchange, form of intangible or tangible.
- Stories, to connect people in the local network.

Table 1. Research themes classifications.

Themes (based on ABCD)	Methods of rice planting	Technologies	Inputs (seeds, fertilizers, etc)
Internal Developed Assets	Rice planting methods that either pass down or developed locally	Technologies that develop locally	Inputs from previous harvest or natural inputs
External Assets Required	Rice planting method developed by external agencies	Technologies bought from external agencies	Inputs purchase or provided by external agencies
External Services Required	Rice planting methods taught by external agencies	Technologies provided by external agencies	Knowledge provided by external agencies about inputs
Local Development Services	Rice planting methods taught by local experts	Technologies provided by local experts (local service providers)	Knowledge provided by local experts about inputs

The methods of the ABCD approach stressing on how we find the local assets by asking the community the following sequenced questions.

1. What can we do?
2. What are the things that we need outside help with?
3. What are the things that we need outside agencies to do for us? (McKnight et al., 2018)

Field observations were later carried out to further capture and observe other information about the research.

## RESULTS AND DISCUSSION

The study revealed that the community has its local assets recorded both in verbal and written form and tends to get shared through communal networks and bartering services. This process has led to knowledge diffusion on farm activities among farmers which translates into locally developed assets within the community. These are the key elements identified by the local community to assess and strategize their technology adoption plans specific to their rice farming landscape and culture. There are efforts from the community to present their rice farming agendas to the agencies, always with a low success rate, as the agencies usually have their own agendas, programs, strategies, and plans for technology selection, adoption, and diffusions to the rice farming communities. The community also questions the technology transfer process which is not usually comprehensive. At the same time, the community has some local initiatives to acquire rice farming machines for ploughing, planting, harvesting, threshing, and milling as helpers in their farm work.

Table 2. Quotes.

Themes (based on ABCD)	Methods of rice planting	Technologies	Inputs (seeds, fertilizers, etc)
Internal Developed Assets	I-“we still use traditional method, and most of us this year still using traditional method, using hand.”	I-“smaller boat ploughing machine” I-“What is traditional, slowly mechanize like bush cutter, later lighter machine”	O-“Local paddy seeds that passing down. Seeds sharing, exchange and purchase from other farmers”
External Assets Required	I-“Taiwan sensor, use only battery, timer is good for watering, timer” I-“There is one snail capture”	I-“5 planter machine. And then maybe even two or three harvesters.” I-“they have a kind of small machine that I think is cheaper compared to the big machine that they use. And I believe that's a sort of machine that can use here, like in Indonesia.”	O-“scientific soil, water, weather profile”
External Services Required	I-“I don't care what method, as long as it is clean”-referring to ploughing. I depend on technology to become helper.”	I-“Whoever is responsible to teach the farmers how to use this machine, I think that is the main problem. Not everybody can use the machine because they are not trained to use it.”	O-“scientific knowledge sharing about soil, water, weather profile”
Local Development Services	O-“Communal work systems in the rice farm”	O-“Bartering system in the rice farm”	O-“traditional knowledge of observing moon phase calendar, birds migrations, planting calendar”

I – Interview, O – Observations

## CONCLUSION

From this case study, mapping local assets of the Kelabits Highlands rice farming community using the ABCD approach can potentially encourage the forming of partnerships with the agencies or outside funders on an equal basis by focusing on internal assets and capabilities first then what is needed, support and help from outside. The study recommends the use of the ABCD approach to map the assets in the community, and the requirements of the technologies, with peer support from the relevant agencies, subsequently leads to successful technology adoption in the community and proposes that the promotion of sustainable Malaysia rice production relies on energizing existing rice farmers, through stakeholder engagement and encouraging bottom-up technology adoption, diffusion, and integration in the policies.

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# Significance of Papaya (*Carica papaya*) WRKY2 Transcription Factor in Salinity Stress Response

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## INTRODUCTION

Papaya (*Carica papaya*) is the third most cultivated tropical crop consumed worldwide due to its high nutritional value and pharmacological benefits. Being tropical crop, papaya is susceptible to environmental stresses such as papaya ringspot virus (PRSV), drought and high salinity stress that affects its growth and productivity and leads to significant yield loss (Zhu et al., 2012). Ineffective and excessive water irrigation and poor drainage may cause salt accumulation, resulting in regional salinization (de Lima-Neto et al., 2016; Hossain, 2019). Decreased osmotic potential caused by salinity stress alters cell metabolism and limits papaya plant growth (Sousa et al., 2019). Thus, scientists are working together to develop salt-tolerant plants. Transcription factors (TFs) play a crucial role in regulating the expression of stress-responsive genes (Ali et al., 2018). As such, WRKY TFs have been reported to play vital roles in the salinity stress responses of various plants (Li et al., 2020). However, the role of CpWRKY genes under salinity stress is not thoroughly studied. Therefore, this study aimed to isolate and examine the role of the CpWRKY2 gene in salinity stress response in transgenic *Arabidopsis thaliana*.

## MATERIALS AND METHODS

### *WRKY2 gene isolation, plasmid construction and Agrobacterium transformation*

Hong Kong papaya seeds were primed in 200 mg/mL gibberellic acid overnight and sown in organic soil. One-month-old seedlings were treated with 200 mM sodium chloride (NaCl) solution for 28 days. Leaves were harvested for RNA isolation using the CTAB method (Yang et al., 2008). The WRKY2 gene was amplified from cDNA using Invitrogen™ Taq DNA Polymerase (Thermo Fisher Scientific) at 95°C for 3 min of initial denaturation and 30 cycles of amplification (95°C for 45 sec, 50°C for 45 sec, and 72°C for 1 min) followed by a final extension at 72°C for 5 min using the primer set: WRKY2\_F: 5'-CCATGGATGTTTTACATGCAG-3' and WRKY2\_R: 5'-AGATCTCTACCTTCTCTTCGA-3' (Pan & Jiang, 2014). WRKY2 gene was cloned into pCambia1304 vector using T4 DNA ligase (Promega Corporation) and transformed into *Agrobacterium tumefaciens* LBA4404 using the freeze and thaw method (Al-Hajaj and Al-Dallee, 2018) and successful transformation was confirmed using colony PCR.

### *Qualitative real-time reverse transcriptase PCR (qRT-PCR)*

The cDNA was synthesized from 1 µg of RNA using of Deoxy HiSpec Reverse Transcriptase kit (Yeastern Biotech Co., Ltd.) at 30°C for 10 mins, 42°C for 40 mins and finally at 70°C for 15 mins. Gene expression was analysed using the CFX96 Real-Time system (Bio-Rad) using the primer WRKY2\_qPCR\_F: 5'-GCAATTGATGCTTCCTCAACC-3' and WRKY2\_qPCR\_R: 5'-GACTCGGATTCATCTCCTTCAC-3' at initial denaturation step of 5 min at 94°C followed by 40 cycles of 10 s at 94°C, 15 s at 60°C, and 15 s at 72°C. The CpEF1a gene was amplified using the CpEF1a\_qPCR\_F: 5'-AACTGGTGTCTGAAGCCTGGT-3' and CpEF1a\_qPCR\_R: 5'-AGAGCCTCGTGGTGCATCTCAA-3' was used as a reference to normalize gene expression across



the samples. Ct values were exported and analysed in MS excel. The relative expression level of *WRKY2* was calculated using the Pfaffl method (Pfaffl, 2001).

### ***Plant transformation, selection of transgenic Arabidopsis and transgene validation***

*A. thaliana* Col 0 was grown at 22°C and 16 h photoperiod until the blotting stage. *Agrobacterium*-mediated transformation with a recombinant vector was performed using the floral dip method (Clough et al., 1998). The plasmid pCAMBIA1304 was used as the positive control. T<sub>0</sub> seeds were harvested from transformed plants and screened on MS agar containing 50 µg/mL kanamycin and 25 µg/mL hygromycin B to generate T<sub>1</sub> plants. A similar selection method was performed for T<sub>2</sub> and T<sub>3</sub> generations. Genomic DNA extraction was performed from the fresh leaf using the PrimeWay Plant DNA extraction kit. The presence of the transgene was validated by amplifying *WRKY2* using the same PCR condition used during gene isolation. GFP was amplified as a positive control at 95°C for 2 mins, followed by 30 cycles of 1 min at 95°C, 30 secs at 45 °C, 1 min at 72°C and a final extension of 4 mins at 72°C.

### ***Salinity stress tolerance assay, physiological and biochemical analysis***

T<sub>3</sub> transgenic and WT *A. thaliana* were treated with 200 mM NaCl for 28 days to induce salinity stress and were monitored daily for morphological changes. At the end of the stress tolerance assay, total chlorophyll (Rajalakshmi and Banu, 2013) and MDA content (Heath and Parker, 1986) were determined.

## **RESULTS AND DISCUSSION**

### ***Isolation of WRKY2 and qRT-PCR analysis***

*WRKY2* isolation was successful with 99.88 % similarity to *C. papaya* probable WRKY transcription factor 2 (LOC110820643). The transformation of recombinant vector, pCAMBIA1304-*WRKY2*, transformed into *A. tumefaciens* was successful. Quantitative RT-PCR analysis revealed that *WRKY2* expression level was significantly upregulated in salinity-treated papaya, which is 9-fold and only 1-fold in the control plant (Figure 1).



Figure 1. Relative quantification of *WRKY2* expression under salinity stress in *C. papaya*.

### ***Generation of transgenic A. thaliana and transgene validation***

The generation of T<sub>3</sub> transgenic *A. thaliana* for stress tolerance successfully detected *WRKY2* and *GFP* genes in transgenic plants and confirmed successful transgene integration into *A. thaliana* genome.

### ***CpWRKY2 overexpression enhances A. thaliana salinity tolerance***

Under salinity stress, leaves of wild-type were yellowish and withered. In contrast, leaves of transgenic *A. thaliana* remained green (Figure 2a). Chlorophyll is used as one of the markers to

identify the degree of salinity stress. Compared to WT, transgenic plants had relatively higher chlorophyll content (Figure 2b). Disruption of chloroplast structure by salinity stress could be a possible reason for reducing chlorophyll content in WT. MDA content is closely related to cell membrane damage under stress. In this study, the MDA content of transgenic *A. thaliana* was significantly lower relative to WT (Figure 2b), indicating that the overexpression of *CpWRKY2* confers tolerance to oxidative associated with salinity stress.

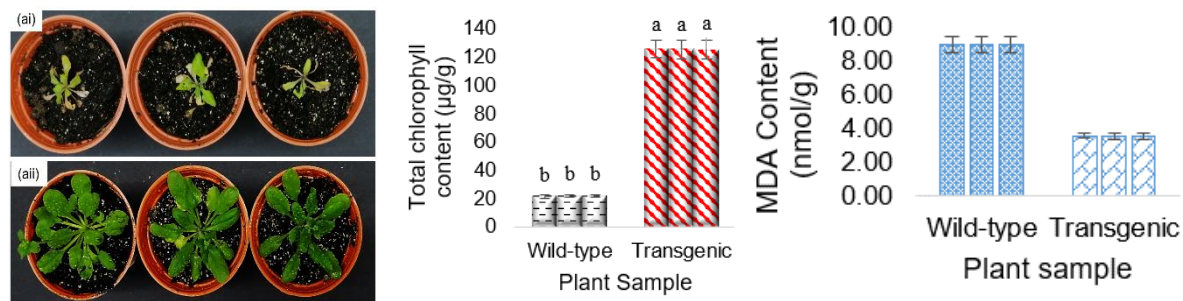


Figure 2. (a) Morphology of T<sub>3</sub> *A. thaliana* at the end of stress tolerance assay; (b) Total chlorophyll content in T<sub>3</sub> WT and transgenic *A. thaliana*, (c) MDA content in T<sub>3</sub> WT and transgenic *A. thaliana*.

## CONCLUSION

In conclusion, *CpWRKY2* was isolated from papaya and up-regulated by salinity stress. Overexpression of *CpWRKY2* in *Arabidopsis* resulted in enhanced tolerance to salinity stress. Morphological, physiological and biochemical analyses revealed that *CpWRKY2* played as a positive regulator in salt stress response and serves as an important candidate gene for salt-tolerant papaya plant cultivation.

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# Predicted Glycaemic Index of Food Formulated using Native and Modified Sago (*Metroxylon sago*) Starches

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## INTRODUCTION

The formulation of starch-based food focus on improving its functional food properties which can benefit human health. This includes the enhancement of starch properties as an ingredient to provide low glycaemic index food. Consumption of this food will benefit obese and diabetic individuals (Bello-Pérez et al., 2021). It can be achieved by producing a starch with low digestibility by increasing the resistant starch (RS) content of the starch. Examples of starches developed to exhibit low glycaemic indexes are maize and cassava starches (Bello-Pérez et al. 2021; Eyinla et al., 2021). As starch is already introduced in food formulations like fish crackers, the addition of RS in food formulation gives a promising alternative in the production of products with better functional food properties. Sago starch which contains a high RS is a promising candidate (Arshad et al., 2018). There is scarce information on sago starch formulated in food products especially its digestibility and potential prebiotic properties. Thus, this study investigated the functional food properties of food formulated with sago starch and its derivatives.

## MATERIALS AND METHODS

### *Materials*

Sago starch was obtained from the CRAUN Research Sdn. Bhd. (Kuching, Malaysia). Chemicals were in analytical grade (HmbG chemicals), while MRS agar and broth from Difco™. Wheat flour, sodium bicarbonate, and vanillin were acquired from local stores.

### *Modification of sago starch*

The starch modification was conducted as described by Zailani et al. (2021) using microwave yielding M5, M10, M15, and M20 where the number indicates the duration of treatment in minutes.

### *Formulation of food*

The formulation of the sago starch-based food product was conducted by replacing a portion of wheat flour with sago starches at concentrations of 25, 50, and 75%. Control was prepared as the other formulation but without replacing the flour.

### ***Determination of resistant starch content***

The resistant starch content was determined using the Megazyme Resistant Starch Assay Kit (K-RSTAR) (Ireland).

### ***In vitro digestion of food products***

Methods described by Lux et al. (2012) and Mandalari et al. (2008) were used to digest the food products. The *in vitro* digestion process was performed in three phases, i.e., oral, gastric, and duodenal phases (Lux et al., 2008).

### ***Predicted glycaemic index of food products***

The determination of the predicted glycaemic index (pGI) was conducted using methods described by Tsai and Lai 2021 and Göni et al. (1997). Briefly, the sample was digested for three hours with an aliquot of mixture sampled every 30 min. The absorbance at 510 nm was measured 20 min after the samples were mixed with the GOPOD reagent (Tsai and Lai, 2021; Göni et al., 1997). The predicted glycaemic index was calculated as described by Göni et al. (1997).

### ***Determination of bacterial growth rates***

A method described by Okolie et al. (2019) was used for the determination of bacterial growth rates. Three bacteria (*Lactobacillus casei*, *Bifidobacterium lactis*, and *Escherichia coli* obtained from the Biology Laboratory, Universiti Malaysia Sarawak) were used with inulin (Orafti®) as the positive control (Okolie et al., 2019).

### ***Statistical analysis***

The data was analysed using One Way Analysis of Variance (ANOVA) and followed by Tukey's test with  $p < 0.05$ . The Pearson's correlation study ( $p < 0.05$ ) was also performed using Statistical Package for Social Sciences (IBM® SPSS® Version 20).

## **RESULTS AND DISCUSSION**

### ***Digestibility of food product***

Based on Figure 1a, the product formulated with 25% starch had similar resistant starch content to the 100% flour formulation except for NS and M20 starches. The other concentrations showed a higher RS content than the 100% flour product. The RS content increases as the amount of starch added increase ( $r = 0.869$ ,  $p < 0.001$ ). The addition of 50% starch was sufficient to increase the RS content which was significantly better than the 100% flour formulation.

### ***Predicted glycaemic index of food product***

The pGI values were between 56 – 120% as shown in Figure 1b. The increase in starch concentration decreases the pGI value of the food product ( $r = 0.621$ ,  $p < 0.001$ ). Formulation using M10 starch with 50% and 75% concentrations had the lowest pGI values. Most of the formulations had moderate pGI values. The pGI values were negatively correlated with the RS content ( $r = -0.741$ ,  $p < 0.001$ ) implying the increase in RS can reduce the pGI value of food products. This was similar to a report in which the RS content increased while the glycaemic index decreased when wheat flour was substituted with a millet flour containing RS (Sharma and Gujral, 2019; Kaimal et al. 2021).

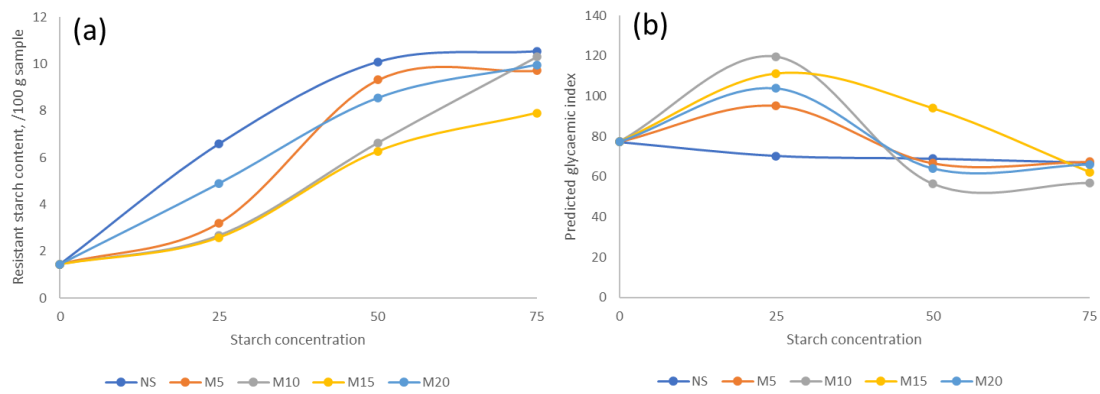


Figure 1. (a) The resistant starch content and (b) the predicted glycaemic index of food product at different starch concentration; NS-native starch; M5-MHT5; M10-MHT10; M15-MHT15; M20-MHT20; starch concentration = 0 is the 100% flour formulation.

### Bacterial growth rates

There were no changes in the growth rates of *L. casei* (Figure 2). Meanwhile, for the growth rates of *B. lactis*, food formulated with 25% M10 (0.298 hr<sup>-1</sup>) and 75% M15 (0.284 hr<sup>-1</sup>) had higher rates than 100% flour. On the other hand, the *E. coli* growth rates displayed had lower rates than the 100% flour formulation. Yet, formulation with 25% M10 had the highest rate. The increase in RS content influences the *E. coli* growth rates ( $r = -0.742$ ,  $p < 0.001$ ). The fermentation of RS by the gut microbiota produces higher butyrate than commercially available prebiotics (Raigond et al., 2015).

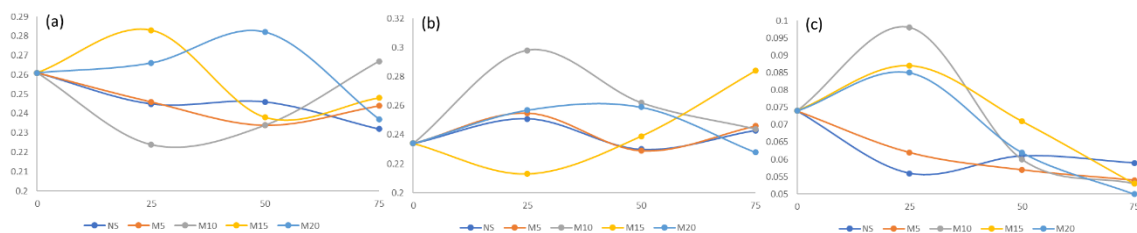


Figure 2. The bacterial growth rates of food formulated using different concentrations of starch; (a) *L. casei*; (b) *B. lactis*; (c) *E. coli*; NS-native starch; M5-MHT5; M10-MHT10; M15-MHT15; M20-MHT20; starch concentration = 0 is the 100% flour formulation.

### CONCLUSION

The formulation of food by replacing wheat flour with native and modified sago starch showed better RS content than the 100% flour formulation. The increase in RS influences the pGI values and the growth rate of *E. coli* in the formulated foods. However, it did not improve the *L. casei* and *B. lactis* growth rates. In conclusion, the increase in starch concentration in the formulation improves the RS content and influences the other characteristics of the food formulated.

### ACKNOWLEDGEMENT

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# Food Insecurity in Rich Resource State: The Case of Sabah

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## INTRODUCTION

This paper examines food policy in Sabah, one of the richest and resource-abundant states in Malaysia. Food and agriculture are both critical in achieving food security. Food policies are significant in defining the direction and strategies for agricultural growth and development as well as food security. Access to adequate infrastructure and facilities, technology and mechanisation, labour, land, and financial resources are critical in driving growth. However, the role of institutions is paramount to any economic development growth including food policy which has direct implications in policymaking that should be considered (Suffian et al., 2022).

The agriculture sector in Sabah contributes significantly to the state's Gross Domestic Product, accounting for approximately RM13 billion (16.5 percent) in 2020. It is the second source of employment after the services sector, with 472 thousand people employed in the sector. The sector's performance is dominated by industrial crops, specifically oil palm which are the most widely cultivated crops in an area of 1.8 million hectares (87.7 per cent) of total agricultural land (2.1 million hectares). While food crops such as paddy (39,426 hectares), vegetables (4,311.8 hectares) and fruits (19,112.5 hectares) are cultivated on only 3 percent of the land.

Issues such as low productivity, low adoption of technology, lack of manpower, limited infrastructure, and the decline of agricultural land have been acknowledged as the causes affecting the growth of agriculture in Sabah ever since the first agricultural policy in the 1990s (Yapp et al., 1999). Additionally, the government has formulated four agricultural policies that are in line with the national policies to accelerate the growth in the agro-food sub-sector, specifically in increasing the production of rice up to 60 percent self-sufficiency to reduce import dependency. The historical data showed that the rice SSL showed a declining trend, with only 22.81 percent in 2020. In 2021, the state's food import bills increased by RM700 million, to RM5.4 billion. The rising trend in food import bills demonstrated the food sector's poor performance. Various policies and mechanisms have been implemented to overcome the issues, but the roles of institutions should be taken into consideration as well in assessing the outcome. This paper is structured as follows. The first section presents the institution and its policies. The second section explains the methodology. The third section examines the results and finally concludes the paper's arguments.

### *Institutions and policy*

Institutions have a major role in the formulation, coordination, and execution of policy (Suffian et al., 2022). The role of institutions can become the constraining or enabling factor to carry out policy. Therefore, any policy issues should start with analysing the institutions. The overall productivity of a country is determined by the quality of its institutions (Hall and Jones, 1999). The fundamental components of economic growth, such as the amount of human capital, physical capital, and technology, are only *proximate causes* of economic development and growth (Acemoglu and Robinson, 2008). Institutions and government policies shape the economic environment in which farmers develop their skills, experience, expertise, knowledge, and capabilities, as well as accumulate their investment in farms with machinery, equipment, and technologies to produce higher levels of output. The success or failures of policies are based on

how policymakers deliberate, configure, and coordinate the factors that lie in the role of institutions (Suffian et al., 2022). This is because institutional configurations influenced by political events, history, and norms can influence policy direction (Suffian et al., 2022).

Institutions are the rules of the game in a society in which it is human who devise constraints that shape human interactions, structuring incentives between economic actors to determine policy outcomes (North, 1992). It consists of formal rules, for example, laws, rules, regulations, and policy, and informal rules such as norms, conventions, culture, and asymmetrical power relations. Many scholars point out that institutional set-ups determine rules and how things are done (Suffian, 2019).

Historical institutionalism claims that institutions are linked to path dependence, a collective decision made by a society in the past on how things are done (Hall 2001, as cited in Suffian, 2019). It can lead to a 'distributional effect', referring to the role of power and asymmetrical relations of power plays between groups (Thelen, 1999). In this context, the agricultural policy in Malaysia has been greatly influenced by the British colonial era, which focused on commodity crops since they provided a higher return to the economy (Suffian et al., 2022).

Political institutions are where decisions are made, preferences are established, and resources are distributed. Politicians, bureaucrats, and private enterprises all have a role in how policies are developed and implemented. The triangular institutional arrangements formed in the policy making domain create policy choices that may influence policy formulation (Suffian, 2021). Public policies and formal institutions are usually designed to be difficult to change, so past decisions encourage policy continuity (Pierson, 2000, as cited in Cerna, 2013). This means that when a country has decided on a certain policy path, it is difficult to change.

In Sabah, there has been limited attention given to the role of institutions when analysing the growth and development in the agriculture sector. Most research in the context of food policy focuses on the policy outcome but not the policy making processes. Understanding the role of institutions is important to knowing how policy making processes are carried out, prioritising and defining preferences. Therefore, the objective of this study is to explore the issues and challenges in the development of the agro-food subsector and to understand the constraints that hinder the development of the agro-food subsector by utilising institutional theory to evaluate the food policy in Sabah.

## **MATERIALS AND METHODS**

This study employed qualitative methods and utilised the theory of institutionalism. Documentary analysis was used by the researcher to analyse written sources including policy documents, government reports, official speeches, media reports, and internet news portals. Primary data collection was the main source of empirical data. The primary data was gathered through face-to-face interviews and focus group discussions using a semi-structured interview approach. The samples were selected based on purposive sampling where respondents were chosen based on their position, knowledge, expert opinion, involvement, and experience in the coordination and implementation of the state's food policy. A total of 35 respondents, including policymakers, policy implementers, farmers, and SMEs were interviewed. This research employed thematic data analysis to analyse the findings. It is a technique for identifying, analysing and reporting themes, patterns or stories within data, and helps to categorizes the data, additionally it helps in interpreting different parts of the research topic from interviews and document analysis (Braun and Clarke, 2006).

## **RESULTS AND DISCUSSION**

The agriculture sector has been a major contributor to the economic growth in Sabah, with oil palm as the major industrial crop worth RM17.3 billion in 2021. However, the agro-food

subsector is not as competitive as the industrial crops. The production of food for local consumption requires importing to fill the gap. The import of food increased 12.9%, or RM5.4 billion, in 2021. There are many constraining factors that caused the imbalance in growth and productivity between industrial commodities and the food crop industry in Sabah. The findings indicate a mismatch in priority, a patronage system, and lack of coordination hinder the development of the sector.

Priority mismatch as a factor leading to low priority for the agro-food subsector. Historically, Malaysia has been prioritising the development of industrial commodities with export values, specifically oil palm, rubber, and cocoa. Sabah, as a state under the Federation of Malaysia, is not excluded. In this case, the policy formulated is responsible for this outcome. In the 1980s, vast areas of degraded forests in Sabah were degazetted and cleared for oil palm cultivation (Jomo et al., cited in Dayang Norwana et al., 2011). Currently, 87.7% of Sabah's agricultural land is covered with oil palm. Policymakers seem to be ignorant of the importance of food crops over industrial crops. This could be challenging for the state to achieve higher self-sufficiency levels. The limitation of land to cultivate food crops and the limited focus given contributed to the amount of supply and higher import dependency.

Another informal institutional factor is the patronage system. It can be defined as relationship involving two parties who are known as the patron and client (Scott, 1972). Generally, a patron will use his resources to provide benefits to clients who, in return will give support to patrons. According to this study, cooperation among state agencies, small and medium-sized businesses, farmers, and politicians is essential. Findings showed that if the client has a good relationship with the patron, they will gain incentives in the form of better monitoring services, subsidies, better infrastructure facilities, and machinery to develop their farm. This situation resulted in uneven distribution of allocated resources to the beneficiaries and ineffectiveness of policy implementation (Suffian et al., 2022).

Additionally, lack of coordination and implementation is another informal institutional factor that has become a limitation to agricultural growth. In this circumstance, government departments and agencies are therefore more inclined to work in silos since they are preoccupied with achieving their own objectives (Suffian and Suffian, 2021). In this context, poor coordination among state agencies related to the issue of seed supply, infrastructure development, incentives and subsidies resulted in a negative impact on the effectiveness of policy implementation.

## **CONCLUSION**

The aim of this research is to explore the processes involved in policy making that resulted in the policy outcome. The formulation of food policy had a significant impact on the growth of the agro-food subsector. The theory of institutions reveals that the role of institutions is critical in any economic development and growth, and it has greatly influenced the effectiveness of policy formulation and policy implementation. Besides the fundamental components of economic growth such as human capital, physical capital, and technology as the significant factors of growth and development, other institutional issues such as mismatch priority, patronage system, and lack of coordination are among the factors that hinder the development of the agriculture sector in the state. The state's agricultural policies are always in line with the national policy but produce different outcomes. It is difficult to solve the problem of development as, according to North (1992), formal rules of institutions can be changed overnight but informal constraints change very slowly. However, the results demonstrate that growth is made challenging by institutions. Therefore, better food first policy will only emerge if the underlying causes of the problems are identified and understood; otherwise, the state's ability to increase food security will be constrained.

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# **The Effect of Different Application Rates of *Trichoderma* Biofertilizer on the Growth Performance of Green Mustard (*Brassica juncea* L.) in Soil Amended with Empty Fruit Bunch (EFB) Compost**

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## **INTRODUCTION**

Green mustard (*Brassica juncea* L.) is a type of leafy vegetable that is favoured by people and consumed by various groups (Siregar et al., 2019) for their nutritional values such as vitamins A, C, and K as well for a source of vitamin. Globally, the production of mustard seeds was 654,112mt led by Nepal, Russia, and Canada in 2019 (Market Intelligence Team, 2021). In Malaysia, the per capita consumption of mustard was about 4.4 kg in 2020 (Department of Statistics Malaysia, 2021). The statistic revealed that the total production of green mustard has reached 142,764mt with an estimated 15,815 ha of land for cultivation (Hanizah Rasad, 2021). Green mustard can be grown on any type of soil that contains high nutrients, loose structure, and good drainage (Hanizah Rasad, 2021). Chemical fertilizers are commonly used in the conventional method of cultivation to boost crop production. However, excessive amounts of fertilizer could bring negative consequences such as environmental contamination (Savci, 2012), human health problems such as food poisoning (Bindraban et al., 2020), and degradation of rhizosphere and nutrients in the soil (Kumar et al., 2019). Hence, the application of biofertilizers, which provides more green technology, has gained more interest from many growers to tackle the negative issues regarding the shortcoming that arise from the application of chemical fertilizer. Moreover, the awareness of health consciousness among consumers has increased, which significantly contributes to the high demand for better quality vegetables in their daily consumption.

Therefore, many attempts for new application or advanced cultivation methods to increase produced has been emerged. One of the alternative farming system adopted by the growers nowadays are organic farming which able to sustain the health of the soil and environments, biodiversity rather than the use of inputs with adverse effects whilst maximizing the yield profit (Bendjebbar and Fouilleux, 2022). The organic farming combines conventional methods, innovative and offers sciences benefit to the environment. Biofertilizers is one of the measures in the organic farming which consist of the living cells of beneficial microorganisms such as *Trichoderma* (Mahmud and Chong, 2021) and has ability to enhance the plant nutrition by mobilizing or increasing the nutrient availability in the soils (Mitter et al., 2021).

*Trichoderma* sp. is one of the useful microorganisms in enhancing the growth and productivity of the plant. The use of *Trichoderma* as a biofertilizer helps in reducing the amount of chemical fertilizers applied in the soil which might be detrimental to the plant and toxicity to the soil. The effect of *Trichoderma* biofertilizer on plant growth performance and productivity has been studied for a large number of plant species mainly in the greenhouse or in pot experiments. However, there is a lack of study on the combined use of *Trichoderma* biofertilizer enriched with EFB compost. Hence, this study was conducted to investigate the effectiveness of *Trichoderma* biofertilizer enriched with EFB compost in promoting the growth of *Brassica juncea* L.



## MATERIALS AND METHODS

### *Experimental site and seedling preparation*

The experimental study was conducted under the rain shelter at the Farm Unit, UiTM Sarawak Branch, Samarahan Campus from May 2022 to July 2022. The viable seeds were obtained by soaking the seeds in the water overnight to break the dormancy and assist the germination. The seeds were sown in peat moss-filled seed trays (9×12 cells) in the nursery and transplanted into the polybags (15cm x 20cm) at four weeks old that contains of a growing media. The growing media are combination of top soil, sand and EFB compost with the ratio of 3:2:1.

### *Experimental design and field trial management*

The experimental study was carried out in Farm Unit, UiTM Cawangan Sarawak, with four treatments viz. T1: Control (Soil: Sand: EFB compost), T2 (Soil: Sand: EFB compost + 100 grams of *Trichoderma* biofertilizer), T3 (Soil: Sand: EFB compost + 200 grams of *Trichoderma* biofertilizer), and T4 (Soil: Sand: EFB compost + 300 grams of *Trichoderma* biofertilizer) laid in a completely randomized design (CRD) with five replications. Hand weeding was carried out weekly to reduce competition for the nutrient. The trial plants received twice a day (300-500ml) of watering to maintain an adequate moisture level in the growth medium.

### *Data collection and analysis*

Mineral contents in the sample was extracted using ash remains from the ash content analysis. The extracted samples were used to quantify nutrient elements such as K, Na, Ca, Mn, Zn, Cu, Fe and Mn using the Atomic Absorption Spectrophotometer (AAS) (AA800 Parkin-Elmer, Germany). Phosphorus was determined using the blue development method as described by Murphy and Riley (1962).

### *Statistical analysis*

The growth performance of green mustard was recorded weekly. The growth parameters recorded included the plant height (cm), leaf length (cm), the width of leaves (cm), and the number of leaves. The freshly harvested plants of each treatment were recorded for their total fresh weight (g), total root weight (g), total shoot weight (g), and the length of root (cm). Root length was measured from the base of the stem to the longest root using a plastic ruler (30cm) while the total fresh weight, shoot weight, and root weight were measured using an electronic scale. ANOVA was carried out at a 5% level of significance. The Tukey's simultaneous test was used to separate the mean comparisons for each treatment at a 5% significance level ( $p \leq 0.05$ ) by using IBM SPSS software version 25.

## RESULTS AND DISCUSSION

In this present study, the analysis of variance (ANOVA) for all agronomic parameters indicates there is no significant different ( $p > 0.05$ ) among all treatments (Table 1). However, plant treated with *Trichoderma* biofertilizer in EFB compost amended medium showed highest mean values for the plant height (32.34 cm), leaf number (9), leaf length (17.20 cm) and leaf width (11.12cm) as compared to the control treatment (EFB compost only) (Table 1). Inonu et al. (2020), stated that the EFB compost has a high potential to improve the soil structure and soil aeration which easier for the roots to grow and increases the ability to absorb more nutrients. Besides that, EFB compost also can be used as an organic fertilizer due to its macro and micronutrients that are essential for the plant to grow. The experiment conducted by Sugianti and Zulheadar (2021), found that all the agronomic parameters under study (plant height, number of leaves, leaf width, canopy width) were not significantly different when treated with different rates (2000kg/ha, 2500kg/ha and 3000kg/ha) of inorganic fertilizer enriched with *Trichoderma* sp. and the control

treatment (without fertilizer). In addition, a study conducted by Mahato et al. (2018), also reported that plant height increased more in the control treatment (only soil) as compared to plants treated with the combination of *Trichoderma* which are 14.5% and 4.6%, respectively.

Table 1. Effects of *Trichoderma* biofertilizer on plant height, number of leaves, leaf length, and leaf width.

Treatments	Plant Height (cm)	Leaves Number	Leaf Length (cm)	Leaf Width (cm)
T1	27.32 ± 3.25 <sup>a</sup>	6.2 ± 1.64 <sup>a</sup>	14.34 ± 3.26 <sup>a</sup>	7.70 ± 1.89 <sup>a</sup>
T2	32.34 ± 0.94 <sup>a</sup>	9.0 ± 1.58 <sup>a</sup>	17.20 ± 0.61 <sup>a</sup>	11.12 ± 1.41 <sup>a</sup>
T3	25.34 ± 4.35 <sup>a</sup>	6.4 ± 2.19 <sup>a</sup>	12.68 ± 2.02 <sup>a</sup>	8.94 ± 3.28 <sup>a</sup>
T4	30.06 ± 6.43 <sup>a</sup>	6.8 ± 1.48 <sup>a</sup>	14.50 ± 3.57 <sup>a</sup>	8.50 ± 2.37 <sup>a</sup>

Mean values in the same row with different alphabets (a>b>c) are significantly different at p<0.05 (ANOVA, Tukey's test). Values are given in means ± standard error: T1: Control (Soil: Sand: EFB compost), T2 (Soil: Sand: EFB compost + 100 grams of *Trichoderma* biofertilizer), T3 (Soil: Sand: EFB compost + 200 grams of *Trichoderma* biofertilizer), and T4 (Soil: Sand: EFB compost + 300 grams of *Trichoderma* biofertilizer).

Table 2 shows plant treated with 100 grams of *Trichoderma* biofertilizer in EFB compost amended medium yielding significantly higher shoot weight (51.62 grams), root weight (3.69 grams), root length (23.48 grams) as compared to other treatments (0, 200 grams, and 300 grams of *Trichoderma* biofertilizer). However, some (root weight and root length) of the parameters show control treatment gives a higher mean value as compared to *Trichoderma* biofertilizer in EFB compost amended medium (200 grams and 300 grams) but there are no remarkable changes (p<0.05). This might be due to the pests and diseases attacked during the study. These results are explained by the research by Ismail et al., (2017) on tomato variety MT11, whereby most of the plants attack by wilt disease after 60 days of application of EFB compost due to certain environmental condition. Moreover, Haque et al. (2012), reported that, sole application of *Trichoderma* biofertilizer did not shows significant changes on the growth and yield of mustard over control treatment. However, it significantly increases the growth and yield of mustard when combine with N fertilizer. In addition, an increased in the root length in plants treated with *Trichoderma* biofertilizer might be one of the important factors for enhancing the shoot weight and increasing the rate of nutrient acquisition by the green mustard.

Table 2. Effects of *Trichoderma* biofertilizer on shoot weight, root weight, root to shoot ratio and root length.

Treatments	Shoot Weight (g)	Root Weight (g)	Root to Shoot Ratio	Root Length (cm)
T1	20.05 ± 8.14 <sup>b</sup>	3.64 ± 1.79 <sup>a</sup>	0.18 ± 0.05 <sup>a</sup>	18.64 ± 5.50 <sup>ab</sup>
T2	51.62 ± 11.12 <sup>a</sup>	3.69 ± 1.19 <sup>a</sup>	0.07 ± 0.02 <sup>bc</sup>	23.48 ± 2.19 <sup>a</sup>
T3	17.56 ± 9.70 <sup>b</sup>	1.45 ± 0.90 <sup>b</sup>	0.08 ± 0.01 <sup>b</sup>	14.50 ± 3.99 <sup>bc</sup>
T4	31.35 ± 18.81 <sup>b</sup>	1.38 ± 0.86 <sup>b</sup>	0.04 ± 0.01 <sup>c</sup>	13.12 ± 2.39 <sup>c</sup>

Mean values in the same row with different alphabets (a>b>c) are significantly different at p<0.05 (ANOVA, Tukey' test). Values are given in means ± standard error.

## CONCLUSION

The use of organic fertilizer as an alternative to increase the growth rate of the plant have been practices around the world. A study on *B. juncea* have been conducted by comparing the effects of its growth after supplied with different rates of *Trichoderma* biofertilizer. Overall, there is no significant difference shown in the agronomic parameters (plant height, leaves number, leaf

length and leaf width) of *B. juncea* with different application rates of *Trichoderma* biofertilizer. However, for the shoot weight, root weight and root length shown that the application of 100 grams of *Trichoderma* biofertilizer in EFB compost gives significantly higher mean values but there are no remarkable changes if the amount of *Trichoderma* biofertilizer increases to 200 and 300 grams. Hence, it is indicated that, with proper management practices *Trichoderma* biofertilizer can improve the growth and quality not only by *B. juncea* but also other horticultural crops.

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# Natural Resources Conservation for Human Well-Being in Gunung Mulu National Park

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## INTRODUCTION

In addition to securing carbon in the forests, holistic park management of natural resources has an impact on local well-being in terms of food security, social equity, health, and income equality (Bowler et al., 2012). A lack of public awareness of conservation will result in unsustainable use of resources, which will cause water pollution, extinction of flora and fauna, and degradation of ecosystems that will affect food production (Pimentel and Burgess, 2013). Indeed, biological knowledge alone is not sufficient to solve conservation problems. Therefore, the role of the social sciences in solving these problems has become increasingly important (Mascia et al. 2003). Attitudes, social influences, and human-nature relationships are the main factors that affect the public awareness of natural resources conservation (Boaitey et al., 2018). Consequently, the purpose of this study is to examine the level of awareness (knowledge, attitude, and experience) of the local community and visitors in GMNP regarding natural resources conservation.

## MATERIALS AND METHODS

There is a total of seven sections in the questionnaire, each representing Section A-knowledge (K), Section B-attitude (A) and Section C-experience or practices (E), and Section D-respondent's demographic background and opinions. Items for K, A, and E are adapted and revised to suit the local study site from Boaitey et al. (2018), Buijs et al. (2008), Dunlap (2008), Soga et al. (2016). The validated questionnaire was disseminated to 99 local communities and 87 visitors from April to July 2021 through convenience sampling. The local communities involved are those who live in the settlement areas around GMNP, including Kampung Batu Bungan, Kampung Long Iman, and Kampung Long Terawan. The online platform Google Form was used due to movement restrictions by the government during the COVID-19 pandemic to get the data. Reliability test showed the knowledge, attitude, and experience questionnaire have reached the minimum acceptable value of Cronbach alpha, 0.5 (Hinton et al., 2014).

## RESULTS AND DISCUSSION

### *Demographic profile of respondents*

Table 1 shows the demographic background of the respondents. There was a total of 186 respondents, 53.2% (n=99) are local community, while 46.8% (n=87) are visitors.

### *Level of awareness between local community and visitors*

Based on Table 2, the visitors' knowledge, attitude, and experience are higher than the local community. The level of knowledge was significantly higher for visitors than for the local community ( $p < 0.001$ ). The knowledge gap between the local community and visitors is very large compared to attitude and experience. This result could be due to the diverse sociodemographic backgrounds. As presented in the table, the level of attitude of visitors is also significantly higher than the local community. This result contradicted the findings of a similar study in Bako National Park (BNP) that the local community has a higher attitude towards natural

Table 1. The demographic background of respondents.

Variable/ Item	Local Frequency (%)	Visitors Frequency (%)
Gender		
Male	60 (60.6)	41 (47.1)
Female	39 (39.4)	46 (52.9)
Race		
Natives	94 (94.9)	63 (72.4)
Chinese	4 (4.0)	16 (18.4)
Indian	0 (0.0)	2 (2.3)
Others	1 (1.0)	6 (6.9)
Ethnics (only for local community)		
Penan	22 (22.2)	N/A
Berawan	45 (45.5)	N/A
Others (e.g. Malay, Iban, Chinese)	32 (32.3)	N/A
Age		
19-25	7 (7.1)	12 (13.8)
26-30	13 (13.1)	16 (18.4)
31-39	25 (25.3)	35 (40.2)
40-50	20 (20.2)	18 (20.7)
More than 50	34 (34.3)	6 (6.9)
Level of education		
No formal education	7 (7.1)	1 (1.1)
Primary education	20 (20.2)	1 (1.1)
Secondary education	58 (58.6)	5 (5.7)
Tertiary education (e.g. PhD, Master, First degree, diploma, certificates)	14 (14.1)	80 (91.9)
Monthly income		
Less than MYR2500	83 (83.8)	23 (26.4)
MYR2,500-MYR4,849	13 (13.1)	27 (31.0)
MYR4,850-MYR7,099	0 (0.0)	0 (0.0)
MYR7,100-MYR10,959	0 (0.0)	23 (26.4)
More than MYR15,039	0 (0.0)	5 (5.7)
Others (e.g. Prefer not to say)	3 (3.0)	4 (4.6)
Observations	99 (53.2)	87 (46.8)

Note: MYR1.00= USD0.23 (Based on currency exchange in July 2022).

resource conservation than visitors (Ibrahim, Adam Assim, et al. 2021). A good sense of belonging to BNP among the local community is a good indicator that encourages them to take care of this area, which has been their identity for a long time. However, in GMNP, the local community might have a lower sense of belonging require stakeholders' attention. The local community of GMNP has a different cultural heterogeneity to the community in BNP. The Penan and Berawan have conflicts over employment opportunities and land ownership in the park area. The dissatisfaction between them and the government is also the cause of the lower level of attitude than visitors to conserve the nature resources. Xu et al. (2022) stated that background factors such as gender, age, income, education, marital status, acceptance to pay, and willingness to reside in the community (for an extended period of time) could influence people's attitudes towards natural resource conservation. The level of experience for the visitors is also significantly higher than the local community. Overall, the public indicates that their level of knowledge and experience is high, mean value of 4.13 and 3.61 respectively. While attitude is still in moderate level with mean value of 3.59.

Table 2. Level of knowledge, attitude, and experience towards natural resource conservation between local community and visitors (n=186).

Construct	Frequency, n	Mean	Cohen's <i>d</i> effect size	Strength of association	<i>p</i> -value
Knowledge					
Local community	99	3.79 (0.65)	1.29	Very large	0.00*
Visitors	87	4.52 (0.47)			
Attitude					
Local community	99	3.44 (0.41)	0.80	Large	0.00*
Visitors	87	3.78 (0.44)			
Experience					
Local community	99	3.33 (0.73)	0.96	Large	0.00*
Visitors	87	3.92 (0.48)			

Note: Figure in parentheses is standard deviation; \*Significant at  $p < 0.01$ .

## CONCLUSION

Based on the awareness constructs, their level of knowledge and experience is high, while attitude portrays a moderate level. The empirical study contributes to the development of a natural resource conservation framework for GMNP that emphasizes human dimensions toward sustainability resource management.

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# Can Online Agriculture Courses Encourage University Students to Practice Urban Agriculture?

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## INTRODUCTION

Urban agriculture has been identified as a key strategy to address problems of current food systems, namely socio-economic, environmental and food security issues (Islam and Siwar, 2012). Urban agriculture refers to agricultural practices in urban environments to produce food and other agricultural products (Kozai et al., 2016). This includes horticulture, animal husbandry and aquaculture. Typically, urban agriculture is rarely technological sophisticated farming nor is it a large-scale practice (Islam and Siwar, 2012). In the past, the practice was only common among poor urban households, serving as a method to reduce food expenditure. However, urban agriculture has slowly gained traction in some countries and is currently serving an important role in the provision of food, employment, and environmental sustainability (Islam and Siwar, 2012).

Urban agriculture shortens the food supply chain while circumventing corporate-controlled food systems that utilize unsustainable agricultural practices. This provides urban population access to fresher and more nutritious food while reducing greenhouse gas emissions associated with supply chains and widescale agricultural practices (Pataki et al., 2006). Studies have also found that urban agriculture improved food security, dietary diversity and access to nutritionally adequate diet in fifteen countries (Shamsudin, 2017). Thus, it is safe to say that urban agriculture plays an important role in the future of urban populations.

The Malaysian government has been actively promoting urban agriculture for the past few years. In 2018, the government launched the “Program Pertanian Bandar 2.0”, which roughly translates to “Urban Agriculture Program 2.0”. The objective was to double the number of urban farming communities around Malaysia (Koris and Ahmad, 2018). These farming systems would not be practiced by urban households or communities as a fulltime occupation, but as a pastime activity that would supplement food source to urban communities. Unfortunately, there is still a lack of interest for urban farming among Malaysians, namely the younger generation. Most young Malaysians shy away from agriculture since they view it as an undesirable and demanding practice (Man, 2012). Therefore, the purpose of this study was to evaluate the potential for agriculture courses to improve the interest of young Malaysians in urban agriculture. More specifically, the study focused on university students who did not have any background in agriculture.

## MATERIALS AND METHODS

This study focused on the “Agriculture and Life” course at Universiti Putra Malaysia (UPM), from August 2020 to January 2021. This course is compulsory, whereby all degree students are required to take the two-credit course that can be completed in one semester. The course consists of tutorials, aimed to teach students the basics of agriculture via lectures, and a practical component, where students can apply the knowledge gained during tutorials.



For this study, we focused on non-agricultural students who had no agriculture experience prior to the course. This is to keep in line with the core objective of this study and to also avoid any bias the students might have towards agricultural practices. For the tutorial component of the course, the students were required to conduct their own urban agriculture projects at their respective homes or communities. They periodically reported the progress of their projects and received continuous guidance from their course lecturers.

The students were asked to complete a pre-course survey (Pre-C) that evaluated their perception and attitudes regarding urban agriculture. More specifically, the survey assessed the students perceived barriers to urban agriculture and the likelihood of them practicing urban agriculture in the future. Students were also asked to provide demographic information. After completing the “Agriculture and Life” course, the students were asked to complete a post- course survey (Post-C) with the same questions as in the Pre-C, to evaluate the effects the course had on their perception and attitudes regarding urban agriculture.

All data were analysed using IBM SPSS Statistics 25 and Microsoft Excel. The study was approved by the university’s ethical community. Students were informed about the study and could choose to not participate in the study at any time for any reason. Students who opted out were still allowed to complete the course without any hindrances. The surveys were conducted anonymously without any participant identifiers.

## **RESULTS AND DISCUSSION**

A total of 114 students (19 to 21 years old) participated in this study, who were pursuing degrees in medicine (N = 94), chemistry (N = 15), physics (N = 4) and biology (N = 1). The majority of these students (N = 50) lived in houses with vacant land or open spaces, such as bungalows or semi-detached houses. There were also a significant number of students (N = 23) who lived in terrace houses with personal green spaces such as front yards, backyards or gardens. The remainder lived in residences without personal green spaces, such as in apartments/condominiums/flats (N = 13), hostels (N = 3) or terrace houses without personal green spaces (N = 25). All students involved in this study were able to complete the “Agriculture and Life” course with passing grades (Figure 1).

Before the course, the majority of the students agreed with two perceived barriers towards practicing urban agriculture (Figure 2). 67.5% of students agreed with the statement “I am against practicing urban agriculture due to it being too time-consuming”, while 65.8% agreed with the statement “I am against practicing urban agriculture due to my lack of knowledge in this field”. There were also a sizeable portion of students who agreed with the other perceived barriers before taking the course. For instance, 44.7% of the students agreed with the statement “I am against practicing urban agriculture due to my lack of interest in this field.”, while 32.5% agreed with the statement “I am against practicing urban agriculture due to a lack of space/land in my area of residence.”. Cost and safety had the lowest number of students who viewed it as a perceived barrier, recording 16.7% and 4.4% respectively. The responses of the students were expected, as researchers have listed misinformation/lack of knowledge, inconvenience, habitual patterns and negative emotions/stress were among the most common perceived barriers (Sjörs et al., 2014). Moreover, as mentioned previously, young Malaysians generally lack interest in agriculture (Man, 2012). This trend appears to be consistent in Southeast Asia. An Indonesian study on young generation’s perception on the agricultural sector found that most youths did not favour agriculture due to it having a poor “warranty of future life” (Widiyanti et al., 2018).

This view was consistent even among farmers’ children, whereby although they had fairly good perceptions in terms of income and convenience of agricultural work, they still did not see



Figure 1. Examples of urban agriculture projects conducted by students. a: Student growing Bok Choy in her home yard, b: Student planting Brazilian Spinach at her apartment, c: Student growing Holy Basil at her apartment's community garden, d: Student harvesting *Ulam Raja* at her backyard, e: Student watering water spinach planted in a community garden.

themselves practicing agriculture in the future. This is especially more prevalent among young urbanites, who mostly migrated to cities to avoid agricultural work (Man, 2012; Widiyanti et al., 2018). There was a statically significant decrease in the number of students who agreed with perceived barriers to urban agriculture after the course (Figure 2). Before the course, 67.5% of students agreed with the statement "I am against practicing urban agriculture due to it being too

time- consuming”, but this figure dropped to only 26.3% after the course ( $p = 0.001$ ). For the statement “I am against practicing urban agriculture due to my lack of knowledge in this field”, the number of students agreeing with the statement reduced by 52.6% ( $p = 0.001$ ). The other perceived barriers also saw a significant reduction; for interest there was a reduction of 31.6% ( $p = 0.001$ ), for cost there was a reduction of 13.2% ( $p = 0.008$ ), while for space/land there was a reduction of 14% ( $p = 0.009$ ). Only safety did not record a significant decrement ( $p = 0.09$ ), but this was due to the number of students agreeing with this perceive barrier was low (4.4%) even before the course.

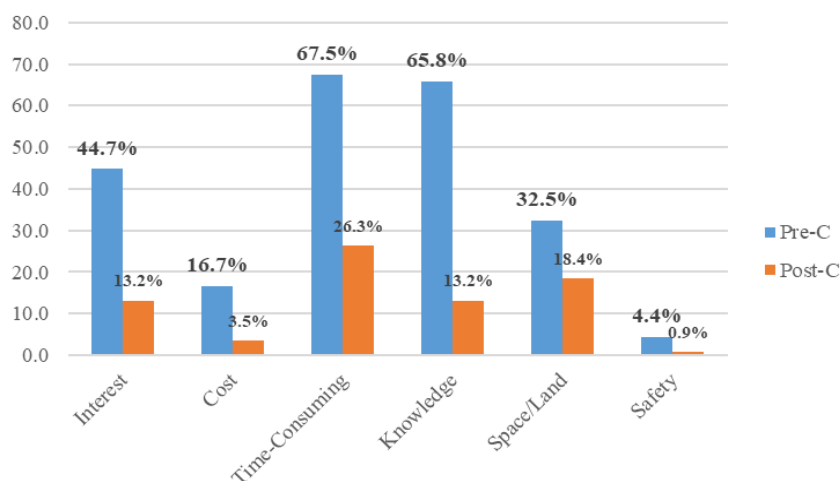


Figure 2. Percentage of students who agreed with perceived barriers to urban agriculture.

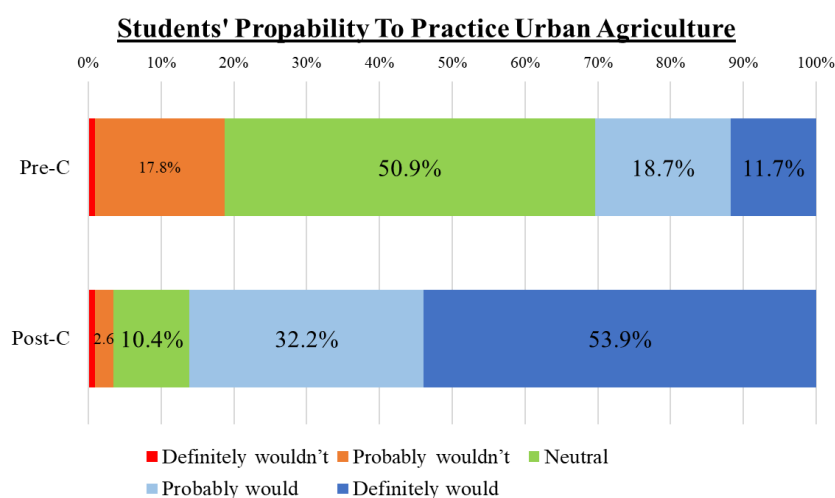


Figure 3. Students’ responses to the statement “I am confident that I will be practicing urban agriculture in the future”, before the agriculture course (Pre-C) and after completing the course (Post-C).

In summary, this study found a statically significant improvement in addressing the perceived barriers of students regarding urban agriculture. The improvement in attitude regarding urban agriculture was also consistent with the students’ confidence in practicing urban agriculture in the future. Before the course, 30.4% of the students believed that they definitely or probably would practice urban agriculture in the future (Figure 3). This is a very promising figure, which meant that a large number of students were open to the idea of practicing agriculture despite having no experience. However, 50.9% of students stated that they were “neutral” while the

remaining 18.7% of students felt that they definitely or probably wouldn't be practicing urban agriculture in the future.

The perception of significantly improved after the compulsory course; 86.1% of the students stated that they definitely or probably would practice urban agriculture in the future, which was an improvement of 55.7%. The number of students that stated they definitely or probably wouldn't practice urban agriculture dropped to 3.5% after the course.

The compulsory agricultural course proved to be effective in increasing the knowledge and interest of non-agricultural students who had no experience in agriculture. This in turn improved the students' confidence in practicing urban agriculture in the future. Appropriate stakeholders will be able to reproduce the results of this study by simply replicating the course and making it available for targeted groups.

## CONCLUSION

When it comes to encouraging the younger generation to practice urban agriculture, or even agriculture in general, the consensus around the globe is consistent; Education and awareness is key. Even single session webinars that engaged youths' concerns regarding agriculture have proven to have a positive impact (Freeman and Mungai, 2018). The research conducted by Widiyanti et al. (2018) in Indonesia drew the same conclusion; education is needed to spark interest to encourage youths to practice agriculture. The first step is to directly engage with the youth's concerns regarding urban agriculture, only then can their perceived barriers be addressed. The next step would be educating them on sustainable urban farming practices. This will provide them with the basic knowhows to become urban farmers themselves. Governmental involvement, in terms of subsidies, educational seminars and land allocations would also have a positive impact (Freeman and Mungai, 2018; Widiyanti et al., 2018).

This study shows a clear benefit in increasing knowledge and awareness regarding urban agriculture. Providing educational opportunities for appropriate stakeholders is a cost-effective model that can make a lasting and valuable impact in the future of urban food security. Limitations of this study include a homogenous study population. They were all university students, thus youth without tertiary education exposure might have different results. Secondly, there is no way to determine the exact number of students that will go on to practice urban agriculture in the future.

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# Rancidity of Ginger Floss (*Serunding Halia*) Cooked with Different Cooking Oils

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## INTRODUCTION

Ginger (*Zingiber officinale*) is the underground rhizomes of a perennial herbs. It belongs to the family Zingiberaceae and originates from South-East Asia. Ginger is an important spice in Malaysia, and it is usually used as an ingredient in local dishes, pharmaceutical, cosmetic, and other industries. Ginger is also useful in treating symptoms such as vomiting, diarrhoea, light-headedness, bloating and decrease in body temperature (Sa-Nguanpuag et al., 2011). Ginger is planted commercially at Pahang, Sabah, and Sarawak. Popular varieties are Bentong, Cina, Tanjung Sepat and Indonesia (Mail et al., 2020). In Sarawak, ginger is commonly used to make floss. Ginger floss is consumed as a snack or mixed with *nasi impit*, *ketupat*, *lemang* and porridge. Ginger floss is an important value-added food product for small and medium-sized enterprises in Sarawak.

In the process of making ginger floss, fresh gingers are shredded into floss, oven dried and fried by stirring continuously. The water activity of ginger floss is ensured to be below 0.60. This is crucial to prevent microbial spoilage and to achieve desired crispiness. Despite its low water activity, it will turn rancid after few months of storage at room temperature. Thus, this study is carried out to determine the effects of different cooking oils towards rancidity of ginger floss.

## MATERIALS AND METHODS

Firstly, fresh gingers were weighed, peeled, and washed. Then, gingers were grated using 4 mm grater. Water was squeezed out from the shredded gingers and weighed. Shredded gingers were dried in the oven at 60 °C for 1 hour. Dried, shredded gingers were fried with Palm oil (T1) until crispy and yellow colour. Volume of Palm oil needed was twice the weight of shredded and pressed gingers. Next, gingers were taken out and anchovies were fried until crispy. The weight of anchovies used is 10% of the weight of gingers. Fried anchovies were divided into half where half of it was blended, and another half was not blended. Crispy gingers and two parts anchovies were mixed well. Finally, ginger floss was ready to be bottled, labelled, and stored at room temperature. The formulation was repeated using three different cooking oils which were Canola oil (T2), Sunflower oil (T3), and Corn oil (T4). Each treatment was prepared in 3 replicates and rancidity was determined every fortnight using Rancimat (892 Professional Rancimat, Metrohm, Switzerland) for up to 4 months.

## RESULTS AND DISCUSSION

Figure 1 showed that the induction time of ginger floss cooked with palm oil is longer than other oils throughout the storage period. This indicates that palm oil is relatively stable to oxidative deterioration. This stability advantage is attributed to low level of linoleic acid (10-11%) and only a trace amount of linolenic acid in palm oil (Siew, 2002). Linoleic acid (18:2) and linolenic acid (18:3) are polyunsaturated fatty acids that highly susceptible to oxidative spoilage (Titoc, 2010). The second cooking oil that is stable to oxidative deterioration is canola oil, followed by corn oil and sunflower oil. This order of edible oil oxidative stability is coherent to literature on induction

period (IP) of oils and blends by Teah (as cited in Gunstone, 2011). Ginger floss cooked with sunflower oil is the least oxidative stable as it undergoes extensive autoxidation in the frying process. This is caused by low level of  $\gamma$ -Tocopherol in sunflower oil that provides oxidative stability against autoxidation (Gupta, 2002). Therefore, sunflower oil is not suitable for making shelf-stable fried foods.

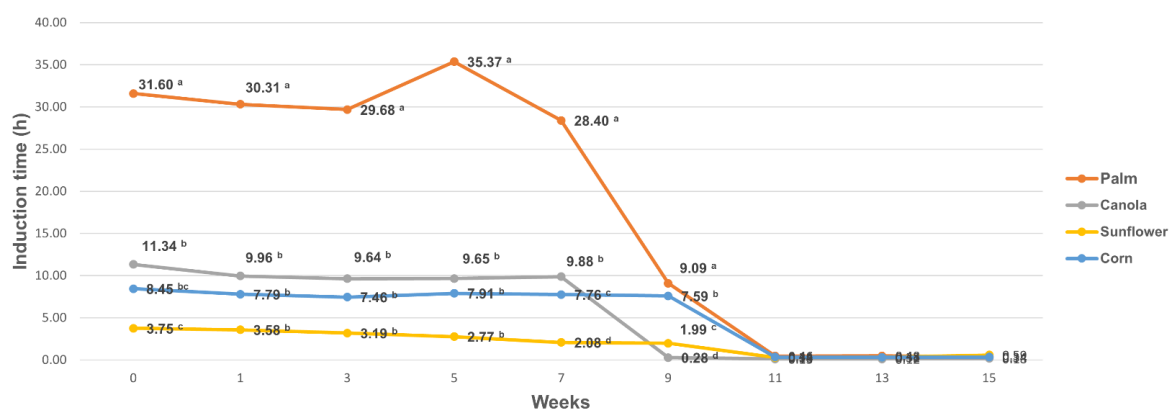


Figure 1. Rancidity of ginger floss (induction time, h) cooked with four different cooking oils (T1=Palm oil, T2= Canola oil, T3=Sunflower oil and T4=Corn oil) for a storage period of 4 months at room temperature. Values followed by the same superscript letter are not significantly different according to Tukey's test at  $\alpha=0.05$ .

## CONCLUSION

Ginger floss cooked with palm oil is shelf-stable to oxidative rancidity as its induction time is highest among other oils. All types of ginger floss need to be removed from the shelves at week 11 as the induction time is 0. Further study is required to prove the correlation between Rancimat results (oxidation stability) and shelf life of food. Sensory evaluation is suggested to be carried out on ginger floss cooked with different cooking oil to assess the effect of different cooking oil towards taste and flavour of ginger floss.

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# Uptake and Distribution of Carbofuran and Its Metabolite in Watermelon (*Citrullus lanatus*)

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## INTRODUCTION

Carbofuran, 2,3-dihydro-2,2-dimethylbenzofuran-7-yl methylcarbamate, is a broad-spectrum carbamate pesticide that kills insects, mites, and nematodes on contact or after ingestion. Carbofuran is harmful to human health which inhibit acetyl cholinesterase, an enzyme vital for the functioning of central nervous system and its usage is banned in the United States, Canada, and the European Union countries (Benicha et al., 2011). Carbofuran is stable and degrades slowly in non-sterile, neutral, or acid aerobic media, with half-lives ranging from one to eight weeks, but easily hydrolyzes under alkaline conditions (WHO, 2008). Carbofuran is rapidly taken up by plants through roots from soil and water, and is translocated mainly into the leaves (FAO, 1979). The uptake and dissipation of carbofuran in corn, groundnut, brinjal and sugarcane have been well documented (Turner and Caro, 1973; Singh and Kalra, 1992; Iqbal et al., 2007; Saini et al., 2020). Its main metabolite in plants is carbofuran-3-hydroxy (FAO, 1979).

In Malaysia, carbofuran is a registered pesticide until year 2023 and widely used in the cultivation of many fruits and vegetables. Recently, the Malaysian Government has announced the banning use of carbofuran in the agriculture sector effective May 1, 2023. However, local information on carbofuran and its metabolite residues in these crops is limited. Therefore, evaluation and comparison between residual levels found in different parts of crops with the MRL established in the Food Legislation are required. The objective of this study was to assess the uptake, distribution, and accumulation of carbofuran residues in watermelon (*Citrullus lunatus*) grown at Agriculture Research Centre, Semongok.

## MATERIALS AND METHODS

### *Chemicals and reagents*

The carbofuran and carbofuran-3-hydroxy standards were obtained from Dr. Ehrenstorfer, Augsburg, Germany while the carbofuran granules (AGRITOX 3G) were purchased from Hextar Chemicals Sdn. Bhd. The acetonitrile (LC grade, >99%), acetone, formic acid (98-100%), sodium chloride (NaCl), acetic acid, anhydrous magnesium sulphate (MgSO<sub>4</sub>), florisil (2% deactivated) were of analytical and residue grade and purchased from Merck, Germany and J.T. Baker, Philipsburg, USA.

### *Field experiments*

The watermelon (Black Lady variety) seeds were sown in a nursery for two weeks. A total of 98 healthy seedlings were transplanted onto 14 raised beds (1.2 x 6 m) with 7 plants/bed in one single row with in-row spacing of 0.8 m and between-row spacing of 2.0 m. The beds were dressed with 1.0 - 2.0 kg/m<sup>2</sup> chicken manure, 100-200 g/m<sup>2</sup> of dolomite and 30 g/m<sup>2</sup> of NPK fertiliser (15:15:15). The raised beds were covered with silver-shine plastic mulch. The plants were watered using a drip irrigation system. Fifty-six plants were treated with carbofuran granules at 17 kg/ha, when the plants began to bear fruits (3 weeks after transplanting) and was applied beside the plant roots. The remaining 42 plants were kept as control. The soil use in the

study was clayey red-yellow podzolic type with pH 6.6 (in water). The soil electrical conductivity (EC) was 123.3  $\mu\text{mhos/cm}$  and the organic matter content was 2.1%.

### ***Samples collection and preparation***

The plants were sampled at 0, 1, 3, 5, 7, 9, 14, 21, 28, 35, 49 and 63 days after carbofuran application. The plants sampled at 0 day were taken after 2 h of carbofuran application. Three watermelon plants were harvested at random from each bed and were separated into fruits, leaves, and stems. The fruits were further divided into skin and flesh samples. The samples were cut and homogenised in food processor (Robot Coupe Blixer®4, Vincennes, France), kept in containers and stored in a  $-18\text{ }^{\circ}\text{C}$  freezer prior to analysis. The homogenised samples were extracted using the modified QuEChERS (quick, easy, cheap, effective, rugged, and safe) method (Chai et al., 2011). Ten grams of homogenised fruit were weighed into PTFE centrifuge tubes and extracted with 20 mL acidified acetonitrile (1%) for 1 min by shaking vigorously with hand followed by vortex mixing (IKA VORTEX 3, Germany) for 1 min. Six grams of anhydrous  $\text{MgSO}_4$  and 1.5 g of NaCl were added and shaken immediately for 1 min using vortex mixer and then centrifuged at 1,127  $g$  for 1 min using multifunction centrifuge (Thermo Jouan Model B4i, Gontier, France). The supernatant was transferred into a centrifuge tube and 3 g of anhydrous  $\text{MgSO}_4$  was added. Florisil was used as an adsorbent in the cleanup process of the final aliquot.

### ***Analysis of carbofuran and its metabolite***

Pesticide stock solutions (500 mg/L) were prepared by dissolving an appropriate amounts of pesticide standards in acetonitrile. The pesticide standard solution mixtures were prepared by diluting the stock solutions to obtain 10.0, 1.0, 0.5, 0.1, 0.05, 0.01 and 0.005 mg/L concentrations. The pesticides in the samples were analysed using Agilent 1290 LC connected to a triple quadrupole mass spectrometer (MS/MS) Agilent 6495 Series. The LC separations were carried out on a 1290 Agilent module using reversed phase column ZORBAX Eclipse XDB-C18 (150mm x 4.6mm x  $5\mu\text{m}$ ). The elution was performed using gradient between acetonitrile and water. The injection volume was 2  $\mu\text{L}$ . A gradient elution was programmed at 1.2 mL/min flow, in which one reservoir contained 5mM ammonium formate and 0.01% formic acid solution in acetonitrile-water (19:1) and the other reservoir contained 5mM ammonium formate and 0.01% formic acid solution in deionised water. The total run time of the method was 3.00 min.

## **RESULTS AND DISCUSSION**

The concentrations of carbofuran residues in all plant parts were observed for 35 days after its application. The carbofuran residues concentrations in the root and the stem increased from Day 1 to Day 9 and gradually decreased to below the quantification level at Day 49 (Figure 1a). The highest carbofuran residues concentrations were recorded on Day 5 for stem at 0.134 mg/kg, Day 9 for root at 0.192 mg/kg and Day 21 for leaf at 0.057 mg/kg (Figure 1a) after its application. The carbofuran residues in the skin were detected at a lesser amount compared with root and stem; and peak on Day 5 before it dropped to below the detection limit on Day 7 (Figure 1a). The carbofuran residues in the flesh was below the quantification level from Day 0 (Figure 1a). These findings agreed with those reported for other crops (Turner and Caro, 1973; Singh and Kalra, 1992; Iqbal et al., 2007 and Saini et al., 2020). The conversion of carbofuran into carbofuran-3-hydroxy was observed in leaves and stems up to Day 35 and Day 28 after its application, respectively. About 80 to 90% of the carbofuran was metabolised to carbofuran-3-hydroxy in the leaves and stems whereas in roots, the carbofuran was still intact (Figure 1b).

After 35 days of carbofuran application, the concentrations of carbofuran residues in all watermelon parts were negligible and below the quantification limit (Figure 1a and Figure 1b). This is likely due to the rapid dissipation and low accumulation of carbofuran and carbofuran-3-hydroxy residues in watermelon. The tropical climate, rainfall, physicochemical properties, and concentration of the active ingredients, leaching and runoff of residues into soil, and

characteristics of the plant are among the factors that may affect the uptake, distribution, dissipation, and accumulation of carbofuran residues in the plant (Wanwimolruk et al., 2015).

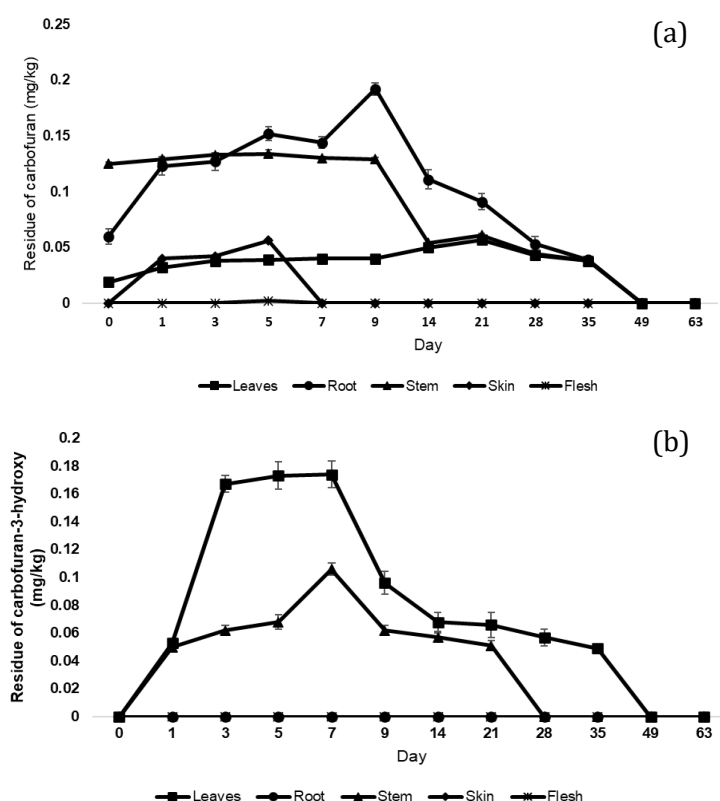


Figure 1. (a) Carbofuran and (b) carbofuran-3-hydroxy residues in leaves, root, stem, skin, and flesh of watermelon plants.

## CONCLUSION

The results suggested that the watermelon fruit harvested at its physiological maturity is safe for consumption with the single application of carbofuran at 17 kg/ha. The carbofuran shall be applied at an early fruiting stage and a strict postharvest interval shall be adhered to. Further study will include the monitoring of carbofuran fate in the soil post its application.

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# Influences of Spacing and Accessions on Growth and Yield Quality of Broccoli under Protected Rain Shelter Fertigation in Lowland

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## INTRODUCTION

Broccoli (*Brassica oleracea* var. *italica* L.) is one of the popular vegetables in Malaysia as cooked vegetables because of its delicious taste and high nutritional value. It is now grown throughout the world, but limited to relatively cool regions, as inflorescence formation is poor in high temperature (Pornsuriya et al., 1997). In Malaysia, broccoli is cultivated in limited areas in Cameron Highlands, Pahang and Ranau, Sabah with total production of 1,283.99 metric tonne in year 2017 (Ariffin et al., 2017).

Normally, broccoli is grown in an open field with the risk of being infected by head rot and soft rot disease from the impact of raindrops or a wet condition (Vodhivanich, 2006). Hence, the successful of its cultivation is highly influenced by the genetic characteristics of the cultivar, adaptive cultivar to climatic and soil conditions and applied nutrients. Various genetic improvement programs have produced disease resistant broccoli cultivars and hybrids that can adapt to high temperature, allowing them to be produced all-year round (Nooprom et al., 2013a). Broccoli is divided into two types: heading and sprouting. The most popular type of broccoli is the heading type, which is closely related to cauliflower and produces a large central head that is in high demand on the market. Italian or sprouting broccoli produces numerous little heads or florets but not a substantial head (Tejaswani et al., 2018).

Due to the rapid demand for imported vegetables, the movement from soil-based growing system to soilless media using fertigation system in lowlands is believed to reduce incidence of soil borne disease and beneficial for plant growth (Yaseer, 2016). The introduction of fertigation has increased crop productivity and quality, reduce soil borne disease and nutrient use efficiency (Romic et al., 2003). However, the initial cost of setting up the infrastructures is costly. To increase yield and cover the cost of production, it is important to utilize the rain shelter's space to the maximum capacity. Normally, broccoli cultivated under soil-based cultivation system at a 60cm x 60cm spacing produced large head size and quality (Ariffin et al., 2017). Optimal plant spacing is vital for crop growth and development, as well as increasing yield, quality, and income for farmers. There are no recommendations available on the suitability of specific varieties and plant spacing for broccoli growth in lowland using a rain shelter fertigation system in Sarawak, Malaysia.

Considering the above factors, the study was carried out to evaluate the suitable broccoli accessions and optimum plant spacing cultivated under protected rain shelter fertigation system in lowland, with the aim of producing high value broccoli in Sarawak.

## MATERIALS AND METHODS

An experiment was conducted in a closed protected rain shelter using fertigation system at Agriculture Research Centre, Semongok. The temperature recorded was within 26 to 35°C. The experiment was designed using a split plot design with three replications. The main plots were two plant spacings: S1-30cm x 30cm and S2-60cm x 60cm with sub-plots being hot summer hybrid broccoli accessions: V1-accession BC114 (Chef 1856), V2- accession BC120 (Green Jade

2034), V3-accession BC123 (Royal Green), V4-accession BC124 (Green Magic) and V5-accession BC126 (V-075).

Seeds were sown in germination trays filled with peat moss. Germinated seedlings were given foliar fertilisers and watered twice a day. Twenty-five days old seedlings were transplanted to the polybags filled with coco peat and arranged inside the rain shelter with two different plants spacing (S1 and S2), respectively (double row planting). Fertigation was carried out automatically to the root zone using EC dosages of 1.5 to 2.0 mS/cm and irrigation dosages of 1-1.5L/day.

Vegetative growth characteristics were measured on a ten random sample of plants, taken from each experimental plot and the following data were recorded: days to head initiation from transplanting, days to marketable head maturity from date of transplanting, plant height and pest and disease incidence. Broccoli heads of each plot were harvested at marketable stage and the following variables were measured: head weight (g/plant), head diameter (cm) and head compactness (yield/diameter, g/cm). Data collected were subjected to analysis of variance (ANOVA) using a statistical software, SPSS version 26. To evaluate the performance differences between broccoli accessions and spacings, the means were compared using Tukey's multiple range test at the significance level ( $P \leq 0.05$ ).

## **RESULTS AND DISCUSSION**

### ***Influences of accessions***

In terms of vegetative growth, Table 2 shows that the shortest plant heights were measured in accessions V1 (45.54 cm) and V5 (46.10 cm), while the tallest plant height was measured in V4 (50.28 cm). Among the accessions, V4 and V5 resulted in significantly the highest head weights and head width (Table 2). This indicates that accessions V4 and V5's higher yield was influenced by their genetic, ability to absorb nutrients from the soil and adaptability to location and environment (Marschner, 1990). Accession V4 and V5 produce significantly higher curd compactness, indicating that both accessions have a solid central head and are demanded by the market (Table 2). It is justified by Tejaswani et al., (2018), which reveals that accessions V4 and V5 belong to the heading type whereas the other accessions were of the sprouting type.

### ***Influences of spacings***

According to Table 2, there were no distinguishable variations between the spacing treatments for broccoli's vegetative development. The shortest plant height was observed using S2 spacing (47.39 cm). Spacing treatments also have no effect on yield performance, with S1 spacing yielding the most head weight (147.53 g), the least curd compactness (12.38 g/cm), and the broadest curd width (13.33 cm). These findings are consistent with the findings of Tejaswani et al., (2018), who discovered that closer plant spacing led in higher plant population per unit area, resulting in an increased yield per area. Broccoli grew well with S1 spacing and it promotes better growth and development while also increasing yield and quality.

### ***Interaction effect of spacing and accessions on Broccoli growth stage***

Referring to Table 3, accession V5 under S1 planting distance (S1V5 treatment) has the significantly longest day to head initiation (61.13 days) and day to harvesting (70.40 days) compared to other treatments. However, the duration of growth development among accessions in the spacing treatment did not affect much on yield quality of accessions (Table 2). In consideration of this, the growth duration for treatment S1V5 was still acceptable.

Table 2. Growth and yield performance of broccoli accessions at different spacings.

Treatment	Plant Height (cm)	Curd Width (cm)	Curd Weight (g/plant)	Curd Compactness (g/cm)
Accession (V)				
V1 (BC 114)	45.54 ± 3.15 <sup>a</sup>	12.28 ± 1.65 <sup>a</sup>	137.23 ± 18.88 <sup>a</sup>	11.27 ± 1.57 <sup>ab</sup>
V2 (BC 120)	48.82 ± 2.98 <sup>bc</sup>	11.75 ± 1.57 <sup>a</sup>	147.97 ± 29.41 <sup>ab</sup>	12.58 ± 1.77 <sup>b</sup>
V3 (BC 123)	46.74 ± 2.66 <sup>ab</sup>	16.79 ± 19.58 <sup>a</sup>	133.37 ± 20.57 <sup>a</sup>	9.93 ± 2.30 <sup>a</sup>
V4 (BC 124)	50.28 ± 3.48 <sup>c</sup>	11.22 ± 1.06 <sup>a</sup>	164.23 ± 21.23 <sup>b</sup>	14.65 ± 1.38 <sup>c</sup>
V5 (BC 126)	46.10 ± 2.25 <sup>a</sup>	11.48 ± 1.19 <sup>a</sup>	166.97 ± 39.36 <sup>b</sup>	14.45 ± 2.40 <sup>c</sup>
Spacing (S)				
S1(30 cm x 30 cm)	47.60 ± 3.55 <sup>a</sup>	13.33 ± 2.59 <sup>a</sup>	147.53 ± 31.36 <sup>a</sup>	12.38 ± 2.77 <sup>a</sup>
S2(60 cm x 60 cm)	47.39 ± 3.27 <sup>a</sup>	12.07 ± 1.65 <sup>a</sup>	132.37 ± 28.49 <sup>a</sup>	12.76 ± 2.46 <sup>a</sup>
Interaction (V x S)	ns	ns	ns	ns

Mean values in the same row with different alphabets (a>b>c) are significantly different at p<0.05 (ANOVA, Tukey' test). Values are given in means ± standard error. NS- not significant.

Table 3. Influences of spacings and accessions on growth stage of Broccoli.

Treatment	Day to Curd Initiation	Day to Harvesting
S1V1	45.00 ± 1.68 <sup>a</sup>	56.53 ± 1.68 <sup>a</sup>
S1V2	46.60 ± 3.31 <sup>a</sup>	58.73 ± 2.26 <sup>a</sup>
S1V3	46.07 ± 2.81 <sup>a</sup>	58.73 ± 2.26 <sup>a</sup>
S1V4	53.40 ± 1.06 <sup>a</sup>	62.47 ± 2.77 <sup>a</sup>
S1V5	61.13 ± 7.26 <sup>b</sup>	70.40 ± 7.26 <sup>b</sup>
S2V1	45.27 ± 2.37 <sup>a</sup>	55.80 ± 3.26 <sup>a</sup>
S2V2	45.53 ± 2.07 <sup>a</sup>	57.13 ± 2.87 <sup>a</sup>
S2V3	45.80 ± 3.09 <sup>a</sup>	57.13 ± 2.87 <sup>a</sup>
S2V4	50.87 ± 5.59 <sup>a</sup>	63.00 ± 6.36 <sup>a</sup>
S2V5	51.13 ± 7.54 <sup>a</sup>	60.20 ± 6.55 <sup>a</sup>

Mean values in the same row with different alphabets (a>b>c) are significantly different at p<0.05 (ANOVA, Tukey' test). Values are given in means ± standard error.

Based on field observations, both accessions and spacing treatments had very low pest incidence (1%) and no disease infestation.

## CONCLUSION

The results reveal that accessions V4 and V5 have the potential to be commercialised in the lowlands with 30 cm x 30 cm plant spacing to achieve high value and yield of broccoli under fertigation system. To increase head size and quality, further research into how broccoli responds to various fertiliser formulations is required.

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# Decipher Lignocellulose Digestion Mechanism of *Coptotermes curvignathus* based on Carbohydrate-Active Enzymes Profile using the Metatranscriptomic Approach

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## INTRODUCTION

As one of the major drivers in global carbon cycle, termites are studied to understand what makes them highly efficient in extracting energy from lignocellulosic materials (Ni and Tokuda, 2013). Lignocellulose, a highly abundant and recalcitrant carbon source is comprised of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are polysaccharides that serve as a primary structural component of plant cell wall and are bound together by lignin. The molecular architecture of lignin that is heterogeneous, phenolic, aromatic, highly branched and polydisperse in nature and makes the entire structure more hydrophobic and resistant to degradation.

Complete biodegradation of lignocellulose requires a combination of multiple enzymes that attack the different moieties of the polymer. This can be completed by termites and its consortium of gut microorganisms that work to co-metabolize lignocellulose. Ke et al. (2012) reported that lower wood-feeding termites could accomplish wood degradation process in hours instead of weeks or months in a fungal system. Termites can selectively modify and decompose the lignin by ~25% and accomplish a maximized utilization of cellulose at >90% and various hemicellulose components at ~60% (Ke et al., 2011).

*Coptotermes curvignathus* Holmgren, an indigenous lower wood termite commonly found in the Indo-Malayan region, is regarded one of the most voracious wood feeding termites in oil palm estates (Chan et al., 2011). Their digestive strategies in decomposing lignocellulosic material are of great interest to scientist at large. This study aims to decipher the lignocellulose degradation mechanisms deployed by *C. curvignathus* through meta transcriptomics approach.

## MATERIALS AND METHODS

The diversity of carbohydrate-active enzymes in the digestive system of *C. curvignathus* was investigated using meta transcriptomic approach. The digestome of *C. curvignathus*, which included the salivary gland, foregut, midgut, hindgut, rectum, and gut content was extracted and immediately flash frozen in liquid nitrogen. Total RNA isolation was conducted using Sepasol RNA1 Super G (Nacalai Tesque, Japan) according to the manufacturer protocol with minor modification where the separation phase using chloroform was repeated twice. Construction of the cDNA library and sequencing was performed using the TruSeq RNA Kit and Illumina HiSeq 2000 sequencing platform (Illumina, USA). All sample libraries were sequenced independently for 100 bp pair-end cycles, and raw sequenced data were pre-processed with the SolexaQA package to obtain high-quality paired-end reads. These reads were concatenated into pool data for *de novo* assembly using the Trinity assembly software (Garbherr et al., 2011).

Quantification of the Trinity assembled transcripts and unigenes were estimated using alignment-based methods, RSEM (Li and Dewey, 2011) and alignment-free methods, Kallisto (Bray et al., 2016) and Salmon (Patro et al., 2017) where the minimum threshold criteria set were all transcripts should have at least one estimated read count in each method to eliminate false positives. All assembled transcripts and unigenes were annotated by searching against the Swissprot and NR (non-redundant protein) database using BLASTx with an e-value of 0.00001, as well as submitted to online search using dBCAN2 for CAZY enzymes annotation (Zhang et al., 2018).

## RESULTS AND DISCUSSION

### *Overview of carbohydrate active enzymes profile of Coptotermes curvignathus digestome*

A total of 326011 unigenes were successfully assembled, and 715 unigenes were predicted as CAZY enzymes, namely 435 glycosyl hydrolases (GH), 200 glycosyl transferases (GT), 30 auxiliary activities (AA), 27 carbohydrate-binding modules (CBM), 13 carbohydrate esterases (CE) and 10 polysaccharide lyases (PL) (Figure 1).

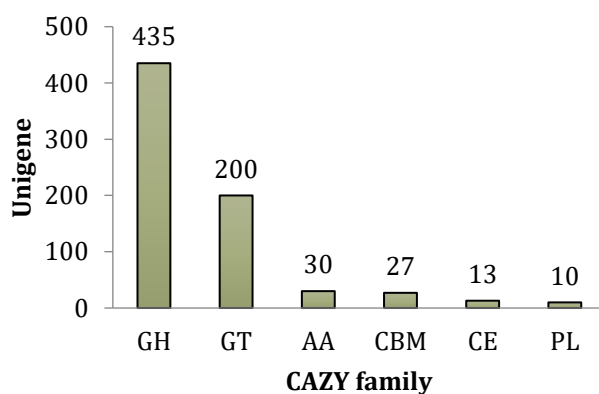


Figure 1. The number of CAZY unigenes defined in the transcriptome of *C. curvignathus* digestome.

### *Deciphering the lignocellulose degradation mechanisms in C. curvignathus digestome*

Glycosyl hydrolases were the predominant CAZY group with 46 members assigned. Cellulases were predominantly expressed in the digestome of *C. curvignathus*. Among them, the most abundant cellulases included GH9 endoglucanases produced by the host, GH5 endoglucanases, GH7 cellobiohydrolases, and GH45 endoglucanases, where the latter three had symbiotic origins (Figure 2). The predominant presence of these cellulases indicated that they are the core enzymes of cellulose degradation. This can be attributed to the aggressiveness of *C. curvignathus* attacking and consuming a vast amount of cellulolytic materials, as observed in the *C. formosanus* (Tokuda et al., 2004).

Compared to cellulases, hemicellulases were less predominant. They are the second predominant group with five members namely GH10, GH8, GH11, GH26, and GH53. GH10 xylanases with symbiotic origins were the core hemicellulase family and their presence was more prominent compared to the other four members, which indicated that the other families played a supporting role in hemicellulose degradation. Both cellulases and hemicellulases can work synergistically and enhance the accessibility to cellulose in the lignocellulose matrix.

The lignin component is considered the rate-limiting component of complete lignocellulose degradation for both natural and industrial processes. In fact, many organisms avoid degrading the lignin and simply change its properties to access the cellulose inside. This is assumably more energy conserving. From the meta transcriptomic analysis on the *C. curvignathus* digestome, this

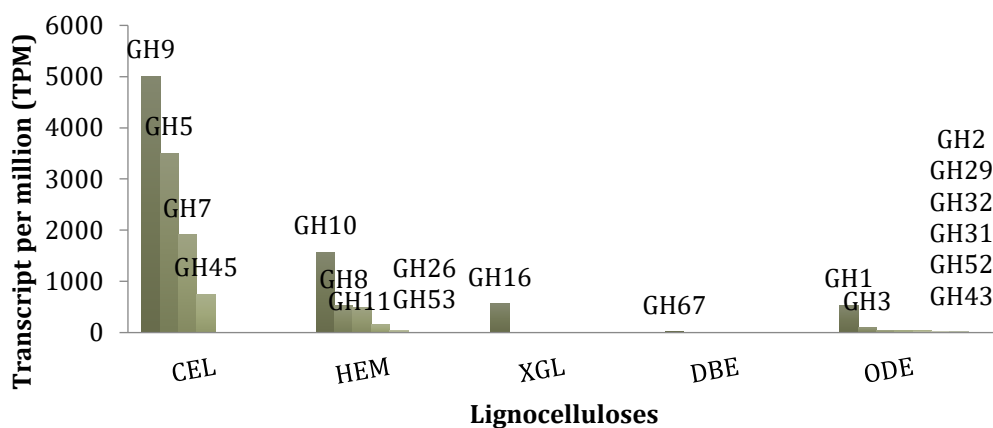


Figure 2. Inventory of glycosyl hydrolase (GH) families for the degradation of cellulose and hemicellulose in the *Coptotermes curvignathus* digestome; CEL cellulases, HEM hemicellulases, XGL xylanoglucanases, DBE debranching enzymes and ODE oligosaccharide degrading enzymes.

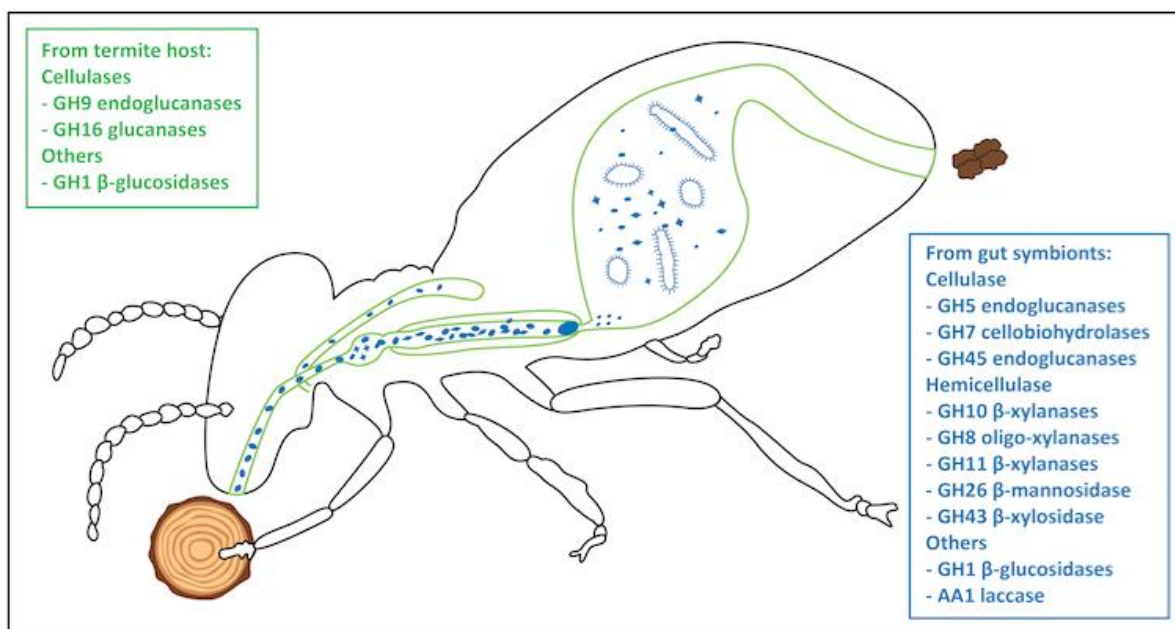


Figure 3. Key enzymes and proposed mechanism for lignocellulose degradation by wood feeding termite, *Coptotermes curvignathus*.

wood feeding termite also deployed this strategy. Lignin modification enzymes such as AA1 laccases and lignin-degrading auxiliary enzymes like AA3 glucose dehydrogenases were found in the termite digestome. These auxiliary activities redox enzymes modify the lignin structure of the lignocellulose materials to enhance the accessibility of cellulolytic and hemicellulolytic enzymes to the cellulose and hemicellulose. This conforming to the findings in Ke et al. that reported much of the lignin consumed by termites remained undigested and excreted through their fecal samples (Ke et al., 2012; Ke et al., 2011).

## CONCLUSION

This study found that the main classes of enzymes that were used by *C. curvignathus* to accomplish lignocellulosic degradation include laccases and carbohydrate active enzymes (CAZymes) and various peroxidases. The tactic in deploying multi-enzyme cocktails used by *C. curvignathus* could be one strategy being explored for future industrial processing.

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# A Case Study for Post-Harvest Losses Assessment in Watermelon Supply Chain for Securing Food Security

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## INTRODUCTION

Watermelon, also known as *Citrulus lanatus*, belongs to the family of *Cucurbitacea*, originally from West Africa and varies in size and shape, commonly in round and oblong. Watermelon is rich in vitamin supply, A B6, C and as a major in lycopene substance. Watermelon was first planted in Malaysia in the early 1950s, and it was identified as one of the main fruits for food security commodities under National Agro-Food Policy 1.0 and 2.0. Watermelon has also been recognized for its importance in the processing industry and is a significant contributor to the country's export. Production data revealed that watermelon had planted almost 4.8% (9,248 hectares) of overall fruit production areas in Peninsular Malaysia in 2020. The major regions are Kelantan (35.5%), Pahang (22%) and Johor (13%) (Table 1). The production of watermelon in 2020 was 135,903 metric tons, an increase of 1.1% from the previous year (134,422 metric tonnes). Watermelon is also the most exported Malaysian tropical fruits amounting to 45,324 metric tons with a value of US\$ 13.24 million (2020).

Table 1: Watermelon production information by state, 2020.

State	Planted Area (hectares)	Production	Percentage of harvested area (%)
Kelantan	2,231	41,615	35.5
Pahang	1,992	18,630	15.9
Johor	1,168	17,247	14.7
Negeri Sembilan	640	13,753	11.75
Others Peninsular State	1,893	25,980	22.2
Total	7,924	117,225	100

Source: Department of Agriculture Malaysia, 2022.

In terms of consumption, watermelon reached the Self-Sufficient Level (SSL) of 139.4% in 2020. However, it was reduced by 15.7% from the previous year (Table 2). Nevertheless, watermelon

Table 2: Selected fruits with 100% and above in Self – sufficiency Level (SSL), 2019-2020.

Fruit	2019	2020
Papaya	153.1	156.0
Watermelon	161.3	139.4
Starfruit	132.8	117.3
Jackfruit	110.5	109.9
Durian	105.2	105.2
Pineapple	105.5	104.7
Banana	98.7	100.1

Source: Department of Statistics Malaysia, 2021.

being the second largest high value of SSL after papaya (156%; 2020) indicates that local production has sufficient supply for domestic demand and is available for the export market. The country's dependency on watermelon imports is low, around 7.7% (2020), which may be imported most for unique watermelon such as Japanese Kumamoto Watermelon.

In mobilizing efforts towards food security issues which includes watermelon, a post-harvest losses element should be considered. Post-harvest losses are defined as the loss from harvesting stage to the consumption stage. Losses will affect food security and nutrition through availability, access, utilization, and stability. Thus, a case study approach was conducted to evaluate post-harvest losses in the watermelon supply chain from farms to the retail levels.

## MATERIALS AND METHODS

This study applied a case study approach using in-depth interviews involving major watermelon industry players (i.e. farmers, wholesalers, and retailers). The data was collected from 1st August 2020 to 25 October 2020. The interview has been done through dual approaches, online and physical. Study samples were obtained from the latest list (2020) of myGAP certificates from the Department of Agriculture. The snowball sampling technique has been used for other respondent groups (wholesalers and retailers), the information from the farmer itself.

Case study approaches are widely used in qualitative research, quantifying, and analyzing the presence, meanings and relationships of particular words, themes, or concepts. The data on the supply chain, issues, and challenges along the supply chain, including production and marketing of fresh watermelon, were then analyzed using Content Analysis. Content analysis using ATLAS.ti 7 has been used in the study.

## RESULTS AND DISCUSSION

Figure 1 shows the flow of watermelon supply chain which involves the main channel from farm to larger wholesaler or collector, flow then to small-scale wholesaler and distributor and to retailer. Overall, distribution time will take from 6 up to 10 days before reaching final consumers. The shelf-life of watermelon is 3 weeks or 21 days after harvest in low temperature of (10°C – 15°C) (USDA, n.a). A post-harvest loss is occurring at all points in the supply chain. Post-harvest activities include harvesting, handling, storage, processing, packaging, transportation, and marketing (Dos Santos et. al, 2020).

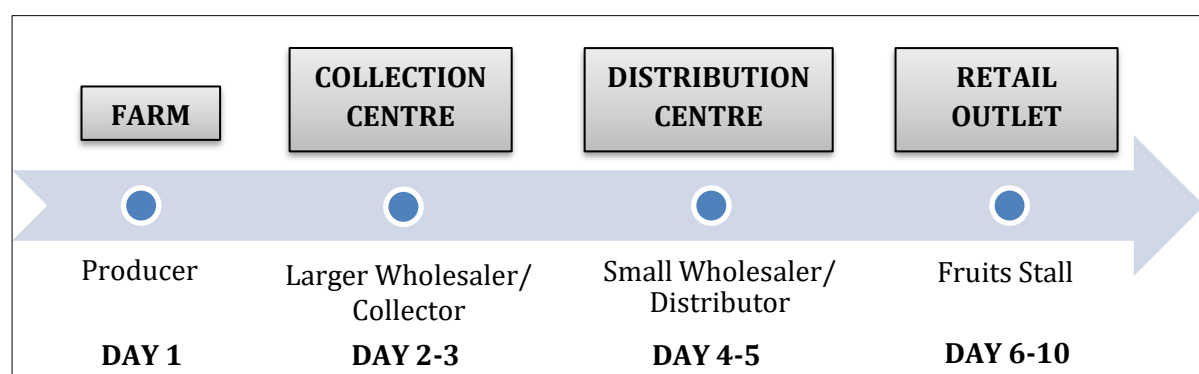


Figure 1. Watermelon supply chain in Malaysia. Source: Field study, 2020.

As shown in Table 3, case study findings revealed that watermelon losses in farms decreased by 33% (from 2.8 metric tons to 2.5 metric tons). While in wholesalers or collection centres, the losses were the biggest at 55.6% (from 2.5 metric tons to 2.0 metric tons), and the last level involved 11.1% distributors and retailers (from 2.0 metric tonnes to 1.9 metric tonnes). The

losses are small at the retail level due to small storage size and selling. Overall, 0.9 metric tonnes or 32% of production was lost along the supply chain. At the farm level, the losses were due to farm harvesting practice, particularly in fruit maturity indices, workers handling which mostly using by hand. Up to the next level, wholesalers and retail are mostly related to storage, transportation, sorting, grading, and handling.

Table 3. Watermelon post-harvest losses rate: Farm – Large Wholesaler – Small Wholesaler-Retailer.

Channel Distribution	Amount of Usable	Amount of Losses	Percentage of Losses (%)
Farm	2.5 Mt	0.3 Mt	33.3
• Production : 2.8 Mt/ acre			
Large Wholesaler	2.0 Mt	0.5 Mt	55.6
• Stock : 2.5 Mt			
Small Wholesaler	1.9 Mt	0.1 Mt	11.1
• Stock : 2.0 Mt			
*Retailer	80 kg	20 kg (or 4 fruits; 5 kg/ fruit)	20
• Stock : min 100 kg			

\*Retailer sample size is at the small-medium case, with an average of 100 kg per one session.

This study does not explain in detail the steps to deal with losses because it is fundamental to assess losses at the supply chain level. Further studies on post-harvest losses at each level in more depth need to be conducted for an exact situation. From the issues obtained, though, preventive action and recommendations should be made.

## CONCLUSION

The reduction of post-harvest losses benefits the supply chain and its ecosystem. It is related to the high impact in improving nutrition and profitability towards food-security issues. Therefore, a study of watermelon post-harvest losses in Malaysia has been done to identify the percentage of losses starting from farms to the retail levels. In supply-chain losses, the critical part is at the large wholesaler, around 55.6% or about 0.5 Mt @ 500 kg). If turning to fruits will equivalence to 100 fruits. The losses are related to mostly storage, transportation, sorting, grading, and handling before passing to the following distributors. However, this study is not completed yet to dictate the issues in detail. Therefore, further study is needed to support the problems with impacted results in future.

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# Challenges Threatening the Profitability of Pepper (*Piper nigrum*) Farmers: A Case Study at Lebu Kulit, Sungai Asap, Belaga, Sarawak

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## INTRODUCTION

Pepper is one of Sarawak's oldest foreign exchange earners, establishing its name beyond Borneo as one of the most well-known and versatile peppercorns (TasteAtlas, n.d.). The spice has been a major export commodity crop of Sarawak since 1840s, and its prominence peaked under the British Rajah rule in the 1900s when the Borneo Company streamlined the pepper trade. Today, Sarawak remains to be the major pepper producer in Malaysia, producing 95% of total pepper export of the country. Although majority of the pepper farmers are smallholders, amounted 33,695 nationwide, yet the pepper industry has sustained the country as the top 5 pepper-producing nations of the world. Most of our pepper export is sent to Japan. Indeed, over 60% of the pepper consumed in Japan comes from Malaysia. Malaysian pepper is also sailed to other countries in Asia, such as China, Korea, and Taiwan, and reaches locations in Europe, Australia, and New Zealand as well. Since Sarawak produces most of the Malaysia's pepper, the world has known Malaysian pepper as "Sarawak pepper".

Sarawak pepper has a protected geographical indication. There are several pepper varieties planted commercially in Sarawak, which are Lada Kuching, Lada India, Lada Semongok Emas, Lada Semongok Aman. Each variety boast a different taste profile, aroma, and heat. Lada Kuching variety is known for its balanced profile of fragrance and heat. This has much to do with the country's tropical climate, Sarawak's hilly slopes, and unique soil composition. Indigenous pepper, such as *Piper sarmentosum*, still grows wild across the island, clinging to massive trees in our ancient rainforest. However, it has merely tiny peppercorn, hence most of its flavour and medicinal value comes from the roots.

The largest pepper-producing regions in Sarawak are Sarikei, Betong, and Serian, which collectively account for about 61% of the state's 17,087 ha total production area in 2017. Due to increased productivity, Malaysia's production of pepper saw a significant increase in 2020. Production increased to 30,804 tonnes from just 24,227 tonnes in 2010. The yield of peppers has increased from 4.4 tonnes to 6.4 tonnes per hectare. The rising demand on the international market (Chen and Mansel, 2017) and the encouraging domestic consumption trend has led to higher prices that encouraged farmers to concentrate on enhancing their crops. However, there are many challenges for Sarawak to entice people to engage in pepper cultivation, amid strong competition from other crops such as palm oil and rubber.

## MATERIALS AND METHODS

In this study, the challenges faced by the pepper farmers at Lebu Kulit, Sg. Asap, Belaga were investigated via structured interview and pest surveillance in the field.



### ***Case study site description***

This study was conducted from March-May 2022 at Lebu Kulit (3° 1' 34" N, 13° 54' 41" E), Belaga district, Kapit division in Sarawak. Lebu Kulit is among the 15 long houses that were established under Sg. Asap resettlement scheme that relocated many Kenyah communities to make way for the largest hydropower project in Southeast Asia, the Bakun dam. In keeping with their cultural norms, Lebu Kulit is managed by a local leader, selected from among the residents and appointed by the local government to protect their culture, ethics, social structure, and values. Sarawak government has also allocated agricultural lands for the villagers to farm as well as to sustain their food and social securities. Majority of the early Lebu Kulit villagers farm pepper (*Piper nigrum*), however, in recent years, many have shifted into oil palms cultivation due to higher profit. This can be seen from the village surrounding that is encircled by oil palm estates, with only few patches of pepper farms scattered on the hill sides.

### ***Structured interview***

A qualitative analysis proceeded by exploring structured interviews was conducted in the month of March 2022 with farmers in Lebu Kulit. Eleven smallholder farmers, that either cultivated pepper or planned to cultivate pepper, were interviewed. Sixteen questions were asked about crop cultivation, agricultural practices, processing, marketing as well as pest and disease status. In this in-depth interview farmers were asked to reflect on how their peppers' growth and production performance were, and what management strategies farmers are familiar with and which they prefer.

### ***Pest surveillance in the field***

A roving survey of pepper farms was conducted in Lebu Kulit during the month of December to record the occurrence and distribution of pest and disease. Five farmers' fields were visited, and the percent pest and disease incidence were recorded. Pepper vines showing typical wilt symptoms were collected in separate paper bags and brought to the laboratory for further investigations.

## **RESULTS AND DISCUSSION**

Among the most distressing issues faced by the local farmers are pest and disease outbreak that diminish the marginal production of pepper. *Phytophthora* blight of pepper had caused 45% of pepper farmers in Lebu Kulit to lose all their crop in their previous planting, while pepper *Fusarium* wilt and root knot nematode disease were still prevalent in most of the farm. Fluctuating pepper price, and lack of organised market are other major concerns of pepper farmers interviewed. In this study, it was also found that some of the farming practice such as zero fertilizer and pesticide application, no proper cleaning of farming tools, and use of undermined source of planting materials can increase the pest and disease risk. Farmers should be encouraged to apply organic soil improving agent to reduce the root knot nematode risk (Babirye et al. 2021). Application of fungicide when the disease incidence is above economic damage threshold should be taught.

## **CONCLUSION**

In order to sustain Sarawak pepper farming industry, there should be adequate financial and institutional supports by government to rural institutions involved in the organising strategic marketing as well as pest and disease management.

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# Towards Bamboo Industry Development in Sarawak: Evaluation on Survivorship and Field Growth Attributes of Four Selected Bamboo Species

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## INTRODUCTION

Bamboo is widely recognised as a highly renewable, fast-growing, and cost-effective raw material. It was traditionally used for making rafts that serve as an important medium of transportation and was used by the communities in agriculture, construction, arts and crafts, and furniture. Specifically, there are 50 species of bamboo in Peninsular Malaysia, 30 species in Sabah, and 20 species in Sarawak. Bamboo grows faster than any other plant in nature, with some species reaching 40 meters in height in just a few months while others can grow faster than one meter per day (Getachew et al., 2021). Sarawak Timber Industry Development Corporation (STIDC) was entrusted by Sarawak State Government to spearhead the development of the bamboo industry in Sarawak. By 2030, Sarawak aims to develop bamboo-based industries to produce food, charcoal, pharmaceutical, pulp and paper, cosmetics, textiles, handicrafts, and engineered bamboo products.

Economically, the bamboo industry has advanced significantly since 2015, when the country's total exports were USD 0.18 million, as opposed to USD 2.09 million in 2019. (INBAR, 2021). INBAR (2021) estimated the global bamboo and rattan sector with a trade value of USD 60 billion based on the current data available, with domestic commerce accounting for the majority of the revenue. The value of foreign exports of bamboo and rattan products in 2017 was USD 1.7 billion, according to data from the UN Comtrade Database. This comprised conventional, handcrafted things, such as woven items, as well as numerous highly processed bamboo and rattan products, such as flooring, panels, and cladding. The majority of bamboo exporting countries are located in tropical and subtropical climates. The majority of bamboo-producing countries are found in Asia, including China. According to the customs data, China is the world leader in the trade of bamboo in 2018, with a value of USD 39 billion. However, a number of regions that do not produce bamboo also export a lot of bamboo goods. For example, bamboo goods are the second-largest export from the European Union (EU) worldwide. The EU imports bamboo raw materials and intermediate products from Asia, processes them, and then exports the high-value-added completed goods to other countries (Amir et al., 2020).

Since research on bamboo in Sarawak, Malaysia has received meagre attention and baseline information on the early survival rate and field growth attributes of bamboo are pivotal for the development of the Sarawak bamboo industry, there is an urgent need to evaluate the survival and field growth. Thus, a study to evaluate the survivorship and field growth attributes of a 3-year-old bamboo was conducted at the Sarawak Bamboo Pilot Project site in Sabal, Sarawak, Malaysia.

## **MATERIALS AND METHODS**

### ***Study area***

The research was carried out at Block 8406B, Sabal Forest Reserve, Simunjan, Sarawak, Malaysia which is about 51 km Southeast of the Serian and at an elevation of more than 20 to 35 m above sea level (Figure 1). Study sites were established at bamboo plantation areas with four different Sarawak local species of bamboo, namely *Bambusa vulgaris* (Buluh minyak), *Gigantochloa levis* (Buluh beting), *Gigantochloa hasskarliana* (Buluh beti), and *Dendrocalamus asper* (Buluh betong). From 2011 to 2020, the study site received 4,134.7 mm of rain annually (Meteorological Department, 2021). The monthly mean air temperature and relative humidity were 26.9°C and 84.1%, respectively (Meteorological Department, 2021).

### ***Planting materials, preparation of growing medium and propagation methods***

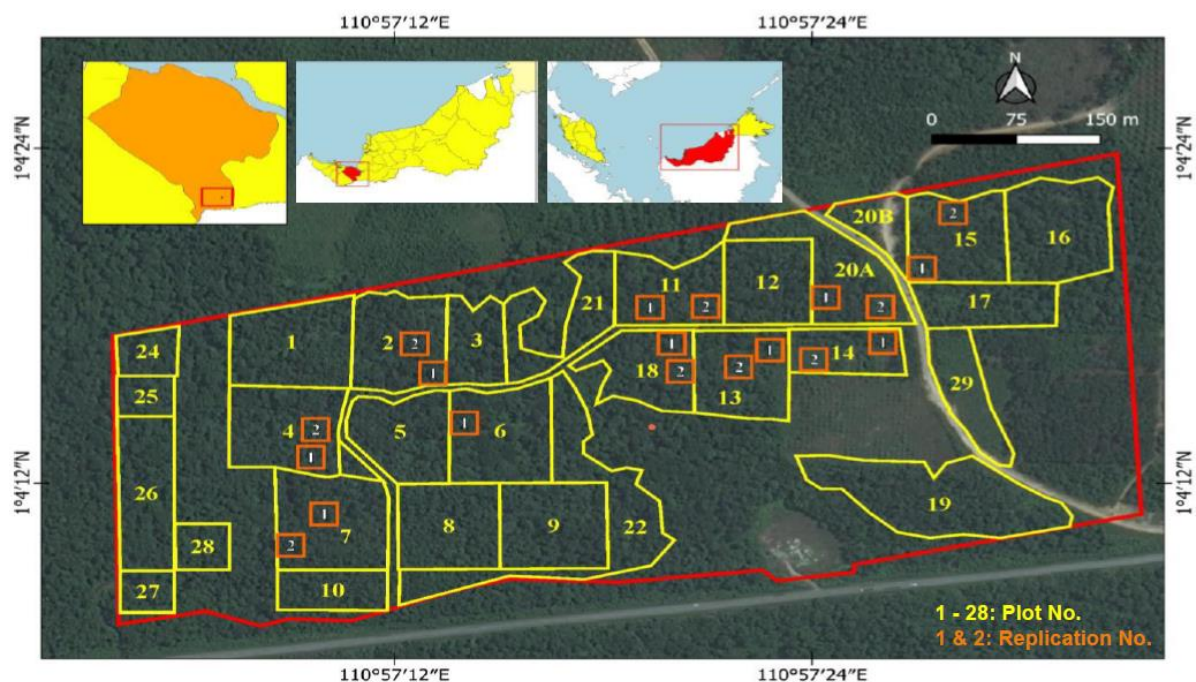
All the bamboo seedlings used in this study originated from Sarawak. The local bamboo was produced from branch cuttings of the mother plant except for *G. hasskarliana*, which was produced from the cuttings of rhizomes. For seedling production in the nursery, topsoil and river sand were mixed in a volumetric ratio of 1:1. The seedlings were transplanted in the field four months later. Fertilisation was performed three times per year at each of the plots following planting. During the early stages of planting, an inorganic fertiliser (NPK) was applied. After three months, organic fertiliser (chicken manure) was applied to the planted bamboo until it reached two years old, or until the bamboo growth conditions in the plantation area were improved. The planted bamboo species were properly maintained during the growth stage.

### ***Survival rate and field growth assessment***

Study sites with the size of 20 m x 20 m (two replications) with 5 m x 5 m planting distance for each bamboo species (Plot No. 2, 11, 13, and 14) were established. Survival rate and field growth attribute in terms of the number of culms per clump, number of new shoots, culm diameter, culm height, mean annual increments of diameter (MAID), and height (MAIH) were measured and quantified quarterly in the year of 2021. Two replications for each bamboo species involving 50 readings (25 readings for each replication) were evaluated. The culm diameter and height were measured using a digital caliper and Vertex Haglof Transponder. The MAID and MAIH were calculated based on the mean values of the seedling diameter and height of the assessed bamboo seedlings with the stand age of the experimental plot.

### ***Data analysis***

The data on the outplanted seedlings were analysed using a one-way analysis of variance (ANOVA). Scheffe's multiple comparison tests were employed to find statistically significant differences between means when the ANOVA was significant. All statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) (IBM, version 24.0 for Windows) (Copyright: SPSS Inc., 2016).



(Source: Mohd Hassan et al. 2022)

Figure 1. Segregation between different bamboo species at STIDC Bamboo Pilot Project area in Sabal Forest Reserve, Simunjan, Sarawak, Malaysia.

## RESULTS AND DISCUSSION

### *Survival rate and field growth attributes*

The percentage of survival rate for planted bamboo species are shown in Figure 2a. The result shows that the survival rate was at 70% and above across all the bamboo species. In November 2021, the highest mean survival rate (88%) was from *G. levis* and the lowest mean survival rate (70%) was from *G. hasskarliana*. Meanwhile, in terms of mean numbers of culms per clump, *G. hasskarliana* depicted the greatest number with 91 culms, followed by *G. levis* with 24 culms, *D. asper* with 17 culms, and *B. vulgaris* with 14 culms (Figure 2b). Based on Figure 2c, in November 2021, the mean number of new shoots for *G. hasskarliana* recorded the highest with 3 shoots as compared to other bamboo species. However, the lowest mean culm diameter was observed in *G. levis* with 2.66 cm and the highest mean culm diameter was observed in *B. vulgaris* with 4.51 cm (Figure 2d). Notwithstanding, *B. vulgaris* remained with the greatest mean culm height of 12.6 m and *G. levis* with the lowest mean culm height of 7.72 m (Figure 2e). Meanwhile, for MAID and MAIH, *B. vulgaris* was significantly higher than that of the other species with 1.69 cm year<sup>-1</sup> and 4.72 m year<sup>-1</sup>, respectively (Figures 2f and 2g).

The survival rate percentage is crucial in order to understand the mechanisms that affect the dynamics of the bamboo population and vital to effectively manage the population and its habitat. In general, the survival rate of different bamboo species in this study is moderately high with approximately  $\geq 70\%$ . In Kenya, a study reported by Were et al. (2017) mentioned that five bamboo species (*B. blumeana*, *B. bambos*, *B. vulgaris*, *D. asper*, and *D. membranaceus*) showed a survival rate of 100% under the prevailing conditions of the tannery soils, except for *D. birmanicus*. The quantity of culms per clump depended on a variety of internal and external conditions. The plant's origin, species, and robust system were internal variables. The type of fertiliser utilised, the slashing methods, and other maintenance schedules for the plantations were all external variables. Our findings show that generally the early growth of culms for all the

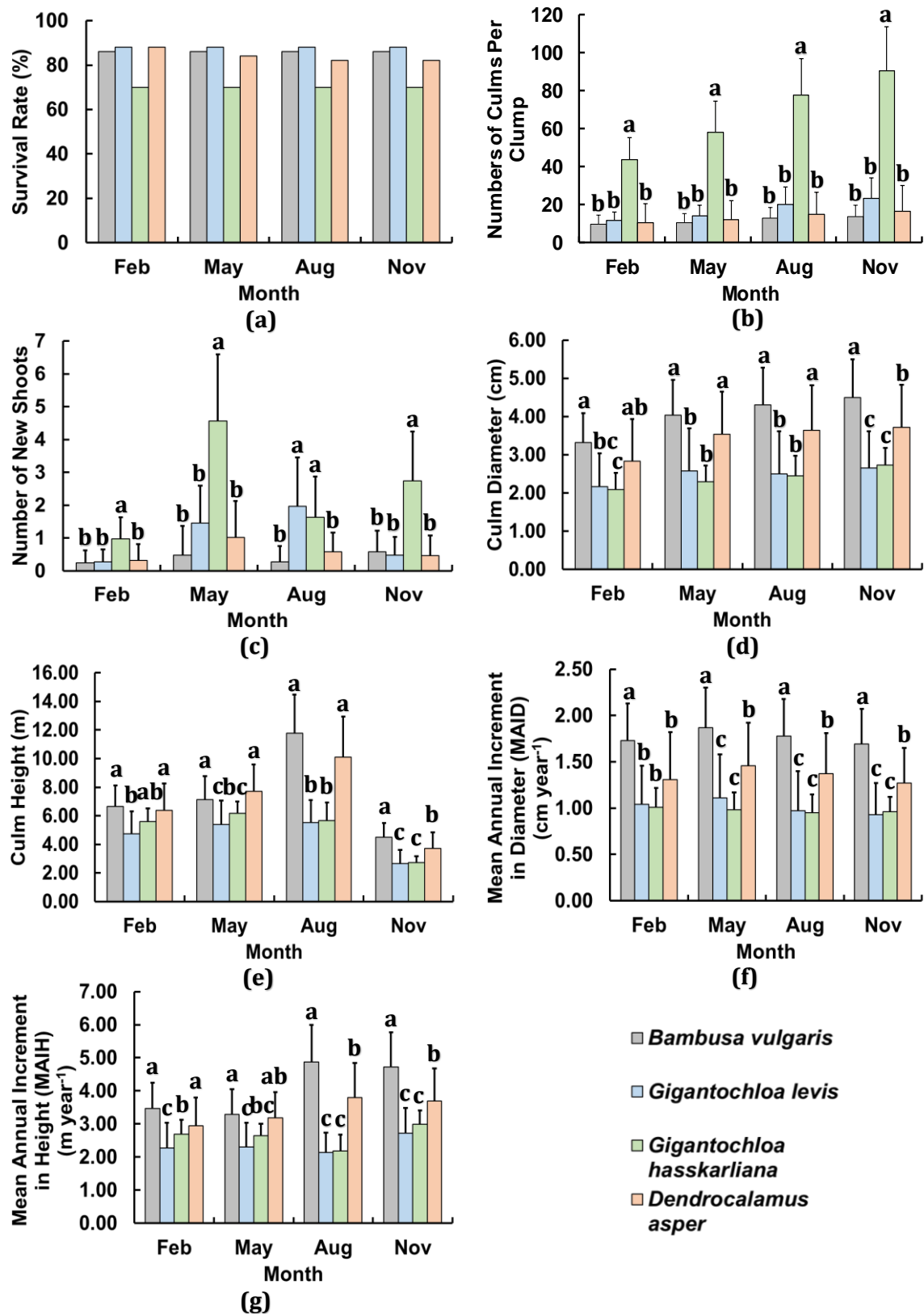


Figure 2. (a) Survival rate; (b) numbers of culms per clump; (c) number of new shoots; (d) culm diameter; (e) culm height; (f) mean annual increment in diameter (MAID); (g) mean annual increment in height (MAIH) among different bamboo species. Bars are means and error bars are standard deviations. Bars with different letters are significantly different at 5% level using Scheffe's multiple comparison test.

species were within the range of results. According to study by Krishnakumar et al. (2017), in the first year (6.46), second year (13.00), third year (19.34), fourth year (26.05), and fifth year (25.05), *B. balcooa* demonstrated its superiority over *B. vulgaris* by generating the most culms (32.37). Understanding which bamboo species are the most active in terms of bamboo growth requires careful observation of the bamboo shoots' production. The first bamboo products that can be sold to the food industry are young bamboo shoots. When the shoots are not picked, they mature into bamboo culms, which alters how bamboo is used in other industries like furniture and charcoal production. In this study, *G. hasskarliana* revealed an active shoot growth in comparison to other bamboo species. On the other hand, field growth attributes in terms of the MAID and MAIH, *B. vulgaris* portrayed the highest increment in growth. It can be deduced that this potential species could be recommended to be planted for larger scale operations towards the development of bamboo industry in Sarawak, Malaysia. According to a previous study by Mohd Hassan et al. (2022) at Sabal Forest Reserve, *B. balcooa* depicted the highest growth increment in diameter and height with 1.84 cm year<sup>-1</sup> and 4.35 m year<sup>-1</sup>, respectively.

## CONCLUSION

Different bamboo species have different growth pattern in terms of survival rate and field growth attributes. *B. vulgaris* clearly showed the fastest MAID and MAIH growth as compared to other Sarawak bamboo species in this study. The scientific information and findings from this study would be beneficial as guidelines for bamboo industry players, managers, nursery practitioners, and policymakers to initiate and undertake the development of the bamboo industry, mainly in Sarawak. It is recommended that, particularly in Sarawak, a long-term monitoring period is required to build a commercial bamboo industry plantation project. However, more thorough research is needed to identify the edaphic elements that can affect the survival and growth performance of planted bamboo in Sarawak using the line planting approach.

## ACKNOWLEDGEMENT

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# Artificial Multiplication Method of Stingless Bee Colony

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## INTRODUCTION

Stingless bees are highly eusocial bees which lack of functional sting and major visitor of flowering plants in tropics (Amano et al., 2000). They can be found throughout the tropical and subtropical parts of the world, such as Australia, Africa, Southeast Asia, and tropical America. In Malaysia, the main species being rear individually or commercially are *Heterotrigona itama* and *Genotrigona thoracica* (Jailani et al., 2019). Aside from providing honey, pollen and propolis, stingless bees are both people and environment-friendly, making them great candidates for commercial pollination to increase agricultural production (Heard, 1999).

The tree trunks or logs are synonymous as their favourite to be used as their place of residence (Vijayakumar et al., 2013). Since hunting wild colonies are not sustainable in the long term, colony propagation will be a good alternative to gain a new colony. Moreover, establishing colonies in artificial hive boxes allows them to be transferred to areas where pollination services are required (Heard, 1999). Colony propagation requires proper techniques and understanding of their behaviour as it involves appropriate time, season, and ability of the personnel to identify the suitable colony to be divided (Jailani et al., 2019). As a result, people who are unfamiliar with the biology of stingless bees may be hesitant to divide the colony. A good practice in colony multiplication is essential for the new colony to survive. Therefore, this study explores three different multiplication method for stingless bee, *H. itama* colony and evaluate their early colony development.

## MATERIALS AND METHODS

### *Study site and colonies selection*

The study was carried out at RH Bee Farm located in Nanga Dap, Sibul, Sarawak. The bee farm is surrounded by *Acacia mangium* trees where 24 *H. itama* colonies in the log were being accommodated for the project purpose.

### *Multiplication hives design*

All the 24 colonies were subjected to division by the following three multiplication methods.

#### Method 1: Direct splitting

The broods in the log were physically split into halves placed into an empty hive box (20 cm x 20 cm x 20 cm) with a topping box. One of the halves had an existing queen in the new hive, and another had queen cells. The split colonies were placed on top of the other. Colony with existing queen were placed on the top while colony with queen cells at the bottom to support the hive without the queen.

#### Method 2: Delayed splitting

Each colony was transferred into an empty hive box made up of two square halves (20 cm x 20 cm x 20 cm + 20 cm x 20 cm x 20 cm) which allows for easy hive splitting later. When the weight

of a colony (excluding weight of hive box) reaches 3 kg, it will be split into two equal parts by separating the two halves hive box with brood and bees in them.

#### Method 3: Eduction

The empty hive box (20 cm x 20 cm x 20 cm), with an entrance hole drilled through the front and back panels, were connected to the original hive entrance at the log via polyethylene pipe, with all gaps and cervices sealed. Then, the original entrance was then removed and attached to the new hive's new entrance. This way, the bees inside the original hive were forced to go out through the new empty hive box, where they might build a new colony.

#### ***Observation of colony development***

The colonies began being evaluated 60 days after the multiplication treatments has been done. The formation of components (i.e., honey pots, pollen pots and brood cells) in the direct splitting method and eduction method were observed. For the delayed splitting method, evaluations were performed by keeping a record of the colonies' weight for a year. No harvesting activity was done during the experimental period.

### **RESULTS AND DISCUSSION**

Colonies are developed relatively well in all methods used. Hive structures, brood cells and honey pots are forming in the new boxes, showing that the colony had survived the division process. In direct splitting method, the colony development was compared in the presence of a queen and in the absence of a queen but with queen cells. It was discovered that in the direct splitting method, there is no significant difference ( $\alpha=0.05$ ) in early colony development between queen and queen cell (Figure 1). This shows that with only queen cells, bee workers can continue with their activities for colony development. The colonies could be propagated successfully by this method provided queen cells are present (Mounika et al., 2019; Mythri et al., 2018).

When using eduction method, the existing nest remains intact in the log and will not be demolished. The new colony was extracted by simply hook an empty box to the original colony in the log. Initially, the colony uses the new box as waste storage space and constructs an unusual cerumen tunnel connecting the new and old entrances with several honey pots. Subsequently, they can accept the new box and started to construct more food stores and brood cells when there is scarcity of space in the log. The early colony development of new colony is compared between the direct splitting method (with queen cells) and the eduction method. The direct splitting and eduction method were shown to have no significant differences ( $\alpha=0.05$ ) in early colony development (Figure 2). The colony may not always accept the additional space supplied to extend the components of its nest, despite the fact that there are no significant differences. The key success in eduction method depends on the scarcity of space for future nest expansion inside the old colony, as well as abundant food resources and a suitable environment (Vijayakumar et al., 2013). Some beekeepers put honey pots from the original nest inside the new box to promote the nest expansion and speed up their construction activities (Vijayakumar et al., 2013).

In delayed splitting method, the hive must be entirely filled before splitting into two equal sections by separating the two portions of the hive box with brood and bees. The increase in weight indicated that the colony's hive contents were developing. Despite there is increment in weight ( $p<0.05$ ), the brood cells, however, did not develop sufficiently, making it unable to divide and produce new colony (Figure 3). It was observed that instead of producing more brood cells, the colony uses the available area to store food.

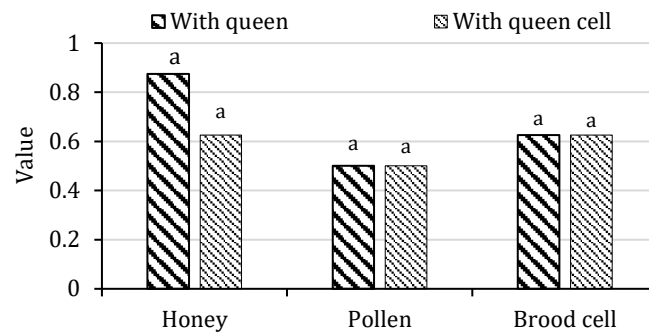


Figure 1. Comparison of early colony development in the Direct Splitting method with Queen vs. Queen Cell ( $n=8$ ). Same letters are not significantly different according to exact McNemar's test at  $\alpha=0.05$ .

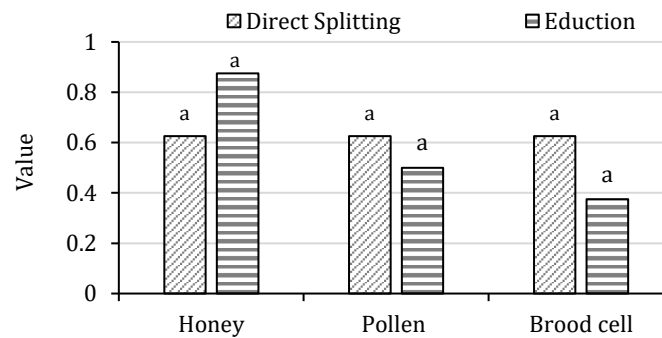


Figure 2. Comparison of early colony development between Direct Splitting method (with queen cells) and Education method ( $n=8$ ). Same letters are not significantly different according to Chi-square test at  $\alpha=0.05$ .

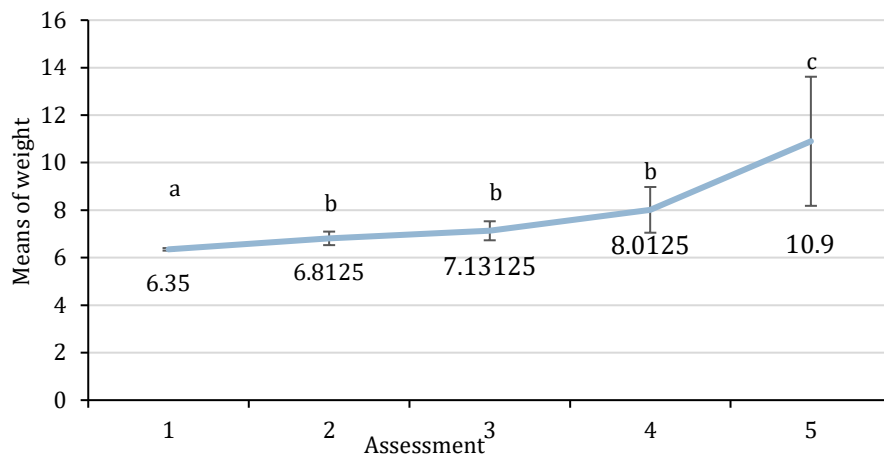


Figure 3. Comparison of colony weight (mean  $\pm$  SD) in the Delayed Splitting method at every assessment ( $n=8$ ). Different letters are significantly different (post hoc test with Bonferroni adjustment) at  $p<0.05$ .

## CONCLUSION

Our preliminary findings indicate that hive models may be a critical factor to be considered in splitting the stingless bee colonies. The direct splitting and education methods showed potential

for establishing a new colony and ensuring the stingless bee colony's long-term sustainability. The colonies in this method will eventually establish, with the presence of a queen, workers, and enough food sources. The delayed splitting method is not necessarily a failure because colonies in the hive model have evolved rather well, but hive remodeling is required to make the division process feasible. To get more reliable findings, more repetition and research on presence of new queen cells and the production of additional broods were required.

## ACKNOWLEDGEMENT

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# Malaysian Good Agricultural Practices (myGAP) Certification Implementation and Pesticide Residues Monitoring: The Scenario in Sarawak

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## INTRODUCTION

Malaysian Good Agricultural Practice (myGAP) certification scheme (Subsector Crop) is a national program implemented by the Department of Agriculture, Malaysia. The main aim of myGAP certification is to ensure safe agricultural produces and sustainable production (Tey et al., 2016). The certification scheme is developed based on sets of standards such as Malaysian Standard, ASEAN GAP, Global GAP, IFOAM and CODEX. In our country, apart from myGAP and myOrganic that certifies farms practicing conventional planting system, the scopes have been extended to Malaysian Good Agricultural Practice Bees and Stingless Bee Rearing Good Agricultural Practices (myGAP.AM) in 2019, Malaysian Good Agricultural Practice *Tanaman Bukan Makanan* (myGAP.TBM) to certify non-edible crops in 2021 and Malaysian Good Agricultural Practice Pesticide Free (myGAP.PF) to certify farms without chemical pesticide application in 2022 to encourage the adoption of Good Agricultural Practise (GAP) by more farm practitioner in agriculture industry.

myGAP certification involves multiple stages such as site inspection, crop produce analysis (pesticide residue, heavy metal and microbial tests), internal and external audits. Pesticide residue test is a vital indicator for food safety and is a key component in myGAP. Currently, monitoring of pesticide residues in agricultural produces is considered as the main measure to assess food safety (Reeves et al., 2019). In Malaysia, pesticide residue monitoring is carried out on both certified and uncertified farms by Department of Agriculture.

This review aims to provide an insight on how the implementation of myGAP in Sarawak influences food safety assurance through GAP and regulated use of pesticide based on farm level case studies. The research methods included a review on myGAP certification process and by comparing pesticide residues in vegetables and fruits samples between myGAP certified and uncertified farms in Sarawak from year 2019 to year 2021.

## MATERIALS AND METHODS

### *Review of myGAP certification*

This review inclusive of information on myGAP certification status, including the number of certified farms in divisions, types of crops certified and its scenario on pesticide residue analysis from year 2019 until 2021 in Sarawak.

### *Samples collection and determination of pesticide residues*

Three thousand one hundred fourteen samples of vegetables and fruits crops were collected from certified and uncertified farms throughout Sarawak from year 2019 until 2021. Samples were extracted according to the modified QuEChERS (quick, easy, cheap, effective, rugged, and safe)

(Chai et al., 2012). Organochlorines and synthetic pyrethroids pesticides were determined by gas chromatography equipped with electron capture detector (GC-ECD). Phosphorus and sulfur containing compounds were determined by gas chromatography equipped with flame photometric detector (GC-FPD). Mass spectrometry were used for the analysis of other pesticides groups using two types of instrumentation methods, an Agilent 1290 LC connected to a triple quadrupole mass spectrometer (MS/MS) Agilent 6495 Series and an Agilent 7890B GC equipped with a triple quadrupole mass spectrometer (MS/MS).

## RESULTS AND DISCUSSION

As of December 2021, a total of 222 farms (Figure 1) have been certified in Sarawak covering approximately 1000 ha of land consists of various crops and stingless bee cultivation with farm size ranging from 0.2 ha to 80 ha. From 2019 until 2021, a total of 77 farms had been certified despite the implementation of the Malaysia Movement Control Order (MCO) during the COVID-19 pandemic, where activities of farmers and department officers were restricted. However, the certified farms only accounted for 6% of the total registered agriculture area in Sarawak. More effort needs to be done to improve food safety and quality assurance in food production through myGAP certification.

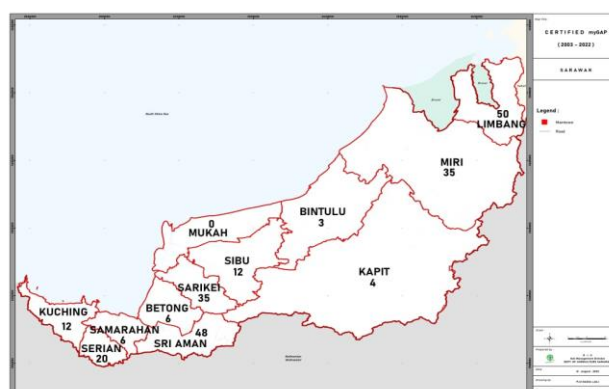


Figure 1. Total certified myGAP farms in divisions in Sarawak as of December 2021.

Over the years, there is growing interest in the implementation of GAP standards by the Governments particularly in Southeast Asia (Amekawa et al., 2017). Figure 2(a) and Figure 2(b) shows the status of myGAP certified farms based on its scope, and types of crops. So far, myGAP

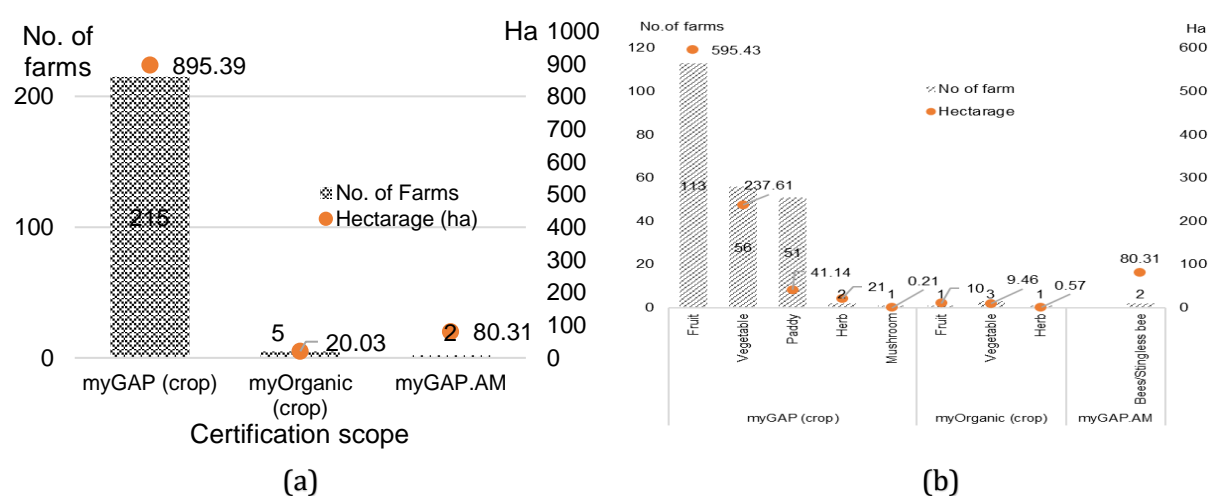


Figure 2. Total (a) certified myGAP farms with hectareage (b) certification based on types of crops in Sarawak as of December 2021.

certification scope on conventional planting recorded the highest number compared to myOrganic which has more stringent standards to comply and myGAP.AM which is newly introduced in 2020. As of to date, the registration of farms for the newly introduced myGAP. TBM and myGAP.PF certification is still in progress. Overall, the majority of certified farms are fruit producing orchards which cover more than half of land size of certified farm.

### ***Pesticide residues monitoring***

Throughout 2019 until 2021, a total of 3,114 samples (534 fruits and 2,580 vegetables) were collected from certified and uncertified farms in Sarawak. 846 samples were taken from certified farms and 2,268 samples from uncertified farms. The uncertified farms (Figure 3a) recorded higher number of violations in crops produce compared to certified farms (Figure 3b) from year 2019 until 2021. The increasing of violation rate in general is due to wider pesticide range tests. In year 2019, the samples were analysed for 60 types of pesticides and increased to 152 types in 2020 onwards. Samples from certified farm showed lower pesticide violation rates of 4%, 14.42% and 10.65%% as compared to uncertified farms (7.94%, 22.76% and 17.29%) from year 2019 until 2021. A significantly lower proportion of certified farms were found to exceed the maximum residue limits (MRLs based on Food Act 1983 and CODEX Alimentarius) in crop produce compared to uncertified farms. Fungicide pencycuron shows the highest frequency of violence for both certified and uncertified farms. Our finding was in accordance with those reported for Q-GAP certified farmers in Thailand, where the farmers exhibited more heedful attitude than uncertified farmers towards the effect of pesticide application (Amekawa et al., 2021). Besides the violation in MRL, residues of unregistered pesticides (based on Pesticides Act 1974) were detected in crop produce from uncertified farms only in year 2019 and 2021. None unregistered pesticides were found in samples from certified farms. The result showed myGAP certified farms are managed and handled better than uncertified farms. Overall, myGAP certified farms exhibited significantly better results than uncertified farms due to its much higher standard of compliance for certification.

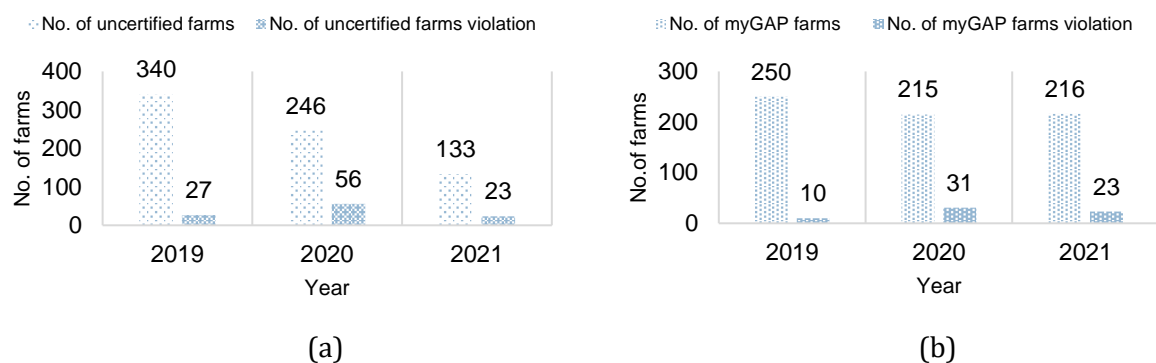


Figure 3. Total of (a) uncertified farms and (b) certified farms that recorded violation of pesticide residues.

### **CONCLUSION**

In this study, the relationship of food safety in terms of pesticide showed that myGAP certification is the way forward. Despite that, the number of myGAP certified farm is still low in Sarawak. Public awareness, policies, incentives, and marketing are some ways that can promote myGAP certification. A more extensive cooperative effort among various parties is needed to ensure better implementation of the program in producing safe and quality produce.

## ACKNOWLEDGEMENT

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# Factors Contributing to Oil Palm Pollinator Weevil *Elaeidobius kamerunicus* Emergence Volume from Post Anthesising Male Inflorescence

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## INTRODUCTION

The oil palm pollinating weevil, *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae) relies on the male inflorescences of oil palm for food and shelter. One of the critical components to ensure the sustainability of *E. kamerunicus* population in the field is to measure the number of progenies that successfully emerge from the spent male inflorescences. This parameter is used in estimating the general health of *E. kamerunicus* population and identifying any possible threats to *E. kamerunicus* in the field. Another important parameter to measure is the sex ratio of newly emerged progenies. Rodríguez-Mun˜oz et al. (2019) suggested slower senescence in a wild insect population over the years with a more female-biased sex ratio.

This paper aims to investigate the differences in the population and number of newly emerging adult *E. kamerunicus* from the male inflorescence in the selected study sites.

## MATERIALS AND METHODS

### *Field sampling*

The field sampling of post anthesising male inflorescences (PAMI) was conducted on monthly basis at three oil palm estates (8 years old plantings) located on tropical peat soils in the region of Roban (N1°53'58" E111°12'19"), Pusa (N1°40'15" E111°13'13") and Kuala Igan (N2°46'27.58" E111°44'54.23"), Sarawak. At each site, an area of 10 hectares was selected for the study. A total of 150 healthy, bunch-producing palms were systematically selected by marking every tenth palm within the study area. Two PAMI aged approximately one-week after anthesis were chosen randomly from each study site for each sampling. From each inflorescence, three spikelets each from the top, middle, and bottom parts of the inflorescences were cut. The spikelets were then brought back to the laboratory and placed in conical flasks covered with a muslin cloth for two weeks until the emergence of adult *E. kamerunicus*. The number of emergence was then sexed, quantified, and recorded. The sex ratio of the emerged weevils (female-to-male) was also calculated.

Analysis of variance and Tukey comparison of means were conducted by using Minitab 17TM software. To better decipher the data, information on the estate management practice was also obtained.

## RESULTS AND DISCUSSION

The mean number of adult *E. kamerunicus* that emerged from the spikelets was recorded and analyzed (Figure 1). Based on the data, Pusa and Roban estates had a comparable number of newly emerged weevils per spikelet, 36- 47 individuals. However, the weevil emergence per spikelet for the Kuala Igan estate was significantly lower compared to the two studied estates, at an average of 20 individuals. The Pusa and Roban data were agreeable to the value suggested by Ming and Bong (2017), who reported that for untreated PAMI spikelets, the average rate of emergence was 43 individuals. Additionally, Syed (1980) suggested a female *E. kamerunicus* was able to produce a progeny of 51. By referring to these previous works, the average weevil emergence recorded at Kuala Igan estate was considerably low.

Another weevils' population health parameter that was measured in this study is the newly emerged adult weevil sex ratio. The sexes of progenies that emerged from the samples were quantified (Figure 2) and the female-to-male ratio was analyzed. In each of the study sites, the sex ratio was female-biased. This agrees with the observation reported by Yue et al. (2015) that the female adult weevils visiting either male or female inflorescences always outnumbered the male weevils. In this study, the female to male ratio for both Roban and Pusa estates (Table 2) was at 3.12- 4.46: 1. However, this ratio is significantly smaller in Kuala Igan estate among the three localities ( $P < 0.05$  ( $P = 0.031$ )), at 2.33: 1.

A higher female to male progenies ratio could be a favorable scenario as it explicitly suggests there are more available females to lay eggs in the future. For *E. kamerunicus*, a higher female to male ratio could leverage pollination efficiency. Male *E. kamerunicus* has a bigger body size and has hairy setae covering its bodies (Dhileepan, 1992; Lumentut and Hosang, 2016), thus able to carry more pollen from anthesising male inflorescences to receptive female inflorescences (Dhileepan, 1992) than their female counterparts. However, female *E. kamerunicus* has the advantage to reach the inner and smaller parts of the female inflorescences due to its' smaller physique, contributing to better pollen delivery to the inner layer of female inflorescences (Prasetyo et al., 2018). Furthermore, phenotypic senescence in males was slower in years when more females were present. Therefore, a higher female: male ratio can be considered as one factor which may leads to higher weevil population level in future (Rodríguez-Muñoz et al., 2019).

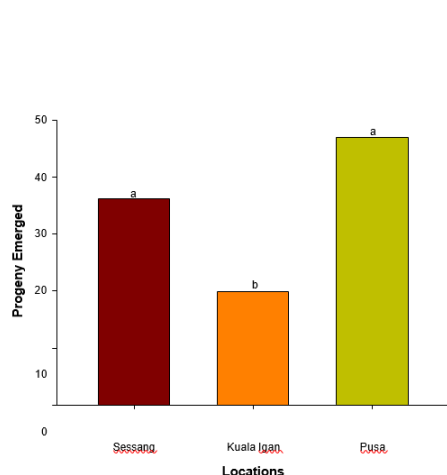


Figure 1

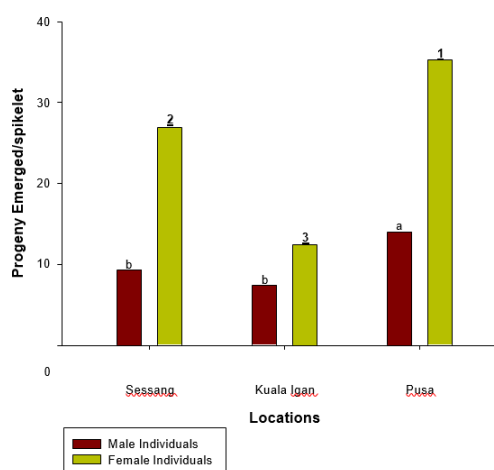


Figure 2

Figure 1. The average number of adult *E. kamerunicus* emerged from spikelets sampled at the study sites. Bars with different letters indicate significant differences ( $P < 0.05$ ).

Figure 2. The average number of adult male and female *E. kamerunicus* emerged from spikelets sampled at the study sites. The significant test was conducted among different study locations. Bars with different letters and numbers indicate significant differences ( $P < 0.05$ ).

The decline in the number of newly emerged weevils from the spent male inflorescences as well as the low female to male ratio indicated the presence of possible threats to the health of the future population of weevils in Kuala Igan. One of the possible threats or factors contributing to the decline in newly emerged weevils in Kuala Igan could be related to pesticide application. Kuala Igan practiced three rounds per year of Cypermethrin and Fipronil application, whereas Pusa used more weevil- friendly pesticides such as *Bacillus thuringiensis*-based pesticide as well as Fipronil to control termites and *Tirathaba* sp. on infected palms; and Roban did not apply any pesticide. Cypermethrin is known to assert a negative impact on *E. kamerunicus* population (Lim, 2012). Thus, vigilant pest and disease management should be implemented at all costs to protect beneficial insects and sustaining the ecosystem quality.

## CONCLUSION

In general, the number of progenies that emerged from the spent male inflorescence was 36-47 individuals per spikelet. The female-to-male ratio for the emergence progenies was 2.33-4.46: 1. There was a significantly lower emergence of *E. kamerunicus* progenies and the female-to-male ratio at Kuala Igan estate as compared to Roban and Pusa. The lower weevil emergence may be associated with Cypermethrin application. Identification of the causes leading to the decline of weevil emergence and female to male ratio is in definitely needed as it will be useful in formulating the proper mitigating action to sustainably maintain the pollinating weevil population in the estate.

## ACKNOWLEDGEMENT

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# The Effects of Set-Aside Forest Patches in Oil Palm-Dominated Landscape on Bird Biodiversity

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## INTRODUCTION

The impact of oil palm plantations on the environment is significant, and the industry has faced many challenges relating to biodiversity loss and climate change (Meijaard et al., 2018). The Malaysian oil palm industry has countered the challenges by increasing scientific research on biodiversity conservation in oil palm production areas through set aside areas (Mohd-Azlan et al., 2019). Malaysia is also promoting sustainable practices by obliging producers to comply with the Malaysian Sustainable Palm Oil Certification Scheme (MSPO, 2021). Recent efforts on establishing set-asides areas (e.g wildlife corridor, forest patches, riparian area) within oil palm plantations are part of the biodiversity conservation initiatives to save wildlife (Lucey et al., 2014). Birds are part of the biodiversity commonly studied and found in oil palm plantations (Yudea and Santosa, 2019). They are sensitive to any habitat disturbance and are widely used for environmental changes indicators in biodiversity conservation evaluation studies. This study investigates how bird species richness and abundance differed between an oil palm plantation and two set aside areas (peat swamp forest and riparian areas) and also investigates how bird species richness and abundance are associated with vegetation characteristics across a gradient of forest-edge-plantations.

## MATERIALS AND METHODS

### *Study site*

The study is located in Sabaju oil palm plantation (SOPP) situated in Bintulu, Sarawak, northern Borneo (N 03° 09.535" E 113° 24.640"), which belongs to Sarawak Oil Palms Berhad (SOPB). This study was located at two study sites: Site A consist of peat swamp forest (PSF) and its adjacent oil palm area (OP-A) and Site B consist of riparian area (RP) and its adjacent oil palm area (OP-B).

### *Field sampling*

Vegetation parameters measured including percentage of canopy cover, shrub cover and herbaceous cover at each plot according to Rodwell (2006). The distance point count technique was used at each plot to observe the bird species (Zakaria et al., 2009). Counts were conducted from 06h30–11h00 am, and each observation plot was sampled for 20 minutes. Only species heard and sighted within the points' counts plot were recorded as present. The information on present bird species was obtained from the *Birds of Borneo* handbook by Myers (2009).

## RESULTS AND DISCUSSION

A total of 3,074 birds belonging to 100 species and 32 families were observed in SOPP (Table 2). Seventy-seven species were recorded in the (PSF) 45 species in the RP, 31 species in the OP-A, and 30 species in the OP-B, including one peat swamp forest species, Hook-billed Bulbul and one endemic to Borneo (Dusky Munia). Overall, twenty conservation priority species were recorded in the SOPP whereby the PSF recorded 15 species, RP with five species, OP-A with four species and OP-B with three species. Interestingly, these set-asides areas, PSF and RP within oil palm landscape provide refuge for threatened species, specialist species to PSF, and bird species endemic to Borneo hence indirectly some of these species also recorded in oil palm plantation area. Plantations that recorded threatened, migratory, forest and wetland species have some conservation value within oil palm dominated landscape (Azhar et al., 2011). These results indicated how forest patches give conservation values in oil palm dominated landscapes and plays an important role as a bird diversity hotspot and refuge for threatened species, thus sustaining biodiversity in Borneo.

Independent one-way ANOVA (Figure 1) showed a significant effect of the different habitat landscape in the oil palm plantations ( $F_{(3,36)} = 50.24, p < 0.001, \omega^2 = 0.787$ ). Hence, set-aside areas do support high number of bird species richness in oil palm plantation. Also, the abundance of birds was showed a significant effect of the different habitat landscape in the oil palm plantations ( $F_{(3,36)} = 13.26, p < 0.001, \omega^2 = 0.479$ ). The high number of species in PSF than RP might be due to the size of PSF which is broad in shape and connected with adjacent forest while RP is narrow-line-shaped along the river line. Narrow-linear shaped forest areas within the oil palm landscape did not support high species richness (Mohd-Azlan et al., 2019). Connecting habitat with the nearby forest is crucial (Hawa et al., 2016) to create wildlife landscape connectivity to support high biodiversity value within oil palm landscapes.

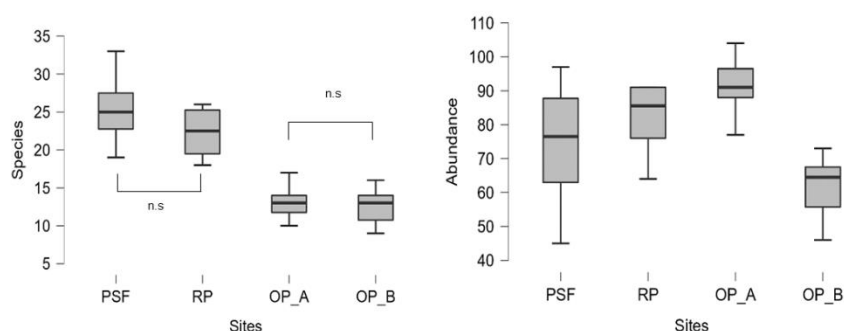


Figure 1. Box plot of bird species and abundance recorded in different landscapes (oil palms, riparian forest and peat swamp forest) within Sabaju oil palm plantation.

The regression results indicate that bird species richness was positively related to canopy and shrub cover but negatively related to herbaceous (Figure 2). Due to the structure of the canopy layer, PSF and RP attracted forest bird species such as trogons, iora, barbet and broadbills and arboreal birds such as pigeons and bee-eater. In addition, some species prefer higher canopy closer to the forest edge for predator birds as they prefer taller trees to lookout for prey (Andersson et al., 2009). Furthermore, the canopy of the PSF provides sufficient sunlight and space for the development of shrub layers such as lianas, epiphytes and hemiepiphytes which may attract forest birds that utilize the different vegetation strata (Hawa et al., 2016). The increasing percentage of herbaceous cover and decreasing percentage of canopy and shrub cover has a higher abundance of birds in the interior of the plantation than in PSF. The low percentage of shrubs than herbaceous in oil palm plantations might be due to the systematic weeding practices (Azhar et al., 2011).

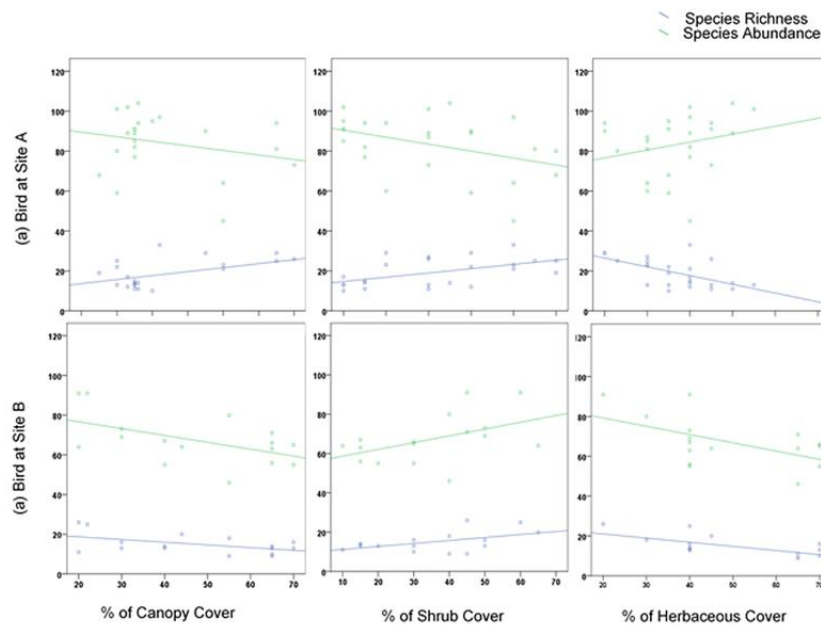


Figure 2. Linear regression analysis of the relationship between bird and vegetation structure including the percentage of canopy cover, percentage of shrub cover and percentage of herb cover at Site A and (b) Site B.

## CONCLUSION

Our results demonstrate a strong effect of set-asides areas; peat swamp forest and riparian reserves supported bird species richness and abundance in overall oil palm dominated landscapes. High canopy and shrub cover by maintaining forest patches in oil palm landscape provides habitat for the forest, wetland, endemic, predator and threatened species of birds. Nevertheless, we found that a high percentage of herbaceous cover may result in high abundance of birds in the oil palm area closed to peat swamp forest. Requirements of protecting and conserving the concerned species are the most important strategies that should be supported through better management of the set-aside areas within oil palm dominated landscape hence producing sustainable palm oil production.

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# Urban Farming Contributes to Shortening the Food Supply Chain

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## INTRODUCTION

The Food and Agriculture Organization of the United Nations (FAO), 2020 defines urban farming (UF) as an agricultural tradition brought to the city, encompassing agricultural production beginning with the cultivation, processing and intensive distribution of the food. Followed by growing crops and livestock in and around the city to prepare fresh food, create jobs, recycle waste and build the city's resilience to climate change. UF has been categorized into two categories: Uncontrolled Environment Agriculture (UEA) and Controlled Environment Agriculture (CEA) (Game and Primus, 2015; Armanda, et al., 2019). UEA includes open space vegetable gardens, rooftop gardens and community gardens, which are widely claimed to play a role in food security in cities or communities worldwide. In contrast, CEA encompasses agricultural practices that apply environmental optimization, usually in conjunction with surrounding urban structures. Examples are greenhouses, indoor farming and plant factory (Game and Primus, 2015; AlKodmany, 2018).

The food supply chain is thus interdisciplinary and highly complex and requires expert analysis to be correctly managed. Toth et al. (2016) emphasized that the food supply chain includes production, processing, retail, distribution and consumption. The issues related to the food supply chain are perishability, seasonality, long production cycles, and variability in quantity and quality. Besides, the variability in logistics costs due to fuel price volatility and political interferences also cause a problem in the food supply chain (Gold et al., 2016).

The implementation of UF can potentially shorten supply chains and thereby reduce dependence on fossil fuels (Perkins, 2000). Charatsari et al. (2018) suggest that short food supply chains are an alternative way to distribute agricultural products that encourage new production and consumption patterns and bring together farmers and consumers. Agricultural activities in UF have been recognized in Malaysia as an efficient approach to food security and have recently been expanded (FAO, 2020). Therefore, research related to the supply chain for UF is crucial as it provides details to meet consumer and producer (farmer) satisfaction and demand, thus ensuring food safety (Zecca and Rastorgueva, 2014). Therefore, this study highlighted the impact of UF, particularly community gardens and plant factories, in shortening the food supply chain in Malaysia.

## MATERIALS AND METHODS

This research involves quantitative methods, and the primary data are collected through a structured face-to-face interview with 154 community garden leaders and nine (9) plant factory producers conducted between September 2021 and January 2022. In addition, the descriptive analysis method is performed for initial analysis to understand the data and to determine the marketing channels of a community garden and plant factory products. The analysis data is performed using Statistical Package for Social Science (SPSS) Version 23 software.

## RESULTS AND DISCUSSION

### *Market channels for community garden products*

The survey on ( $n=154$ ) community gardens shows that 78.6% have successfully marketed their products. Of this total, 62% market their products directly to consumers. A small number of these community gardens have also marketed their products to groceries (14%), wet markets (12%), wholesale markets (8%) and supermarkets (4%) (Figure 1). This community garden is observed to change the supply chain to more of a community in nature. A sustainable community garden is believed to contribute to productivity elevation to fulfil the rising demand. This new supply chain of a community nature considers the importance of producers and buyers being coordinated with the cost, risk and return being shared. For instance, the buyer is more involved in every stage of operation. They are informed comprehensively, have a partial influence on business decisions, contribute to finance and participate in production, storage and distribution.

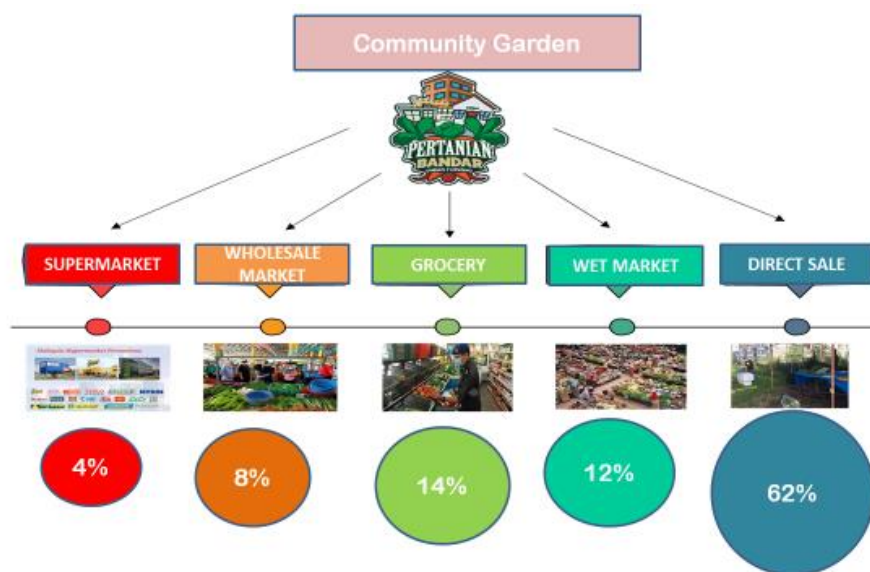


Figure 1. The supply chain of community garden products.

### *Market channels for plant factory products*

The marketing system and plant factory supply chain are still new in Malaysia. The cost of production through plant factory cultivation is higher than conventional due to higher operational costs, with an average monthly cost of more than RM 5000. Therefore, the marketing strategy for ( $n=9$ ) plant factories are targeting a premium market, for example, fine-dining restaurant (33%), premium supermarkets (22%) and direct sales (77%) (on their premise and online). Therefore, marketing strategies online using social media platforms like Facebook, Instagram, and websites are more effective in promoting plant factory products. Figure 2 shows the plant factory marketing channel, which comprises a One-Level channel and Direct Channel or Zero Channel. Zero Channel is a channel from manufacturers straight to the final consumers.

## CONCLUSION

In conclusion, both concepts of the community garden and plant factory have successfully shortened the supply chain where both concepts benefited the end-user. UF is presented in this paper mainly (community garden and plant factory) as a potential method to address the urban risks stemming from the lack of food planning and as a tool to shorten supply chains. However, UF has recently been motivated in terms of ecosystem services and sustainable development to

use UF's multifunctionality for more than just food production but to address various other urban inefficiencies and issues.



Figure 2. The supply chain of plant factory products.

## ACKNOWLEDGEMENT

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# Optimization of Different Auxin and Cytokinin Combination in Nutrient Medium for Establishment of Optimal *in vitro* Multiple Plantlet in *Ficus carica* L. cv Siyah Orak

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## INTRODUCTION

The edible fig plant (*Ficus carica* L.) is a member of the Moraceae family and is native to the Middle East and South Asia, with importance in many cultures around the world. Fig fruits can be consumed as fresh or dried. Several *Ficus* spp. are utilized as medicine in Ayurvedic and traditional Chinese medicine, in addition to being used as a food source (Lee et al., 2022). Fig tree is usually propagated through conventional methods like cuttings, air layering and grafting due to the seeds being non-viable. However, these methods are susceptible to diseases, pest, virus, fungus, and bacterial infection. It is also time consuming and has low survival rate (Chan Hong et al., 2020). Tissue culture techniques will produce disease and virus free cultures. It also takes relatively shorter duration, allows mass propagation and grown in a control environment (Sriskanda et al., 2021). Hence, this study was carried out to select the optimal Murashige and Skoog (MS) media strength, hormone combination and explant type to produce multiple plantlets of *Ficus carica* L. cv Siyah Orak.

## MATERIALS AND METHODS

### *Plant material*

*Ficus carica* L. cv Siyah Orak were obtained from HighTech Nursery, Miri, Malaysia. The plants were potted and placed outside of the Tissue Culture and Cryopreservation Laboratory, Universiti Putra Malaysia, Bintulu Campus, Sarawak. Healthy leaf, nodal segments and apical buds were used as explants. The explants were excised from the mother plant washed properly using tap water, followed by washing using Dettol and Tween 20 and rinsed thoroughly using tap water thereafter. All explants were cultured in different media composition incubated under 25±2 °C under florescent lamp at 16 h photoperiods for 3 weeks. All experiment consisted of 6 replicates per treatment, with 5 samples of explant each.

### *Evaluation of the media strength types for highest callus induction*

Leaf segment and thin cell layer (TCL) explants were cultured on different MS medium strength (1/4, 1/2, 3/4, 1 MS). Each media was supplemented with 20 g/L sucrose, 2.75 mg/L gelrite and 1.0 mg/L activated charcoal. Results on callus induction percentage and callus proliferation were taken for leaf segments. As for thin cell layer, callus induction, friable callus percentage and number of leaves were assessed for both transverse (tTCL) and longitudinal (lTCL), respectively.

## ***Evaluation of different hormone concentration for callus proliferation of leaf segment, shoot and rooting system for apical buds***

Callus proliferated from previous experiment of leaf segments were subcultured to  $\frac{3}{4}$  MS media supplemented with different concentration of hormone TDZ (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L). Results on the weight of callus proliferated were taken. Apical buds were cultured on 1 MS media supplemented with different concentration of hormone BAP (0, 1.0, 1.5, 2.0, 2.5, and 3.0 mg/L). Results on the number of leaves and shoot length were taken. Apical buds from previous experiment were then subcultured into 1MS media supplemented with 2.0 mg/L BAP with different concentration of IAA (0, 1.0, 1.5, 2.0, 2.5 and 3.0 mg/L). Result on the percentage of root formation and number of roots were taken.

### ***Statistical analysis***

All data was analysed by one-way variance analysis (ANOVA). Significant differences ( $p < 0.05$ ) between treatment methods were tested using the Duncan comparison test at a 5% probability using the Statistical Analysis System (SAS) version 9.4.

## **RESULTS AND DISCUSSION**

### ***Effect of MS media strength on callus induction of leaf segment***

Significant difference in the callus induction percentage of leaf segments was observed among different MS media strength after 4 weeks of incubation (Table 1). The highest callus mean weight was noted on leaf segment cultured in  $\frac{3}{4}$  MS (875±0.03 mg) media supplemented with 1.0 mg/L BAP with 0.2 mg/L IAA. Based on observation, explants cultured on 1 MS media showed slow growth and turned brown after 3 weeks of incubation. According to Wani et al. (2014), the growth of callus can be affected by the different MS media strength. High amount of nutrient could cause the explant to die due to stress, while too little will inhibit the growth. In this study, leaf segment cultured on  $\frac{3}{4}$  MS media produced optimal callus formation.

Table 1. Callus weight obtained in relation to different MS media strength.

Media strength (MS)	Callus weight (mg)
$\frac{1}{4}$	697±0.047 <sup>bc</sup>
$\frac{1}{2}$	762±0.036 <sup>b</sup>
$\frac{3}{4}$	875±0.036 <sup>a</sup>
1	660±0.037 <sup>c</sup>

### ***Effect of different hormone concentration on callus proliferation of fig leaf segments***

Based on Table 2, there are significant difference in the mean of callus weight for leaf segments cultured on media supplemented with different concentration of hormone. From our findings,  $\frac{3}{4}$  MS media supplemented with 2.0 mg/L TDZ produced the highest callus mean weight percentage (920±0.04 mg) for shoot proliferation. Sa'adan and Zainuddin (2020) reported that when they

Table 2. Callus weight obtain in relation to different concentration of TDZ.

TDZ concentration (mg/L)	0	0.5	1.0	1.5	2.0	2.5	3.0
Callus weight (mg)	320 ± 0.035 <sup>e</sup>	412 ± 0.037 <sup>d</sup>	626 ± 0.034 <sup>c</sup>	728 ± 0.012 <sup>b</sup>	920 ± 0.03 <sup>a</sup>	798 ± 0.025 <sup>b</sup>	766 ± 0.045 <sup>b</sup>

tested the callus induction from leaf explant of *Ficus deltoidea* var *kunstleri*, the callus formation of the leaf explant was bigger and healthier as the concentration of BAP used increased. The same observations were seen from this study when using TDZ. In short, the higher concentration of TDZ used, the higher tendency for the explant to induce callus. Hence, there is significant correlation between TDZ concentration with formation of callus.

#### ***Effect of MS media strength for tTCL and ITCL nodal segment***

Optimization of the different media strength revealed significant difference on the callus induction (%), friable callus induction (%) and number of leaves for both tTCL and ITCL techniques. Results revealed that ¼ MS strength had the highest callus induction percentage for tTCL (100%±0), and ITCL (96.7%±0.15) segments. Significantly friable callus was obtained for tTCL segments cultured on ½ MS media (63.33%±0.55) and ITCL segments cultured on ½ MS media (76.67%±0.15). Cultures on ¼ MS media produced the most number of leaves both tTCL (0.83±0.28) and ITCL (1.00±0.33) (Table 3). This study shows that TCL worked best when using lower MS media strength due to the explant has greater surface area contact resulting in efficient transport of medium compared to other type of explant (Texeira da Silva, 2019). Hence, using higher MS media strength could cause death to explant due to stress.

Table 3. Callus induction, friable callus induction percentage and number of leaves for tTCL and ITCL segments.

Media strength (MS)	Callus induction(%)		Friable callus(%)		Number of leaves	
	tTCL	ITCL	tTCL	ITCL	tTCL	ITCL
¼	100±0 <sup>a</sup>	96.7±0.15 <sup>a</sup>	26±0.26 <sup>b</sup>	76.67±0.50 <sup>a</sup>	0.83±0.0.28 <sup>a</sup>	1.00±0.33 <sup>a</sup>
½	86±0.30 <sup>ab</sup>	76±0.43 <sup>b</sup>	63.33±0.55 <sup>a</sup>	46±0.55 <sup>bc</sup>	0.33±0.19 <sup>b</sup>	0.50±0.20 <sup>b</sup>
¾	70±0.36 <sup>bc</sup>	60±0.36 <sup>b</sup>	53±0.28 <sup>a</sup>	40±0.28 <sup>bc</sup>	0.16±0.15 <sup>c</sup>	0.37±0.15 <sup>c</sup>
1	56±0.33 <sup>c</sup>	50±0.43 <sup>c</sup>	0±0 <sup>c</sup>	0±0 <sup>d</sup>	0±0 <sup>d</sup>	0±0 <sup>d</sup>

#### ***Effect of different BAP concentration on shoot formation of fig apical buds***

Apical bud cultured on 1MS media supplemented with 2.0 mg/L BAP produced higher number of leaves (3.50±0.20) and shoot length (13.73 mm±0.65) than other treatments (Table 4). This finding is in agreement to that of the micropropagation of *F. carica* L. cv Black Jack reported by Parab et al. (2021). Both experiment had shown treatment using BAP was successful for induction of multiple shoot on apical buds explant. However, Parab et al. (2021) used Woody Plant Medium (WPM). In this study, media supplemented with 2.0 mg/L BAP is the optimal concentration for shoot formation and shoot length.

Table 4. Number of leaves and shoot length of *in vitro* apical buds.

BAP concentration (mg/L)	0	1.0	1.5	2.0	2.5	3.0
Number of leaves	1.16±0.23 <sup>c</sup>	2.16±0.21 <sup>bc</sup>	2.33±0.30 <sup>b</sup>	3.50±0.20 <sup>a</sup>	2.67±0.30 <sup>b</sup>	2.33±0.30 <sup>bc</sup>
Shoot length (mm)	9.35±0.37 <sup>d</sup>	10.50±0.35 <sup>cd</sup>	11.37±0.57 <sup>cb</sup>	13.73±0.66 <sup>a</sup>	12.50±0.51 <sup>ab</sup>	11.8±0.88 <sup>bc</sup>

### **Effect of different IAA concentration on percentage of root formation and number of roots produced from apical buds**

Different concentration of IAA in 1MS media supplemented with 2.0 mg/L BAP induced different percentage of root formation (Table 5). Apical bud cultured on media supplemented with 2.0 mg/L BAP+2.5 mg/L IAA had the highest root formation ( $10\% \pm 0.31$ ) and root number ( $0.83 \pm 0.50$ ). Noticeable root formations were absent on all the other treatments tested. This could be due to the observation was carried out too soon after a short incubation (2 weeks). However, it was observed that explants had shown sign of browning and this could indicate presence of inhibitors for growth. Oxidation of phenolic compounds which are abundant in fig trees could result in browning and hinder the explant establishment (Dhage et al., 2015). Therefore, in this study, 1MS media supplemented with 2.5 mg/L IAA is regarded the most optimal hormone concentration for both root formation percentage and root number produce.

Table 5. Root formation percentage and number or root produced of apical buds.

IAA concentration (mg/L)	0	1.0	1.5	2.0	2.5	3.0
Root formation (%)	$0 \pm 0^c$	$0 \pm 0^c$	$0 \pm 0^c$	$3.33 \pm 0.15^b$	$10 \pm 0.31^a$	$0 \pm 0^c$
Number of roots	$0 \pm 0^c$	$0 \pm 0^c$	$0 \pm 0^c$	$0.33 \pm 0.30^b$	$0.83 \pm 0.50^a$	$0 \pm 0^c$

### **CONCLUSION**

The results concluded that that the overall regenerations of *F. carica* L. showed the best shoots growth performance when using apical buds cultured on 1MS media supplemented with 2.0 mg/L BAP + 2.5 mg/L IAA.

### **ACKNOWLEDGEMENT**

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# Physical Properties of Safawi, Sukkari and Medjool Dates

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## INTRODUCTION

Date palm (*Phoenix dactylifera* L.) is one of the oldest cultivated plants and source of nutrition in most arid and semi-arid regions. Saudi Arabia, which is one of the major exporters of dates, has planted approximately 7 to 8 million palm trees (Al-Hooti et al., 1997). Dates are well recognised for having high functional dietary fibre and polyphenols that contribute to bioactive agents such as antimutagenic, antioxidant, anticarcinogenic and anti-inflammatory (Maqsood et al., 2020). Dates can be freshly eaten after harvesting or processed to obtain by-products such as date syrup and date sugar (UNCTAD, 2018) as well as dehydrated date powder, and date-mixed high fructose.

Agricultural goods such as dates come with many varieties and have their own unique characteristics. A thorough understanding of dates physical attributes is necessary for effective agricultural material handling, storage, and processing (Stroshine, 1998). Therefore, this study aims to investigate the physical properties of three types of Middle East date varieties, which are Safawi, Sukkari, and Medjool.

## MATERIALS AND METHODS

### *Fruit samples*

A total of three types of dates were used in this study which were Safawi, Sukkari, and Medjool as shown in Figure 1. The samples were purchased at Makkah & Almadinah Market, Wholesale Yemen Trading Sdn. Bhd., Bangi, Selangor, that sells Arabic products mostly from Madinah, Saudi Arabia. Ten samples of each type of dates were randomly chosen from a total of hundred dates to be used in this study.

### *Physicochemical analysis*

The moisture content of the dates was measured using the recommended method by AOAC (2005). About 5 g of each type of dates flesh were weighed and dried using an oven at 105°C (24



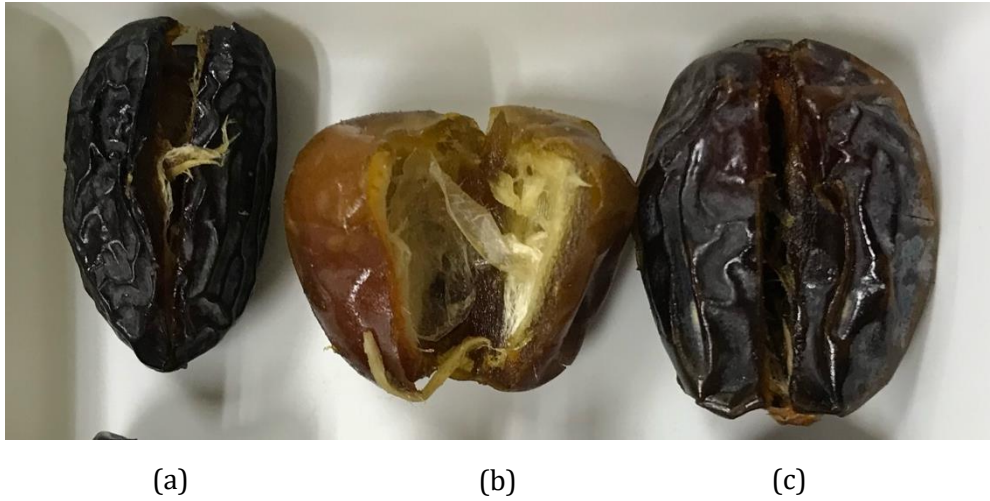


Figure 1. The flesh of (a) Safawi, (b) Sukkari, and (c) Medjool dates.

h) and then weighed again. The moisture content (%) of the dates were calculated using equation 1.

$$\text{Moisture content (\%)} = \frac{(M_1 - M_2)}{M_1} \times 100 \quad (1)$$

Where  $M_1$  is the initial weight of samples and  $M_2$  is the final weight of samples after drying in oven.

For total soluble solids content (TSS) of the dates, the chopped fruit was added with distilled water (1:1) and triturated in a ceramic mortar. Then, the solution was filtered and the TSS content was estimated using the Hand-Held Refractometer (ATAGO CO. LTD., Japan) by placing a drop of the solution on the refractometer.

The dimensions of the fruit in terms of length ( $L$ ), width ( $W$ ) and thickness ( $T$ ) were measured using a vernier callipers. Geometric mean diameter ( $D_g$ ) and sphericity index ( $\phi$ ) were calculated using the equation 2 and 3, respectively (Kabas et al., 2006; Golmohammadi and Afkari-Sayyah, 2013).

$$D_g = (LWT)^{1/3} \quad (2)$$

$$\phi = \frac{D_g}{L} \quad (3)$$

The fruits were weighed individually using an analytical weight balance. Then, the pit was removed, and the weight of the fruit flesh and pit were recorded subsequently. The fruit density was determined by using equation 4. The fruits were weighed in air and lowered into a beaker filled with water (Mohsenin, 1986).

$$\rho_f = \frac{M_a}{M_a - M_w} \rho_w \quad (4)$$

Where  $\rho_f$  and  $\rho_w$  are fruit and water densities ( $\text{g/m}^3$ );  $M_a$  and  $M_w$  are mass of date in air and water, respectively. The bulk density was measured as the ratio of weight of fruits to the volume occupied by the same fruits (Hazbavi et al., 2015).

## RESULTS AND DISCUSSION

Table 1 shows the physical properties of Safawi, Sukkari, and Medjool dates. Moisture content is one of the critical parameters in food product that can affect the quality and shelf life of the food. In this study, the moisture content of Sukkari showed the highest value followed by Medjool and Safawi (Table 1). The dates were categorized into three groups based on the moisture content, which are soft, semi-soft and dry dates. The three varieties of dates in this study were therefore categorized as semi-soft dates as their moisture content was in the range of 10 to 30% (Habib and Ibrahim, 2011). The TTS content of Medjool had the highest value compared to Safawi and Sukkari.

The determination of dates physical parameters in terms of their dimensions is crucial for dates processing such as drying and storage studies. From Table 1, the length of Medjool had significantly highest value ( $43\pm3.4$  mm) compared to other samples. Studies by Abdul-Hamid et al. (2020) also found that the length of Medjool was higher than Sukkari. Medjool exhibited the highest width value compared to other samples, while Sukkari had the highest value in thickness. In terms of geometric mean diameter which is important in designing of separating, harvesting, sizing and grinding machine (Desai et al., 2019), Medjool showed the highest value followed by Sukkari and Safawi.

Table 1. Physical properties of Safawi, Sukkari, and Medjool dates.

Variables	Safawi	Sukkari	Medjool
Moisture content (%)	$23.8\pm0.2^c$	$28.8\pm0.6^a$	$25.2\pm0.4^b$
Total soluble solids ( $^{\circ}$ Brix)	$36.3\pm0.1^b$	$35.1\pm0.1^c$	$37.9\pm0.1^a$
Length (mm)	$38.3\pm2.2^b$	$33.5\pm1.5^c$	$43.0\pm3.4^a$
Width (mm)	$21.1\pm0.9^b$	$25.9\pm1.8^a$	$26\pm2.6^a$
Thickness (mm)	$17.9\pm1.8^b$	$23.3\pm2.7^a$	$22.3\pm2^a$
Geometric mean diameter (mm)	$24.4\pm1.1^c$	$27.2\pm1.4^b$	$29.2\pm1.8^a$
Fruit weight (g)	$8.8\pm1.7^c$	$12.2\pm1.5^b$	$17\pm1.7^a$
Flesh weight (g)	$7.9\pm1.6^c$	$10.9\pm1.3^b$	$15.8\pm1.7^a$
Pit weight (g)	$0.9\pm0.1^b$	$1.2\pm0.3^a$	$1.2\pm0.1^a$
Bulk density (g/cm <sup>3</sup> )	$0.388\pm0.021^b$	$0.380\pm0.001^b$	$0.454\pm0.02^a$
Sphericity	$0.64\pm0.03^b$	$0.81\pm0.05^a$	$0.68\pm0.05^b$

Different superscripts <sup>a-e</sup> in the same row indicates significant differences ( $p\leq0.05$ ) between different types of dates.

As Medjool had the highest dimensions than Sukkari and Safawi, it was observed that the fruit and flesh weights of Medjool were also greater ( $17\pm1.7$  g and  $15.8\pm1.7$  g, respectively). This result is in accordance with Siddiqi et al. (2020) who found that the dimensions of the dates were related to their fruit and flesh weights. The bulk density of all samples was in the range of 0.38 to 0.454 g/cm<sup>3</sup> with Medjool having the highest value. For the sphericity value, Sukkari had the highest value than Medjool and Safawi. This factor is crucial in the design of fruit handling equipment as it indicates the fruit shape (oval to cylinder) (Dasei et al., 2019) which is important in calculations of heat and mass transfer, screening and grading of fruits. The results in this study showed that all dates varieties have a cylindrical shape, considering the close value between width and thickness of the dates.

## CONCLUSION

The selected date varieties namely Safawi, Sukkari, and Medjool exhibited differences in terms of moisture content, TSS content and the physical properties. Therefore, the information on the physical characteristics of dates from this study is important to spur the development of technologies that are suitable for processing them.

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# An Overview of the Key Ingredients Commonly Utilized in Commercially Available Sports Energy Gels

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## INTRODUCTION

Over the past two decades, food hydrocolloids have gained much attention in the food science and technology research field as they play a crucial role in structure, processing, stability, flavour, nutrition, and health benefits of foods (Lu et al., 2020). They also play an important role in pharmaceutical formulations application (Manzoor et al., 2020). Energy supplement is used in keeping the energy levels required for training, athletes, and physical activities, including dietary supplements based on carbohydrate as to maintain glucose levels during the physical activities. Carbohydrate supplements can be found in many forms such as tablets, capsules, powder, drinks and gels. Carbohydrate supplement in the form of drinks is conventional to the society. However, there is a concern that taking liquid sports drink may cause indigestion and stomach discomfort to the athletes and sportsman. Besides that, the athletes have to carry more weight with them when exercising as electrolyte drinks have to be consumed on average of 250-450 mL to replenish the glucose loss (Tharnpichet et al., 2019). Applying energy supplement in gel form may avoid gastric discomfort and it is a practical alternative (Alves et al., 2012). The potential of carbohydrate energy gels to sustain blood glucose and lactate concentrations may facilitate athletes' endurance performance when engaging in prolonged workouts or increase performance in sports (Tharnpichet et al., 2019; Harper et al., 2015; Patterson and Gray, 2007; Kingsley et al., 2013). Energy gel development is currently expanding due to the incorporation of different formulation ingredients. Preservatives, acid regulators, and caffeine are frequently added to sports energy gel formulations to prolong shelf life and promote alertness as well as providing sources of adrenaline rush during physical activity.

In a review work by Zhang et al. (2015), the authors claimed that the extreme variations of energy gels that are commercially produced are meant to fulfil all customers taste and demands. A review based on 23 brands of energy gels by Zhang et al. (2015) and his team found that the mean value of the serving size, energy density, energy/gel, total CHO, free sugars/gel and osmolality of energy gels are  $50 \pm 22$  g,  $2.34 \pm 0.70$  kcal/g,  $105 \pm 24$  kcal,  $25.9 \pm 6.2$  g,  $9.3 \pm 7.0$  g and  $4424 \pm 2883$  mmol/kg, respectively. Nevertheless, their study did not explore the key ingredients of the energy gels that could provide an insight for the consumers or energy gels manufacturers to produce nutritious and higher value energy gels. To date, no standard regulation has been established that

cause extreme variations of energy gels made from wide varieties of ingredients available in the market. The commercially available energy gels are made from a few primary components. Thus, the purpose of this study is to highlight a few key components of energy gels that are commonly utilized in the surveyed energy gels.

## **MATERIALS AND METHODS**

The energy gels from the following 32 brands were surveyed and evaluated: Nilofa, Hammer, Koda, High5, GU, Spring Energy, Science in Sport (SiS), Gatorade, Maurten, Huma, Clif, Ucan Edge, Honey Stinger, PNG, Torq, Accelerade, CNP, Maxim, USN, Mule, Multipower, Nectar, Carb-Boom, Power Bar, Lucozade, Shottz, Dextro, Kinetica, Zipvit, Maxifuel, Squeezy and OTE. All energy gels in this study were formulated and intended as energy booster. Gels that are designed for beauty care and delivery of protein and carbohydrate mixtures were not included in this overview study. Data relating to nutritional ingredients and serving size were collected from the packaging materials and website of individual manufacturers.

## **RESULTS AND DISCUSSION**

### ***Key ingredients of commercial energy gels***

From the survey (Table 1), 23 out of 32 brands used maltodextrin as the main source of the carbohydrate in the energy gels. Maltodextrin is a polysaccharide that has long carbohydrate chains with 2-3% glucose and 5-7% maltose which lead to slightly sweet flavour and available in the form of white hygroscopic spray-dried powder (Parikh et al., 2014). As maltodextrin can be easily digested into a simple carbohydrate that can be converted into instant energy, it is commonly used in the energy supplements for the athletes' endurance performance.

Like any other foods and drinks products, salts (benzoates and sorbates) of benzoic acid (E210) and sorbic acid (E200) were also included in the energy gels ingredients. In Table 1, more than 50% of the surveyed energy gels used either potassium sorbate or sodium benzoate, or both as the preservatives are known to act as antibacterial and antifungal agents, and also have a very low mammalian toxicity (Piper and Piper, 2017). According to Piper and Piper (2017), they are generally recognized to be innately devoid of carcinogenicity but have the ability to transform into potential mutagens if they are consumed excessively. Acceptable daily intakes of potassium sorbate and sodium benzoate are 0-25 mg/kg body weight/day and 0-5 mg/kg body weight/day, respectively (Mischek and Krapfenbauer-Cermak, 2012). Many studies have clinically proven that pregnant women should avoid consuming foods containing sodium benzoate as the usage of this food additive might cause DNA damage and increase micronuclei formation. As a solution, producers might instead prioritise preservative-free energy supplements to ensure a safe product that can be ingested by all levels of the community.

Acidification is one of the food preservation methods that is perceived as a minor parameter but very important in maintaining appropriate pH to ensure food safety and longer shelf life. Based on Table 1, 68% (22 out of 32 brands) of the commercial energy gels used citric acid (E330) as the acid regulator. Other than that, citric acid has the capability to provide aroma, sour taste, increase gel consistency and also decrease enzymatic browning in fruits and fruits products that assist in colour retention. Citric acid also plays a key role in the metabolic process that converts carbs, lipids, and proteins into carbon dioxide and water to provide energy in the tricarboxylic acid cycle (TCA) or Krebs cycle or citric acid cycle (Dhillon et al., 2011). Therefore, it is clear that citric acid plays a significant role in the energy gels, justifying its inclusion as part of the energy gels ingredients.

Based on Table 1, 53% of the surveyed sports energy gels contain caffeine in different types of flavour. However, the sources of the caffeine used might not be clearly stated by the manufacturers either on the packaging or on the websites. Apart from synthetic caffeine, the

extracts from guarana leaves are often used as the natural source of caffeine in the energy drinks (Temple et al., 2017). In Table 1, one brand (Maxim) claimed to use natural guarana leaves extract as its caffeine source. More than 400 mg/day, 100 mg/day, and 2.5 mg/kg/day of caffeine could lead to caffeine toxicity in healthy adults (more than 19 years old), healthy adolescents (12-18 years) and healthy children (less than 12 years old), respectively (Nawrot et al., 2003; Seifert et al., 2011). Moreover, Reissig et al. (2009) stated that for the energy drinks, the total amount of caffeine contained in some energy drinks can exceed 500 mg which is equivalent to 14 cans of

Table 1. Key ingredients of the commercially available energy gels.

Source of carbohydrate	<p>Maltodextrin (23 out of 32 brands)  <i>Hammer, Koda, High5, GU, Science in Sport (SiS), Gatorade, Clif, PNG, Torq, Accelerade, CNP, Maxim, USN, Multipower, Nectar, Carb Boom, Power Bar, Shottz, Kinetica, Zipvit, Maxifuel, Squeezy and OTE.</i></p> <p>Other sources of carbohydrate:  Rice syrup, fruits syrup, fruit concentrates, sugars (fructose, glucose and sucrose)</p>
Preservatives	<p>Potassium sorbate (20 out of 32 brands)  <i>Hammer, High5, GU, Science in Sport (SiS), Ucan Edge, PNG, Torq, CNP, Maxim, USN, Multipower, Nectar, Carb Boom, Power Bar, Lucozade, Kinetica, Zipvit, Maxifuel, Squeezy, OTE.</i></p> <p>Sodium benzoate (11 out of 32 brands)  <i>Koda, High5, GU, Science in Sport (SiS), PNG, Maxim, Multipower, Carb Boom, Power Bar, Shottz, Kinetica.</i></p>
Acids regulators	<p>Citric acid (22 out of 32 brands)  <i>Hammer, Koda, High5, Spring Energy, Science in Sport (SiS), Gatorade, Huma, Clif, Honey Stinger, PNG, Torq, Maxim, Multipower, Nectar, Carb Boom, Power Bar, Shottz, Dextro, Kinetica, Zipvit, Maxifuel, and Squeezy.</i></p> <p>Other acid regulators used:  Sodium citrate  Potassium citrate</p>
Caffeine content	<p>(17 out of 32 brands)  <i>Hammer: 2 flavours (25&amp;50 mg)</i>  <i>Koda: 3 flavours (80 mg)</i>  <i>GU: Contain green tea (leaf) extract</i>  <i>Spring Energy: 3 flavours (10-50 mg)</i>  <i>Maurten: 1 flavour (100 mg)</i>  <i>Huma: 4 flavours (25&amp;50 mg)</i>  <i>Clif: 5 flavours (25-100 mg)</i>  <i>Honey Stinger: 2 flavours (32 mg)</i>  <i>Torq: 3 flavours (89 mg)</i>  <i>Accelerade: 2 flavours (20&amp;100 mg)</i>  <i>Maxim: 1 flavour (12 mg) (Guarana leaves extract)</i>  <i>Mule: 2 flavours (50&amp;100 mg)</i>  <i>Power Bar: 2 flavours (50 mg)</i>  <i>Shottz: 2 flavours (80 mg)</i>  <i>Dextro: 2 flavours (50 mg)</i>  <i>Maxifuel: 1 flavour (100 mg)</i>  <i>OTE: 1 flavour (50 mg)</i></p>

common caffeinated soft drinks or 5 cups of coffee. Since there is no established standard regulation for the dosage of caffeine in energy gels, the trends in caffeine concentration in energy gels are a concern, especially for children and young adults. The highest caffeine content among surveyed energy gels is about 100 mg (5 brands) which is equivalent to 1-2 cups of coffee. As the

energy gels might be consumed in more than one sachet during vigorous exercises, the consumers are exposed to the caffeine toxicity.

## CONCLUSION

As a conclusion, this study provides an insight into the choices of the key ingredient of the energy gels and the necessity of a formulation for the end customers that is preservative-free, less processed, and highly nutritious to comply a healthy living lifestyle and can be safely consumed by all community levels.

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# Physicochemical and Texture Profile Analysis of Gummy Candy made of Nutritive and Non-nutritive Sweeteners

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## INTRODUCTION

The global confectionery market is projected to grow from \$194.37 billion in 2021 to \$242.53 billion in 2028 at a CAGR of 3.8% in the forecast period. While around 65.5% of packaged products had added sugars or sweeteners, white sugar was reported to be the most widely used (50.6%) food-grade sweetener (Bayram and Ozturkcan, 2022).

Commercial gummies are made from sugar-gelling ingredients, artificial flavouring, and colouring (Marfil et al., 2012). Due to its firm and elastic nature, gelatine is often used as a gelling agent in many gummy products. However, the gelling process is affected by various factors, including concentration, pH, and temperature used during the preparation. In addition, the type of sweeteners used can modify the gelation properties of gelatine, increasing the gel strength and melting points (Oakenfull and Scott, 1984). This is due to different sweeteners having different stereochemical structures. For example, sucrose and glucose syrup can interact with gelatine to enhance the stability of conformationally ordered junctions in gelatine gels, which affect the end product structure, strength and stability (Burey et al., 2009).

There are three sweetener types: added sugars, free sugars, and non-milk extrinsic sugars (Scapin et al., 2017). It is determined based on their presence in nature rather than physiological or chemical-structural categories. The added sugars could be further divided into two categories known as nutritive and non-nutritive. While non-nutritive sweeteners have extremely few calories, nutritive sweeteners give the body calories. The Brix value, rate of caramelisation and/or Maillard reaction and sol-gel transition behaviour in the food products are depended on the types of sugars used (Kurt et al., 2021).

Yet, less is known about the effect of physicochemical and texture properties on gummies from different sweeteners. Thus, choosing a suitable sweetener is crucial as it could affect the sensory properties, quality, and viscosity of the aqueous phase and the water activity of the main product (Čižauskaite et al., 2019). The evaluation of types of sweeteners on formulated gummies is important as it could be a reference to formulate gummies with acceptable qualities (Mahat et al., 2020).

## **MATERIALS AND METHODS**

### ***Materials***

Five commonly used nutritive-added sweeteners and three non-nutritive-added sweeteners were purchased from the local market. The nutritive added sugars are white sugar, brown sugar, honey, molasses, and coconut sugar, while the non-nutritive added sugars are stevia, sucralose, and monk sugar. The other ingredients used were bovine gelatine 200 Bloom, citric acid anhydrous, and glucose syrup.

### ***Formulation of gummies***

The formulation was carried out by referring to Charoen et al. (2015), with slight modifications on the variety of sweeteners used. About 8 g of gelatine, 3 g citric acid, 24.5 ml of water and 32.25 g of glucose syrup, and 32.25 of each sweetener were mixed accordingly. The concoction was then poured into a silicon mould and left at 4°C for 12-24 hours.

### ***pH, moisture and water activity***

The pH of gummies was checked using SERICO Laboratory Benchtop pH Meter Model PH600L. While, the moisture content was determined using a moisture meter (MA 160 Sartorius Electronic, Germany) at 105°C. A portable water activity kit (AQUALAB Pawkit Water Activity Meter, Greece) was used for the water activity analysis. Data was prepared in triplicates.

### ***Texture profile analysis***

Texture Analyser (TA-XT Plus, United Kingdom) was used to measure the texture of formulated gummy based on hardness, adhesiveness, springiness, cohesiveness, gumminess, resilience, and chewiness in triplicates. The test was performed at room temperature with pre-test speed 1 mm/s, test speed of 5 mm/s, distance at 5 mm/s, trigger force of 1 g and delay time at 5 sec.

### ***Statistical analysis***

Data obtained were analysed using Minitab Statistical Software Version 21.1.0. Significance difference between different types of sweeteners towards gummies on pH, moisture and water activity was measured using One-way ANOVA with 95% confidence level. Tukey HSD test was performed after the normality and standard deviation and homogeneity test.

## **RESULTS AND DISCUSSION**

### ***Moisture content, water activity, and pH***

Water elements and pH are crucial as they could influence the taste, texture, and stability of the confectionery product. This includes the potential for food spoilage in future. Table 1 represents the moisture content, water activity and pH of different sweeteners.

The highest moisture content was recorded at  $55.08 \pm 0.50\%$  when sucralose was used as the substitute sugars while the lowest was brown sugar at  $29.44 \pm 0.57\%$ . The moisture content recorded can be classified as shelf-stable food which is between 20-50% moisture ( $a_w$  is between 0.60 and 0.85) (Bozoglu and Erkmen, 2016). However, there is a dissimilar pattern observed on types of sugars on moisture content and water activity. This is due to the isotherm of moisture-sorption and water activity which are non-linear, as well as differences in temperature, humidity, and types of the food.

Table 1. The types of sugars on moisture content, water activity, and pH.

Types of sugar/ Analysis	Moisture (%)	Water Activity	pH
Nutritive sweeteners			
white sugar	31.67±0.79 <sup>d</sup>	0.82±0.02 <sup>a</sup>	2.59±0.01 <sup>b</sup>
brown sugar	29.44±0.57 <sup>d</sup>	0.76±0.01 <sup>c</sup>	2.62±0.03 <sup>ab</sup>
molasses	37.50±1.04 <sup>c</sup>	0.75±0.02 <sup>b</sup>	2.75±0.03 <sup>ab</sup>
coconut sugar	36.64±0.48 <sup>c</sup>	0.73±0.02 <sup>b</sup>	2.71±0.01 <sup>ab</sup>
honey	45.58±0.88 <sup>b</sup>	0.81±0.02 <sup>a</sup>	2.67±0.04 <sup>ab</sup>
Non-nutritive sweeteners			
stevia	48.59±0.86 <sup>b</sup>	0.66±0.02 <sup>c</sup>	2.77±0.19 <sup>ab</sup>
sucralose	55.08±0.50 <sup>a</sup>	0.73±0.02 <sup>b</sup>	2.80±0.01 <sup>a</sup>
monk sugar	30.99±3.04 <sup>d</sup>	0.73±0.01 <sup>b</sup>	2.80±0.02 <sup>ab</sup>

\*The data are presented in triplicates (±SE). Different letters in the same column indicate significant differences between the samples, based on Tukey's test ( $p \leq 0.05$ ).

Conversely, the nutritive added sugars of molasses, honey, white and brown sugar showed higher water activity compared to the non-nutritive added sugars of the gummies. The highest water activity was detected in honey gummies with  $0.81 \pm 0.02$  and the lowest water activity was in stevia at  $0.66 \pm 0.02$ . This is due to honey alone having a high-water activity which is up to  $0.788 \pm 0.002$  (Yap et al., 2019), while stevia itself was reported to have water activity below than 0.4 (Ruiz-Ruiz et al., 2015). The low water activity represents in nutritive sugars indicate the low amount of unbounded water present, which could decrease the susceptibility of microbial growth and improve the shelf life of the food.

The low pH values were observed in all the gummies, which are between 2.59 to 2.8. This is in line with gummies from Sumonsiri et al. (2021), which in between 2 to 3.7. The similar condition was observed when sugar lowered the pH of gummies as studied by Lopez et al. (1999). In this study, different types of sweeteners used have contributed to the pH disparities. This is because every sweetener has a distinct pH level.

### ***Texture profile analysis***

In the sensory assessment and creation of a novel food product, texture analysis is crucial. It is a characteristic of food that is linked to the sensation of touch, or the mouth feel experience. Table 2 represents the textural properties of the gummies formulated from eight different added sweeteners.

Based on Table 2, it was recorded that the types of sweeteners gave different textural properties toward the gummies. Except for the monk sugar, the non-nutritive added sugar of stevia and sucralose have higher hardness and gumminess of the candy. This might because the sucralose had a small structure which could disperse into the gel structure and strengthen the gel strength (Pattarathitiwat, 2020). The harder the gummy will give a rigid the structure. The gummy will also appear to be chewier and gummier (Mahat et al., 2020). Thus, food with small scale hardness and high springiness ( $4.15 \pm 2.74$ ) as shown by brown sugar is preferred as it could give a good chewiness ( $1314.27 \pm 823$ ) property (Kek et al., 2013). According to Mahat et al. (2020), the authors mentioned the preferred gummies should also has high cohesiveness and springiness. These characteristics were observed with formulated gummies of brown sugar. The springiness was appeared to be  $0.99 \pm 0.00$  and cohesiveness up to  $0.98 \pm 0.04$ , respectively. In addition, the adhesiveness of preferred gummies should be minimum as high adhesiveness indicates the potential of it to stick to the teeth, palate, and tongue. Referring to the data obtained, all the sweeteners have low adhesiveness, thus suitable as gummies (Sumonsiri et al., 2021). In this

study, although the brown sugar's gummy has higher adhesiveness, the rate is still minimal. In addition, adhesiveness is usually an undesirable feature of gummies, as it indicates the potential of it to stick to the teeth, palate, and tongue. Referring to the data obtained, all the sweeteners have low adhesiveness, thus suitable as gummies (Sumonsiri et al., 2021). This variation happened due to the -OH groups of sugar and sugars alcohol which could influence the water molecules surrounding the biopolymers (Shimuzu and Matubayasi, 2014).

Table 2. The textural properties of gummy candies.

Sweeteners /TPA	Hardness (N)	Springiness (mm)	Chewiness (J)	Gumminess (N)	Cohesiveness	Adhesive (Ns <sup>-1</sup> )
Nutritive sweeteners						
White Sugar	992.44±	0.87±	659.54±	755.42±	0.76±	-23.14±
	87.9 <sup>bc</sup>	0.02 <sup>b</sup>	37.2 <sup>a</sup>	58.4 <sup>bc</sup>	00.0 <sup>cd</sup>	0.4 <sup>a</sup>
Brown Sugar	337.12±	0.99±	1314±	331.26±	0.98±	-0.44±
	50.5 <sup>e</sup>	0.00 <sup>a</sup>	823 <sup>a</sup>	35.9 <sup>d</sup>	0.04 <sup>a</sup>	0.6 <sup>a</sup>
Molasses	501.30±	0.98±	440.82±	447.27±	0.89±	-4.19±
	25.4 <sup>e</sup>	0.00 <sup>a</sup>	27 <sup>a</sup>	25.9 <sup>d</sup>	0.00 <sup>ab</sup>	2.1 <sup>a</sup>
Coconut Sugar	559.63±	0.98±	497.52±	504.94±	0.90±	-5.09±
	88.9 <sup>de</sup>	0.00 <sup>a</sup>	86 <sup>a</sup>	87.8 <sup>cd</sup>	0.01 <sup>a</sup>	0.3 <sup>a</sup>
Honey	637.05±	0.99±	833.43±	924.31±	0.71±	-12.73±
	304 <sup>cde</sup>	0.00 <sup>a</sup>	6 <sup>a</sup>	13.4 <sup>d</sup>	0.06 <sup>cd</sup>	0.8 <sup>a</sup>
Non-nutritive sweeteners						
Stevia	1312.96±	0.90±	833.43±	924.31±	0.71±	-12.73±
	134 <sup>b</sup>	0.01 <sup>ab</sup>	6.01 <sup>a</sup>	13.4 <sup>b</sup>	0.06 <sup>cd</sup>	0.84 <sup>a</sup>
Sucralose	2473.53±	0.66±	1018.57±	1625.72±	0.67±	-155.24±
	154 <sup>a</sup>	0.09 <sup>c</sup>	315 <sup>a</sup>	149.2 <sup>a</sup>	0.01 <sup>d</sup>	31 <sup>b</sup>
Monk sugar	936.07±	0.88±	650.51±	736.07±	0.78±	-13.58±
	81 <sup>abc</sup>	0.00 <sup>b</sup>	46 <sup>a</sup>	55 <sup>bc</sup>	0.04 <sup>bc</sup>	2.9 <sup>a</sup>

\*The data are presented in triplicates (±SE). Different letters in the same column indicate significant differences between the samples, based on Tukey's test (p≤.05).

## CONCLUSION

Based on the physicochemical analysis on moisture content, water activity and pH, together with texture profile analysis, all formulated gummies have distinguishable characteristics. The variation is influenced by the types of sweeteners used in the formulation. However, based on data obtained, the brown sugar offers less moisture content and medium range of water activity and pH, respectively. In addition, formulated gummies of brown sugar has shown a preferable texture analysis.

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# Preparation of Invert Emulsion Containing *Metarhizium anisopliae* as a Biocontrol for Red Palm Weevil

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## INTRODUCTION

The Red Palm Weevil (RPW), also known as *Rhynchophorus ferrugineus*, is currently the most destructive pest of significant farmed palms, particularly coconut palms. The RPW causes significant damage to coconuts in Malaysia, notably in the east coast states of Peninsular Malaysia, including Terengganu and Kelantan. However, in 2016, the RPW has been reported in three other states, which are Perlis, Kedah, and Penang (DOA, 2016). The existence of holes in petioles and chewed up plant fibres with a bad, fermented odour are further indications. RPW attacked the coconut palms in three different ways. The first route is via the stalk and directly to the coconut's cabbage. The second route is through the trunk, where adults of RPW either create holes in the trunk or use holes made by other insects. *O. rhinoceros* (Coconut rhinoceros beetle) often creates the holes. The third route is through the root system, where adults of RPW have excavated the soil and tunnelled into the root system of the coconut palm (Azmi et al., 2017). The drooping of dried leaves, forming an umbrella-like shape, is one of the most severe indicators of infested coconut palms. However, this symptom is regarded as trivial and does not cause the death of the palms for six to eight months.

In Malaysia, strategies for managing the RPW have resorted to cypermethrin spraying and soil drenching to prevent the spread of this pest. In addition, the Department of Agriculture (DOA) employs pheromone trapping using the aggregation pheromone to combat the RPW infestation. However, the efficacy of the control techniques is yet unknown, as infestations have steadily increased over the past decade (Azmi et al., 2017).

In recent years, natural parasites of RPW have been investigated intensively to determine their potential as biological control agents. In comparison to most insect pathogens, fungi infect their hosts through direct touch, piercing the insect cuticle. Both direct treatment and transmission of inoculum from treated insects or cadavers to untreated insects or following developmental stages via the new generation of spores can infect the host (Quesada-Moraga et al., 2004). Due to these distinctive characteristics, entomopathogenic fungi are particularly useful for the management of RPW. One of the naturally occurring entomopathogenic fungi, *Metarhizium anisopliae* (*M. anisopliae*), has been the subject of extensive investigation to ascertain its potential as a biological control agent.

Water-in-oil invert emulsions are the most commonly used type of invert emulsion. They consist primarily of two liquid phases: a dispersed phase (water) and a continuous phase (oil). Emulsifiers that are added to these emulsions are typically included in the ingredients of the two phases prior to their mixing; they may be oil-soluble or water-soluble (Batta, 2016). The present study aimed to develop an invert emulsion-based formulation containing *M. anisopliae* as a biocontrol against RPW.

## MATERIALS AND METHODS

### Chemicals and materials

The *M. anisopliae* used in the present work was obtained from Universiti Malaysia Terengganu (UMT). The inverted emulsion consists of two phases: (i) water or aqueous phase comprising sterile distilled water, glycerine, and water-soluble emulsifier; and (ii) oil phase comprising oil (preferably of plant-origin) and oil-soluble emulsifier. Potato dextrose agar (PDA), Tween-20 from Sigma Aldrich (USA), ultrapure water (Direct Q3, Millipore, Billerica, MA, USA) were used throughout the study. Five food-grade plant oils, including palm oil, sunflower oil, olive oil, canola oil and soybean oil, were bought from a supermarket in Putrajaya.

### Preparation of invert emulsion containing *M. anisopliae*

An invert emulsion consisting of *M. anisopliae* was produced according to Batta, 2016. The oil phase consisted of a mixture of canola oil (24% w/w) and sunflower oil (24% w/w), and surfactant (Tween 20) (2% w/w) were stirred for 2 minutes. Then, the water phase (42.25% w/w) containing glycerine (4% w/w), water soluble emulsifier (0.75% w/w) and *M. anisopliae* ( $1 \times 10^6$  spore  $\text{mL}^{-1}$ ) were added to the oil phase and stirred again using vortex for another 5 minutes at  $2000 \times g$  to form a coarse emulsion. The fine emulsion was prepared by homogenizing the coarse emulsion using a homogenizer at  $3000 \times g$  for 1.5 minutes at room temperature ( $27^\circ\text{C}$ ). The produced invert emulsion from this process proceeded for stability test, polydispersity (PDI), size of the emulsion droplets characterization by dynamic light scattering method using Zetasizer (Brookhaven Instruments, USA) and the ability of the invert emulsion formulation containing *M. anisopliae* to grow on PDA.

## RESULTS AND DISCUSSION

Table 1. Physicochemical properties of the invert emulsion containing *M. anisopliae*.

Characteristic	Value
Appearance	Milky white
Droplet size	79.1 nm
PDI	0.208
Storage stability	No phase separation up to 6 months

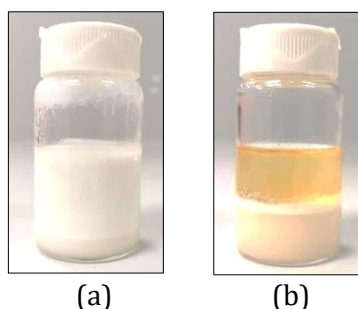


Figure 1. Observation on (a) a stable invert emulsion formulation without phase separation and (b) an example of unstable formulation showing 2 phases of separated formulation.

Other than that, the ability of *M. anisopliae* spores to grow on PDA was also be used to determine the efficacy of the developed formulation. The viability of *M. anisopliae* is essential, as the preparation process may have killed the fungus by affecting its viability. The result in Figure 2 showed that *M. anisopliae* spores in the invert emulsion were successfully cultured on PDA, indicating that they were viable and suitable for use as a biocontrol agent for RPW.



Figure 2. Observation of the growth of *M. anisopliae* on PDA.

When sprayed on RPW, the *M. anisopliae* spores in the invert emulsion will adhere to the cuticle with the aid of the oil in the formulation. The outer layer of *M. anisopliae* spores is composed of interwoven fascicles of hydrophobic rodlets that adhere to the insect cuticle due to non-specific hydrophobic forces (Gindin et al., 2006). The spores will eventually germinate and invade the interior, infecting the cuticular membrane of the host.

## CONCLUSION

Early tests conducted in our laboratory on the efficacy of formulated of *M. anisoplia* in invert emulsion revealed that the tested formulation was more effective than their unformulated. The advantage of using invert emulsion in the formulation of entomopathogenic fungi is that these emulsions contain the water necessary for the germination of fungal conidia during or after application, thereby enhancing the fungi's efficacy during application against targeted insects.

## ACKNOWLEDGEMENT

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# Plant Growth Promoting Activities of Endophytic and Epiphytic *Methylobacterium* sp. Isolated from Palm Oil (*Elaeis guineensis*) Leaves

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## INTRODUCTION

*Methylobacterium* sp. are pink-pigmented facultative methylotrophs (PPFMs) that can promote plant growth due to several physiological characteristics, for instance, the ability to produce phytohormones such as auxins and cytokinins and effective roles in phosphate solubilization and nitrogen fixation process. Association of endophytic and epiphytic *Methylobacterium* sp. were reported to regulate cell division and elongation of plant's shoots and roots and improve plant resistance toward abiotic and biotic stress (Zhang et al., 2021). These characteristics made *Methylobacterium* sp. a potential candidate for biofertilizer. In 2018, a new genus, *Methylobacterium* gen. nov., was proposed to accommodate 11 species previously held in *Methylobacterium*. These include *M. extorquens*, *M. zatmanii*, *M. populi* and *M. salsuginis* (Green and Ardley, 2018). This study aimed to elucidate the plant growth promoting activities of endophytic and epiphytic *Methylobacterium* sp. isolated from palm oil leaves by evaluating the production of indole-3-acetic acid (IAA), solubilization of inorganic phosphate and determination of seed vigour index (SVI).

## MATERIALS AND METHODS

### *Chemicals and materials*

### *Molecular identification of bacterial isolates by 16S rRNA gene analysis*

16S rRNA gene were amplified from the bacterial isolates using degenerate primers; 68F (5' TNA NAC ATG CAA GTC GAR 3') and 1392R (5' ACG GGC GGT GTG TRC 3'). Amplification was conducted with 30 PCR cycles using the following temperature profiles (initial denaturation, 95°C for 5 minutes; denaturation, 95°C for 1 min; annealing, 52°C for 30 seconds; elongation, 72°C for 1 minute and 30 seconds; final elongation, 72°C for 5 minutes). PCR products were sent for sequencing and DNA sequences were analysed using BLASTn software at [www.ncbi.nlm.nih.gov](http://www.ncbi.nlm.nih.gov).

### *3-indole-acetic-acid (IAA) assay*

IAA assay was conducted based on the method by Goswami (2014). Bacteria isolates were inoculated into ammonia mineral salts (AMS) broth and incubated at 36°C for 7-10 days until they reached OD<sub>600</sub> = ±0.4. 1000 µg/ml of L-tryptophan was added to the culture respectively and the cultures were further incubated for 7 days. After 7 days, the cultures were centrifuged at 9000 rpm for 15 minutes. The supernatant was filtered using a 0.22-µm membrane filter and mixed with 2 ml of Salkowski reagent and incubated in the dark at room temperature for 25 minutes. Then, the absorbance of the mixture solution was quantified spectrophotometrically at 530 nm.

### *Phosphate solubilization assay*

Bacterial isolates were inoculated into Pikovskaya's broth and incubated at 37°C for 7 to 10 days until the growth reached optical density (OD<sub>470</sub>) ±0.4. The cultures were then centrifuged at 9000

rpm for 15 minutes and the supernatant was filtered using a 0.22- $\mu$ m membrane filter. The supernatant was dissolved with 0.25 mL of vanadate-molybdate reagent and incubated for 10 minutes. After 10 minutes of incubation, colour changes started to develop. The absorbance of samples was quantified spectrophotometrically at 470 nm.

### Seed vigour index (SVI)

Mung bean seeds were surface sterilized, and 30 seeds were soaked overnight in 7 days old culture of each bacterial isolate. Then the seeds were placed on 0.7% solidified agar and incubated for 3-5 days. All experiments were carried out in triplicates. Germination % and seed vigour index (SVI) were calculated as the formula below:

Germination (%) = (Number of seeds germinated in a sample/Number of seeds germinated in the control)  $\times$  100%

Vigour index = Germination (%)  $\times$  (Mean root length + Mean shoot length)

## RESULTS AND DISCUSSION

As depicted in Table 1. *Methylobacterium* sp. ENPM1, *Methylobacterium* sp. ENPM2 and *Methylobacterium* sp. EPPM1 show the highest similarities to *Methylobacterium zatmanii* while *Methylobacterium* sp. ENPM3 shows the highest similarity to *Methylobacterium populi*. The genus *Methylobacterium* which previously contained the genus *Methylobacterium* were reported to be associated with several plant species. For instance, the presence of *Methylobacterium extorquens* MM2 isolated from mustard leaf was shown to promote the growth of tomato plant (Pattnaik et al., 2017).

Table 1. BLASTn analysis of 16S rRNA gene amplified from *Methylobacterium* sp. isolates.

Isolates	Highest Hit in NCBI Database	% Identity	Accession Number
<i>Methylobacterium</i> sp. ENPM1	<i>Methylobacterium zatmanii</i>	85%	NR_041031.1
<i>Methylobacterium</i> sp. ENPM2	<i>Methylobacterium zatmanii</i>	98%	NR_041031.1
<i>Methylobacterium</i> sp. ENPM3	<i>Methylobacterium populi</i>	87%	NR_074257.1
<i>Methylobacterium</i> sp. ENPPM1	<i>Methylobacterium zatmanii</i>	99%	NR_041031.1

*In vitro* plant growth-promoting activities of the endophytic and epiphytic *Methylobacterium* sp. were evaluated based on the production of phytohormone, 3-indole-acetic acid (IAA) and its ability to solubilize inorganic phosphate. As result shown in Figure 2, all the isolates demonstrated relatively low levels of IAA production with only *Methylobacterium* sp. ENPM2 demonstrating an IAA concentration of more than 0.1 mg/ml. A study by Senthilkumar and Krishnamoorthy (2017) reported the production of IAA by *Methylobacterium* sp. isolated from tomato leaves up to 10.2 mg/ml.

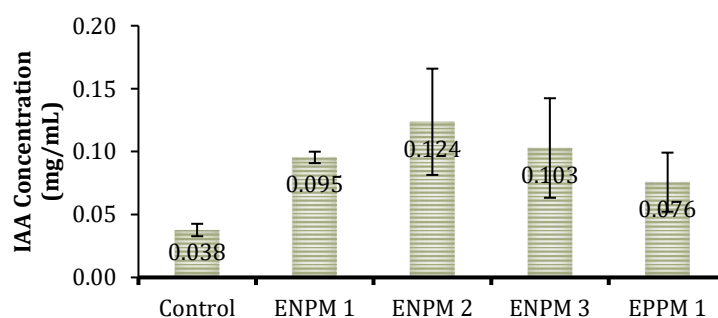


Figure 1. Production of 3-indole-acetic acid (IAA) by bacterial isolates.

Phosphate solubilization activity was determined quantitatively by using vanadium-molybdate assay. As shown in Figure 2, *Methylobacterium* sp. ENPM1 recorded the highest phosphate solubilizing activity followed by *Methylobacterium* sp. ENPM3, *Methylobacterium* sp. EPPM1 and the lowest by *Methylobacterium* sp. ENPM2. Phosphate solubilizing activity demonstrated by the *Methylobacterium* sp. isolates was relatively higher than other reported plant growth promoting *Methylobacterium* sp. (Grossi et al., 2020).

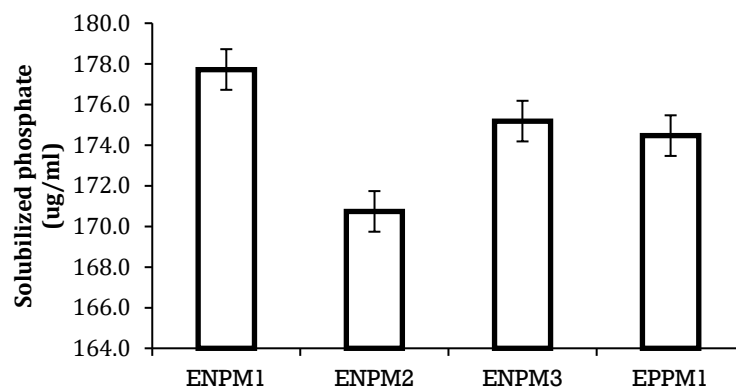


Figure 2. Phosphate solubilizing activity of bacterial isolates.

The ability of the bacterial isolates to enhance seed germination and increase roots and shoot length were determined by seed vigour index (SVI) and the result is tabulated in Table 2. All the four isolates showed SVI values higher than control with *Methylobacterium* sp. ENPM1 showing a higher SVI value of 1578 suggesting an improved seed germination rate and the ability to promote roots and shoot development. The range of SVI values demonstrated by all the isolates is higher than other reported *Methylobacterium extorquens* and *Methylobacterium zatmanii* (Senthilkumar and Krishnamoorthy, 2017; Shubaswaraj et al., 2017). This result can be provided as preliminary data for further *in vivo* analysis.

Table 2. Seed Vigour Index (SVI) of *Methylobacterium* sp. isolates.

Isolates	Germination Rate	Germination %	Vigour Index
<i>Methylobacterium</i> sp. ENPM1	28	100	1578
<i>Methylobacterium</i> sp. ENPM2	29	100	1374
<i>Methylobacterium</i> sp. ENPM3	27	100	1476
<i>Methylobacterium</i> sp. ENPPM1	27	97	1323
Control	27	96.7	1151

## CONCLUSION

Based on the observed results, *Methylobacterium* sp. ENPM1 has the potential to be exploited as a plant growth promoter since it shows relatively the highest phosphate solubilization activity and seed vigour index (SVI) compared to other bacterial isolates. However, further investigation such as pot study is needed to elucidate its plant growth-promoting activity *in vivo*.

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# Sustainable Sime Darby Plantation Palm Oil Mill Economic Circularity Potentials

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## INTRODUCTION

Sustainability is pledged via commitments towards the global palm oil certifications to ensure the sustainable food supply chain. Sime Darby Plantation (SDP) for instance revised policies in 2020 which were launched earlier namely Responsible Agriculture Charter (launched in 2016) and Human Rights Charter (published in 2017), believing in implementing sustainable practices and commitment of 'No Deforestation, No Peat and No Exploitation' (NDPE) (Sime Darby Plantation Berhad, 2020; 2021). These SDP pursuits are in line with the Sustainable Development Goals (SDG) (United Nations, 2022) with the sustainability purposes for people, planet and prosperity, ensuring a better society contribution, a minimised environmental harm and a delivered sustainable development, respectively. SDP embraces SDG 12 and 17 contributing approaches of Responsible Consumption and Production and Partnerships for the Goals, respectively (Sime Darby Plantation Berhad, 2021).

SDP has been an active player in reviewing the Malaysian Sustainable Palm Oil (MSPO) certification scheme (MSPO standard (Salleh et al., 2020)) besides itself being also 100% certified POM by the Roundtable on Sustainable Palm Oil (RSPO), all in Malaysia, Indonesia and Solomon Islands, contributing 14.7% of the global Certified Sustainable Palm Oil (CSPO) (Sime Darby Plantation Berhad, 2021). In addition to that, SDP is also 100% Malaysian Sustainable Palm Oil (MSPO) and Indonesian Sustainable Palm Oil (ISPO) certified for the upstream.

Generally, to gain the global credibility on palm oil sustainable productivity, carbon footprint programs (environmental related) towards economic circularity are adopted by the palm oil industrial players. Environmental sustainability ranges from biodiversity, deforestation, environmental pollution and peatland conversion, involving sustainability practices and implementation as well as planning and policy making which are beneficially crucial for sustainability certifications (Tang and Al-Qahtani, 2020). With this it means that not only the sustainability of the environmental aspect is ensured but also on the social and economic performance (Tang and Al-Qahtani, 2020) covering from plantations and smallholders to productions involving POM (upstream) and refinery (downstream) operations. Thus, in this article however, only on the upstream technological advances (future or recent) particularly on POM operations aligning with the SDG, sustainable policy and POM economic circularity are presented.

### ***Palm Oil Mill (POM) economic circularity***

The POM economic circularity covers the aspects on how the resources (i.e. 9.2 Million Metric Tonne fresh fruit bunch (Million MT FFB)/2021) are managed and on how the products/by-products are used afterwards in SDP. Figure 1 shows the SDP value chain for the upstream and downstream of SDP. In relation to economic circularity, particularly, the reusability of any by-products (B or C) back to the POM operations (A) can be regarded as options in net zero strategy and towards economic circularity.

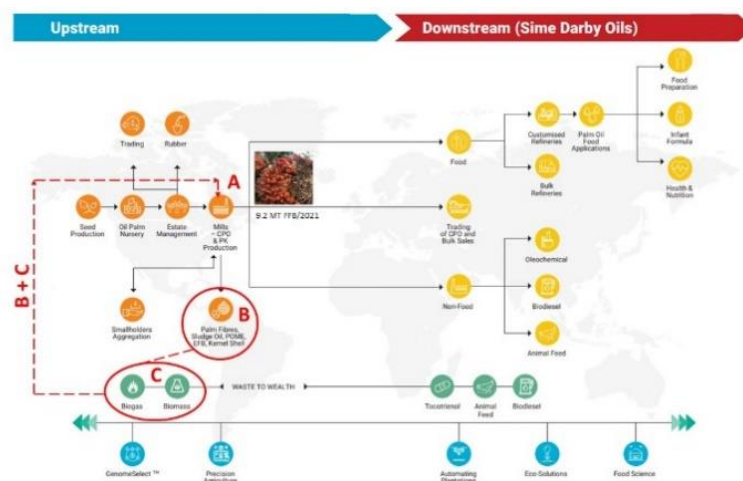


Figure 1. Sime Darby Plantation (SDP) value chain for upstream and downstream (Sime Darby Plantation Berhad, 2021).

In POM, useful solid or liquid organic by-products such as palm oil mill effluent (treated POME reuse for field irrigation), mesocarp fibre, palm kernel shells (fibre and PKS reuse as fuel for boilers), and empty fruit bunch (EFB reuse as plantation compost) wastes contain high energy/nutritional contents. While, utilisation of the liquid POME for electricity production to be used back to POM operation is adopted via biogas development in SDP. In comparison to 2019, a slight decreasing volume of the processed FFB [MT] (2020 and 2021) due to the COVID-19 restrictions was observed (Figure 2), in which, however, a slight increase in the average POME intensity for Malaysia of 0.65 (2020) and 0.64 (2021) cubic meters POME per Metric Tonne of processed FFB ( $\text{m}^3/\text{MT FFB}$ ), respectively.

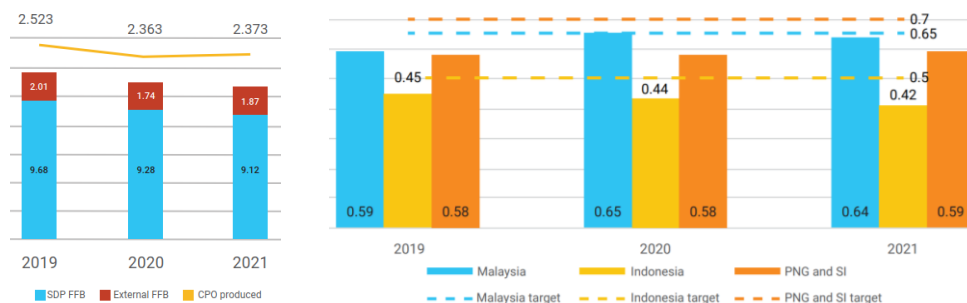


Figure 2. Estimated million tonnes of processed Fresh Fruit Bunch (FFB) and Crude Palm Oil (CPO) [Million MT] produced (left) and average POME intensity [ $\text{m}^3/\text{MT FFB}$  processed] (right), for the year 2019 to 2021 (Sime Darby Plantation Berhad, 2021).

The SDP history of biogas initiative has started since early 2000 with focus work on the utilisation of the biogas in 2010 (for 3 years). A collaborative research project initiated between Sime Darby Plantation Research Sdn. Bhd. (SDPR) and SIRIM to develop the first bio-natural gas pilot plant in Pulau Carey using POME via methane fermentation (biological anaerobic digestion) which underwent purification and enrichment for methane storage and dispensing. Ever since, the initiative has led to newer biogas development for electricity for POM operations. In addition to the MPOB new regulation on biogas development effective January 2014, visioning by 2020, mandatory implementation at all POMs to have biogas trapping facilities, SDP has so far as of December 2021, 12 operational biogas plant across Malaysia. SDP has been strategizing for carbon footprint reduction of up to 40% with the planned operation of 60 biogas plants by 2030. The latest target so far has increased up to 50% reduction of carbon footprint which is equivalent to Greenhouse Gas (GHG) emission intensity of 0.53 Metric Tonnes of carbon dioxide per Metric

Tonne of Crude Palm Oil (CPO)/palm kernel(PK) [MT CO<sub>2</sub>e/MT CPO/PK] (Sime Darby Plantation Berhad, 2021).

Figure 3 indicates the SDP digester tank in a biogas plant in POM (left) and the achieved MT CO<sub>2</sub> reduction in 2020 and 2021 (right). A total of 9 and 12 biogas plants were installed by SDP in the year of 2020 and 2021, theoretically achieving an estimated 428,603 and 499,617 MT CO<sub>2</sub>e being avoided yearly, respectively. Another challenge is to sequester the significant emission source of 66%, 12%, 8% and 6% MT CO<sub>2</sub>e (Figure 4) (left) from POM processing methane emission from treating effluent, POM boilers, fertiliser use at plantation and purchased electricity, respectively, in support of the net zero strategy in SDP. Increasing production is always synonymous to energy usage and GHG emission. Figure 4 (right) indicates that renewable energy contributes of more than 80% of the energy consumption in gigajoules [GJ] since 2017 to 2021 in SDP, namely from biomass, biodiesel and other liquid biofuels (supporting economic circularity), increasing the revenue and profit before interest and tax (data not shown).

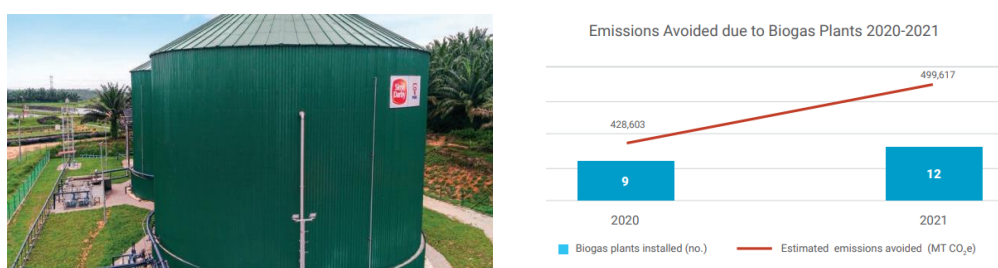


Figure 3. Sime Darby Plantation (SDP) digester tanks for biogas plant in palm oil mill (POM) (left) and the consequent Metric Tonnes carbon dioxide of total emission [MT CO<sub>2</sub>e] reduction via biogas plant (right) (Sime Darby Plantation Berhad, 2021).

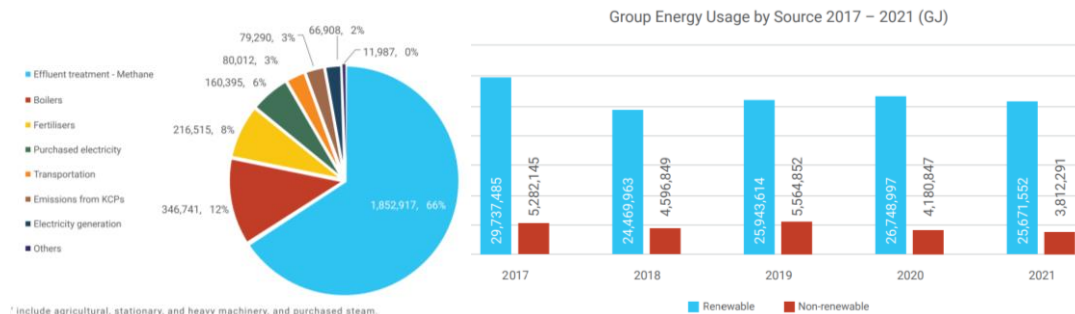


Figure 4. Emission source calculated by Greenhouse Gas (GHG) Protocol methodology (left) and upstream usage of energy in gigajoule [GJ] from renewable and non-renewable energy (right) (Sime Darby Plantation Berhad, 2021).

### ***Other palm oil mill (POM) efficiency projects***

SDP Tennamaran Experimental Station (TEST), pilot plant scale for POM operation, was designed to run investigations on milling of FFB for CPO production. Few engineering projects for instance the CPO production effectiveness (via biocatalyst), optimization of CPO production (digester and screw press engineering parameters), artificial intelligence (AI) technique of the reduction of fresh fruit losses for POM pressing and skimming effectiveness for CPO production are planned. The engineering and process control full automation are also visioned for upgrades to increase the POM economic circularity in SDP.



## CONCLUSION

POM economic circularity with engineering sustainable solutions being actively investigated and proposed are crucial to support the SDP net zero strategy and aspirations, vision to support SDG and SDP profit gains while reducing the existing operational costs.

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## NOTES



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