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The 4th International Conference in Agriculture, Animal Sciences, and Food Technology (ICAFT 2025)

Guest Editors

Azman Azid, Veryl Hasan, and Isa Baba Koki

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Preface

The 4th International Conference on Agriculture, Animal Sciences, and Food Technology (ICAFT 2025) serves as a vibrant platform where researchers, academicians, and industry professionals convene to share insights, innovations, and solutions that advance sustainable practices in agriculture and food systems. The conference, proudly organised by the Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin (UniSZA), Malaysia, gathers global participants at the Paya Bunga Hotel, Kuala Terengganu, Malaysia, on 11–12 August 2025.

The papers presented at the ICAFT 2025 are now published in the Proceedings of ICAFT 2025, hosted under the *Pertanika* Journal of Tropical Agricultural Science. This publication encapsulates the spirit of the conference theme — "Sustainable Synergies: Bridging Agriculture, Food Innovation, Animal Science, and Environmental Sustainability." It highlights the interdisciplinary collaboration and research advancements that drive transformation across agricultural and food-related sectors in facing future global challenges.

Research contributions such as "Isolating and Characterising Phosphate-solubilising Bacteria from Oil Palm and Forest Soils for Improved Agricultural Practices", "Palm Kernel Expeller and Empty Fruit Bunch Inclusion in Beef Cattle Feed Formulation and Rumen Fermentation", "Indoor Hydroponic Growth of *Labisia pumila* Clone", and "Nutritional Composition and Protein Quality of the *Keropok Lekor* By-products" reflect the conference's commitment in fostering innovative research with practical implications for sustainable resource management, animal nutrition, and food technology development.

This proceedings is a testament to the collaborative spirit of our scientific community — a convergence of minds dedicated in advancing agricultural and environmental sciences through research and innovation. We extend our sincere appreciation to *Pertanika* and the UPM Press Centre for their support in realising this publication, and our heartfelt gratitude to all keynote speakers, reviewers, participants, and the organising committee for their commitment, hard work, and intellectual contributions that have made the ICAFT 2025 a success.

Guest Editors
Azman Azid (Associate Professor Dr.)
Veryl Hasan (Dr.)
Isa Baba Koki (Dr.)

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Auxin Priming Promotes Seed Germination and Seedling Growth of Spinach (*Amaranthus tricolor*)

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ABSTRACT

Seed priming is a successful method to promote germination in agriculture production. The result of auxin priming in promoting the germination and seedling growth of *Amaranthus tricolor* remains to be examined. Therefore, the aim of this research was to examine the effects of auxin priming on seed germination and seedling growth of *A. tricolor*, a leafy vegetable with high nutritional value and potentially contribute to food security, which in line with SDG 2 (Zero Hunger) and SDG 12 (Responsible Consumption and Production). In this study, 50 seeds of *A. tricolor* were soaked in each Petri dish contained three different concentrations of auxin [A0- 0 µM (control), A1- 50 µM, and A2- 100 µM] for eight hours before germinate on the germination paper. The Petri dishes were laid out based on a completely randomised design (CRD) with five replicates. Data for seed germination and seedling growth of *A. tricolor* were recorded. The data were analysed using the one-way analysis of variance (ANOVA) followed by the Duncan's post-hoc test. Results showed that A1 and A2 significantly enhanced germination percentage and germination index meanwhile only A2 significantly increased seedling length and hypocotyl length compared to the control. These finding suggests that auxin priming promotes seed germination and seedling growth of *A. tricolor*, potentially improving agricultural productivity.

Keywords: Auxin priming, *A. tricolor*, indole-3-butyric acid, germination, seedling growth

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INTRODUCTION

Seed germination happens when the radicle protrudes from seed with the presence of optimum phytohormones, water, light and temperature (Farooq et al., 2022). The process of germination happens in three phases; first is imbibition, second is activation phase and third is root protrusion (Zulfiqar, 2021). The process of seed germination involves two kinds of hormones, first is abscisic acid (ABA) and second is gibberellic acid (GA) which act antagonistically (Farooq et al., 2022).

Auxein in Greek word meaning to grow (Gomes & Scortecci, 2021) or to expand (Jing et al., 2023). Auxin is a phytohormone acts as a key regulator of plant physiological processes (Cohen & Strader, 2024) and promotes the formation of plant organs (Gao et al., 2024). A few studies reported that auxin able to alleviate abiotic stress (Yu et al., 2022). On the other hand, auxin crosstalk with other hormones like abscisic acid (ABA) and jasmonic acid (JA) antagonistically (Ali et al., 2025).

In this study, seed priming method was implemented to improve seed germination rates (Pappalettere et al., 2024). Priming is classified into different categories depend on the priming agents (Liu et al., 2022). Current study used auxin as hormonal priming method. This method has been successfully implemented to stimulate and coordinate germination (Pereira et al., 2021).

Amaranthus tricolor L., a purple-red-green leafy vegetable belongs to the genus *Amaranthus* in the family Amaranthaceae (Jahan et al., 2022) and order Caryophyllales (Wang et al., 2023) is widely called as Bayam Separa Merah in Malaysia. It was reported that *A. tricolor* contains high carbohydrate (39.80%), protein (26.60%), potassium (1080.02 mg/100g), calcium (39.76 mg/100g) (Jahan et al., 2022), phenolic content (TPC), and total flavonoid content (TFC) (Sarker et al., 2024). Hence, these specialities attract food industry player interest to invent supplement benefits to human health (Sarker et al., 2022).

There are several reports on the effects of auxin priming on seed germination and seedling growth of *Gossypium hirsutum* L. (Zhao et al., 2020) and Abelmoschus esculentus L. (Sarath et al., 2022). However, the study on auxin priming in promoting the germination and seedling growth of A. tricolor remains to be examined. The objectives of current research are to (1) determine the effects of auxin priming on seed germination and (2) investigate the effects of auxin priming on seedling growth of *A. tricolor*.

MATERIALS AND METHODS

Experimental Design

This research was conducted in the Biology Laboratory, UCYP University. *A. tricolor* seeds (Crop Power, Malaysia) and auxin (SERBAJADI, Malaysia) were used in this experiment. Fifty of *A. tricolor* seeds were soaked in different Petri dishes containing three concentrations of auxin [A0- no auxin (control), A1- 50 µM (8.76 mg/L of auxin)

and A2- 100 µM (17.52 mg/L of auxin)] for eight hours before sowing (Lyalina et al., 2023). Each of the Petri dishes was arranged based on a completely randomised design (CRD) with five replications (Table 1). After priming, *A. tricolor* was rinsed with water three times and was germinated on wet germination paper (Zhao et al., 2020). The seeds were maintained under temperature (24 ± 2 ºC) and relative humidity (60 ± 10 %) (Abdullahi et al., 2025). Data for seed germination and seedling growth were recorded from the first to the seventh day after sowing (DAS).

Table 1
Completely Randomised Design (CRD) with five replicates

A0R1	A1R2	A2R2
A1R3	A2R3	A0R2
A2R1	A0R3	A1R4
A0R5	A1R5	A2R4
A1R1	A2R5	A0R4

Determination of Seed Germination Parameters of *A. Tricolor*

Determination of Germination Percentage (G %)

Germination percentage (G %) of *A. tricolor* seeds were recorded on the first DAS based on the formula (Ellouzi et al., 2024):

$$G\% = \left(\frac{Number\ \ of\ Germinated\ \ Seeds}{Total\ Number\ \ of\ Seeds\ Sowed}\right) \times 100$$

Determination of germination index (GI)

The germination index (GI) was determined based on the formula below to measure the speed and uniformity of seed germination (Ismaeil et al., 2022):

$$GI = \sum \left(\frac{Gt}{T\tau}\right)$$

Where, Gt = Number of seeds germinated on first to third DAS; Tt = Number of days from first to third DAS

Measurement of Seedling Growth Parameters of *A. Tricolor*

Measurement of Seedling Length and Hypocotyl Length

Seedling length was measured starting from the tip of the plumule to the end of the radicle (Ali et al., 2021) meanwhile hypocotyl length was measured from the cotyledon to the radicle (Yaakob et al., 2020) on seventh DAS by using ruler.

Statistical Analysis

The collected data were subjected to one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test by using Statistical Package for the Social Sciences (SPSS) version

29. The mean values were considered significant when $p < 0.05$. All data were presented as mean ± standard deviation (STD).

RESULTS AND DISCUSSION

Effects of Auxin Priming on Seed Germination Parameters of *A. Tricolor*

Germination Percentage (G %)

The effects of auxin on germination percentage of *A. tricolor* were presented (Figure 1A). Different letters show there are significantly different ($p<0.05$). The germination percentage of *A. tricolor* treated with A1 (98.4%) and A2 (99.2%) increased significantly compared to the treatment A0 (32%). However, treatment A1 and A2 showed no significant differences. The results of current study align with previous report that highlight the critical role of auxins in seed germination and early plant development (Mekonnen et al., 2024). Moreover, germination percentage of hormonal primed *D. carota* seed was the second highest (84%) compared to hydro primed (85%) and nutri primed (77%) (Dessalew et al., 2022). In contrast, other study reported that high concentration (50, 100 and 150 ppm) of auxin inhibits the germination of onion seeds (Song et al., 2020).

Germination Index

According to Figure 1B, *A. tricolor* treated with A1 (90.87) and A2 (91.27) were significantly higher compared to treatment A0 (56.87). However, the germination index of treatment A1 and A2 have no significant differences. Earlier study has presented that auxin is also involved in the transformation of seed from dormant stage to germination (Wu et al., 2020). Instead of that, biopriming with seaweed and microbes increased germination index of *Abelmoschus esculentus* (L.) Moench (Makhaye et al., 2021). Conversely, germination index treated with sheep manure was high compared to pig and chicken manure due to high ammonium nitrogen content (Wang et al., 2022).

Effects of Auxin Priming on Seedling Growth Parameters of *A. Tricolor*

Seedling Length

Auxin priming effects on seedling length are displayed in Figure 1C. Seedling length treated with A1 (2.25 cm) was insignificantly higher than A0 (1.91 cm) meanwhile A2 (2.35 cm) was significantly higher than A0. Earlier study reported that hormo priming with gibberellic acid enhanced seedling length of *Tanacetum parthenium* (L.) Sch. Bip. Auxin might increase enzyme activity and enhance plasma membrane integrity (Alizadeh et al., 2022). Furthermore, bio priming with *Trichoderma virens* increased seedling length of *Glycine max* L. The increment of seedling length may be due to the increment of zinc and iron uptake (Dhal et al., 2022).

Hypocotyl Length

Figure 1D presented that the hypocotyl length treated with A2 (1.08 cm) is significantly higher compared to A1 (0.81 cm) and A0 (0.62 cm). Auxin promotes hypocotyl elongation (Yu et al., 2023) by enhancing the BZR1, a central component of the brassinosteroid (BR) signalling pathway which is vital for plant growth (Yu et al., 2023). The production of antioxidant in sprouts treated with plant hormone increased compare to the untreated sprouts (Yan et al., 2024). In contrast, hypocotyl length of hormo and nutri primed *L. sativa* seed was decreased than control (Adhikari et al., 2022).

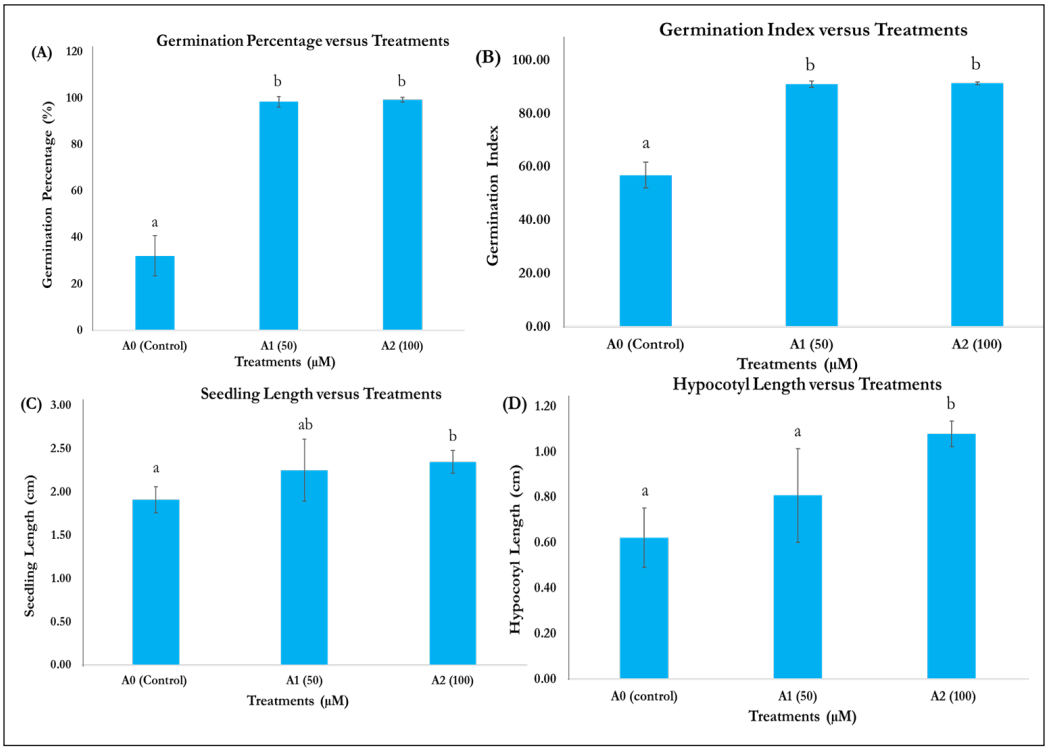

Figure 1. Effects of auxin priming on germination percentage (A), germination index (B), seedling length (C) and hypocotyl length (D) of *A. tricolor*. The results are indicated as mean value ± standard deviation. Different letters show there are significantly different ($p<0.05$)

CONCLUSION

The results of this study emphasize that auxin priming could enhance seed germination and seedling growth parameters of *A. tricolor*. This study suggests that 100 µM IBA is the optimum concentration for enhancing germination and seedling growth. It is recommended that further research conduct in field condition to validate the effects of auxin priming under abiotic stress on other important parameters.

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Estimating the Cost of Rearing Charolais Mixed Breed (Lembu Sado) for Small-scale and Semi-scale Farms in Terengganu and Kelantan

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ABSTRACT

Charolais mixed breed, or Lembu Sado, is a beef cattle breed popular in Malaysia, valued for its high meat quality and adaptability. However, many Lembu Sado farmers lack awareness of the exact rearing costs, which leads to poor cost management and affects their profitability. Therefore, this study aims to assess the socioeconomic status of Lembu Sado farmers and estimate the cost of rearing Lembu Sado in Terengganu and Kelantan. A quantitative approach was employed using a structured questionnaire divided into three sections: demographic information, farm characteristics, and cost and revenue. Data were collected through a convenience sampling, with 86 respondents from Terengganu and 50 from Kelantan, facilitated by DVS officers in both states. The study population comprised Lembu Sado farmers in these regions, and the data were analysed descriptively using IBM SPSS 2.0 and Microsoft Excel. The findings indicate that the average Lembu Sado farmer is 40 years old, has six years of farming experience, and has a household size of three. Most farmers have attained a secondary level of education, engage in off-farm income activities, and receive limited government support. The estimated cost of rearing Lembu Sado for small-scale farms is RM9,750.07 in Terengganu and RM7,540.04 in Kelantan. For semi-commercial farms, the estimated cost is RM42,806.05 in Terengganu. In conclusion, there is a cost difference between the two states. Therefore, the findings assist policymakers and agricultural agencies develop new strategies and guidelines, as well as implementing solutions such as providing subsidies, and training programmes, tailored to the specific needs of small-scale and semi-commercial Lembu Sado farmers.

Keywords: Charolais cattle, rearing cost, socioeconomics, Lembu Sado, mixed breed cattle

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INTRODUCTION

Livestock farming is vital in supporting the sustenance and economic livelihood of more than 1.3 billion people (Aguirre et al., 2024). Lembu Sado, a local term for mixed-breed cattle. In 2023, Malaysia's beef utilisation for food amounted to 224,110.3 tonnes, which can be interpreted as the level of domestic demand (Department of Veterinary Services, 2024). With a self-sufficiency ratio of 15.9%, local production was at 38667.2 tonnes, while imports reached 205,246.9 tonnes to bridge the gap (Department of Veterinary Services, 2024). These figures highlight Malaysia's heavy reliance on external sources, with more than 80% of beef demand met through imports. On a consumption basis, the per capita beef intake was recorded at 6.7 kg per person per year, equivalent to just 18.4 grams per person per day (Department of Veterinary Services, 2024). Nevertheless, the growing demand relative to stagnant production raises important implications for food security, particularly in the face of global price fluctuations and supply chain disruptions.

LITERATURE REVIEW

Breed Characteristics

The indigenous Kedah-Kelantan (KK) cattle are well adapted to Malaysia's tropical climate and valued for beef production due to their heat tolerance and disease resistance (Islam et al., 2022). To reduce imports, crossbreeding programmes have been promoted in Terengganu and Kelantan (Department of Veterinary Services, 2022). Lembu Sado, a Charolais–KK cross, mature earlier, produce more milk, and wean heavier calves but require higher energy intake (Radzil et al., 2023). Mature males weigh 500–1000 kg and females 300–600 kg (Hisham et al., 2022).

Cost Studies

A cost study examines all expenses in production; here, it refers to rearing Lembu Sado. Fixed costs, such as land rental, remain constant, while variable costs, such as feed, fluctuate with production. Although a Lembu Sado can sell for up to RM30,000, rearing costs are high. Larger producers with cheaper prices pose a serious threat to small-scale farmers, and many investors would rather import beef than help out local breeding farmers (Radzil, 2024).

MATERIALS AND METHODS

This study categorised Lembu Sado farms into small-scale and semi-commercial. Small-scale farms, typically with fewer than five cattle, rely on family labour, minimal inputs, and produce mainly for household use or local sales. In contrast, semi-commercial farms, with 10 or more cattle, target market sales, adopt supplementary feeding and housing, employ veterinary services, and use both family and hired labour to generate sustainable income.

Study Area

This quantitative study was conducted in Terengganu and Kelantan, where agriculture and livestock are key economic activities. Terengganu hosts a major Lembu Sado breeding centre, around 5,000 farmers (Hisham et al., 2022), while Kelantan has around 5,500 farmers and 24,291 cattle (Alias, 2023). These states were therefore suitable study areas.

Method of Data Collection and Research Sampling

Data were collected from 86 respondents in Terengganu and 50 in Kelantan using convenience sampling, with farmer lists provided by the DVS. GPower 3.1.9.4 indicated a minimum sample size of 98, and 136 valid responses were obtained. A structured questionnaire, reviewed by experts, covered three sections: demographics, farm characteristics, and cost/revenue, using both open- and closed-ended questions.

Method of Data Analysis

Data were analysed using Statistical Package for the Social Sciences (SPSS) to assess farmers' socioeconomics and Excel to estimate rearing costs for small- and semi-scale farms in Terengganu and Kelantan (Table 1).

This study calculates the production costs according to different farm scales and areas. The production costs were grouped into two costs, namely variable costs and fixed costs. The data on beef production costs were;

1. Total variable costs (TVC) - Consisting of feed, fertilizer, pesticide, grass seed, chemical, salary, veterinary, vaccine, deworming, medicine, AI cost, utilities, petrol, rental, transportation, and maintenance.
2. Total fixed costs (TFC) - Including the depreciation cost of the building, equipment, and machinery.
3. Total revenue (TR) - Consisting of sales of calves, slaughtered, and bred.

Table 1
Variables in socioeconomics and their units

Variables in Socioeconomics	Units
Age	Years
Education	No Education, Primary, Secondary, Diploma, and Degree
Experience	Years
Household Size	Number of Persons
Distance from Home	Kilometres (KM)
Off-farm Income	Malaysian Ringgit (MYR)
Government Support	Malaysian Ringgit (MYR)

4. Gross profit - Revenue – Variable Costs (measures earnings before fixed costs).
5. Net profit - Revenue – Total Costs (Variable + Fixed Costs) (final earnings after all expenses).

Ethical Issue of the Study

This study has followed standard research ethics protocols. Respondents were informed about the purpose of the research, their right to refuse or withdraw, and the confidentiality of responses. Participation was entirely voluntary, and results were reported without revealing any personally identifiable information. Farmers were informed with the assistance of DVS Terengganu and Kelantan officers, before the study instruments were distributed. The data were used solely for academic purposes.

RESULTS AND DISCUSSION

Socioeconomics of Lembu Sado Farmers in Terengganu and Kelantan

Table 2 shows that most farmers are in their 40s, with an average of 6.9 years of experience and a household size of three. Education levels are modest: 66.2% completed secondary school, 23.5% a diploma, and 5.9% a degree. Farms are generally close to homesteads (average 1.75 km, up to 20 km). Most farmers (85.3%) rely on off-farm income, while only 18.4% receive government support.

Table 2
Socioeconomic characteristics of Lembu Sado farmers in Terengganu and Kelantan (n = 136)

Characteristics	Min	Max	Mean	Standard deviation	Frequency	Percentage
Age	23 years old	65 years old	40.13	9.608		
Education:	0	4	2.30	0.683211		
0) No Edu					3	2.2
1) Primary					3	2.2
2) Secondary					90	66.2
3) Diploma					32	23.5
4) Degree					8	5.9
Experience	2	18	6.88	4.893		
Household Size	0	10	3.07	2.457		
Distance from Home	0	20	1.75	3.565		
Off-farm Income			0.85	0.355	No – 20 Yes - 116	14.7 85.3
Government Support			0.18	0.389	No – 111 Yes -25	81.6 18.4

The Cost of Rearing Lembu Sado for Small-scale and Semi-commercial Farms in Terengganu and Kelantan

Table 3 shows that a sold Lembu Sado can earn RM20,912.42 for a small-scale farm in Terengganu and RM16,794.30 in Kelantan. A farm generates income through sales, which forms the basis of profitability. While costs can affect the profitability, variable costs (e.g., feed, labour, veterinary expenses) and fixed costs (e.g., depreciation) will reduce the revenue that turns into profit. A total of RM7,642.18 is calculated in Lembu Sado rearing in Terengganu and RM3,715.58 in Kelantan for variable costs, with salary being the highest contributor towards variable costs, followed by feed, and maintenance for Terengganu and utilities for Kelantan traditional farm. Contrary to a study by Lumenta et al. (2024) the most significant cost in the cattle farming business in Sangkub district is feed, amounting to RM4,849.14, equal to 18,208,074 IDR. The fixed costs include depreciation of vehicles, buildings, and machinery, with a total of fixed costs of RM 2,107.89 per head in traditional farm in Terengganu and RM 3,824.46 in Kelantan. The small-scale farm net margin is RM12,171.75 in Terengganu and RM9,254.26 for Kelantan, indicating a difference of RM2,917.09 compared to Terengganu. The lower net margin from Kelantan farms is primarily due to revenue. The differences in revenue mean that Kelantan earns less than Terengganu, even though Kelantan farms' operating costs are lower than those of Terengganu farms, which conforms to the findings from Radzil et al. (2023), a profit of less than RM10,000 per year (39.8 percent) was made. Revenue is closely associated with the pricing market. Selling price is determined based on the product market price, and it is often affected by demand for the product and the number of demands (Lumenta et al., 2021). In contrast, semi-commercial farms earned RM13,976.18 in revenue but faced much higher costs (RM27,794.39) in variable (mainly salaries, feed, AI) and RM15,011.66 in fixed leading to a gross margin of RM13,818.21 and a net loss of RM28,829.87. This indicates severe larger scale production generating significant losses.

Comparison of Small-scale and Semi-commercial Farms

Kelantan's small-scale farms had the lowest variable costs, giving a total cost of RM7,540.04, which was RM2,210.03 less than Terengganu's small-scale farms and RM35,266.01 less than semi-commercial farms. Their fixed costs were higher than Terengganu's due to building depreciation, but still manageable. Overall, Kelantan's small-scale farms achieved a net margin of RM9,254.26 and a profit of RM13,078.72, while Terengganu's small-scale farms, despite higher costs, earned an even higher margin of RM12,171.35. In contrast, Terengganu's semi-commercial farms generated only RM13,976.18 in revenue and recorded large net losses, showing that larger scale does not guarantee profitability. Profitability depends on efficient cost management, with unpaid family labour helping reduce expenses (Radzil, 2024).

Table 3
Enterprise budget of the small-scale and semi-commercial farms in Terengganu and Kelantan

Category	Unit (Head)	Terengganu Small-scale Farm		Unit (Head)	Kelantan Small-scale Farm		Unit (Head)	Terengganu Semi-commercial Farm	
A. Revenue		**Total (RM)**	**Total RM (per-head)**		**Total (RM)**	**Total RM (per-head)**		**Total (RM)**	**Total RM (per-head)**
Slaughtered	207	1,201,300	5,803.38	65	304,160	4,679.38	30	100,000	3,333.33
Breed	146	1,490,700	10,210.27	130	1,085,800	8,352.31	20	150,000	7500
Sale of calves	103	608,500	5,907.76	101	380,024	3,762.61	35	110,000	3,142.85
Total gross revenue			20,921.42	65	304,160	16,794.30			13,976.18
B. Variable cost									
Feed	L/S	1,486.54		L/S	644		L/S	6,541.11	
Fertilizer	L/S	337.54		L/S	249.92		L/S	983.33	
Pesticide	L/S	60.46		L/S	13.6		L/S	14,600	
Grass seed	L/S	49.39		L/S	57		L/S	233.33	
Chemical	L/S	78.76		L/S	18		L/S	66.66	
Salary	L/S	2,939.02		L/S	1,116		L/S	16.66	
Veterinary	L/S	89.15		L/S	116.6		L/S	733.33	
Vaccine	L/S	38.98		L/S	94.7		L/S	1,106.66	
Deworming	L/S	157.65		L/S	178.86		L/S	746.66	
Medicine	L/S	241.24		L/S	170.8		L/S	1,766.66	
AI cost	L/S	304.04		L/S	69.9		L/S	166.66	
Utilities	L/S	389.5		L/S	313.4		L/S	500	
Petrol	L/S	459.52		L/S	135.8		L/S	333.33	
Rental	L/S	118.07		L/S	159				
Transportation	L/S	307.97		L/S	183				
Maintenance	L/S	584.35		L/S	195				
Total variable cost (RM)			7,642.18			3,715.58			27,794.39

Table 3 *(continue)*

Category	Unit (Head)	Terengganu Small-scale Farm	Unit (Head)	Kelantan Small-scale Farm	Unit (Head)	Terengganu Semi-commercial Farm
C. Fixed cost						
Depreciation of the vehicle	L/S	635.29	L/S	165.96	L/S	5,100
Depreciation of the building	L/S	1,118.31	L/S	2,090.52	L/S	9,183.33
Depreciation of machinery	L/S	354.29	L/S	1,567.98	L/S	728.33
Total fixed cost (RM)		2,107.89		3,824.46		15,011.66
D. Total cost (RM) *B+C*		9,750.07		7,540.04		42,806.05
E. Gross margin (RM) *A-B*		14,279.24		13,078.72		-13,818.21
F.Net margin (RM) *A-D*		12,171.35		9,254.26		-28,829.87

CONCLUSION

In conclusion, Lembu Sado farmers in Terengganu and Kelantan are on average 40 years old, with six years of experience, and small households. Most have secondary education, off-farm income, and little government support. The estimated net margin is RM12,171.35 in Terengganu and RM9,254.26 in Kelantan, while semi-commercial farms face heavy losses (−RM28,829.87) due to high costs. Overall, profitability depends more on efficient cost management than farm size, for instance small-scale farms in Kelantan showing better financial outcomes. These findings highlight the need for improved cost management, financial support, and policies to ensure Lembu Sado farming remains viable.

Limitations of the Study

This study has faced some limitations. Firstly, the research was carried out on a limited sample of Lembu Sado farmers in specific areas, which may not accurately reflect the entire population of beef farmers in Terengganu and Kelantan. Secondly, no pilot test was conducted prior to data collection due to limited time of the study. However, despite these limitations, the study provides valuable insights into the socioeconomic profile and production challenges of Lembu Sado farmers in Malaysia.

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Isolating and Characterising Phosphate-solubilising Bacteria from Oil Palm and Forest Soils for Improved Agricultural Practices

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ABSTRACT

Phosphate-solubilising bacteria (PSB) are important players in plant growth promotion. This study aimed to screen functionally active phosphate-solubilising bacteria (PSB) from oil palm and forests near oil palm plantations. The microbial composition of the two soil types was compared, revealing distinct differences. In forest soil, *Streptomyces* (31%) and *Bacillus* (23%) were the dominant genera, whereas in oil palm soil, *Burkholderia* (27%) was the most abundant, followed by *Streptomyces* (21%). Phosphate Solubilisation screening on Pikovskaya's agar identified 30 isolates producing halo zones indicative of tri-calcium phosphate Solubilisation. Dual-culture assays against *Ganoderma boninense* (PER71) shows six isolates with dual activity, exhibiting phosphate solubilising indices (2.30–4.22 cm) and radial growth inhibition of 50–70%. Further test on plant growth-promoting trait evaluation demonstrated (PSM 1, PSM 4, PSM 5, PSM 6, PSM 8 and PSM 9) produced Indole-3-Acetic Acid (IAA) in the range of 8.7-22.46 µg/mL. The findings highlight efficient PSB strains with dual functions in nutrient Solubilisation and pathogen suppression, offering a sustainable and eco-friendly approach for enhancing oil palm growth, disease management, and yield improvement.

Keywords: Phosphate Solubilising Bacteria (PSB), oil palm, *Ganoderma boninense,* plant growth promotion, soil microbial diversity

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INTRODUCTION

Phosphorus is an important macronutrient that essential for plant growth, together with nitrogen and potassium (Glaser & Lehr, 2019). Phosphate-solubilising bacteria (PSB) play a crucial role in converting soil-insoluble phosphates into bioavailable forms through various

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mechanisms, including the secretion of organic acids, enzyme production, and siderophore excretion (Puri et al., 2020).

Pathogens pose a significant threat to plant health by reducing plant yield and negatively impact food quality. Usage of chemical fertilisers for controlling phytopathogens has shown several side effects due to which different biocontrol agents have emerged as an efficient tool to protect the plant from pathogens (Fallahzadeh-Mamaghani et al., 2009; Yaqub & Shahzad, 2011). The term biocontrol means controlling the disease or reducing the number or effect of the pathogen infestation in the host (Choudhary & Johri, 2009).

Problem Statement

Oil palm cultivation relies heavily on chemical fertilizers, but excessive usage of degrades soil health and disrupts microbial diversity. High nitrogen inputs are further linked to increased basal stem rot (BSR) caused by *Ganoderma boninense*, which the most destructive oil palm pathogen. Identifying native microbes with nutrient-solubilising and biocontrol capacities offers a sustainable integrated nutrient and disease management strategy.

Research Questions

Do isolated microbial strains possess nutrient-solubilising abilities (such as phosphorus and potassium) and antagonistic activity against *Ganoderma boninense*, the pathogen responsible for basal stem rot in oil palm?

MATERIALS AND METHODS

Site Description and Sampling Design

A series of sampling was carried out in two types of soils (oil palm: Alluvial soil; forest adjacent oil palm) in the area of Segamat (2°10’29.5”N 103°00’03.7”E)and Keratong, Johor (2°46’34.2”N 102°55’13.2”E). Five samples were collected at each location. Each sample was taken at the depth of 15 cm, 30 cm and 45 cm with a hand auger and placed in plastic bags with appropriate labelling and brought to the laboratory for further analysis.

Isolations of Phosphate Solubilising Bacteria (PSB)

Soil samples from three depths were pooled, serially diluted up to 10^{-5}, and spread on Pikovskaya’s (PVK) agar. After 5 days of incubation at 28°C, colonies producing clear halo zones were purified on Nutrient Agar and re-inoculated onto PVK agar in triplicate. Pure phosphate-solubilising bacteria (PSB) isolates were then preserved at −80°C for further analysis.

Assessment of the Solubilising Activities of PSB Strains on Solid Media

The phosphate-solubilising ability of bacterial isolates was evaluated on PVK agar containing tricalcium phosphate (TCP). After 7 days of incubation at 28°C, the Solubilisation index (PSI) was calculated.

Determination of the Production of Indole Acetic Acid

Phosphate-solubilising Bacteria (PSB) were assayed for their capacity to produce indole acetic acid (IAA) (Kumar et al., 2015). Absorbance was measured at 530 nm using UV-Vis Spectrophotometer (Shimadzu, UV-1800, Japan) and the quantity of IAA was determined from a standard curve and expressed as µg/mL. These experiments were carried out in triplicate for each isolate.

DNA Extraction, PCR Amplification, and Sequencing

The screened PSB were then subjected for the bacterial genomic DNA extraction by using Plant DNeasy Mini Kit (Qiagen, Germany). The PCR was done by using bacterial 16SrRNA primer with GoTaq® Green Master mix (Promega, USA) (Amri et al., 2023). The amplified products were purified and sequenced by Apical Scientific Sdn. Bhd. (Malaysia).

RESULTS AND DISCUSSION

Composition of Phosphate Solubilising Bacteria (PSB) Community and Molecular Identification

The comparative relative abundance of phosphate-solubilising microbes (PSMs) in forest soil and oil palm soil is presented in Figure 1.

Phosphate Solubilisation Index

The ability of PSB to solubilize phosphate is visually confirmed by the formation of clear halos around bacterial colonies on PVK agar plates. A higher SI suggests a more effective

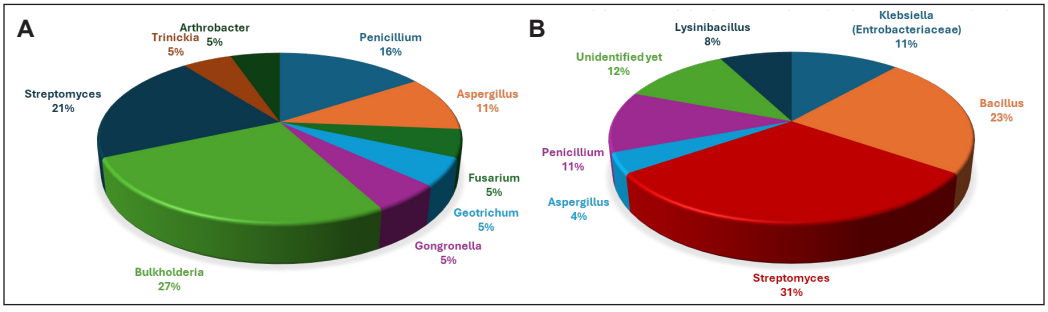

Figure 1. Relative abundance of the different species of phosphate-solubilising Microbes (PSB) in the studied oil palm (A) and forest soil (B)

Table 1
Phosphate solubilising index

No	Isolates ID	Colony Diameter (mm)	Solubilisation diameter (mm)	Solubilisation index (SI)
		Forest Soil		
	PSM5	0.48 ± 0.04	0.68 ± 0.01	2.42[a]
	PSM4	1.03 ± 0.11	1.63 ± 0.04	2.59[a]
		Oil Palm		
	PSM6	5.99 ± 0.04	19.25 ± 5.52	4.22[b]
	PSM1	7.10 ± 0.14	21.88 ± 5.69	4.08[b]
	PSM8	11.0 ± 1.91	24.73 ± 1.17	3.25[b]
	PSM9	8.10 ± 0.67	10.53 ± 0.67	2.30[ab]

Note. Means with same letter are not significantly different at $P>0.05$ using Turkey test

phosphate Solubilisation potential. The average solubilising index (SI) of the selected isolates is presented in Table 1.

Determination of the Production of Indole Acetic Acid

Indole-3-Acetic Acid (IAA) production varied significantly among the phosphate-solubilising bacterial isolates. PSM 6, PSM 8, and PSM 9 showed the highest absorbance, indicating greater IAA biosynthesis compared to other strains. These isolates demonstrate strong potential as plant growth-promoting rhizobacteria (PGPR) by enhancing root development and elongation.

CONCLUSION

In conclusion, this study indicates that certain microbial isolates, particularly PSM 1, PSM 6 and PSM8 have high potential for phosphate Solubilisation.

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Effects of Palm Kernel Expeller and Empty Fruit Bunch Inclusion in Beef Cattle Feed Formulation on *In Vitro* Gas Production and Rumen Fermentation

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ABSTRACT

Oil palm by-products offer significant potential as ingredients in beef cattle feed formulations, providing an alternative to imported feed ingredients. This study evaluated the effects of incorporating palm kernel expeller (PKE) and empty fruit bunches (EFB) at varying levels in beef cattle feed formulations on *in vitro* gas production and rumen fermentation. Six treatments were formulated: T1 = 70% PKE + 5% EFB, T2 = 70% PKE + 10% EFB, T3 = 70% PKE + 15% EFB, T4 = 65% PKE + 5% EFB, T5 = 65% PKE + 10% EFB, and T6 = 65% PKE + 15% EFB. Rumen fluids collected from slaughtered cattle were used for 48-hour incubations. T5 had the highest gas production (P = 0.0001), with 99.25 mL/500 mg, exhibited high values for *in vitro* dry matter degradability (*iv*DMD) and *in vitro* organic matter degradability (*iv*OMD), at 55.11% and 69.45%, respectively. Volatile fatty acid (VFA) analysis showed that T1 had a significantly higher (P = 0.0001) total VFA (64.29 mM), acetic acid (15.01 mM) and propionic acid (25.34 mM) concentration, respectively. Higher EFB inclusion (T3 and T6) resulted in lower VFA production. Incorporating PKE and EFB in beef cattle feed provides nutritional adequacy and supports favourable rumen fermentation profiles. These findings suggest that incorporating oil palm by-products can improve feed sustainability and reduce feed costs without compromising nutritional quality. Further *in vivo* studies are recommended to assess the impact of these feed formulations on beef cattle performance.

Keywords: Beef cattle nutrition, oil palm by-products, ruminant feedstuffs

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INTRODUCTION

The growing demand for beef production has intensified the search for sustainable and cost-effective feed ingredients, particularly in regions where corn and soybean meal

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are costly or limited. In Malaysia, palm oil by-products such as palm kernel expeller (PKE) and empty fruit bunches (EFB) are promising alternatives for ruminant diets. Although their nutritional potential has been individually studied, limited research has explored their combined effects on rumen fermentation and nutrient degradability. This study evaluates the *in vitro* fermentation characteristics of beef cattle feed pellets containing varying levels of PKE and EFB which could determine their feasibility in sustainable ruminant feeding systems.

Problem Statement

Combining PKE and EFB at optimal ratios could balance energy and fibre supply, improving degradability and fermentation efficiency in ruminant nutrition.

Research Questions

How does the inclusion of PKE and EFB at different levels in beef cattle feed affect *in vitro* gas production, rumen fermentation and VFA production?

MATERIALS AND METHODS

Six diets were formulated using FORMAT software: T1 = 70% PKE + 5% EFB, T2 = 70% PKE + 10% EFB, T3 = 70% PKE + 15% EFB, T4 = 65% PKE + 5% EFB, T5 = 65% PKE + 10% EFB, and T6 = 65% PKE + 15% EFB. Rumen fluid was collected from slaughtered beef cattle and maintained at 39°C under anaerobic conditions. The inoculum was prepared by mixing strained rumen fluid with artificial saliva (Menke, 1988). Gas production was recorded at throughout 48 h. At 48 h, pH was measured, and residues were analyzed for *in vitro* dry matter degradability (*iv*DMD), organic matter degradability (*iv*OMD) and VFA.

RESULTS AND DISCUSSION

Proximate analysis of the developed feed formulations with different inclusion levels of PKE and EFB is shown in Table 1. All of the formulations were isonitrogenous and isocaloric. Crude fibre content was higher in T3 and T6 which contained the highest EFB inclusion level (15%).

Figure 1 shows *in vitro* gas production of the different feed formulations with varying inclusion levels of PKE and EFB. T5 had shown the highest gas production, reaching 99.25 mL/500 mg at 48 h. Inclusion of 65% PKE and 10% EFB offers an optimal fibre-to-fat balance, resulting in maximal microbial degradation and gas production (Kum & Zahari, 2011).

Table 2 shows the effects of PKE and EFB inclusion on *in vitro* rumen fermentation and VFA profiles. T6 showed the highest *iv*DMD (58.40%), while T5 recorded the highest *iv*OMD (69.45%), indicating potential benefits for feed efficiency. T1 produced the highest

Table 1
Proximate analysis of developed feed formulations with different inclusion levels of PKE and EFB

Proximate Analysis	T1	T2	T3	T4	T5	T6
Moisture content (% DM)	8.97 ± 0.05^{a}	9.21 ± 0.22^{a}	8.14 ± 0.18^{c}	7.49 ± 0.24^{d}	8.54 ± 0.24^{b}	8.31 ± 0.09^{bc}
Ash (% DM)	5.26 ± 0.03^{b}	5.25 ± 0.02^{b}	5.23 ± 0.09^{b}	5.68 ± 0.18^{a}	5.71 ± 0.04^{a}	5.19 ± 0.01^{b}
Crude fat (% DM)	8.49 ± 0.11^{c}	8.84 ± 0.06^{bc}	9.25 ± 0.12^{b}	10.80 ± 0.21^{a}	7.94 ± 0.48^{d}	8.93 ± 0.31^{bc}
Crude protein (% DM)	15.99 ± 0.39^{ab}	16.07 ± 0.07^{ab}	15.37 ± 0.83^{b}	16.60 ± 0.40^{a}	15.67 ± 0.23^{b}	15.46 ± 0.20^{b}
Crude fibre (% DM)	18.26 ± 0.53^{c}	20.87 ± 0.24^{ab}	22.58 ± 1.32^{a}	21.50 ± 0.49^{a}	19.25 ± 1.20^{bc}	22.00 ± 1.45^{a}
Gross energy (cal gram^{-1})	4861 ± 6.68^{b}	4910 ± 9.15^{ab}	4890 ± 13.46^{ab}	4922 ± 41.43^{a}	4813 ± 24.73^{c}	4906 ± 9.42^{ab}

Note. T1 = 70% PKE, 5% EFB; T2 = 70% PKE, 10% EFB; T3 = 70% PKE, 15% EFB; T4 = 65% PKE, 5% EFB; T5 = 65% PKE, 10% EFB; T6 = 65% PKE, 15% EFB; DM = dry matter; cal gram^{-1} = calorie gram^{-1}

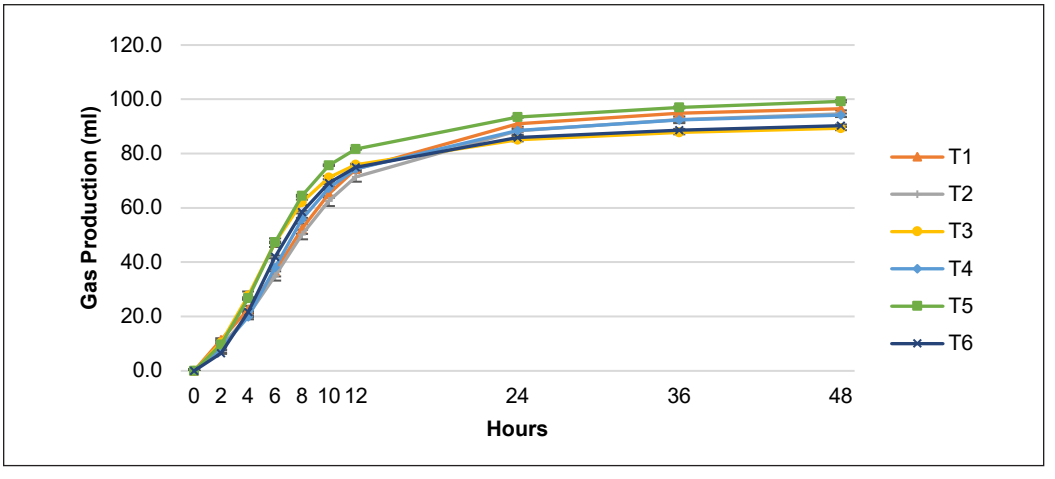

Figure 1. *In vitro* rumen gas production according to different treatment groups

total VFA (64.29 mM) and propionic acid (25.34 mM), suggesting favourable fermentation. Total VFA reflects rumen activity and energy availability, whereas propionate supports gluconeogenesis and reduces methane (Bergman, 1990; Ungerfeld, 2020). Lower VFA in T3 and T6 indicates reduced fermentability at higher fibre levels, emphasizing the importance of optimizing PKE–EFB ratios for efficient energy supply.

CONCLUSION

Incorporating PKE and EFB in beef cattle feed enhances nutritional value and rumen fermentation. The 65% PKE + 10% EFB (T5) diet showed superior gas production,

Table 2
Effect of PKE and EFB in cattle feed on in vitro *rumen fermentation and VFA profile*

Treatment	T1	T2	T3	T4	T5	T6
Rumen Fermentation						
pH	6.67 ± 0.02	6.72 ± 0.01	6.74 ± 0.02	6.76 ± 0.01	6.79 ± 0.01	6.81 ± 0.02
*iv*DMD (%)	52.60 ± 4.19	50.95 ± 2.52	53.73 ± 2.13	53.91 ± 3.71	55.11 ± 2.67	58.40 ± 1.56
*iv*OMD (%)	68.23 ± 1.39	65.78 ± 1.07	65.64 ± 0.90	66.54 ± 1.54	69.45 ± 1.23	68.64 ± 0.27
VFA profile						
Acetic	15.01 ± 0.96 [a]	10.84 ± 0.32 [e]	11.09 ± 0.70 [d]	11.55 ± 0.18 [c]	12.54 ± 0.30 [b]	10.27 ± 0.27 [d]
Propionic	25.34 ± 0.66 [a]	19.52 ± 0.55 [b]	15.81 ± 1.16 [e]	18.90 ± 0.36 [c]	19.48 ± 0.44 [b]	17.07 ± 0.30 [d]
Iso-Butyric	1.46 ± 0.08 [a]	0.74 ± 0.03 [d]	0.74 ± 0.03 [d]	0.82 ± 0.01 [b]	0.77 ± 0.02 [c]	0.79 ± 0.02 [c]
Butyric	18.27 ± 0.77 [a]	14.55 ± 0.27 [b]	11.93 ± 1.06 [c]	12.55 ± 0.21 [c]	11.64 ± 0.18 [d]	12.61 ± 0.31 [c]
Iso-Valeric	1.26 ± 0.08 [a]	0.66 ± 0.02 [c]	0.64 ± 0.05 [c]	0.73 ± 0.01 [b]	0.69 ± 0.02 [c]	0.67 ± 0.03 [c]
Valeric	1.79 ± 0.03 [a]	1.32 ± 0.03 [b]	1.11 ± 0.10 [c]	1.29 ± 0.01 [b]	1.33 ± 0.03 [b]	1.24 ± 0.03 [b]
Caproic	0.14 ± 0.01	0.12 ± 0.02	0.13 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.13 ± 0.01
Total VFA	64.29 ± 2.57 [a]	47.73 ± 0.31 [b]	42.11 ± 3.09 [b]	45.40 ± 0.75 [b]	46.57 ± 0.98 [b]	42.78 ± 0.89 [b]

Note. T1 = 70% PKE, 5% EFB; T2 = 70% PKE, 10% EFB; T3 = 70% PKE, 15% EFB; T4 = 65% PKE, 5% EFB; T5 = 65% PKE, 10% EFB; T6 = 65% PKE, 15% EFB. Means (± standard error) in the same row with different superscripts indicate statistically significant difference at $p<0.05$

digestibility, and good VFA profile balance. This approach supports sustainable feeding by reducing reliance on conventional feeds, lowering costs, and repurposing palm-based by-products.

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Evaluation of the Anti-microbial Properties of Kelulut-derived Lozenges on Oral Pathogens

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ABSTRACT

Kelulut honey is documented to exhibit numerous pharmacological advantages, including antibacterial, antioxidant, anti-inflammatory, and anti-apoptotic properties. This study evaluated a newly formulated Kelulut -based lozenge for its antimicrobial efficacy against five oral bacteria: *Streptococcus mutans*, *Streptococcus sobrinus*, *Staphylococcus aureus*, *Actinomyces viscosus*, and *Escherichia coli*, using the agar well diffusion assay method. The findings indicated that the Kelulut-based lozenges exhibited antimicrobial efficacy against all tested bacteria, with the exception of *A. viscosus* and *S. aureus*. The zones of inhibition ranged from 23.00 to 31.5 mm, following this strength sequence of *S. mutans* > *E. coli* > *S. sobrinus*. The results indicated that the Kelulut-based lozenges possess antimicrobial properties against the studied oral pathogens, warranting additional investigation to assess their potential as an alternate product for combating oral pathogens.

Keywords: Antimicrobial, kelulut, lozenges, oral pathogen, stingless bee

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INTRODUCTION

The human oral cavity has more than 700 bacterial species, some of which are pathogenic and may lead to gastrointestinal disorders and tooth loss (Bumm et al., 2021). Oral pathogens also cause dental pulp disease and fungal infections (Santosh et al., 2021) and may lead to both oral and systemic disorders (Bui et al., 2019). Therefore, agents that may specifically suppress oral bacteria are essential to avert disorders induced by oral pathogens.

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Oral care is considered a crucial preventive measure for sustaining oral health and, by extension, the overall well-being of the human body (Dudek et al., 2023). Oral health may be preserved by the use of numerous items, such as gels, drinks, or lozenges. Lozenges are flavoured solid dosage forms often used to alleviate local infections and associated symptoms in the oral or oropharyngeal regions. They are extensively used as a support in the treatment of upper respiratory tract infections or as a dietary supplement. Lozenges composed of natural substances are increasingly favoured owing to the diverse pharmacological properties of the molecules within their composition.

In recent years, products produced from natural resources have been the focus of research due to the declining effectiveness of current antibiotics and the alarming rise of increasingly aggressive and resistant microorganisms. This situation has required the scientific community to explore medicinal alternatives derived from natural sources.

Stingless bee honey or famously known as Kelulut honey is known to have high nutritional and therapeutic value. The antimicrobial activity of Kelulut honey, besides helping to preserve the honey itself or some foods, has been useful for wound and burn care, skin, eye, and mucosal infections such as throat diseases and gastrointestinal infections in humans (Cabezas-Mera et al., 2024). The microbiota associated with probiotics and the various combinations of chemicals, including phenols, flavonoids, terpenes, and alkaloids, in stingless bee honey enhance its distinctive biological properties (Machado et al., 2023).

In this context, it may be beneficial to explore the efficacy of Kelulut honey in controlling oral pathogens. Thus, in this study, we examined the antimicrobial activity of a newly developed Kelulut-based lozenge against five oral bacteria: *S. mutans, S.sobrinus, S. aureus, A. viscosus,* and *E. coli.* This preliminary study involves the development of Kelulut honey lozenges, and the evaluation of anti-microbial potential using the agar well diffusion assay.

MATERIALS AND METHODS

Preparation of Kelulut-based Lozenges

The Kelulut honey used in this study was obtained from a local Kelulut farmer in Ketereh, Kelantan. The composition of the Kelulut lozenges comprises sucrose (35%), glucose syrup (33%), Kelulut honey (30%), and water (2%). The mixture of sugar, glucose syrup, and water was heated to 150°C. Subsequently, the Kelulut honey was incorporated into the mixture and heated to 140°C prior to slight cooling, followed by rapid stirring to eliminate bubbles, and poured into moulds, where it was allowed to solidify. For the antimicrobial experiment, each lozenge was dissolved in phosphate-buffered saline (pH 7) at a ratio of 1:1. The lozenge solution was then filtered through a 0.22 μm syringe to ensure sterility before further use.

Preparation of Test Microorganisms

The tested microorganisms for antimicrobial activity were *S. mutans, S.sobrinus, S. aureus, A. viscosus,* and *E. coli.* The bacterial inoculum was maintained and subcultured on Mueller-Hinton agar (MHA) and incubated at 37°C for 24 h. For the antimicrobial experiment, bacterial inocula were produced from overnight cultures.

Antimicrobial Assay—Agar Well-diffusion Assay

The *in vitro* antimicrobial efficacy of Kelulut-based lozenges was assessed using the agar well-diffusion method. The surface of the Muller-Hinton agar plate was inoculated by evenly distributing a volume of the tested microbial inoculum throughout the whole agar surface using a sterile cotton swab. A circular hole is aseptically created in the agar using a sterile cork borer, into which about 100 µL of Kelulut lozenges solution is added. The agar plates were incubated at 37°C to facilitate the diffusion of the Kelulut lozenges solution into the agar medium. After 16 hours, the plates were examined for the zone of inhibition, and the diameters of these zones were measured in millimeters. A clear inhibition zone around the well was the indication of the presence antimicrobial activity and the average was evaluated based on classification by the Clinical and Laboratory Standards Institute (2020).

RESULTS AND DISCUSSION

An inhibition zone or ring that forms around the well after the incubation indicates antimicrobial activity possessed by the studied Kelulut lozenges solution. The observed antimicrobial activity of the lozenge solution against tested oral pathogens is presented in Table 1. The Kelulut lozenges exhibited the greatest inhibitory effects against *Streptococcus mutans* measuring 31.50 ± 0.71 mm (Figure 1A). The inhibitory effect against *S. mutans*, a Gram-positive, facultative anaerobic microorganism is particularly noteworthy, given its pivotal role in the initiation of dental caries which has been a challenge for decades (Ravi et al., 2017).

The Kelulut lozenges also demonstrated considerable inhibitory effects against *E. coli* and *S. sobrinus* with inhibition zones measuring 26.50 ± 0.71 mm and 23.00 ±

Table 1
Antimicrobial susceptibility of Kelulut honey-based lozenges

Test microorganism	Antibacterial activity (zone of inhibition in mm)
Streptococcus mutans	31.50 ± 0.71
Escherichia coli	26.50 ± 0.71
Streptococcus sobrinus	23.00 ± 0.00
Actinomyces viscosus	14.00 ± 0.00
Staphylococcus aureus	-

0.00, respectively (Figures 1B and 1C). Although *E.coli* is frequently linked to intestinal infections, it can also be an oral pathogen, although this occurs less frequently than other bacteria such as *Streptococcus. S. sobrinus* is another member of the *Streptococcus* genus that is one of the primary causal pathogens of early dental caries. This is due to their capacity to generate insoluble glucan and fructan and to adhere to the tooth surface (Lee & Kim, 2014). *Streptococci* are also recognized for their ability to aggregate and generate oral biofilms (Jeong et al., 2018). Therefore, inhibiting biofilm formation and decreasing the size of cariogenic bacterial populations are essential approaches to the prevention of oral diseases (Lin et al., 2015). Although Kelulut lozenges exhibited significant inhibition against these pathogens, *A. viscosus* and *S. aureus* exhibited resistance to the lozenges (Figure 1D and 1E).

The observed differential antimicrobial efficacy is likely the result of intricate interactions between the bioactive compounds in the Kelulut honey and the intrinsic resistance mechanisms of each bacterial species (Al-kafaween et al., 2023). As described by Aspar et al. (2020), it is possible that the variation in the antimicrobial efficacy of Kelulut honey against different bacteria is due to its distinctive composition of peroxide and non-peroxide activities, which interact differently with different bacterial strains, thereby affecting their susceptibility to the antibacterial properties of honey. In addition, the mixtures of numerous compounds such as

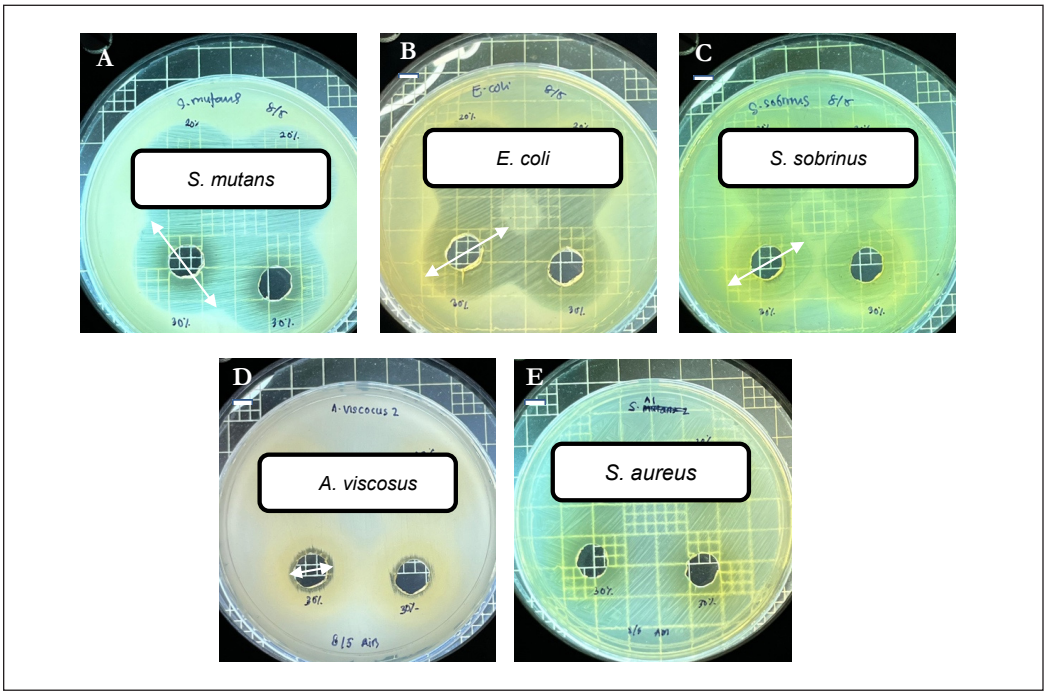

Figure 1. Antimicrobial susceptibility of Kelulut-based lozenges against *Streptococcus mutans, Streptococcus sobrinus, Staphylococcus aureus, Actinomyces viscosus,* and *Escherichia coli*

phenols, flavonoids, terpenes, and alkaloids within Kelulut honey contribute to their unique antimicrobial properties (Machado et al., 2023).

CONCLUSION

According to these findings, Kelulut-based lozenges possess antimicrobial properties against *S. mutans, E. coli,* and *S. sobrinus*, with the exception of *A. viscosus* and *S. aureus,* as evidenced by the inhibition zone's manifestation in the agar well diffusion assay. Nevertheless, additional assessment is necessary to ascertain the Kelulut lozenges' potential as a supplement or alternative for the management of oral pathogens.

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Proximate, Functional, and Sensory Properties of Dried Okra (*Abelmoschus esculentus* L. Moench) Slices

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ABSTRACT

Drying is a widely recognised preservation technique used to reduce the perishability of vegetables such as okra. A comprehensive evaluation is needed to assess how different drying methods affect okra's proximate composition, functional properties, and sensory attributes. Research indicates that drying significantly influences these qualities, with methods like freeze-drying or hot air drying at specific temperatures showing better preservation of nutritional and functional properties compared to other methods. The purpose of this study is to close this knowledge gap by thoroughly evaluating the nutritional composition and sensory qualities of dried okra, which will help to better comprehend its potential as a preserved food product. After being cleaned with clean water, the okra samples were pre-treated for 30 seconds in boiling water. Three portions of the samples were then separated, cut into pieces that were 4 mm thick, and dried at 50 and 60 degrees Celsius. Every analysis was conducted using accepted practices. The proximate composition of the dried okra slices varied significantly ($p<0.05$) among the three drying techniques, with the exception of the fibre content, which stayed mostly constant. The highest emulsion (48.39%) and swelling (41.38%) capacities were recorded in the sample which was dehydrated at 50°C. Oven drying at 50°C produced the maximum emulsion stability (38.66%). The most effective method was drying at 50°C with a dehydrator for preserving the functional and nutritional properties of okra, with oven drying at 60°C and solar drying performing less well, especially in emulsion stability and swelling capacity. Sensory tests also showed a strong preference for oven dried and dehydrator dried okra compared to the solar dried okra, suggesting the dehydrator method produces a superior product.

Keywords: Dehydrator, functional, okra, oven drier, proximate, sensory, solar drier

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INTRODUCTION

Okara (*Abelmoschus esculentus* L. Moench) is a highly valued vegetable that is grown all over the world because of its many culinary uses and rich nutritional profile. More of a diet food than a mainstay is okra (National Research Council, 2006). Okra, a vegetable widely cultivated in tropical and subtropical regions, is a nutritional powerhouse with numerous health benefits. Okra is a good source of vitamins A, C, K, and B6. Vitamin A, derived from beta-carotene, is crucial for healthy vision and skin. Vitamin K is essential for bone health and blood clotting (Daniluk, 2020). Okra known as *Ila* (Hussein et al., 2018).

Okra is well known for having a lot of vital nutrients. Having a sticky mucilage that promotes better digestion, it belongs to the Mallow family and used to treat stomach ulcer, gastritis, and liver and gall bladder cleansing (Dailuk, 2020). On a small scale, okra seeds are used to produce oil, and the fatty components enhance the nutritional and sensory qualities of nearly every diet (Vermerris & Nicholson 2020).

Dehydrated okra, or dried okra, is used in a variety of recipes. It is a popular method of preserving okra that increases its stability in storage and makes transportation easier. However, okra's nutritional qualities might be impacted by drying.

Problem Statement

Fresh okra has a high moisture content, which makes it prone to rapid spoiling, quality degradation, nutrient loss, and decreased storage stability despite its nutritional advantages. Effective preservation methods are therefore essential to extending the shelf life of okra while maintaining its nutritional content and flavour characteristics.

Significant of the Study

The research focuses on figuring out the best drying methods for okra to retain its nutritional value, taste, texture, and other beneficial properties. The research will help create culinary techniques that highlight the healthful qualities of okra, guide food manufacturers in developing products that strike a balance between nutritional value and sensory appeal, and inform dietary recommendations for consumers looking for increased nutritional benefits.

MATERIALS AND METHODS

Sample Preparation

A Nigerian okra farm in Ikole Ekiti provided the fresh okra material. Analytical-grade chemicals and reagents were acquired from the Food Science and Technology Department of Federal University Oye-Ekiti.

Dried Okra Slices Preparation

The dried okra slices were prepared in dryers according to the methods described by Hussein et al. (2018). The drying experiment was carried out between April and May, 2024. After being cleaned with fresh water, the okra samples were pre-treated for 30 seconds in boiling water and then drained. After that, the sample were separated into three sections, each of which was manually cut with a vernier calliper to a thickness of around 4 mm. One kilogramme of sliced okra was initially placed in a single layer within a wire mesh tray and dried in a dehydrator set to 50 and 60 degrees Celsius. One kilogramme of the second piece was dried at 50 and 60 degrees Celsius in a hot-air oven (Model: TO 008GA-34, Akal-Tokiyo, Japan). Hussein et al. (2017) used a hybrid photovoltaic sun drier with a solar collector as only heating source to dry the remaining 1 kg at 50°C and 60°C for roughly 10 hours every day. The moisture content was determined according to the methos of Association of Official Analytical Chemists (2004).

Proximate Analysis of the Dried Okra Slices

The Association of Official Analytical Chemists (2010) standard method was used to determine the dried okra slices' proximate composition, which included protein, fat, crude fibre, moisture, and ash content. The carbohydrate content was determined by using another method.

Dried Okra Slices Functional Properties

Emulsion stability and capacity were determined according to the methods of Onwuka (2005). Okra samples swelling capacity was assessed using the techniques outlined by Adepeju et al. (2014).

Sensory Analysis of Okra Slices

After screening for familiarity with the okra samples, thirty (30) untrained panellists who were students at Federal University, Oye Ekiti, assessed the sensory profile of the dried okra slice samples. A 9-point hedonic scale was used to assesed taste, color, flavour/aroma, texture, appearance, and general acceptability of slices okra according to method of Iwe (2010).

Statistical Analysis

A one way ANOVA was used to statistically analyze the acquired data. The Duncan Multiple Range test (SPSS version 23 computer software) was performed to differentiate the means at a 95% confidence level.

RESULTS AND DISCUSSION

Nutrient Composion of Dried Okra Slice

Figure 1 displays the findings of the dried okra slices' proximate composition. Okra that was sun-dried had the highest moisture content (11.27%), whereas okra that was oven-dried at 60°C had the lowest (8.08%). The moisture content of wheat and starch dried foods is around 10% (Ogunlakin et al., 2012). The protein content varied substantially ($p < 0.05$) between samples, with solar-dried okra slices having the lowest value (14.78%) and dehydrated (18.91 %) and oven-dried slices (18.91 %) at 50°C. Okra dried in a dehydrator at 50°C had a considerably ($p<0.05$) greater fat level (2.67%) than okra dried at other temperatures and methods, while oven-dried okra slices at 60°C and solar-dried okra slices had the lowest fat content (2.10% and 2.09%, respectively). There were no significant differences in the fibre content across the various approaches. The okra that was dehydrated at 50°C had the highest ash percentage (8.67%), whereas the sample that was sun-dried had the lowest. The carbohydrates content was significantly higher in oven dried and solar dried okra slices with a value of 56.02% and 55.35% compared to the other methods.

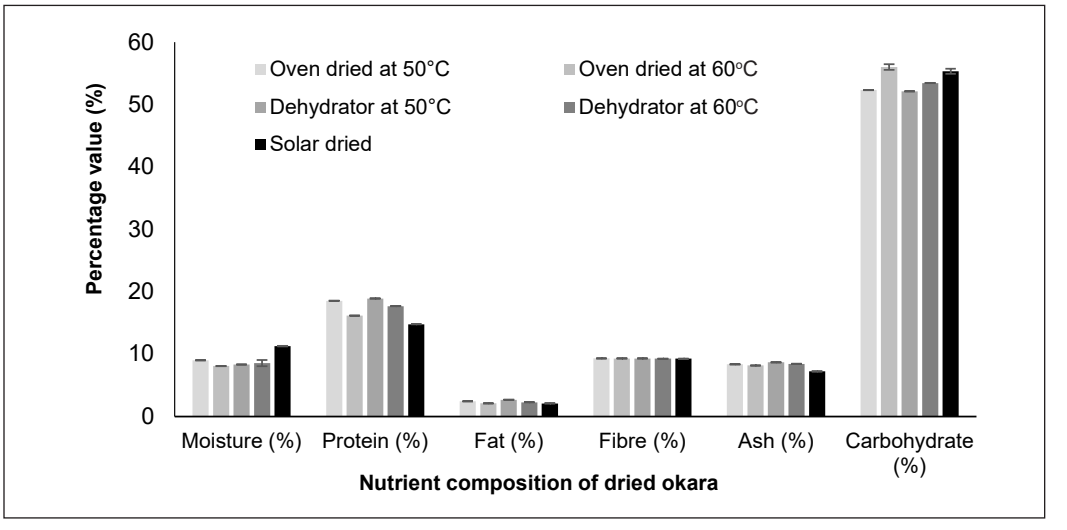

Figure 1. Nutrient content of dried okra slices

Dried Okra Slices Functional Properties

Table 1 shows the functional property results for the dried okra. When compared to other drying techniques, the highest emulsion capacity (48.39 %) and swelling capacity (41.38 %) were recorded in dehydrated okra (50°C), demonstrating superior functional qualities. Okra slices dried in a dehydrator at 50°C (37.15%) and oven-dried at 50°C (38.66%) had

the maximum emulsion stability. Particularly in terms of emulsion stability (34.92%) and swelling capacity (38.90%), solar drying did not perform well. Functional property values were consistently lower after oven drying at 60°C.

Table 1
The effects of drying methods on functional property of okra slices

Sample	Emulsion capacity (%)	Emulsion stability (%)	Swelling capacity (%)
Oven dried at 50°C	46.45±0.23[b]	38.66±0.51[a]	39.76±0.30[ab]
Oven dried at 60°C	45.00±0.41[b]	34.55±0.09[c]	38.52±0.20[b]
Dehydrated at 50°C	48.39±0.032[a]	37.15±0.24[b]	41.38±0.37[a]
Dehydrated at 60°C	45.97±0.08[b]	35.26±0.19[c]	39.31±0.43[ab]
Solar dried	45.49±0.011[a]	34.92±0.10[c]	38.90±0.26[b]

Note. Values in each column with same letters are not significantly different at 5% level of significant

Dried Okra Slices Sensory Attributes

In sensory evaluations, okra slices dried by an oven or dehydrator generally received significant ($p<0.05$) preferences higher scores for attributes like colour, texture, and flavour, while solar-dried okra is rated significantly lower across all parameters (Table 2). The differences in sensory quality can be attributed to the drying conditions, such as temperature, air circulation, and drying time, which affect the degradation of pigments and volatile compounds

Table 2
Sensory attributes of okra as affected by drying methods

Sample	Appearance	Brittleness	Texture	Aroma	Overall Acceptability
Oven dried at 50°C	8.10±0.22[a]	7.58±0.65[a]	7.36±0.62[a]	8.21±0.25[a]	8.47±0.21[a]
Oven dried at 60°C	8.45±0.14[a]	7.70±0.44[a]	7.49±0.42[a]	8.47±0.12[a]	8.43±0.20[a]
Dehydrated at 50°C	8.41±0.25[a]	7.45±0.14[a]	7.27±0.11[a]	8.38±0.27[a]	8.15±0.29[a]
Dehydrated at 60°C	7.97±0.42[a]	7.68±0.36[a]	7.45±0.32[a]	7.99±0.46[a]	8.08±0.53[a]
Solar dried	6.23±0.70[b]	6.11±0.24[b]	6.12±0.21[b]	6.22±0.72[b]	6.013±0.22[b]

Values in each column with same letters are not significantly different at 5% level of significant

CONCLUSION

It can be concluded that the nutrient composition of different dried okra slices varied significantly ($p<0.05$). However, there were no appreciable differences in the fibre content between the various approaches. The best way to maintain dried okra's useful qualities was to dry it in a dehydrator set to 50°C. This critical review emphasizes how crucial it is to choose the right drying techniques in order to optimize the nutritional value and practical

uses of dried okra in food products. Okra that had been oven-dried or dehydrated was significantly ($p<0.05$) preferred above samples that had been sun-dried. The biochemical characteristics of the dried okra slices should be investigated.

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The Impact of Different Concentration of Starch on Starch-based Hydrogels Loaded with *Clitoria ternatea* Extract

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ABSTRACT

Anthocyanins are natural pigments that's available is plant such as *Clitoria ternatea* (Butterfly Pea Flower, BPF) and are widely used as colourants in food industry. Despite their attractive colour and health benefits, anthocyanins are relatively unstable and prone to degradation limiting their application. Starch-based hydrogel (SH) is gaining popularity as a sustainable and cost-effective medium for encapsulating and protecting sensitive compounds like anthocyanins. *Ipomea batatas* (white sweet potato) contains the highest starch content compared to purple and yellow varieties. Starch, a natural polymer that has unique properties that make it suitable for forming hydrogels. This study explores the use of SH derived from white sweet potato starch to encapsulate anthocyanins extracted from BPF. Four formulations of SH ranging from 0% to 6% starch concentration were prepared. This study aims to investigate the physical properties and encapsulation efficiency of these hydrogels. Results showed no significant difference in encapsulation efficiency between hydrogels with the highest starch concentration (96.07 ± 3.1^{A}) and without starch (98.52 ± 0.99^{A}). In conclusion, SH effectively enhances the recovery and stability of anthocyanins and holds promising potential for application in the food industry as natural, functional ingredients.

Keywords: Anthocyanin, *Clitoria ternatea,* encapsulation, *Ipomea batatas*, starch-based hydrogel

INTRODUCTION

Starch-based hydrogel (SH) are three-dimensional networks capable of absorbing water and are widely used in fields such as food and beverages (Cui et al., 2022). Anthocyanins, natural pigments found in flowers, are visually appealing but highly unstable during processing. To enhance their stability and functionality, natural carriers

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like starch have been explored (Schlindweinn et al., 2022). Chen et al. (2022) stated that SH may offer potential for encapsulating anthocyanins, improving their stability and nutritional value by providing a suitable matrix. This study aims to investigate the influence of varying starch concentrations on the physical properties of starch-based hydrogels loaded with *Clitoria ternatea* extract.

MATERIALS AND METHOD

Materials

Dried *Clitoria ternatea* was sourced from Superbee Enterprise, country while the fresh *Ipomea batatas* was obtained from WP Online Groceries, country

Isolation of Starch, Extraction of *Clitoria Ternatea* and Starch-based Hydrogel Production

Starch was isolated using a method by Hazrati et al. (2021) with slight modification. *Clitoria ternatea* was extracted using distilled water at 60°C for 2 h. Starch-based hydrogels were prepared by following a modified method from Apostolidis et al. (2021).

Water Holding Capacity (WHC), Anthocyanin Encapsulation Efficiency (AEE), Colour Profile and Texture Profile Analysis (TPA)

Water holding capacity (WHC) was measured using a modified method from Liu et al. (2018). Anthocyanin encapsulation efficiency (AEE) was evaluated following the method from Liew et al. (2020). The colour profile and t**exture profile analysis** (TPA) of the hydrogel were measured using a modified method from Huang et al. (2024).

Statistical Analysis

The data were analysed using One-way ANOVA (Minitab Version 20).

RESULTS AND DISCUSSION

Water Holding Capacity (WHC) and Anthocyanin Encapsulation Efficiency (AEE)

Table 1 shows the physical properties of starch-based hydrogel (SH). Water holding capacity (WHC) was significantly influenced by starch concentration. Although hydroxyl groups in starch enhance water retention (Yang et al., 2021), the denser gel networks formed at higher starch levels may reduce the availability of free water. There was no significant differences found in anthocyanin encapsulation efficiency among SH with 0%, 4%, and 6% of starch concentration, however, it significantly varied with different starch concentration. This supports the finding of Schlindweinn et al. (2022), who reported that starch-based encapsulation can improve the stability of *Clitoria ternatea* extract.

Table 1
The result of physical properties for starch-based hydrogel

Sample	0% starch	2% starch	4% starch	6% starch
WHC (%)	2.33±1.37^{B}	17.15±7.20^{A}	16.82±1.42^{A}	6.31±4.86^{AB}
AEE (%)	98.52±0.99^{A}	88.62±2.39^{B}	95.00±2.05^{A}	96.07±3.11^{A}
Hardness (g)	4483.10±180.50^{A}	2518.00±238.00^{BC}	2788.20±213.00^{B}	2416.40±149.10^{C}
Gumminess	3883.60±143.90^{A}	2141.90±185.90^{BC}	2345.70±192.20^{C}	2027.80±125.70^{C}
L*	15.56±0.08^{C}	25.56±1.91^{A}	20.23±0.05^{B}	23.80±0.12^{A}
a*	3.87±0.06^{A}	1.28±0.08^{D}	2.02±0.08^{C}	2.60±0.08^{B}
b*	0.86±0.02^{A}	-1.66±0.24^{B}	-6.30±0.08^{C}	-9.72±0.30^{D}

Note. Different superscript letters within row indicate significant difference (P<0.05) between treatments

Texture Profile Analysis and Colour Profile

The hardness of the SH was significantly affected by starch concentration (Table 1). Starch-based hydrogel (SH) with 0% starch had the highest hardness and gumminess. Starch-based hydrogel (SH) with 2% starch showed no significant differences compared to 4% starch. In colour profile, significant differences in b* value indicate variation in blue intensity, with the 0% SH appearing to be the darkest. Decrease in a* and b* values at higher starch concentration (4% and 6%) may indicated opacity in the SH.

CONCLUSION

This study showed that starch-based hydrogels have the potential to enhance the encapsulation and physical stability of anthocyanin extracts from *Clitoria ternatea*. Higher starch concentrations affected the hydrogels water holding capacity, texture, and colour properties. Although WHC decreased with increased starch, encapsulation efficiency remained high. Overall, SH is a promising natural carrier for anthocyanins, supporting its application in functional food systems.

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Moderating Effects of the Market Environment between Government Intervention and Comparative Advantage of Coconut Farming in Malaysia

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ABSTRACT

The study analysed the moderating effects of the market environment between government intervention and comparative advantage. The research used in this study is quantitative. The information was gathered from coconut growers in 12 Malaysian states. Respondents received the questionnaires through their WhatsApp numbers. Out of the 160 respondents who completed the questionnaires, 130 were selected at random to be analysed using the partial least squares (PLS) and Statistical Package for the Social Sciences (SPSS). The Partial Least Squares Structural Equation Modelling (PLS-SEM) was used to test the study's framework. The hypothesis testing was aided by bootstrapping. The hypothesis testing was supported by t-statistics and path coefficients. This study revealed that the moderation effect of the market environment on the relationship between government intervention and comparative advantage was significant at 10%. These findings offer practitioners actionable frameworks for improving competitiveness, emphasising the importance of both internal decision-making and external environmental factors.

Keywords: Coconut farming, Malaysia, comparative advantage, government intervention, market environment

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INTRODUCTION

According to Nayar (2016), the most beneficial plant species for humans is the coconut palm (Cocos nucifera L., family Arecaceae). Since very early times, the entire palm has been used for productive commercial purposes. The coconut has been affected by the start of the Anthropocene

Age, especially for people who live in areas where it is seen as the "staff of life," even though the coconut palm's standing has largely remained unchanged in areas where it is significant. The coconut palm is the most common plant in the littoral zones of the more than 30,000 islands that make up the ancient world's tropical and subtropical oceans. It has been integral to the mythology, folklore, ethnobotany, and everyday lives of the people who live in these vast regions since ancient times (Nayar 2016). 61.4 million metric tonnes of coconut fruit are produced year on 12.3 million hectares of coconut cultivation worldwide (Statista, 2020). The main phase of the planting of coconut trees to export copra and oil was the time between 1900 and 1930 when its price was extremely high. With a 538,685 mt capacity in 2018, Malaysia was the world's 12th-largest producer of coconuts [Selected Agriculture Indicator (SUA 2019)]. Therefore, the study analysed the moderating effects of the market environment between government intervention and comparative advantage.

Theory of Market Failures and Government Intervention

A broader school of Keynesian welfare and macroeconomics, which included the development of the theory of market failure, appeared in the middle of the twentieth century. Among the notable contributors were Paul A. Samuelson, William Baumol, Francis Bator, and Arthur C. Pigou. These theorists were interested in how the results of free markets correlated with the optimisation of social welfare. The "invisible hand" or duality theory of classical economics posits that Pareto optimality and laissez-faire market performance are mutually exclusive. In response to price signals, producers and consumers decide how to manufacture the product, whether to buy it, and whether to sell it. Correcting market failures can lead to more efficient allocation of resources and better cost-benefit ratios for public investments. Government policies can promote competition, provide information, and support marketing infrastructure to enhance marketing efficiency. By addressing market failures, government intervention can increase the social returns on public investments and private sector activities. The theory suggests government should intervene only when the benefits outweigh the costs and risks of failure. Governments can establish regulatory frameworks that promote fair competition, prevent market power abuse, and ensure that firms operate efficiently and effectively. This can lead to a comparative advantage in industries where firms can adapt more efficiently to changing market conditions. Governments can provide subsidies or tax incentives to firms to invest in specific projects or industries, which can enhance their comparative advantage by reducing costs and increasing competitiveness.

Development of Hypothesis

Market Environment Moderates the Relationship between the government intervention (subsidy) and dependent variable (Comparative Advantage) (Figure 1). Mizik (2021)

argues that the most important factor influencing domestic producers' competitiveness is favourable law and policy, which is followed by more complex and high-value items as well as extremely profitable and efficient production. According to Nagy and Jambor (2019), producing highly processed goods and specialising in producing one or a small number of the competitive goods are the primary drivers of a substantial competitive advantage.

Agricultural policies are a comprehensive set of laws and policies created by the government to accomplish goals outlined in plans for agricultural growth. These objectives are meant to promote greater production to attain food security, self-sufficiency, and a decrease in imports from overseas, which necessitates the payment of foreign funds that could be invested domestically and the improvement of added value to raise revenue and improve the income of workers involved in this activity (Al-Khazraji & Ahmed, 2023). Interestingly, when compared to their closest competitors, India, Pakistan, and Indonesia have consistently improved their fish export competitiveness (Md Ali et al., 2024). The National Shrimp Policy (NSP) has had a short-term detrimental effect on export potential, even if it has succeeded in reaching its objectives of sustainable production and market expansion.

The return on investment obtained with each transaction using the Ichimoku-based technique improved by roughly 8 to 9% in comparison to the pre-pandemic period. The Ichimoku-based approach could somewhat increase the cumulative return approach is less risky than the buy-and-hold approach (Che-Ngoc et al., 2023). Nandini and Samal (2020) assessed the profitability of technical indicators such the Simple Moving Average, Exponential Moving Average, and Moving Average in connection to COVID19. For the Malaysian stock market, the performance of six moving average (MA) criteria was examined by Lee (2020). They use utility indicators, return on equity, and policyholder internal rate of return to evaluate which market configurations, under low interest rates, optimise the return tradeoff for both stakeholders.

Higher contrarian profitability is associated with times when the market is trending upward, when market volatility and liquidity are higher, and when macroeconomic uncertainty is lower. Considering this, dates account for a sizable amount of Iraq's

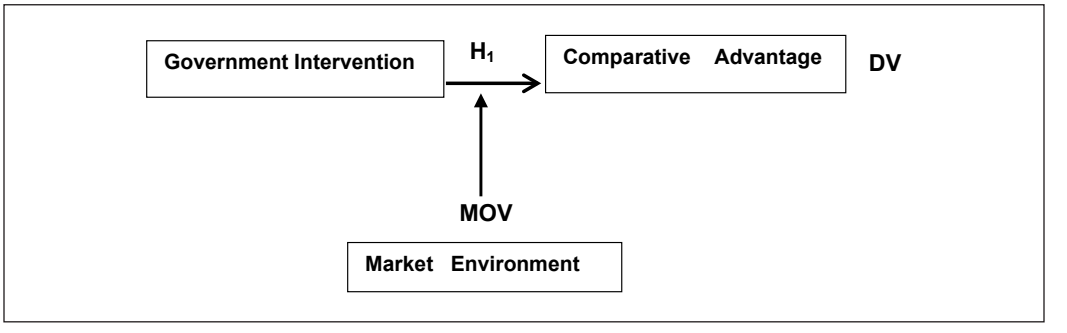

Figure 1. Conceptual framework

agricultural exports. If crop production is impacted by shocks that affect both the quantity and quality of output, such as pest epidemics, unfavourable weather, or changes in prices on the global market, both agricultural exports and the agricultural trade balance could be at risk (Al-Wasity et al., 2023). Even in the face of environmental legislation and factor endowments, strong institutions boost relative exports in clean industries (Shapiro, 2023).

H_1: Market environment moderates the relationship between government intervention (subsidy) and comparative advantage

MATERIALS AND METHODS

Using quantitative data gathering techniques, this study examines the moderating effects of market environment between mediating variable (government intervention (subsidy)) and Comparative Advantage of coconut farming in Malaysia. To gather quantitative data, a standardised questionnaire was created. The intended respondents, coconut farmers, were taken into consideration when creating the questionnaire. Getting the opinions of three research professionals on the study's topic area was the first step in creating the questionnaire's questions. The pertinent questions were subsequently revised after taking these remarks into account. Pre-testing of the study tools allowed for the identification and modification of items that respondents frequently misunderstood, ignored, or responded to incorrectly.

The Department of Agriculture Malaysia supplied a list of 160 coconut growers, from which information pertaining to coconut production was gathered. The G Power software developed by Edgar Erdfelder, Franz Faul, and Axel Buchner was used to calculate the study's sample size. In this case, 113 people make up the sample size for the 160-person population. For this reason, the researcher decided that 113 would be the ideal sample size for this investigation. However, as recommended by (Safara & Keshavarz, 2015), the study increased the sample size by 15% to account for incomplete questionnaires and sampling error.

To account for potential non-cooperative subjects and lost questionnaires, the researcher incorporated an extra 17 questioners (Bartlett, 2001), resulting in a total distribution of 130 questionnaires (113 + 17) for data collection. Nevertheless, a total of 123 questionnaires were recovered, yielding a 94.61% response rate—common in this kind of research. The survey's participants are the coconut farmers in Malaysia's coconut-growing states. The partial least squares structural equation modeling (PLS-SEM) technique, which creates composite reliability to emphasize the indication with greater reliability levels, was used in this work to examine the variables' reliability (Becker et al., 2012). For the variables in PLS-SEM to be considered significant, their outer loading must be greater than 0.60. Unless experts believe they can be kept, any variables with values less than 0.60 will be seen strong candidates for elimination (Fornell & Larcker 1981; Hair et al., 2012).

RESULTS AND DISCUSSION

The Results of the Structural Model Analysis

Hypotheses Testing Results

Normalcy of the data is not required because partial least squares is a non-parametric technique. Consequently, there is a chance that the T-values will either rise or fall, leading to a type 1 error. The bootstrapping approach is therefore recommended (Wong, 2013). To calculate bootstrapping standard errors, 5,000 subsamples are extracted from the original sample using replacement. T-value approximations for the structural path's significance test are generated by this process (Wong, 2013). The literature also recommends a variety of bootstrapping values, including 1000. The findings of the structural model employed in this study are shown in Figure 2.

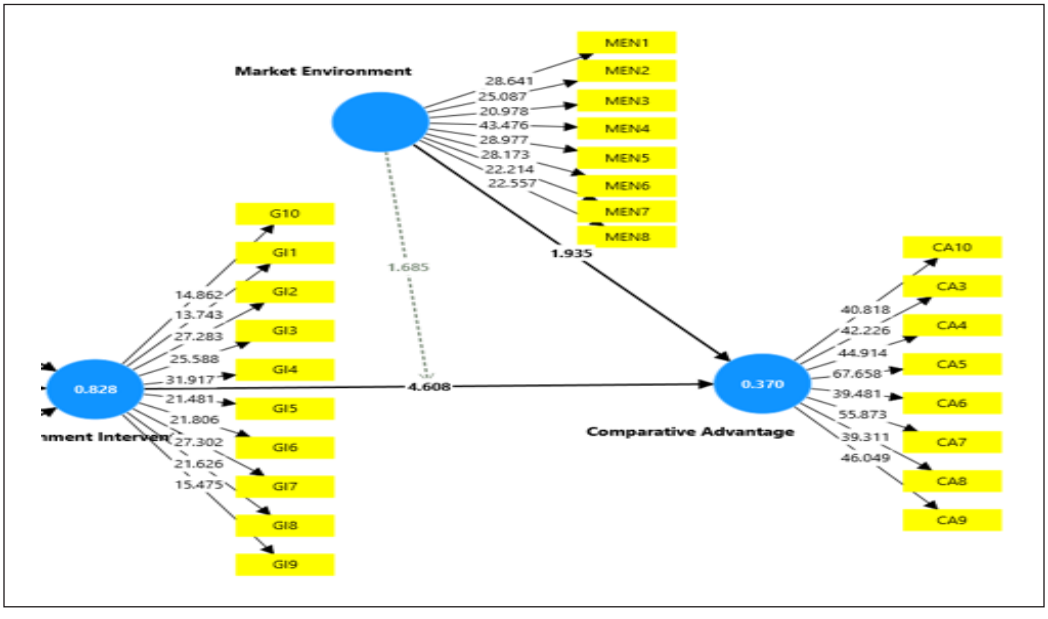

Figure 2. Results of structural model

The Moderation Analysis

In the current study, market environment was the moderator variable. H1 states that the market environment moderates the relationship between government intervention (subsidy) and Comparative Advantage. To test moderation, researcher has used the orthogonalisation approach as suggested by Becker et al. (2012), Results of the moderating test were discussed in Table 1.

H1: Market Environment Moderates the Relationship between Mediating Variable (Government Intervention) and Dependent Variable (Comparative Advantage)

Table 1
Indirect effect for moderator

Hypothesis	Relationship	Indirect Effect (β)	Sample mean (M)	Standard deviation (STDEV)	T statistics (|O/STDEV|)	P values
H1	ME x GI -> CA	-0.09	-0.083	0.053	1.685	0.093

Note. P values - *, **, *** represent significance at 1%, 5%, 10 % respectively

Researchers hypothesised that the market environment moderates the relationship between comparative advantage and government intervention (Table 2). Findings revealed that the moderation effect of the market environment between government intervention and comparative advantage was significant at 10% (β -0.09, t-value = 1.685, p-value = -0.093).

The p-value of 0.093 indicates that while the result is not statistically significant at the conventional 5% level ($p < 0.05$), it is close to significance at the 10% level. The findings imply that there is a statistically significant moderating influence of the market environment on the association between comparative advantage and government intervention, but this effect is only moderately strong (significant at the 10% level). The t-value indicates how many standard deviations the coefficient is from zero. The prevailing market environment moderates the effectiveness of government intervention, which can play a critical role in enhancing comparative advantage.

The t-value (1.685) indicates how far the beta coefficient is from 0 in terms of standard errors. Generally, a t-value above 1.65 at a 10% significance level suggests a trend worth considering, although it's weaker compared to higher significance levels. The findings suggest that when the market environment becomes more prominent, the positive effects of government intervention on comparative advantage weaken. The statistically significant at the 10% level indicates that the effect is not very strong, but it's still meaningful enough to warrant attention.

Table 2
Hypothesis summary (moderator relationship test)

	Hypothesis	Result
H1	Market environment moderates the relationship between government intervention (subsidy) and comparative advantage	Supported

CONCLUSION

The results indicate that the moderating effect of the market environment on the relationship between government intervention and comparative advantage is significant at 10%. The findings imply that there is a statistically significant moderating influence of the

market environment on the association between comparative advantage and government intervention, but this effect is only moderately strong (significant at the 10% level).

This study also revealed that the moderating effect of the market environment on government intervention and comparative advantage was significant at 10%. These findings offer practitioners actionable frameworks for improving competitiveness, emphasising the importance of both internal decision-making and external environmental factors.

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Starch-based Nanoemulsion of *Andrographis paniculata*: Prolonging Fruit Shelf Life by Reducing Postharvest Spoilage

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ABSTRACT

Post-harvest deterioration is a major contributor to food loss, estimated to affect up to 40% of global fruit and vegetable supplies, with the problem being particularly severe in regions with elevated temperature and humidity. These environmental factors hasten microbial activity and physiological breakdown, resulting in decreased market value, food availability, and nutritional quality. The accelerated spoilage in tropical climates underscores the necessity for environmentally responsible and economically viable preservation approaches, especially as worldwide populations grow and demand for fresh produce increases. Without effective intervention, ongoing losses threaten both the agricultural economy and broader food security. This investigation centres on novel starch-based edible coatings, which have been enriched with nanoemulsified extracts of *Andrographis paniculata* (NAP) across various concentrations (5–20% w/v). Among the tested formulations, the 20% NAP-coated film (NAP_{20}) achieved optimal nanoemulsion properties with a minimal droplet size of around 201 nm, ensuring consistent integration of bioactive compounds throughout the polymer matrix. Incorporation of NAP not only upgraded moisture resistance and structural stability but also enhanced the protective features of the coating when applied to bananas, resulting in reduced softening and extended firmness suitable for post-harvest storage. By demonstrating substantial improvements in the shelf life of fruit, this study establishes nanoemulsion-infused starch films as a sustainable and scalable solution to post-harvest spoilage. This approach provides an innovative pathway for minimising waste and supporting growers, retailers, and consumers in maintaining the quality of fresh produce.

Keywords: Andrographis paniculata, antimicrobial, nanoemulsion, starch-based coating

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INTRODUCTION

Bananas such as Pisang Emas (*Musa acuminata*) typically remain fresh for only around 5 days, as swift ripening from ethylene production leads to texture softening and heightened risk of spoilage (Singh et al., 2014). High humidity and temperature in tropical climates exacerbate this postharvest loss, which may reach up to 40%. Conventional methods such as controlled atmosphere storage and refrigeration slow ripening but can be costly and less accessible for small-scale producers (Musa et al., 2022). Recently, edible films incorporating natural bioactive compounds have emerged as sustainable alternatives to protect fruit quality by reducing moisture loss, microbial growth, and oxidation (Bizymis et al., 2024; Lopes et al., 2024). This study develops a starch-based coating loaded with nanoemulsified *Andrographis paniculata*, known for its antimicrobial and antioxidant effects. Combining nanotechnology with the film-forming ability of starch aims to extend banana shelf life in an eco-friendly, cost-effective manner, supporting food security, and reducing waste. This approach aligns with recent advancements in postharvest technology emphasising natural preservation techniques (Nuamduang et al., 2024; Paulo et al., 2021; Romero et al., 2022; Singh et al., 2014).

MATERIALS AND METHODS

Chemicals

Ethanol, methanol and Tween 80 were purchased from Chemiz (Malaysia). The antioxidant reagent 1,1-diphenyl-2-picrylhydrazyl (DPPH) was supplied by Sigma-Aldrich (USA). Whereas tapioca starch, glycerol, and coconut oil were obtained through Dchemie (Malaysia).

Extraction of *Andrographis paniculata*

To prepare the extract of *Andrographis paniculata*, stem material was dried, finely powdered, and subjected to extraction using a 70% ethanol solution, as outlined by Bennour et al. (2020). For extraction, 1 g of powdered stems was combined with 40 mL of ethanol, followed by filtration to remove insoluble fractions. The filtrate was then concentrated under reduced pressure at 45 °C with continuous agitation at 100 rpm for four hours using a HEI-VAP rotary evaporator (Germany).

Nanoemulsion Preparation and Evaluation of *Andrographis paniculata*

Nanoemulsions (100 g) were formulated by mixing *A. paniculata* extract at concentrations of 5%, 10%, 15%, and 20% w/v relative to oil, designated as NAP_5, NAP_{10}, NAP_{15} and NAP_{20}, using an oil:Tween 80:water ratio of 10:10:80. Based on Sundararajan et al. (2018), the mixture was homogenised at 14,000 rpm for 15 minutes and particle size was analysed with a Zetasizer Nano ZS (Malvern Instruments Ltd, UK).

Firmness Analysis of Bananas Coated with NAP Solution

The nanoemulsion with 20% A. paniculata extract exhibiting the smallest particle size was selected for firmness testing. Tapioca starch at 2% (w/v) concentration was dissolved in distilled water at 80 ºC and combined with 1% (w/v) glycerol. The coating solution was prepared by stirring a 1:1 mixture of nanoemulsion and starch solution for 30 minutes. Firmness of coated bananas was measured over 10 days using a Texture Analyzer TA.XT plus (Stable Micro Systems, UK).

Statistical Analysis

Data were analysed using the SPSS version 26.0 employing one-way ANOVA followed by the Duncan's test at a significance level of $p \leq 0.05$.

RESULTS AND DISCUSSION

Characterisation and Optimisation of *Andrographis paniculata* Nanoemulsion

Examination of the *Andrographis paniculata* nanoemulsions showed a distinct decrease in droplet size with increasing extract concentration, as depicted in Figure 1. For instance, average droplet size reduced from 293.9 nm in the NAP_5 formulation to 201.5 nm observed with NAP_{20}. This outcome can be explained by the presence of natural surfactant compounds like andrographolide and flavonoids, which lower interfacial tension and support the stability of finer droplets. Utilising higher extract levels leads to the formation of a denser interfacial layer, greatly reducing droplet merging and slowing Ostwald ripening, thereby creating a more stable and uniform nanoemulsion (Pornpitchanarong et al., 2024). The improved stability and decreased size distribution indicate promising physical characteristics suited for formulation purposes. Given its

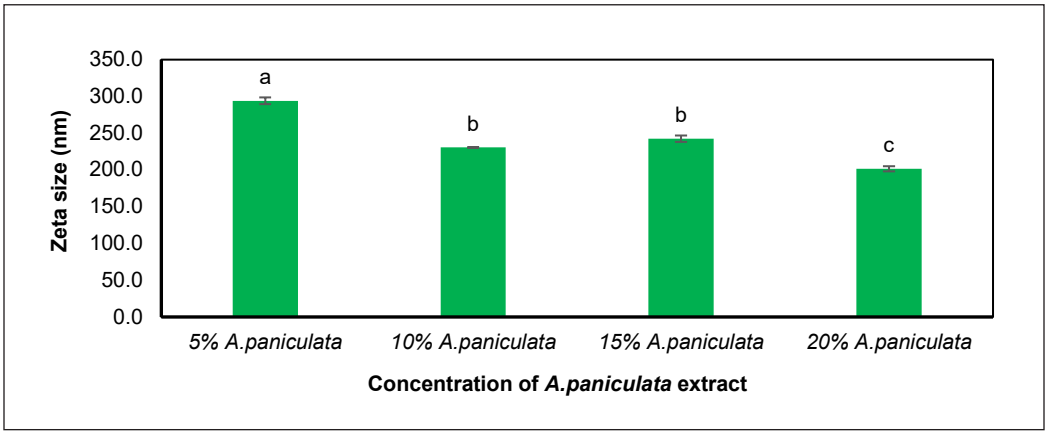

Figure 1. Zeta diameter of NAP prepared with varying *A. paniculata* extract concentrations
Note. Distinct superscripts denote significant differences ($p < 0.05$)

advantageous particle size and expected stability, the nanoemulsion containing 20% extract was chosen for further tests, specifically to assess its effectiveness in maintaining banana firmness throughout storage.

Effect of Nanoemulsion Coating on Banana Physical Quality

During the ripening process, bananas tend to soften largely due to water loss and the breakdown of cell walls. Application of a 20% NAP coating markedly preserved the firmness of bananas compared to uncoated samples, as depicted in Figure 2. After ten days, coated bananas retained a firmness of 483.24 g/mm, significantly higher than the 219.09 g/mm recorded for the control group. The nanoemulsion layer efficiently curtails moisture loss, manages gas exchange, and decelerates ripening. In addition, bioactive agents present in Andrographis paniculata contribute antimicrobial properties that safeguard the fruit's cellular structure and delay the onset of softening.

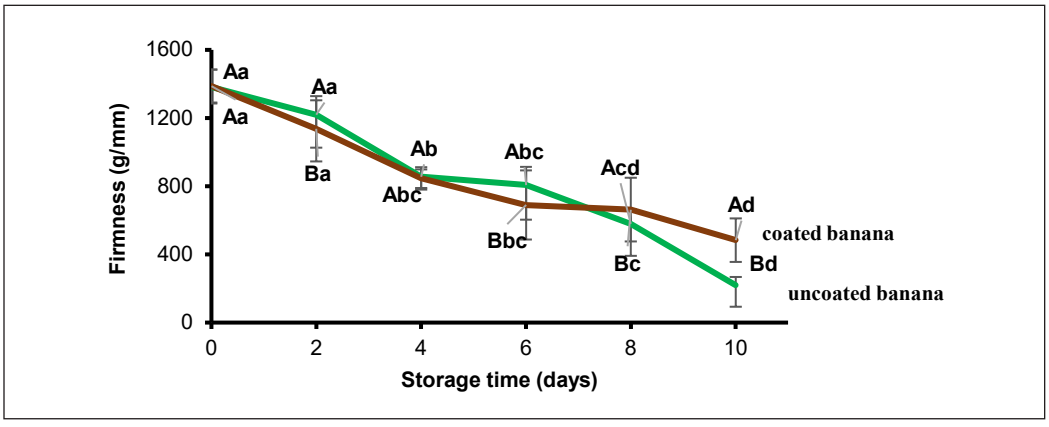

Figure 2. Firmness of NAP-coated bananas over 10 days at ambient temperature
Note. Uppercase letters denote significant differences between sample types ($p < 0.05$), while lowercase letters reflect significant changes across storage days ($p < 0.05$)

CONCLUSION

The application of NAP coating effectively decelerated the spoilage of bananas and improved the protective capacity of starch and enhanced the film's antimicrobial resistance. Edible films based on nanoemulsions can serve as a biodegradable substitute for synthetic coating, reducing postharvest losses.

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Triploidy Seeds Development in Watermelon *Citrullus lanatus* (Thunb) Matsum. & Nakai

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ABSTRACT

Watermelon, *Citrullus lanatus*, is a member of the family Cucurbitaceae; it originated from Africa and extensively grown in the tropical regions. In Nigeria, seedless watermelon is preferred by consumers; however, the cytogenetic and breeding factors are less understood leading due to limited genotypes. This study investigated the breeding and cytogenetic factors influencing the production of triploid seeds in watermelon. Diploid seeds were purchased from Technisem Agritropic Limited, Nigeria. After three weeks, the resulting seedlings were induced using colchicine concentrations. The successfully induced tetraploids were hybridised with the diploid parents, while chromosomal study was conducted using Orcein. Data were collected and subjected to one-way ANOVA at $P<0.05$. The colchicine treatment revealed 0.2% as the LD50. The hybridisation results showed successful formation of triploid seeds. Chromosomal analysis showed the chromosomal numbers: (i) diploid variety has 2n=22, (ii) tetraploid variety has 4n= 44 and the triploid has 3n=33. The triploid seeds and the established protocols are inevitable for the sustainable production of seedless watermelon.

Keywords: Hybridisation, seedless watermelon, tetraploidy, triploidy

INTRODUCTION

Watermelon, *Citrullus lanatus*, is a member of the family Cucurbitaceae; it originated from Africa and widely grown in the tropical and sub-tropical regions. Watermelon is an important herbaceous crop often grown for its sweet and juicy fruit; the crop is grown in warm climates the world over (Dube et al., 2021).

Seedless watermelons are triploid fruits due to their ploidy level. Seedless watermelon was first unravelled at Kyoto University Japan by Kihara in 1951 (Padhan & Kumar, 2022). Under natural condition

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watermelons exist as diploid plants with chromosomal number 22. The genus *Citrullus* is known to include the following species- *Citrullus lanatus, C. ecirrhosus, C. colocynths* and *C. rehmii* (Achigan-Dako et al., 2021).

Polyploidy in organisms is said to occur when there are multiple sets of chromosomes in the somatic cells other than the usual diploid numbers (Venkatesh, 2021). The cytogenetic and breeding factors for the development of the seedless watermelon are less understood; additionally, there are limited genotypes of the cultivars in Nigeria, hence the seeds of seedless watermelon are not available. The aim of the study is to investigate the breeding and cytogenetic factors influencing the production of triploid seeds in watermelon *Citrullus lanatus* (Thunb) Matsum. & Nakai.

MATERIALS AND METHODS

Source of Materials

Kaolack variety of watermelon was used for the research. Seeds were purchased from the company Technisem Agritropic limited Nigeria, Jos, Plateau State, Nigeria.

Tetraploidy Induction via Colchicine

Seedlings were induced by dropping method using cotton wool placed on the apical shoot to keep it moist. 0.2% Colchicine solution was used to induce tetraploidy on the seedlings (Wehner, 2022).

Cytological Studies

Cytological studies were conducted to affirm chromosomal number and stomata concentration. Chromosome number count using flower bud: flower bud was collected in the morning (7.00-9.00 am) and evening (4.00-6.30 pm). The sample was kept in fixative (3 Ethanol: 1 Acetic Acid) for 24 h to arrest cells and maintain them at the current stage of cell division. The flower bud was pressed for the pollen mother cell to emerge and then subjected to cytological procedures (Tong & Wang, 2024). Stomata size: Nail varnish was used to make an imprint on the lower surface of the leaf. The coated portion was carefully removed and placed on a slide containing a drop of water. The set up was covered with a cover slip and observed under light microscope. The stomata sizes were measured on Canva Software and calculated accordingly. Stomata Size= (SLXSWXK) Where SL=Length of stomata, SW= width of Stomata and k=0.78524.

Morphological Parameter Evaluations and Hybridisation

The morphological parameters (tendril length, stem diameter, number of leaves and leaf area) evaluations and hybridisation experiment were carried out in this section. Reciprocal

pollination was conducted manually with hand through cap and recap method between diploid and tetraploid plants to effect hybridisation between the diploid and the tetraploid plants (Popelka et al., 2019). The collected data were analysed using IBM SPSS version 22.0, and analysis of variance (ANOVA) at $P \leq 0.05$ level of significance.

RESULTS AND DISCUSSION

Treatment of Seedlings for Tetraploid Induction

The treatment of watermelon shoots with 0.2% colchicine concentrations successfully induced tetraploidy. Section 3.4 showed results on some cytological parameters were used to confirm tetraploidy induction. Similarly, Handayani et al. (2018) also reported that the induction of tetraploidy using 0.2% colchicine solution was effective.

Cytological Studies

Cytological study on stomata sizes showed result ranges of 47.11 µ to 54.97 µ in the tetraploids and 15.70 µ to 28.27 µ in the diploid. This showed that the tetraploids have stomata larger in size compared to that of diploid. This result agrees with the report from Handayani et al. (2018) who reported significant differences in stomata sizes between diploids and tetraploids in Watermelon. The chromosomal number showed the tetraploid plants have n=22, while the diploids have n=11 in the meiotic cells. The doubling number of chromosomes in this result confirmed the tetraploidy induction in the plant.

Morphological Parameter Evaluations and Hybridisation

The morphological parameters showed that the tendril length, number of leaves and leaf area increased significantly. There was no significant increase in stem girth. Tetraploid lines showed high growth rate over the diploids, in consonant with findings by Cui et al. (2017). Hybridisation conducted between the tetraploid and the diploid lines showed fruits formation. The tetraploids have larger size of flowers than the diploid. Triploid seeds were successfully developed: 3n=33, n=11. Triploid seeds were obtained from the resulting fruits of the crosses. In similar research, pointed Gourd revealed triploid seeds as reported by Hassan et al. (2020).

CONCLUSION

The study succeeded in the development of triploid seeds from crosses between tetraploids and diploids. The development of triploid seeds of watermelon has added to the gene bank of watermelon in Nigeria. With the triploid seeds, the production of seedless watermelon becomes seamless.

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Unleashing the Antioxidant Potential of Local Indonesian Bay Leaf, *Syzgium polyanthum*

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ABSTRACT

Daun salam, or Indonesian bay leaf with the scientific name *Syzygium polyanthum,* is a traditional medicinal plant rich in bioactive compounds. The plant is almost unknown and unrecognised by local people. The leaf was actually more popular in Indonesia due to the wide usage in cooking and was even drunk as tea. This study aimed to evaluate the antioxidant activity and phenolic composition of its leaf extracts. The bay leaf drink was prepared by boiling it until the final volume was reduced to two-thirds of its initial volume. Antioxidant capacity was assessed using the Ferric Reducing Antioxidant Power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assays, and quantification of total phenolic content (TPC) was measured via the Folin–Ciocalteu method. Ultra-Performance Liquid Chromatography (UPLC) was also employed to profile individual phenolic constituents. The extracts demonstrated the scavenging effect of the DPPH radical in bay leaf was 71.18% inhibition, while the FRAP was 0.148 mM$FeSO_4$, and total phenolic content (TPC) was 177µgGAE/ml. Based on the results, DPPH showed strong free radical activity and high TPC associated with high DPPH scavenging. UPLC profiling identified compounds such as gallic acid, catechin, and ellagic acid as major phenolics. The bay leaf aqueous extract exhibited notable antioxidant activity, evidenced by high DPPH, moderate total phenolic content, indicating its potential as a natural source of antioxidants.

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Keywords: Antioxidant, bay leaf, *Syngium polyanthum*

INTRODUCTION

Antioxidants play a vital role in combating oxidative stress, a major contributor to chronic diseases such as cardiovascular disorders, neurodegeneration, and cancer. Among natural sources, plant-based antioxidants are

preferred for their safety and efficacy (Sharma et al., 2021). *Syzygium polyanthum* (Wight) Walp., commonly known as Indonesian bay leaf or *daun salam*, is a tropical species of the Myrtaceae family native to Southeast Asia, including Malaysia, Indonesia, Thailand, Vietnam, and the Philippines (Quattrocchi, 2012; Wilson, 2011). Despite its resemblance to the Mediterranean bay laurel (*Laurus nobilis*), it is taxonomically distinct (Dalimartha, 2005; Renfrew & Sanderson, 2005).

In Malaysia, particularly in Kelantan and Terengganu, *S. polyanthum* is an integral part of traditional leaf used to flavor dishes such as *nasi kerabu* and herbal soups (Anuar, 2019) and traditional medicine for ailments like diarrhea, fever, and skin irritation. Recent studies have begun to validate its pharmacological potential: ethanolic extracts exhibit strong antioxidant activity (Yunarto et al., 2022), while essential oils show antimicrobial properties (Universiti Putra Malaysia, 2020). Halim et al. (2022) further identified it among Malaysia's top antioxidant-rich herbs.

This study, therefore, investigates the antioxidant activity and phenolic composition of *S. polyanthum* aqueous leaf extract to scientifically support its traditional uses and explore its potential for functional food and nutraceutical development.

PROBLEM STATEMENT

Although *S. polyanthum* (Indonesian bay leaf or *daun salam*) is widely used in Southeast Asian cuisine and traditional medicine, its scientific evaluation in Malaysia remains limited. While previous studies have shown antioxidant and antimicrobial properties in ethanolic and essential oil extracts, data on its aqueous extract reflecting traditional preparation are scarce. The absence of detailed profiling of its phenolic compounds and antioxidant capacity hinders its development as a functional food or nutraceutical. This study, therefore aims to evaluate the antioxidant activity and phenolic composition of *S. polyanthum* aqueous leaf extract using validated analytical methods.

RESEARCH QUESTIONS

This study aimed to evaluate the antioxidant capacity of *S. polyanthum* aqueous leaf extract through DPPH, FRAP, and TPC assays, and to characterise its phenolic composition using UPLC (Table 1). Additionally, the research sought to establish the relationship between its phenolic profile and potential applications in functional food and nutraceutical formulations.

Table 1
Antioxidant activity and total phenolic content of Syzygium polyanthum *aqueous extract*

Daun Salam	DPPH	FRAP	Total Phenolic Content
	71.18%	0.148 mM$FeSO_4$	177µgGAE/mL

Note. Values are the mean and standard deviation of triplicate independent runs

This study evaluated the antioxidant capacity of *Syzygium polyanthum* (Indonesian bay leaf) aqueous extract using DPPH, FRAP, and TPC assays, and identified its phenolic profile via UPLC. The extract showed strong antioxidant potential, consistent with traditional use and earlier findings. DPPH radical scavenging activity reached 71.18%, indicating high free radical neutralisation, while the FRAP value of 0.148 mM $FeSO_4$ equivalents reflected moderate reducing power. The total phenolic content (177 µg GAE/mL) correlated well with antioxidant performance, confirming phenolics as key contributors (Poojary et al., 2021). UPLC detected eleven phenolic acids, with gallic acid (12.38 ± 0.46 ppm) as the most abundant, followed by chlorogenic and 2,5-dihydroxybenzoic acids. These compounds, known for their antioxidant, anti-inflammatory, and antimicrobial properties (Chen et al., 2020; Upadhyay & Dixit, 2015), collectively enhanced the extract's bioactivity. The presence of gallic, caffeic, ferulic, and p-coumaric acids suggests synergistic effects that strengthen antioxidant efficacy (Chung et al., 1998; Pereira et al., 2009). Overall, the diverse phenolic composition underscores the strong antioxidant potential of *S. polyanthum* and confirms its ethnomedicinal use, highlighting its promise as a natural antioxidant source for functional food and nutraceutical applications. Table 2. Phenolic acid composition of local *Indonesian* bay leaf, *S. polyanthum* aqueous leaf extract, as determined by UPLC.

Table 2
Phenolic acid composition of local Indonesian bay leaf, Syzygium polyanthum *aqueous leaf extract as determined by UPLC*

Phenolic Acid	Amount (ppm)
Quercetin	0.0564±0.0
2,5-dihyrobenzoic acid	0.66±0.03
Chlorogenic acid	0.93±0.07
Caffeic acid	0.1255±0.01
p-coumaric acid	0.28±0.02
Ferulic acid	0.31±0.06
Sinapic acid	0.1442±0.004
Gallic acid	12.38±0.46
Protocatechic acid	0.54±0.01
4-Hydroxybenzoic acid	0.18±0.001
Vanillic acid	0.08±0.004

Note. Values are the mean and standard deviation of triplicate independent runs

CONCLUSION

This study confirmed the antioxidant potential of the local Indonesian bay leaf, *S. polyanthum*. The aqueous extract exhibited strong DPPH radical scavenging activity (71.18%), moderate ferric reducing power (0.148 mM $FeSO_4$), and a high total phenolic content (177 µg GAE/mL). UPLC analysis identified gallic acid as the major phenolic compound, along with chlorogenic, caffeic, and ferulic acids as key bioactive constituents. These results validate the traditional use of *S. polyanthum* in food and medicine and highlight its promise as a natural antioxidant source for functional foods, dietary supplements, and therapeutic formulations. Further studies should examine its bioavailability, in vivo efficacy, and synergistic interactions among its phytochemicals to enhance its application potential.

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Object-based Image Analysis (OBIA) for Bamboo Area Classification using Unmanned AerialV (UAV): A Case Study in Koperasi Kariah Masjid Kundur Ulu (KOMASKU), Rembau

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ABSTRACT

Bamboo cultivation in Rembau, Peninsular Malaysia, presents opportunities for community-based marketing, particularly as a food supply for Giant Pandas, with the potential to enhance local income. Bamboo plantations can serve as both productive landscapes and leisure spaces for villagers and visitors, underscoring the need for accurate area assessment. In Model 1 ("vegetation" vs. "non-vegetation"), subsets A, B, and C achieved overall accuracies of 97.79%, 94.10%, and 99.08%, with Kappa values of 0.60, 0.84, and 0.84, respectively. In contrast, Model 2 ("bamboo" vs. "non-bamboo") showed poor performance, with accuracies of 85.71%, 56.71%, and 52.50%, and Kappa values of 0.67, 0.15, and –0.01. The results highlight UAV based on OBIA as effective for general vegetation mapping but less robust for bamboo-specific classification and larger area. Spectral similarity by other landscaped combined with bamboo and mixed vegetation, infrastructure reduce the stability, reliability, and consistency of the classification conducted in the study. Conclusively, bamboo classification maps are valuable not only for methodological advancement but also for supporting agricultural and agroforestry planning, including plantation management, yield estimation, and land-use mapping.

Keywords: Bamboo, community, fibre strength, vegetation indices, world fibre

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INTRODUCTION

To begin with, bamboo, widely known as the "poor man's timber" or "green gold," is a key forest resource distributed across tropical and subtropical regions (Alamerew et al., 2024). Specifically, most of the bamboo resources in Asia originates from

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South and Southeast Asia which amounted to 17.87 million hectares followed by East Asia about 7.00 million hectares (Tahir et al., 2023). Meanwhile, bamboo is planted around the globe because its unusual fibre characteristics and longevity. In terms of growing capability, bamboo is known as multifunctional and fastest-growing plant on Earth (Ahmad et al., 2021). Beyond Asia, bamboo is distributed mainly in Brazil, Columbia, Venezuela, Panama, Argentina, and many more countries located in tropical region countries (Ruiz-Sanchez et al., 2021).

Due to its huge benefits and rapid growth small holders are adopting bamboo in their small plantation nearby their home. To date, UAV-based image classification has become well established, frequently being applied for segmenting forest, non-forest, agricultural, and urban tree canopy. In terms of methodology, the best digital land cover maps to date have been produced using OBIA, which usually achieves good accuracies. In fact, the study highlighted that the lack of direct transferability is an important limitation of OBIA methods since, once calibrated for one image, the OBIA settings are not directly portable to other images (e.g., to different areas, extensions, radiometric calibrations, background colour, spatial and spectral resolutions, or different sizes or shapes of the target objects).

As a result, this research aims to evaluate imaging classification techniques for bamboo monitoring to provide up-to-date mapping tools. Specifically, to develop an efficient bamboo classification model for distinguishing bamboo from non-bamboo vegetation. Finally, the study assessed the accuracy of the classification model compared for smallholder bamboo plantation mapping, that indicate land use types for the study area.

MATERIALS AND METHODS

Study Area

This study was conducted in the Betong (*Dendrocalamus asper*) bamboo plantation area in Rembau District, Peninsular. The study received prior consent and cooperation from the cooperatives, which manages the plantation as part of its community-based economic activities. The plantation is owned by Koperasi Khariah Masjid Kundur Ulu (KOMASKU), a local cooperative comprising residents of Kampung Kundur Ulu, which manages small-scale economic activities such as a local food restaurant and the *Pusat Jualan Produk IKS Kundur* Figure 1. showed study site for this study.

Data Acquisition

UAV flights were implemented to obtain high-resolution raw raster data on RGB cameras in the study area in May 2024. For this purpose, the DJI Matrice 300 RTK (M300 RTK), using Zenmuse L2 camera, that integrate LiDAR sensor with an integrated RGB camera for 3D mapping and forestry applications (DJI, 2025) was deployed. This UAV is a professional-grade quadcopter that integrates Real-Time Kinematic (RTK) positioning

Figure 1. (Left) Orthomosaic of the KOMASKU bamboo plantation site; (Middle) Exact location of the OBIA classification area; (Right) Team set-up the UAV flight

technology, enabling centimetre-level accuracy in aerial surveys, which assists farmers to obtain accurate information Figure 2 showed the team setting up the flight mission, with the UAV positioned for launch.

OBIA Workflow

Geographic OBIA also can be referred to OBIA, as explain in (Chen et al., 2018). Accurately, it refers as a classification technology sets adjacent pixels as an objects to identify interested spectral elements, and makes full use of spatial, texture and spectral information of high-resolution panchromatic and multi-spectral data to segment and classify, and outputs high-precision classification results or vectors (Zhao et al., 2020). OBIA is particularly valuable as it moves beyond a land-cover centric view that relies only on the spectral characteristics of pixels and instead integrates both spectral and spatial (contextual) information (Blaschke, 2010; Ma et al., 2017). Classification is carried out using OBIA through CATALYST Professional Version 2 (Figure 2). Segmentation is the important steps in this technique, where it refers to as a bridge between raw pixel data and meaningful interpretation (Riabko, 2023). The segmentation process was completed using the defined parameters (Scale = 150, Shape = 0.5, Compactness = 0.5), after that attribute calculations were subsequently performed (Figure 3). The classification model was developed for Model 1: General vegetation (vegetation and non-vegetation) and Model 2: Bamboo (bamboo and non-bamboo). The main objective of the model segmentation process is to remove irrelevant features such as roads, rivers, houses, shops, and mosques. by site visit information and ground-truth validation.

For this study, Support Vector Machine (SVM) algorithm was employed. The study employed Radial Basis Function (RBF/Gaussian kernel) (Sawarkar et al., 2023). The details of the training site editing process was presented in Figure 4. The selection of classes is based on attribute information calculation conducted prior of the training site editing. The SVM is well known classifier and produced an overall accuracy of 85%

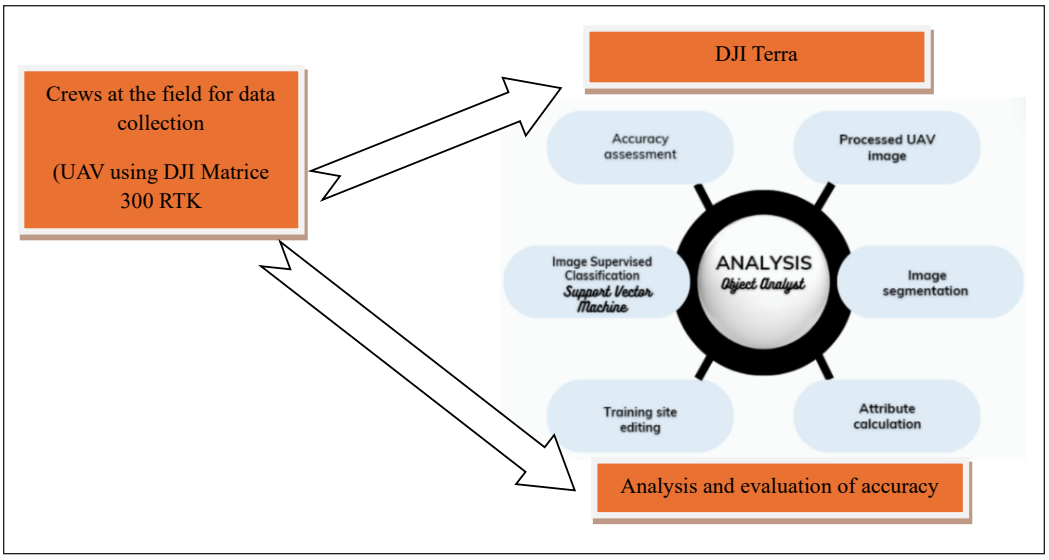

Figure 2. Workflow of UAV bamboo mapping at the KOMASKU plantation site

and kappa coefficient value of 0.74 when compared with conventional classifier (Kamarulzaman et al., 2022). These results highlight the distinct spectral separability of structured plantation crops compared to heterogeneous vegetation types (Razali & Lion, 2021), such as demonstrated in the study.

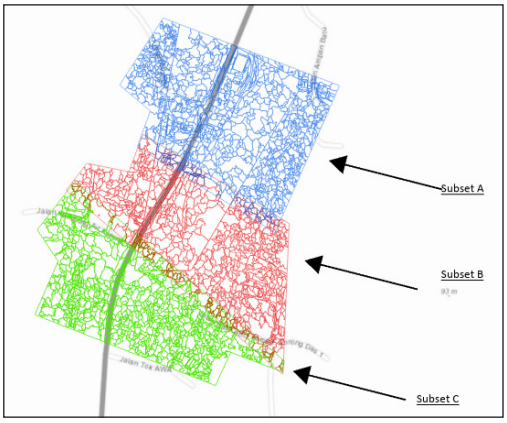

Figure 3. Overview of all the segmentation segmentation into three subsets (A – blue, B – green, and C – red) to facilitate image analysis

RESULTS AND DISCUSSION

The segmentation is evaluated based on visual evaluation with expert judgment, based on ArcGIS Pro base maps. Field reference employed as supports materials for features identification during overlaid process. Based on Figure 3, the subsets produced reveal that, for Model 1, the data show a strong skew toward the "vegetation" land-use category, which comprises most entries—approximately 95%. This suggests that "vegetation" is the dominant category within this subset A. The results show that "vegetation" dominates the category with 31,907 counts, while "non-vegetation" has only 323 counts, indicating a strong ability of discrimination between the two classes. Meanwhile, subset C showed the bar chart has a significantly higher count of 17,817 for "vegetation", compared to "non-vegetation", which has a count of 181.

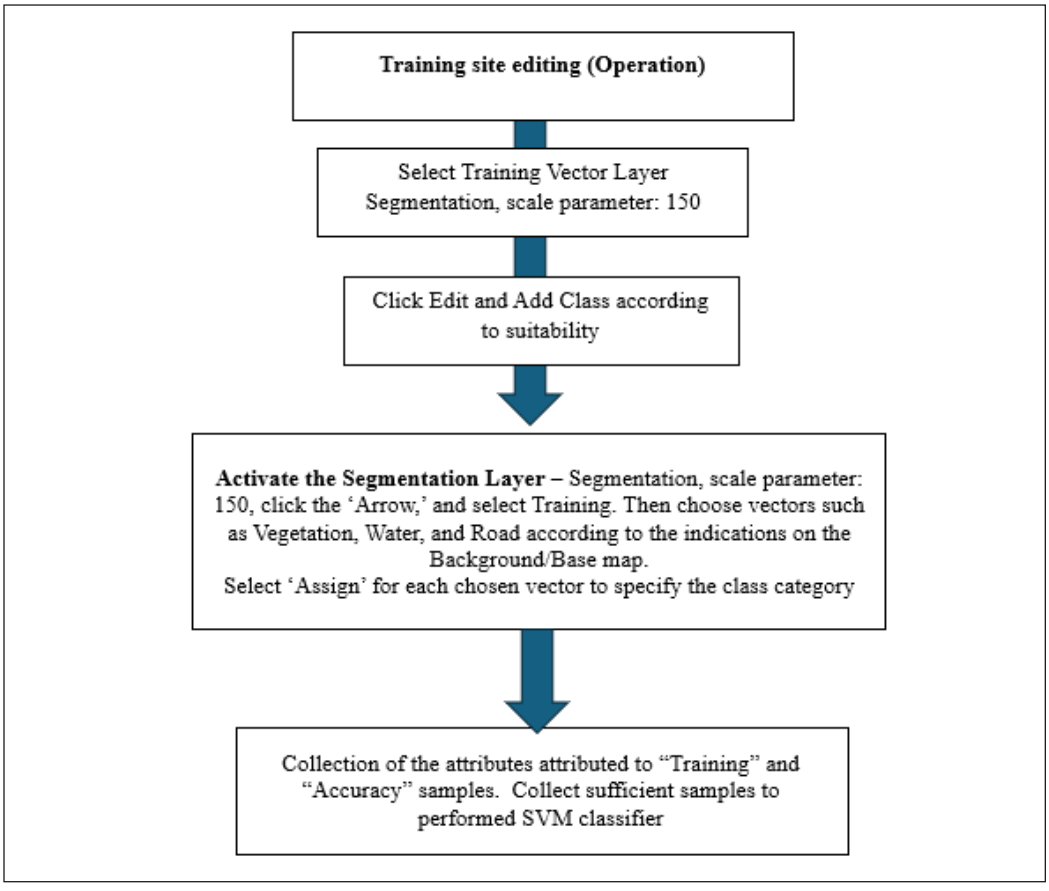

Figure 4. Workflow of the training site editing for the classification

Models' Classification Scheme and Data Sampling

The model classification was developed for the models. The designed scheme outlined rules and criteria for selecting training samples for training site editing, which the study employed SVM techniques for classification (Table 1).

To evaluate the effectiveness of this classification framework, an accuracy assessment was conducted that presented in table below (Table 2). Training and accuracy samples collected in the training site editing procedure.

Data Accuracy Assessment

The kappa coefficient, like most correlation measures, can range between -1 and +1 (Chicco et al., 2021). When measuring the accuracy, a value of Kappa greater than 0.75 might be deemed (arbitrarily) as "excellent" agreement, whereas a value less than 0.4 indicates "poor" agreement (Table 3).

Table 1
Classification rule for training site editing

Model	Land use type	Classification rules
1	Vegetation	All vegetation features selected are other than infrastructure such as houses and shops. They also do not include rivers and main roads or village roads.
	Non-vegetation	Including all non-vegetation features using a combination of true colour UAV visualization of the image.
2	Bamboo	Perform pre-collection before non-bamboo features to avoid overlapping feature selection. Most of the bamboo trees seen in this UAV data have shadows. Shadows are selected as non-bamboo. Bamboo shows small leaf opening and clumps therefore the clump shape is easy to see from above.
	Non-bamboo	This sample is easier for non-plant types, such as roads, village roads, rivers, buildings including village houses and mosques. Plants other than bamboo are easier to sample by only picking bamboo that shows the shape of "broccoli" and is present without grouping.

Table 2
Training sample collected for the accuracy assessment

Model	Land use type	Training	Accuracy
1	Vegetation	5915	3360
	Non-vegetation	151	194
2	Bamboo	579	258
	Non-bamboo	482	287
Total sample	7127		4099

Table 3
Accuracy assessment of Model 1 and Model 2 across polygon subset area (A, B, and C), showing overall accuracy, allocation disagreement, and Kappa coefficients

Model	Subset area	A	B	C
1	Overall accuracy (%)	97.70	94.10	99.05
	Allocation disagrees (%)	0.90	28.12	0.78
	Kappa	0.60	0.84	0.84
2	Overall accuracy (%)	85.71	56.77	52.50
	Allocation disagrees (%)	10.88	28.13	40.00
	Kappa	0.67	0.15	- 0.01

The accuracy assessment indicates Model 1 demonstrates high overall accuracy across all polygon subsets, with percentages ranging from 94.10% to 99.05%. Its Kappa coefficients (0.60 to 0.84) indicate moderate to excellent agreement, particularly for subsets B and C, which show strong agreement (Kappa > 0.75). In contrast, Model 2 showed more variable and generally lower performance. Subset A achieved reasonable

accuracy (85.71%) with a Kappa value of 0.67, showed a moderate agreement. However, performance declined significantly found in Subset B (56.77% accuracy, Kappa = 0.15) and Subset C (52.50% accuracy, Kappa = –0.01), indicating little to no agreement between classification and ground reference. The final map for "bamboo" and "non-bamboo are presented below (Figure 5).

These lower accuracies can be attributed to spectral confusion between bamboo and other vegetation types, as well as shadow effects from UAV imagery that obscure bamboo clump structures (Blaschke, 2010; Ma et al., 2017). These findings highlight a key limitation in applying Model 2 for bamboo classification. The low performance of the Model 2 particularly in subsets B and C, can be attributed to spectral confusion between bamboo and other vegetation types, especially in heterogeneous landscapes. In this aspect, based on other study higher classification accuracy was recorded in the case study of UAV data *Fallopia japonica* and *Portulacaria afra* due to higher availability of training (Soltani et al., 2022) data, which unlike the Model 2. Lower training and accuracy samples mislead the classification and results in inappropriately identified the "bamboo" and "non-bamboo" across the subset. Consequently, the homogeneity of bamboo species could also be due to low overall accuracy, hence low kappa statistics for Model 2 in subset C. This task is very challenging to bamboo area with similarity to forest understory (Liu et al., 2021) , bushes and various herbs and lianas, that occurred in the study area.

RELEVANCE TO AGRICULTURE APPLICATION

In particular, the bamboo classification maps are not only important from a methodological perspective but also have direct implications for agricultural and agroforestry applications. The Table 3 the high accuracy observed in Model 1 ("vegetation" vs. "non-vegetation") demonstrates the reliability of UAV-based OBIA in delineating cultivable land versus non-productive areas, for example "banana" and "non-banana". In addition, the models

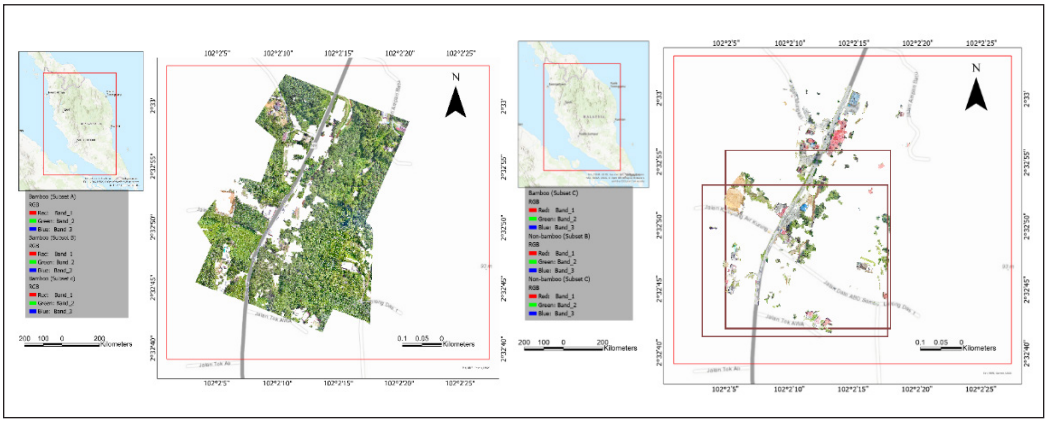

Figure 5. Bamboo final maps based developed based on OBIA classification techniques for the study area

developed from the study is fundamental in plantation establishment and expansion planning that can be employed in agricultural company such as SD Gutherie for Sime Darby Plantation for oil palm plantation area mapping.

However, when the focus shifts to bamboo-specific mapping (Model 2), the results reveal limitations. The lower overall accuracy and Kappa values in Subset C suggest that bamboo is not easily distinguished from other woody vegetation. In the meantime, now a days new methodology of Convolutional neural network (CNN)-based methods have been widely used to predict crop types according to UAV remote sensing imagery, which has excellent local feature extraction capabilities (Dersch et al., 2023; Xiang et al., 2023; Zhang et al., 2022).

CONCLUSION

This study demonstrates the potential of OBIA for bamboo mapping in both productive landscapes and leisure spaces for villager's areas. Model 1 successfully differentiated vegetation from non-vegetation with consistently high accuracy, which confirmed by many studies. However, Model 2 revealed the limitations of bamboo-specific classification, where spectral similarity with low growth from surrounding vegetation and subset variability reduced classification stability. Despite these challenges, the findings is so practical and relevance for the forestry, agricultural and agroforestry sectors.

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Growth Performance of Redclaw, *Cherax quaricarinatus* through Pineapple Waste Utilisation

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ABSTRACT

The Australian Redclaw crayfish (*Cherax quadricarinatus*) has significant potential for aquaculture production. This study aimed to produce pellets incorporating pineapple waste and to evaluate the growth performance of redclaw based on body weight, body length, survival rate, and total number of molts. The experiment consisted of four dietary treatments: Diet 1 (0% pineapple waste), Diet 2 (5% pineapple waste), Diet 3 (10% pineapple waste), and Diet 4 (15% pineapple waste). A total number of 80 juveniles were used in the study were 4.7–7±0.69 cm in body length and weighed 3–9 ± 1.53 g. Results indicated that the percentage of pineapple waste in the diet influenced total weight, length, and weight gain. Notably, the pellets containing 10% pineapple waste achieved the highest average growth, with a body weight of 15.26 g and a body length of 8.46 cm, outperforming the other diets. Besides, the survival rate was 100 % during the period of study for 60 days. The total number of molts observed for the diets containing 5%, 10%, and 15% pineapple waste was a cumulative total of 10 times. In conclusion, the use of pineapple waste pellets significantly enhanced the growth of redclaw, demonstrating an effective way to utilise this agricultural byproduct.

Keywords: Growth performance, pineapple waste, pellet, redclaw, survival rate

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INTRODUCTION

Pineapple is a rapidly expanding agricultural product in Malaysia, with various processed goods such as juice, jam, and beverages derived from it. During the production process, by-products including the stem, crown, core, and peel are discarded. This raises concerns about the disposal of pineapple waste, which is considered

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agricultural waste. Each year, approximately 150,000 kg of pineapple waste is generated (Selvanathan et al., 2020). Studies have shown positive effects of pineapple waste on animal growth, with significant improvements in growth rates compared to control groups (Sukri et al., 2022). Building upon this, the current research focuses on developing feed pellets for redclaw, *Cherax quadricarinatus* using pineapple waste as a primary ingredient. *Cherax quadricarinatus* was introduced to Kluang, Johor, Malaysia from Australia in 1990 for aquaculture purposes (Naqiuddin, 2020). The first wild population was recorded in 2012 in Parit Sulong, Johor, and Bintulu, Sarawak (Naqiuddin, 2020). This species has since been classified as invasive in Malaysia due to its environmental and economic impacts (Dali et al., 2023). Although widely cultivated for food, Redclaw has escaped from aquaculture facilities and spread into natural water bodies across the country (Dali et al., 2023). One major challenge in Redclaw farming is the limited availability of specialised compound feeds, which often results in nutritional imbalances, slow growth, and high mortality rates during culture (Chen et al., 2024).

The first wild record of *C. quadricarinatus* on the East Coast of Peninsular Malaysia involved six specimens identified through morphological and molecular analysis. These were captured in their natural habitat in Terengganu, an area with no nearby Redclaw aquaculture operations. This suggests the possibility of accidental release by aquarium hobbyists (Norshida et al., 2021). Further research is necessary to understand the spread and ecological impact of this non-native species along the East Coast and surrounding regions.

Previous studies, such as the investigation into the use of pineapple waste in the diet of Nile Tilapia (*Oreochromis niloticus*), have demonstrated positive outcomes. The fish showed optimal weight gain, improved specific growth rate, and no adverse effects on fillet texture (Sukri et al., 2022). Inspired by these findings, the current study aims to determine whether similar benefits can be observed in *C. quadricarinatus*. The growing interest in pellet production using agricultural and animal waste has also contributed to the motivation behind this research. The objective is to evaluate the effectiveness of pineapple waste-based pellets on Redclaw growth and to identify any potential positive or negative impacts, with a view toward future improvements.

The redclaw was chosen to observe the growth after consuming pineapple waste. Additionally, the aim is to gain knowledge of redclaw feeding behavior and the nutritional protein needed for their growth. Redclaw displays several physical, biological, and commercial attributes that make them suitable aquaculture candidates. Other positive characteristics include gregariousness, non-aggressive and non-burrowing behavior, and tolerance to relatively high stocking densities. This species can tolerate wide ranges of water quality conditions including low oxygen concentrations (> 1 ppm), wide ranges of hardness and alkalinity (20 to 30 ppm), and pH (6.5 to 9). Regarding pineapple waste, the crown part was selected. It contains higher amounts of cellulose, hemicellulose, and lignin.

The wastes are rich in fibre and bioactive substances and can be used as energy sources as well as digestible feedstuffs. The waste is typically non-toxic and represents a source of plant crowns, and stems that have a rich supply of bromelain along with additional cysteine proteasesPellet production specifically for *C. quadricarinatus* remains limited in Malaysia. As a bottom-feeding species, Redclaw typically consumes available zooplankton in its environment. In indoor culture systems, suboptimal growth and increased cannibalism are common issues. This study also addresses environmental concerns by exploring the potential of pineapple waste pellets to reduce agricultural waste pollution. Furthermore, findings from this research may support sustainable aquaculture practices and enhance Redclaw cultivation for local production. The ultimate aim is to develop effective pineapple waste-based pellets for *C. quadricarinatus* and assess their impact on growth performance and survival.

MATERIALS AND METHODS

Site Selection and Experimental Design

The experiment was taken place at the Fish Propagation House, located in Politeknik Jeli, Kelantan (N 5.712074, E 101.850085) The experiment was lasted for a period of three months. The starting point involves creating the pellet (Figure 1). Prior to pellet processing, it is necessary to prepare both the material and machine. Following that, four tanks are required for a trial experiment involving pellets. These tanks include a control tank (without any pineapple waste), treatment tank 1 (with 10% pineapple waste), treatment tank 2 (with 20% pineapple waste), and treatment tank 3 (with 30% pineapple waste). Each tank contains varying percentages of pineapple waste crude protein content as this experiment aims to investigate the impact of pineapple waste on *C. quadricarinatus*. Furthermore, each tank has 20 specimens of *C. quadricarinatus*, commonly known as red claw, measuring 2 inches in length, b for each sample. A total of 80 juvenile *C. quadricarinatus* tails, were used for the samples.

Culture Quality Control and Feeding Method

Various types of substrates, such as PVC pipes, bricks, and palm leaves, are used to protect the aquatic animal (redclaw) from predators. These substrates serve dual functions: stabilising the pH of the water and providing shelter for redclaw. Additionally, nursery nets are employed to shield redclaw from threats and reduce stress. This control system helps maintain the quality of the aquatic animal and minimise the risk of death. The method of feeding is 2 times a day, morning and evening. Food is given at a rate of 10% of total biomass. The feeding weight was changed every 10 days after sampling the redclaw (Fatihah et al., 2020; Fatihah & Cheng-Ann, 2022).

Figure 1. Utilisation of pineapple waste for pellet production: (a) Pineapple waste was collected for the drying process; (b) After drying process, the pineapple waste was ground by using a blender; (c) Mixed all the ingredients, mix it up and shape it into a circle; (d) Put it in the grinder to form pellets; and (e) Dry it out under the sunlight and in the oven (70-100 °C)

Data Collection and Analysis

The project to be developed is the effectiveness of pineapple residue on redclaw to help the process of growth and maturation of Red Claw. The data of this project is analysed depending on the objectives that have been set. Among the objectives is to produce high quality and healthy pellets for *C. quadricarinatus*. In addition, to obtain the growth performance and molting frequency on *C. quadricarinatus* uses the pellet from the waste of pineapple. This data helps us to obtain the effectiveness of pineapple waste on Red Claw. Data was collected on survival rate, body weight (g), body length (cm), and total number of moults for *C. quadricarinatus*) every 10 days over the 60-day experimental period.

RESULTS AND DISCUSSION

Figure 2 showed the survival of *C. quadricarinatus* throughout the study period. The rate of molting occurs when changing the water in each treatment tank and this results in the new of *C. quadricarinatus* after molting preferring to be in the substrates such as PVC

pipes. *C. quadricarinatus*'s skin after molting is still soft and weak so handle carefully to avoid death. Figure 3 showed the total number of molting on each sampling day. While, Figure 4 and Figure 5 showed the means body weight (BW) and body length (BL) for *C. quadricarinatus* until days 100.

A comparable study is the research on mulberry leaves (*Morus alba*) as a potential protein source in tilapia feed. In that study, fish were weighed weekly over a five-week period, while *C. quadricarinatus* in the current experiment were sampled every ten days. The results indicated that mulberry pellets yielded a higher absolute growth rate compared to commercial pellets, with a lower feed conversion ratio (FCR) of 2, as opposed to 3 for commercial feed. Both pineapple waste and mulberry-based pellets demonstrated effectiveness in promoting growth in tilapia and *C. quadricarinatus*.

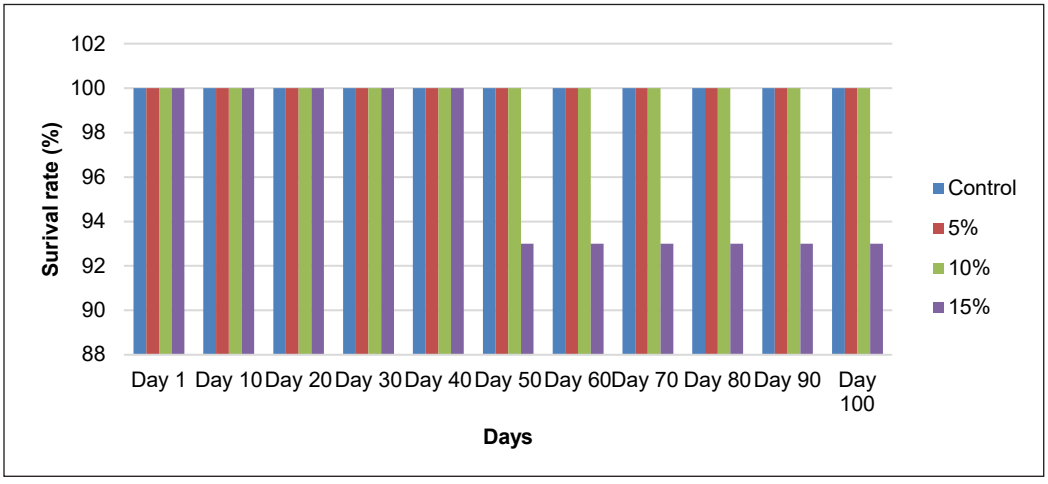

Figure 2. Survival rate for redclaw, *Cherax quadricarinatus* until days 60

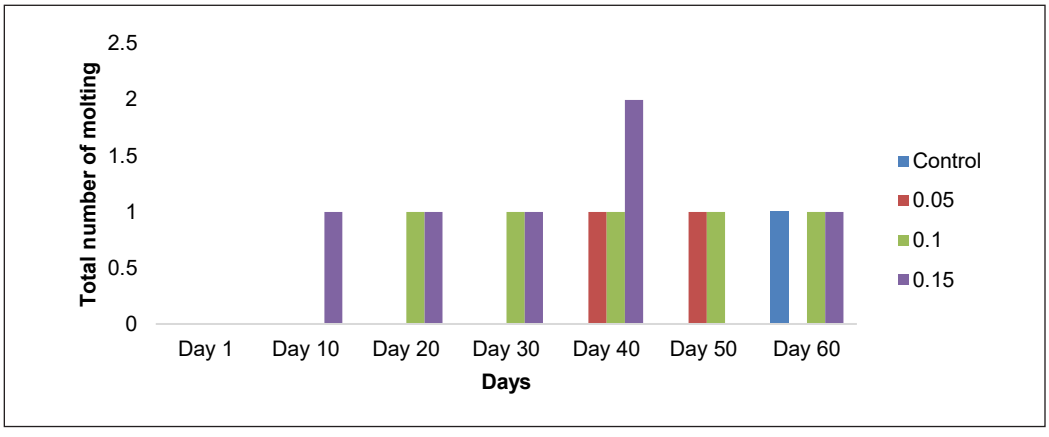

Figure 3. Total number of molting for redclaw, *Cherax quadricatinatus* until days 60

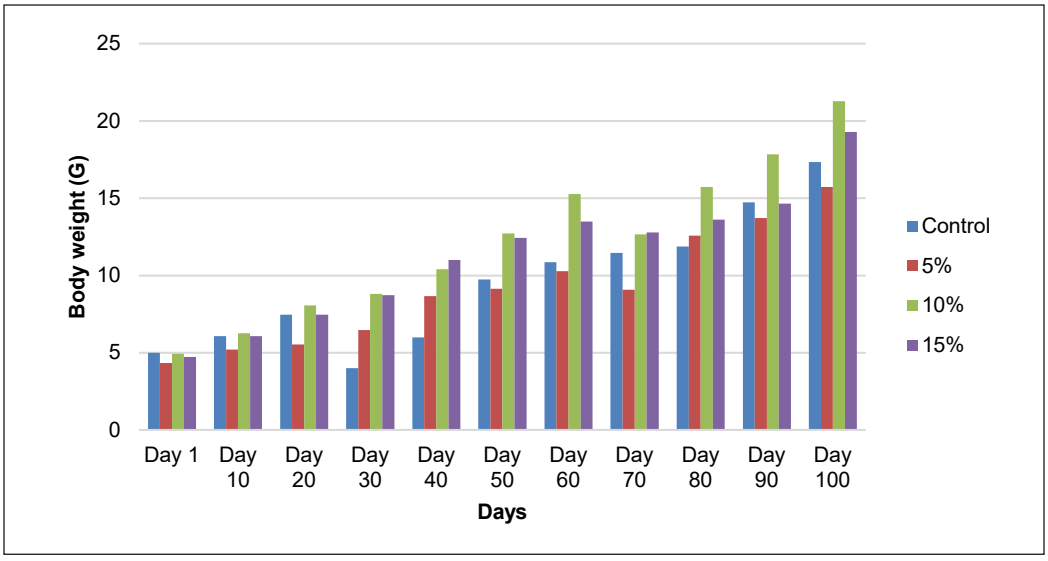

Figure 4. Means body weight (BW) for redclaw, *Cherax quadricarinatus* until days 60

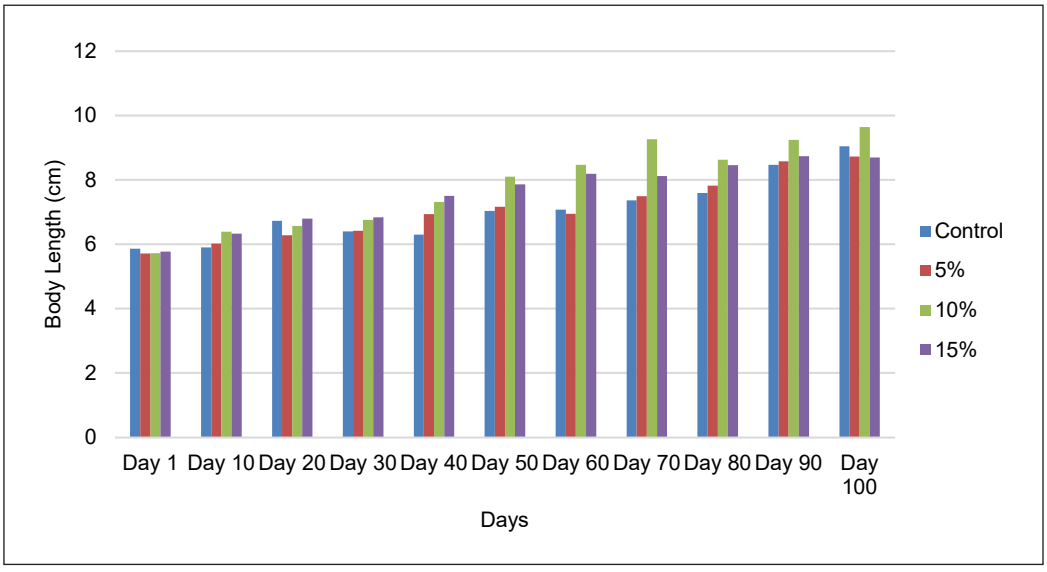

Figure 5. Means body length (BL) for redclaw, *Cherax quadricarinatus* until days 60

Previous research supports these findings. Fatihah et al. (2020) conducted a 60-day study on juvenile Redclaw using various substrates to assess their effects on growth, survival, and molting. Sukri et al. (2022) examined the influence of pineapple waste on growth, texture quality, and flesh color in Nile tilapia (*O. niloticus*) fingerlings. Similarly, Khumsrisuk et al. (2022) evaluated the use of pineapple waste to improve growth and resistance to *Aeromonas*

hydrophila in *O. niloticus*. Besides, study by Fatihah and Cheng-Ann (2022) showed the culture the C. quaricarinatus in aquaponics system and it was significant in growth performance of *C. quadricarinatus* when cultured in aquaponics system.

CONCLUSION

The study successfully achieved its objectives, as demonstrated by data indicating that the application of pineapple waste in pellets can enhance growth performance in *C. quadricarinatus.* The experiment was conducted over a two-month period, with data collected every ten days by measuring weight, length, and molting frequency. Pineapple waste pellets proved effective even without the addition of probiotic ingredients. Four different tank setups were used: one with commercial pellets, and three with 5%, 10%, and 15% pineapple waste inclusion, respectively. After 50 until 100 days, the tank with 10% pineapple waste showed the highest average values in weight, length, and molting rate. Additionally, all tanks recorded a 100% survival rate, although growth performance varied between groups. Despite these promising results, the study has several limitations that should be addressed to improve pellet quality. Recommendations include adding attractant flavours to increase Redclaw's appetite for the pellets, enriching the formulation with essential nutrients such as calcium, magnesium, and carbohydrates to support optimal growth, and incorporating more natural substrates like sand and gravel to enhance habitat conditions. Beyond pellet formulation, packaging also plays a critical role in maintaining pellet quality by preventing damage and fungal contamination, which can cause disease in *C. quadricarinatus* and increase treatment costs. Furthermore, selecting a research location with minimal disturbances is advised to reduce environmental stress on the specimens.

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Physical Characterisation and Surface Morphology of Hybrid Oil Palm Trunk and Corncob Biofuel Briquettes with Paper Pulp Waste as Binder

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ABSTRACT

A blend of oil palm trunk (OPT) waste with corncob (CC) is proposed to produce solid biofuel briquettes. This study focus on characterising the performance of OPT and CC blend briquettes in terms of physical properties and surface morphology. The 100% OPT and 100% CC briquettes were considered as the control samples, while the blend of 50% OPT and 50% CC (OPT/CC) briquettes was considered as the test samples. Physical characterisations included moisture content (MC), water resistance (WR), density, and shatter index. Surface morphology was examined using a Scanning Electron Microscope (SEM) to observe and compare the microstructures of the samples under 50×, 100×, and 300× magnifications. The CC samples possess a higher MC (10.24%), lower WR (86.20%), and lower density (0.35 g/cm^3) than OPT samples (MC = 9.75%, WR = 93.20%, density = 0.43 g/cm^3). Observations from the SEM uncovered the presence of large cavities and pores for the CC samples and the test samples, which was largely missing from OPT samples. These observations confirmed the results from physical characterisation. Samples with large cavities tend to store more water, which leads to higher moisture content, lower water resistance, and lower density, which are undesirable qualities for briquettes.

Keywords: Briquettes, corncob, oil palm trunk, physical characterisation, surface morphology

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INTRODUCTION

Oil palm trunk (OPT) remains the least utilised waste for bioenergy production due to its lack of availability and accessibility in the oil palm fields. Therefore, a blend of OPT with other biomass waste such as corncob (CC) is proposed to solve this issue. The physical, mechanical, chemical, and combustion properties of OPT/CC blend briquettes were thoroughly investigated in Kpalo et al. (2020, 2021). Past investigations revealed that the OPT/CC briquettes performed as well as other biomass waste briquettes such as coffee husk, rice husk, rice bran, rice straw, sawdust, neem powder, pine needle, and banana waste.

However, the surface morphological assessment of the OPT/CC blend briquettes is yet to be covered. Surface morphology is essential to observe the fragmented microstructure of biomass briquettes (Gan et al., 2021). These observations are needed to explain the findings reported on the physical properties of OPT/CC blend briquettes, namely moisture content, water resistance, density, and shatter index (Kpalo et al., 2020, 2021).

METHODOLOGY

The samples were prepared, and characterisations were conducted based on the methods explained by Kpalo et al. (2020, 2021). The 100% OPT and 100% CC briquettes were considered as the control samples, while the blend of 50% OPT and 50% CC (OPT/CC) briquettes was considered as the test samples. The microstructures of the briquettes were examined with Scanning Electron Microscopy (SEM) at the Material Characterisation Laboratory, Faculty of Engineering, University Putra Malaysia (UPM) with 50×, 100×, and 300× magnifications operating at an accelerating voltage of 10 kV.

RESULTS AND DISCUSSION

Table 1 shows that CC briquettes possessed the highest moisture content, followed by the OPT/CC and the OPT briquettes. In contrast, the CC briquettes exhibited the lowest water resistance, followed by the test and the OPT briquettes. In terms of density, the CC briquettes were the lowest among all samples, followed by the test and the OPT briquettes. On the other hand, the CC briquettes recorded the highest shatter index compared to the test and the OPT briquettes. However, the LSD analysis revealed that no significant differences between the moisture content and the shatter index of all control and test briquettes. Nevertheless, significant differences were observed between the water resistances and the densities of the briquettes.

SEM images in Figures 1-3 uncovered the presence of large particles of OPT and CC biomasses in all briquette samples. This observation was expected due to the application of low densification pressure (< 7 MPa) to form the briquette cakes. Figures 1-3 show

Table 1
Physical quantifications of the OPT/CC samples and the Least Significant Difference (LSD) test

Parameter	CC + binder (100% CC)	OPT + binder (100% OPT)	CC + OPT + binder (50% CC, 50% OPT)
Moisture content (%)	10.24[a]	9.25[a]	9.75[a]
Water resistance (%)	86.20[b]	93.20[a]	88.30[b]
Density (g/cm^3)	0.35[a]	0.43[c]	0.39[b]
Shatter index (%)	99.20[a]	99.05[a]	98.16[a]

Note. Means with the same letter(s) in a row for each parameter are not significantly different ($P < 0.05$)

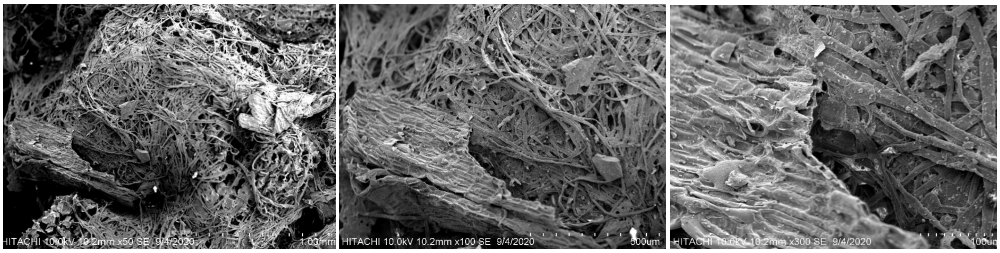

Figure 1. SEM images of CC briquettes (left: 50×, center: 100×, right: 300×)

Figure 2. SEM images of OPT briquettes (left: 50×, center: 100×, right: 300×)

Figure 3. SEM images of OPT/CC hybrid briquettes (left: 50×, center: 100×, right: 300×)

long fibres whichare thought to be wastepaper fibers. Large cavities found for the CC and the hybrid briquettes may explain high moisture contents, low water resistances and low densities recorded for both briquettes (Table 1). The presence of large cavities indicates that higher porosity in these briquetteswhich increases the capacity to absorb more water compared to OPT the briquettes.

CONCLUSION

The CC and hybrid briquettes exhibited higher moisture contents, lower water resistances, and lower densities compared with OPT briquettes. SEM analysis revealed the presence of large cavities in both CC and OPT/CC samples, which suggested high porosities for both briquettes. High porosity leads to high moisture contents, reduce water resistances, and lower densities of both samples. In addition, large particles of biomass and wastepaper pulp fibres were also observed in both samples.

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Effects of Melatonin Seed Priming in Waxy Corn on Germination under Salinity Stress

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ABSTRACT

Salinity stress is a major abiotic stress affecting corn germination and growth. Melatonin, known to regulate physiological functions in animals, is now identified as a plant hormone that reduces abiotic stress by regulating antioxidant defense and improving osmotic balance. However, its role in waxy corn germination is unclear. This study investigated the effects of seed priming with melatonin on waxy corn germination under salinity stress. Seeds were primed with melatonin (0, 100, 200, 300 and 400 µM) for 24 h and germinated under 100 µM NaCl stress for six days. Melatonin at 200 µM enhanced waxy corn germination, showing greater coleoptile length, radicle length, biomass, germination energy, and vigor index. This study highlights melatonin's potential as an affordable and sustainable method to reduce salinity stress in waxy corn production, potentially increasing agricultural output in salinity-affected areas.

Keywords: Germination, melatonin, salinity stress, seed priming, waxy corn

INTRODUCTION

Waxy corn cultivation in saline soils faces significant challenges due to reduced germination rates and inconsistent yield (Askari et al., 2023). Salinity impacts vital metabolic functions like protein synthesis and lipid metabolism due to ionic and osmotic stress. Strategies to mitigate salinity stress focus on preserving osmotic homeostasis and ion balance (Wang et al., 2013). Melatonin, an indoleamine

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compound, has recently become a major focus for overcoming abiotic stresses in plants, promoting resilience to salt, drought, heat, and cold (Tiwari et al., 2020).

Problem Statement

Current use of melatonin application results in unpredictable and inconclusive outcomes.

Research Questions

What is the lowest effective concentration of melatonin to improve waxy corn germination under salt stress?

MATERIALS AND METHODS

Seed Priming with Melatonin

Fungicide-treated waxy corn seeds were soaked in melatonin solutions (0, 100, 200, 300, and 400 μM) for 24 h at 25°C in the dark with gentle stirring.

Germination and Salinity Stress Induction

Primed seeds were germinated on sterile filter paper moistened with 100 μM NaCl and germinated for 6 days under 12:12 hours of light/dark conditions at 25°C.

Germination Characteristics Determination

Coleoptile and radicle length were recorded manually. Biomass accumulation was determined by drying at 80°C for 24 h. Germination parameters—mean germination time (MGT), germination rate index (GRI), germination percentage (GP), germination energy (GE), and vigor index (VI)—were calculated using standard formulas (Yang et al., 2021).

Statistical Analysis

Data were analysed using SPSS20 with one-way ANOVA ($P \leq 0.05$). Tukey's HSD was used for multiple comparisons.

RESULTS AND DISCUSSION

Melatonin Sustained Early Growth under Salinity Stress

Early growth data showed coleoptile and radicle lengths increased significantly at 200 μM under both stressed and unstressed conditions, indicating melatonin's stimulatory effects on cell division and elongation (Table 1). This supports previous research that melatonin increases auxin-related gene expression, promoting plant elongation (Zhang et al., 2022).

Table 1
Early germination growth parameters of waxy corn seeds treated with melatonin (MEL) with and without application of NaCl (n=20, P≤ 0.05)

MEL (µM)	Coleoptile length (cm)		Radicle length (cm)		Fresh weight (g)	
	-Nacl	+Nacl	-Nacl	+Nacl	-Nacl	+Nacl
0	1.33±0.10^{a}	0.40±0.00^{a}	0.41±0.00^{a}	0.24±0.03^{a}	3.00±0.00^{a}	1.20±0.20^{a}
100	2.67±0.10^{b}	0.50±0.00^{a}	0.42 ±0.01^{ab}	0.25±0.02^{a}	3.40±0.26^{a}	2.03±0.06^{b}
200	3.13±0.06^{bc}	0.63±0.06^{b}	0.43±0.01^{bc}	0.34±0.03^{b}	4.97±0.15^{b}	2.27±0.12^{b}
300	4.40±0.36^{c}	0.83±0.06^{c}	0.44±0.01^{c}	0.36±0.01^{b}	5.30±0.10^{b}	3.00±0.20^{c}
400	5.57±0.33^{d}	1.23±0.06^{d}	0.46±0.01^{d}	0.38±0.01^{b}	8.07±0.25^{c}	3.70±0.20^{d}

Fresh weight (biomass) was consistently lower under stress, highlighting NaCl's adverse effects. However, melatonin application as low as 100 µM significantly improved biomass, possibly due to its role in regulating carbon and nitrogen assimilation (Qin et al., 2023).

Melatonin-primed Seeds Possessed Better Germination Qualities

Germination parameters were obtained from four replicates of five seeds each. Mean germination time (MGT) remained consistent (3.5-3.7 days), suggesting melatonin may not speed up germination but promotes consistency under stress (Chen et al., 2021). Germination rate index (GRI)results were inconsistent, indicating melatonin improves germination efficiency and uniformity rather than rate (Zeng et al., 2022). No significant difference was found in GP while GE showed no clear pattern. However, VI showed significant differences at all melatonin concentrations, with or without NaCl. This suggests that melatonin may strengthen the antioxidant defense system.

CONCLUSION

Melatonin effectively mitigated the negative effects of salinity stress in a dose-responsive mannerwith a concentration of 200 µM was sufficient to exert positive effects.

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Potential of *Paenibacillus* sp. and *Bacillus* sp. as Biofertiliser for Soil Fertility Improvement in Lembah Bidong Oil Palm Plantation

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ABSTRACT

Oil palm is a vital crop in Malaysia, but continuous use of chemical fertilisers has degraded soil quality, reducing fertility and yields. This study evaluated the potential of *Paenibacillus* sp. and *Bacillus* sp. as biofertilisers to improve soil fertility in the Lembah Bidong oil palm plantation. Soil treated with microbial inoculants was analysed using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES). The results demonstrated significant improvements in soil properties. Soil pH increased and reduced acidity, while nutrient availability improved, with nitrogen, phosphorus, and potassium increasing by 25%, 18%, and 30%, respectively. These outcomes highlight the role of beneficial microbes in nutrient solubilisation, soil health restoration, and plant growth promotion. This study suggests that *Paenibacillus* sp. and *Bacillus* sp. have strong potential as biofertilisers for sustainable oil palm production. Their use could improve soil conditions, enhance crop performance, and promote environmentally friendly practices. Further research should focus on large-scale field trials and integration with organic amendments to optimise long-term effectiveness.

Keywords: Biofertiliser, *Paenibacillus* sp., *Bacillus* sp., soil fertility, oil palm

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INTRODUCTION

Oil palm (*Elaeis guineensis*) is a key tropical crop with high oil yield and economic importance. Malaysia and Indonesia lead global production, where palm oil has boosted rural employment and reduced poverty (Chew et al., 2021). Maintaining soil fertility is essential for sustainable yields, yet prolonged chemical fertiliser use has caused degradation, acidity, and nutrient imbalance

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(Woittiez et al., 2017). Biofertilisers using beneficial microbes offer a sustainable alternative. *Paenibacillus* sp. fixes nitrogen and solubilises phosphorus, while *Bacillus* sp. provides biocontrol and stress tolerance (Al Methyeb et al., 2023). Both are widely distributed in soil and vital for nutrient cycling. This study evaluates their potential to improve soil fertility in Lembah Bidong oil palm plantations to reduce chemical inputs and enhance crop performance.

Problem Statement

The Lembah Bidong oil palm plantation (5°29'02.7"N 102°58'59.5"E) faces declining crop growth from reduced soil fertility, even though most areas have peat soils rich in organic matter. Continuous chemical fertiliser use has increased acidity, disrupting nutrient availability and microbial activity, while excessive pesticide use has worsened soil degradation and pollution (Woittiez et al., 2017). Monocropping has further lowered soil health and productivity. These issues underscore the need for sustainable alternatives. Biofertilisers, especially *Paenibacillus* and *Bacillus* sp., can enhance nutrient cycling, improve soil health, and promote plant growth. This study examines their use to address fertility problems in Lembah Bidong.

MATERIALS AND METHODS

Culturing of *Paenibacillus* sp. and *Bacillus* sp.

Strains of *Paenibacillus* sp. and *Bacillus* sp. were obtained from Universiti Sultan Zainal Abidin, Besut, Malaysia. The isolates were cultured on Nutrient Broth (NB) and incubated at 37°C for two days. Bacterial granules were prepared by mixing broth culture with potato flour and kaolin, followed by drying and grinding into uniform particles. These granules were applied to soil samples. Soil fertility and plant growth responses were monitored after 3 months of treatment.

Soil Sampling and Analysis

Soil samples (Kuah – clay soil, Gondang – peat soil, and Rusila – BRIS soil) were collected from Lembah Bidong oil palm plantation at a depth of 0–15 cm using a soil auger. Samples were analysed for pH using standard meters. Macronutrient contents were determined using Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) following microwave digestion with concentrated HNO_3.

Statistical Analysis

Data were analysed using the Analysis of Variance (ANOVA) to test the significance of treatment effects on soil chemical and fertility parameters. Mean differences among treatments were compared, and significance was determined at $p < 0.05$.

RESULTS AND DISCUSSION

Soil Fertility Analysis

Soil samples from Kuah, Gondang, and Rusila showed wide chemical variation. pH ranged from strongly acidic (3.27 in Gondang A) to moderately acidic (5.78 in Rusila D) (Table 1). Total nitrogen was highest in Gondang (up to 1.65%) and lower in Kuah and Rusila (<0.20%). Organic carbon was greatest in Gondang (45.59–51.10%), compared to Kuah (1.29–11.55%) and Rusila (0.99–12.25%). Available phosphorus ranged from 2.536 mg/kg in Kuah A to 159.0 mg/kg in Rusila A. Exchangeable potassium, magnesium, and calcium also varied, with Rusila A showing the highest Exc-K (2.002 meq/100) and Exc-Mg (1.883 meq/100), while Kuah and Gondang were moderate (Table 1). Overall, Gondang was richest in organic matter and nitrogen, and Rusila was richest in phosphorus.

Table 1
Soil fertility analysis

Estate/ Division/ Block		pH	Total-N (%)	Org-C (%)	Av-P (mg/ kg)	Exc-K (meq/100)	Exc-Mg (meq/100)	Exc-Ca (meq/100)
Kuah	A	4.16	0.15	1.29	2.536	0.214	0.332	2.051
	B	4.83	0.17	1.77	12.13	0.113	0.317	2.152
	C	3.86	0.19	11.55	18.82	0.148	0.527	3.179
	D	4.10	0.18	8.76	15.11	0.149	0.432	2.880
Gondang	A	3.27	1.46	45.59	14.94	0.419	0.472	2.879
	B	3.28	1.65	48.58	34.73	0.114	0.499	2.863
	C	3.37	1.62	49.90	28.82	0.156	0.684	3.348
	D	3.39	1.43	51.10	17.36	0.030	0.552	3.798
Rusila	A	3.96	0.26	12.25	159.0	2.002	1.883	4.091
	B	5.02	0.11	3.18	33.53	0.433	0.330	2.236
	C	5.41	0.14	1.42	17.32	0.089	0.193	2.139
	D	5.78	0.10	0.99	35.79	0.176	0.244	2.346

Note. Control (A), *Bacillus* (B), *Paenibacillus* (C), and *Consortium* (D)

DISCUSSION

Soil origin and treatments (*Bacillus*, *Paenibacillus*, and Consortium) strongly influenced soil fertility. Acidic soils such as Gondang and Rusila showed high organic carbon and nitrogen, indicating substantial organic matter accumulation. In contrast, soils from Kuah exhibited lower organic carbon and nitrogen, suggesting lower biological activity and fertility potential. Microbial inoculants generally improved soil chemical properties by enhancing nutrient availability and cation exchange capacity (CEC). Treatments with *Paenibacillus* and the Consortium tended to increase available phosphorus and exchangeable bases, contributing to improved soil health and nutrient balance (Al Methyeb

et al., 2023). Their effectiveness, however, varied with soil type and organic matter content, highlighting the need for tailored management to achieve sustainable productivity.

CONCLUSION

This study shows that *Paenibacillus* and *Bacillus* sp. effectively improve soil fertility in Lembah Bidong oil palm plantations by raising pH, enhancing nutrient availability, and promoting plant growth. Their use reduces reliance on chemical fertilisers, supporting more sustainable and eco-friendly practices. Future research should validate these results through large-scale field trials under varied conditions.

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Effects of Homogenised Coffee Extract Concentration on the Structural Properties and Solution Stability of Cellulose Nanocrystal (CNC)

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ABSTRACT

This study investigates the possibility of using spent ground coffee extract (SGCE) as a natural stabiliser for cellulose nanocrystals (CNC). SGCE, rich in polyphenols and polysaccharides, was extracted before being mixed with microcrystalline cellulose (MCC) at concentrations of 1%, 5%, and 10 % (w/v). Dynamic Light Scattering (DLS) and FTIR spectroscopy were conducted to investigate the formulations. Results showed that 5% SGCE created the most stable CNC solution, with lower hydrodynamic diameters (~229 nm), and shorter particle lengths than control samples. FTIR spectra revealed intermolecular interactions between SGCE and MCC, indicating a successful integration. These findings highlight SGCE as a sustainable alternative to synthetic stabilizers with potential applications in food packaging and biopolymer systems.

Keywords: Biopolymer stabilisation, food packaging, hydrogen bonding, nano whiskers, spent coffee grounds

INTRODUCTION

Cellulose nanocrystals (CNC) are renewable and biodegradable nanomaterials with high strength, large surface area, and biocompatibility. They are commonly synthesised from microcrystalline cellulose (MCC) using acid hydrolysis or related methods, producing rod-like particles with tuneable size and surface chemistry (Huang et al., 2020). Cellulose nanocrystals (CNC) have promising applications in food

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packaging, biomedical materials, and nanocomposites. However, the stability of CNC suspensions remains a key challenge. Conventional stabilizers such as polyvinyl alcohol (PVA) provide dispersion but are synthetic, non-biodegradable, and mostly unsuitable for food applications (Cheng et al., 2024).

Spent coffee grounds are an abundant agro-industrial by-product that often ends up in landfills. Extracts from these residues are rich in polyphenols and polysaccharides with antioxidant and emulsifying properties (Campos-Vega et al., 2015; Mohammadi et al., 2021). Previous studies have shown that plant-derived polysaccharides such as guar gum, alginate, and arabinogalactans had improved the CNC dispersion, while polyphenols enhance colloidal stability through hydrogen bonding interaction (Li et al., 2021). Despite these advances, limited research has evaluated the effect of spent ground coffee extract (SGCE) concentration on CNC stability and structure. This study addresses this gap by investigating SGCE as a natural dispersant for CNC suspensions, with potential applications in sustainable food packaging and biopolymer composites.

Problem Statement

Conventional stabilisers for CNC suspensions are predominantly synthetic surfactants, which are often non-biodegradable and environmentally hazardous. SGCE contains abundant in polyphenols and polysaccharides, presents a viable option for CNC stabilization while reducing agro-industrial waste.

Research Questions

How does SGCE concentration affect the structural features and particle size distribution of CNC? Can SGCE replace synthetic stabilizers while maintaining CNC suspension stability?

MATERIALS AND METHODS

Materials

Spent ground coffee, mixture of Arabica from Brazil and Papua New Guinea and Robusta from Java, Indonesiawas supplied by QAWA Coffee, Kuala Terengganu, Terengganu, Malaysia. Food grade microcrystalline cellulose (MCC) particle size of ~50 µm was purchased from Sigma-Aldrich, USA.

Spent Ground Coffee Extract (SGCE) Preparation and CNC Synthesis

Spent coffee grounds were oven dried overnight at 60ºC to standardise the moisture content of the spent coffee grounds. Next, the sample was homogenized with distilled water, and extracted at 1%, 5%, and 10 % (w/v). Extracts were centrifuged to obtain liquid spent coffee grounds extract (SGCE).

SCGE was mixed with 0.4 g MCC at constant time for 15 min and speed at 10,000-29,000 min-1 using handheld homogeniser (WiggenHauser D-500, Germany). The bottom layer of the solutions was removed after being centrifuged at room temperature for 10 min at 5,000 rpm. To increase the concentration of CNC, the top clear layer of the solution was removed, and the solution was reduced to 30 mL. Remaining solution was designated as CNC solution and further analysed.

CNC Suspension in SGCE Solution Analysis

Particle size was measured by Dynamic Light Scattering (DLS) via Particle Size Analyser (PSA) (Anton Paar Litesizer DLS 500, Austria). Molecular interactions were assessed by FTIR spectrometer IRPrestige-21 (Shimadzu, Japan). Data were analysed using one-way ANOVA with Duncan's test at $P < 0.05$.

RESULTS AND DISCUSSION

Dynamic Light Scattering Analysis

Spent ground coffee extract (SGCE) greatly reduced particle aggregation, since non-homogenized suspensions had huge diameters (>15,000 nm), whereas SGCE-based systems had much smaller hydrodynamic sizes (229-291 nm) as shown in Figure 1. 5% SGCE sample had the smallest particle size, indicating excellent stabilization in comparison to other samples. This is consistent with recent observations in which polysaccharides and polyphenols improved CNC dispersion through steric hindrance and hydrogen bonding (Adam et al., 2021; Wang et al., 2024).

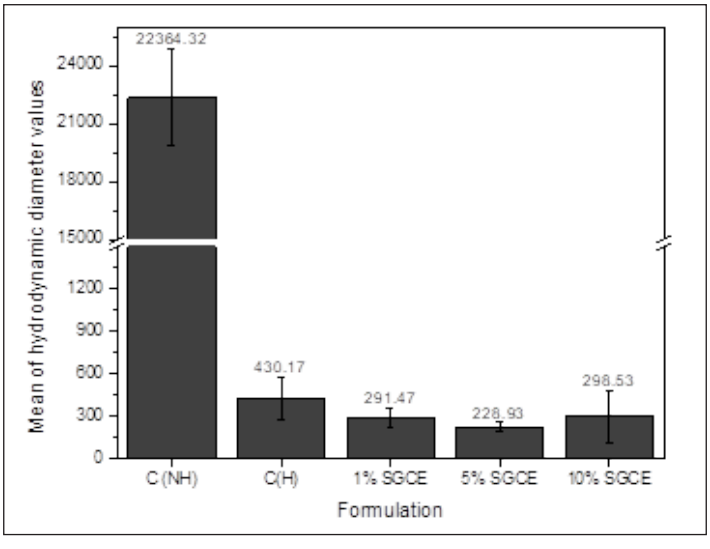

Figure 1. Mean of hydrodynamic diameter value of CNC suspension in SGCE solution

FTIR Spectroscopy Analysis

The FTIR peak analysis in Table 1 discovered changes in the hydroxyl and carbonyl peaks, demonstrating hydrogen bonding between SGCE chemicals and CNC surfaces. Due to limitation of cost, the FTIR analysis was conducted for three samples only - Control, 1% SGCE and 5% SGCE. Comparing between 1% SGCE and 5% SGCE, increasing amount of coffee extract shows absorbance increment of hydrogen bonded hydroxyl group (O-H), aliphatic (C-H) chains, carbonyl group (C=O) bending, and ether group (C-O) stretching. Such interactions improve dispersion by lowering interparticle attraction, which is consistent with previous studies on polyphenol-cellulose interactions in colloidal systems (Xu et al., 2013).

Table 1
Area under curve for selected functional groups

Formulation	Area under curve (cm^{-1})			
	O-H 3,600 cm^{-1}	**C-H 2,900 cm^{-1}**	**C=O 1,100 cm^{-1}**	**C-O 1,700 cm^{-1}**
Control (No SGCE)	594.40	N/D	221.65	191.11
1% SGCE	149.27	10.40	74.43	102.64
5% SGCE	213.06	29.23	101.10	115.84

* *N/D = not detected*

CONCLUSION

Spent ground coffee extract (SGCE) at 5% concentration was the most effective for stabilizing CNC suspensions, minimizing particle aggregation, and improving molecular interactions. Using used coffee grounds as a stabilizer promotes waste reutilization and circular economy concepts, providing a sustainable alternative to chemical stabilizers. Differences in coffee variety and composition may affect SGCE's stabilizing performance. Future study should investigate these aspects, as well as applications in food packaging systems.

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Nutritional Composition and Physicochemical Properties of Different Parts of Powdered Immature Japanese Muskmelon

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ABSTRACT

The "One-fruit per plant" cultivation system of Japanese muskmelon generates waste. To address this issue, the present study evaluated the nutritional and physicochemical properties of powdered immature Japanese muskmelon (*Cucumis melo* var. Casanova), focusing on pulp, peel, and seeds. The fruit parts were separated and processed into fine powders. Proximate and physicochemical analyses were conducted. The results showed significant differences (P<0.05) among fruit parts. The pulp recorded the highest moisture content, total soluble solid, and total titratable acidity. The peel displayed the highest ash content, and water activity, with marked greenness and yellowness attributed to chlorophyll and carotenoids but showing the lowest lightness value. Seeds demonstrated the highest crude protein, crude fat, crude fiber, available carbohydrate, caloric value, and pH. These findings highlight the potential valorisation of immature Japanese muskmelons into functional ingredients, providing a sustainable strategy to reduce food loss in muskmelon production.

Keywords: Food waste valorisation, functional ingredients, Japanese muskmelon, physicochemical properties, proximate composition

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INTRODUCTION

Japanese muskmelons (*Cucumis melo* L.) are cultivated in Malaysia as premium fruits, with the Casanova variety grown under greenhouse conditions at Melon Manis

Terengganu Farm, Besut, Terengganu. In muskmelons cultivation, farmers meticulously select only one well-developed fruit each plant to reduce competition for nutrients. Consequently, approximately 45% by weight of immature muskmelons are discarded, causing a signigicant amount of waste. With increasing concerns about sustainability, food security, and reducing waste, it is important to investigate strategies to use these leftover fruits and reduce losses after harvest. Previous studies show that fruit wastes such as mango, banana, citrus, apple, and pineapple residues are abundant in pigments, phytochemicals, antioxidants, dietary fibre, vitamins, and minerals, offering value-added potential (Ong et al., 2022; Shahidan et al., 2022). Immature Japanese muskmelon, particularly the Casanova variety, may exhibit distinct nutritional values and physicochemical properties across different parts such as pulp, peel, and seeds, compared to other muskmelon cultivars. However, there is currently no published information on these aspects. Therefore, this study evaluates the nutritional composition and physicochemical properties of pulp, peel, and seed of Japanese muskmelons cultivated in Malaysia.

MATERIALS AND METHODS

Preparation of Samples

Immature Japanese muskmelons (*Cucumis melon* var. Casanova) were washed, separated into pulp, peel, and seeds, and treated with sodium metabisulphite seeds underwent mucilage extraction with hydrochloric acid. All parts were then dried at 50ºC for 24 h, ground and sieved for further analysis.

Proximate and Physicochemical Analyses

Proximate analyses were conducted according to Association of Official Agricultural Chemists (AOAC) method. Colour was measured using a Hunter Lab colour meter, total soluble solids with a digital refractometer, pH with a calibrated pH meter, and water activity with a dew point hygrometer. Total titratable acidity was determined by titration method.

RESULTS AND DISCUSSION

Proximate Composition

The proximate composition of immature Japanese muskmelon revealed seeds as the most nutrient-dense part (Table 1). Seed moisture was higher than *Cucumis melon* seeds (Saeed et al., 2023) but comparable to Melon Manis Terengganu (Shahidan et al., 2022). Peel ash exceeded pulp and seeds. Seeds contained notable crude protein though lower than *C. melo* seeds (Saeed et al., 2022), and had the highest crude fat, consistent with reports of melon seeds being oil-rich (Mallek-Ayadi et al., 2018), suggesting their suitability for oil extraction. The seeds also had highest levels of crude fibre, available carbohydrate and

caloric value, aligning with earlier findings for *C. melo* and *C. lanatus* seeds (Saeed et al., 2023). These characteristics further enhance their potential for inclusion in dietary fibre-enriched products such as bakery items, pre-mixed beverages, and energy bars.

Table 1
*Proximate composition of Japanese muskmelon (*Cucumis melon *var. Casanova)*

Proximate Composition	Pulp	Peel	Seed
Moisture	16.24 ± 0.02 ^{c}	8.47 ± 0.15 ^{b}	5.51 ± 0.09 ^{a}
Ash	9.58 ± 0.38 ^{b}	10.14 ± 0.30 ^{b}	3.75 ± 0.46 ^{a}
Crude protein	13.31 ± 0.43 ^{a}	15.61 ± 0.07 ^{b}	16.84 ± 0.56 ^{c}
Crude fat	0.56 ± 0.12 ^{a}	3.98 ± 0.27 ^{b}	8.70 ± 0.83 ^{c}
Crude fibre	10.32 ± 1.39 ^{a}	28.98 ± 0.97 ^{b}	48.33 ± 1.07 ^{c}
Available carbohydrates	60.31 ± 0.32 ^{a}	61.78 ± 0.42 ^{a}	65.20 ± 1.21 ^{b}
Calorie value	299.55 ± 2.05 ^{a}	345.47 ± 0.83 ^{b}	406.48 ± 4.74 ^{c}

Data are presented as mean ± SD (n=3). Different superscripts within the same row indicate significant differences (P<0.05)

Physicochemical Properties

The physicochemical attributes of immature Japanese muskmelon showed significant differences among pulp, peel, and seeds (Table 2). Seeds recorded the highest L*, followed by pulp and peel. The peel exhibited strong greenness and yellowness due to chlorophylls and carotenoids (Ong et al., 2022), suggesting its potential use as a natural food colourant. All parts showed positive b* values, indicating yellow tones linked to carotenoid pigments in melon tissues (Fundo et al., 2018). Pulp contained the highest TSS. Seed pH closely resembling cantaloupe seed values (Fundo et al., 2018). Water activity for all parts was below the growth threshold for pathogenic bacteria (Ong et al., 2022). Total titratable acidity was greatest in the pulp, consistent with its stronger organic acid content (Fundo et al., 2018).

Table 2
*Physicochemical properties of Japanese muskmelon (*Cucumis melon *var. Casanova)*

Parameter	Pulp	Peel	Seed
L*	80.53 ± 0.68 ^{b}	60.92 ± 1.00 ^{a}	85.76 ± 0.29 ^{c}
a*	-5.37 ± 0.04 ^{b}	-12.57 ± 0.12 ^{a}	0.80 ± 0.01 ^{c}
b*	21.59 ± 0.19 ^{a}	25.29 ± 0.43 ^{b}	24.53 ± 0.09 ^{a}
Total Soluble Solid (^{o} Brix)	2.16 ± 0.00 ^{b}	1.19 ± 0.00 ^{a}	1.19 ± 0.00 ^{a}
pH	5.97 ± 0.02 ^{a}	6.25 ± 0.01 ^{b}	6.39 ± 0.02 ^{c}
Water Activity	0.47 ± 0.00 ^{b}	0.53 ± 0.00 ^{c}	0.42 ± 0.01 ^{a}
Total titratable acidity (%)	0.08 ± 0.02 ^{b}	0.03 ± 0.01 ^{a}	0.03 ± 0.01 ^{a}

Data are presented as mean ± SD (n=3). Different superscripts within the same row indicate significant differences (p<0.05)

CONCLUSION

Immature Japanese muskmelon seeds were nutrients-rich, the peels were the darkest in colour, and the pulp had highest in TSS and acidity. All parts demonstrate potential for sustainable application in food products such as bakery product, noodle, and functional beverages.

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Enhancing BRIS Soil Sustainability through Biological Agent-drive Composting Approaches

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ABSTRACT

Beach Ridges Interspersed Swales (BRIS) soils are among the least fertile soil types in Malaysia due to poor nutrient content, low cation exchange capacity, and weak water-holding ability. At the same time, increasing organic food waste presents environmental challenges if unmanaged. Composting offers a dual solution by improving soil fertility and reducing waste accumulation. This study compared four composting approaches whereas: control, Vermicomposting, Black Soldier Fly (BSF) composting, and Effective Microorganisms (EM) composting by using a Randomised Complete Block Design (RCBD). The parameters that were observed are temperature, pH, and moisture content and monitored across the composting phases of mesophilic, thermophilic and curing stages. The findings revealed that BSF composting was the most effective technique by producing stable compost with favourable nutrient content (potassium and phosphorus) and faster decomposition. Vermicomposting ranked second, while EM and control treatments showed moderate improvements. Overall, these findings highlight the role of biological agents such as BSF larvae and earthworms in accelerating the composting process and improving the quality of the final product. Accordingly, BSF and vermicomposting are recommended for sustainable strategies for organic waste management and improving BRIS soil fertility.

Keywords: BRIS soil, composting, BSF, vermicomposting, effective microorganism, food waste management

INTRODUCTION

The global rise in organic food waste poses significant environmental and agricultural challenges. In Malaysia, food waste contributes substantially to municipal solid waste, while BRIS soils limit agricultural productivity due to their sandy texture (>90%), poor water retention, and low

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fertility (Yusoff et al., 2017). Composting represents a sustainable solution, reducing environmental burdens while enhancing soil fertility.

The efficiency of composting depends on multiple factors, including temperature, moisture, and pH. Optimal microbial activity requires moisture levels of 50–60% (Ameen et al., 2016), while temperature not only accelerates decomposition but also eliminates pathogens (Hafeez et al., 2018). A slightly alkaline pH promotes microbial decomposition, whereas acidic conditions slow it down. Biological agents such as earthworms and BSF larvae have shown potential to accelerate composting and improve nutrient enrichment.

This study systematically compares the effectiveness of BSF composting, vermicomposting, and EM composting in enhancing BRIS soil fertility.

Problem Statement

BRIS soil is unsuitable for sustainable agricultural practices. At the same time, the increasing generation of organic food waste poses a significant environmental challenge if left unmanaged. Composting has emerged as a promising approach to address both issues by converting organic waste into nutrient-rich material that can enhance soil fertility. However, the efficiency and quality of compost are highly dependent on the composting method employed. Biological agents such as BSF larvae and earthworms have demonstrated potential in accelerating decomposition and enhancing compost quality. Despite these advantages, limited research has systematically compare the effectiveness of different composting techniques, particularly in relation to their impact on BRIS soil fertility. This highlights the need for a comprehensive evaluation of composting methods to identify the most effective strategy for improving BRIS soil productivity while simultaneously promoting sustainable food waste management.

MATERIALS AND METHODS

Experimental Setup

The study was conducted at Universiti Sultan Zainal Abidin (UniSZA), under sheltered 10m2 structure to minimise weather influence. A Randomized Complete Block Design (RCBD) with four treatments (control, vermicomposting, EM, and BSF composting) replicated three times was used (12 units total) based on Karmegan et al. (2023).

Composting Treatments

Four composting treatments were applied in this study. In the vermicomposting treatment, kitchen waste was layered with BRIS soil and dried leaves at a 3:1 carbon-to-nitrogen ratio. Earthworms (Eisenia fetida) were introduced, and moisture was maintained through periodic watering to support their activity. For the Black Soldier Fly (BSF) composting

treatment, 150 larvae of Hermetia illucens were introduced into organic waste layered with BRIS soil, with continuous monitoring of moisture and larval health to ensure optimal decomposition. The Effective Microorganisms (EM) composting treatment involved the preparation of an EM solution consisting of 1 L of EM, 1 L of molasses, and 10 L of water. This solution was applied to layered organic waste, and the piles were turned regularly to promote aeration and ensure even microbial distribution. Finally, the control treatment relied solely on natural microbial decomposition without the addition of biological agents.

Data Collection

Temperature, pH, and moisture were recorded twice daily across mesophilic, thermophilic, and curing phases. Nutrient analysis was performed using ICP-OES.

RESULTS AND DISCUSSION

Composting Phases

All treatments followed typical composting phases. The mesophilic stage (Day 1–14) showed temperature rises as microbes decomposed readily available organic matter. Control bins exhibited the sharpest rise, while vermicomposting showed moderated increases due to worm activity. BSF composting displayed moderate heat increases, reflecting larval mechanical breakdown of waste.

In the thermophilic phase (Day 15–17), temperatures exceeded 40°C. Control reached the highest peak (35.5°C), followed by EM (34.3°C), vermicompost (33.8°C), and BSF (33.3°C). Moisture from vegetable-rich waste likely reduced oxygen, suppressing aerobic microbial activity (Jahan & Chowdury, 2015).

During curing (Day 19–35), vermicompost maintained gradual declines, BSF compost cooled rapidly as larval activity ceased earlier, and EM exhibited steady declines to ~27°C, indicating stable microbial decomposition.

Relationship between the Composting Technique and Each Parameter

Correlation analysis revealed strong positive relationships among composting parameters. Temperature trends across treatments were aligned, BSF and vermicomposting maintained more stable stable moisture and pH ranges. EM composting maintained smoother transitions, while control showed higher fluctuations.

Nutrient Enrichment

BSF and vermicomposting produced compost with improved nutrient profiles, notably potassium and phosphorus. Control treatment showed higher concentrations of certain macronutrients but lacked stability. The inclusion of eggshells also improved calcium availability for BRIS soils.

CONCLUSION

This study demonstrates that BSF composting is the most effective method for rapid decomposition and nutrient stabilisation, followed by vermicomposting. EM composting provided moderate benefits, while control treatment was least effective. Biological agents, particularly BSF larvae and earthworms, significantly enhance compost quality and decomposition rate. Integrating BSF and vermicomposting offers a sustainable strategy for managing food waste and improving BRIS soil fertility.

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Evaluation the Effect of *Azolla microphylla* and *Trichanthera gigantea* Supplementation on Broiler Starter Growth Performance

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ABSTRACT

High feed costs and limited availability are among the major challenges currently faced by poultry farmers. In response, this study evaluated *Azolla microphylla* and *Trichanthera gigantea* (Ketum Ayam) as natural, sustainable alternatives to soybean meal and fishmeal in broiler starter diets. A 14-day feeding trial was conducted using sixty broiler chicks (1–2 days old) to evaluate the effects of supplementing starter diets with 10%, 20%, and 30% *A. microphylla* and *T. gigantea* on feed intake, body weight gain, average daily gain (ADG), and feed conversion ratio (FCR). Results demonstrated that moderate supplementation at approximately 20% inclusion significantly improved body weight gain and feed efficiency compared to both the control (0% inclusion) and higher supplementation (30%) groups. The moderate inclusion level yielded the lowest FCR, signifying enhanced feed conversion without adverse effects on growth. These findings suggest that incorporating moderate levels of *A. microphylla* and *T. gigantea* into broiler starter diets provides practical, economic, and sustainable benefits by effectively replacing conventional protein sources without sacrificing performance. This approach supports the development of eco-friendly and cost-effective poultry production systems.

Keywords: Azolla microphylla, broiler starter, *trichanthera gigantea,* poultry feed, protein sources

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INTRODUCTION

In broiler production, soybean meal and maize are widely used as the main protein and energy sources in poultry diets. However, increasing costs and competition with human food demand have driven the search for alternative, sustainable feed ingredients. The starter phase of broiler

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production is crucial because young chicks are fragile and need balanced nutrients and high-quality protein to grow quickly, develop strong immunity, and use feed efficiently (Zhang et al., 2022).

However, few studies have looked at alternative protein sources that meet the specific nutritional needs of broilers in the starter phase (references). Azolla sp., a fast-growing aquatic fern, and *Trichanthera gigantea*, a high-protein forage plant, have been recognized for their rich protein content and essential nutrients, making them promising alternatives to conventional protein sources in poultry diets (AL-Shwilly, 2022; Nasarudin et al., 2024). However, their combined potential in broiler starter diets remains underexplored.

Therefore, this study evaluated the effects of different levels of Azolla sp. and *T. gigantea* in broiler starter diets on growth performance, emphasizing on average daily weight gain and feed conversion ratio. These findings provide practical insights for reducing reliance on soybean meal and fishmeal, supporting sustainable and cost-effective starter broiler production.

MATERIALS AND METHODS

Study Site

This study was conducted at Pasir Akar Farm, Universiti Sultan Zainal Abidin (UniSZA), Malaysia The Pasir Akar Farm is in Jerteh, Terengganu, Malaysia (coordinates: 5°38’37 “N 102°28’16” E). The region experiences a tropical climate, with an average temperature of 26.9°C and humidity levels between 80% and 95%, depending on the season.

Plant Samples Collection and Animal Samples Preparation

Plant samples (*Azolla microphylla* and *Trichanthera gigantea*) were collected from Ladang Pasir Akar farm. Sixty 1–2-day-old broiler chicks (40–50 g) were then randomly divided into four groups and fed different supplementation levels for 14 days to evaluate growth performance.

Feeding Treatments Preparation and Experimentation

Sixty broiler chickens were divided into four groups and given different feed treatments: Control (basal feed), T1 (5% *Azolla microphylla* + 5% *Trichanthera gigantea*), T2 (10% + 10%), and T3 (15% + 15%) as shown in Table 1. Feed was provided twice daily (8 a.m. and 6 p.m.), with intake recorded every 3 days and weekly consumption calculated. Feeders were cleaned regularly to prevent fungal growth and ensure fresh feed.

Table 1
Experimental design of feeding treatments

Group of Animals	Inclusion (%)	Feeding Treatments
C	0	Basal Diet
T1	10	Basal Diet + 5% *Azolla microphylla* + 5% *Trichanthera gigantea*
T2	20	Basal Diet + 10% *Azolla microphylla* + 10% *Trichanthera gigantea*
T3	30	Basal Diet + 15% *Azolla microphylla* + 15% *Trichanthera gigantea*

Data Collection for Growth Performance

Feed intake and body weight were measured every 3 days. Weight gain was calculated as final minus initial weight. Average daily gain (ADG) was total gain divided by days, and feed conversion ratio (FCR) was feed intake divided by weight gain, with lower FCR showing better efficiency (Kamaruddin et al., 2024).

Data Analysis

Growth performance was analyzed using one-way ANOVA, with means and standard deviations calculated. Significant differences between treatments were identified at $P < 0.05$, providing a clear statistical interpretation of treatment effects on growth.

RESULTS AND DISCUSSION

Feed Intake

Table 2 shows feed intake was highest in T1 (273.0 ± 25 g), followed by T2 (256.8 ± 28 g), T3 (189.4 ± 22 g), and the Control (100.8 ± 20g). While T1 (10% inclusion) consumed the most feed, T2 (20% inclusion) provided a better balance, combining adequate intake with optimal nutrient utilization. The decline in T3 (30% inclusion) likely resulted from higher fiber content reducing palatability. These findings suggest that moderate supplementation improves growth performance and feed efficiency, consistent with previous studies (Libatique et al., 2021; Samad et al., 2020).

Table 2
Average feed intake (g) of broiler chickens under different dietary treatments

Group of Animals	Average Feed Intake (g)
C	100.8 ± 2^{0}c
T1	273.0 ± 25^{a}
T2	256.8 ± 2^{8}a
T3	189.4 ± 22^{b}

Note. Values are means ± SE. Different superscript letters within the column indicate significant differences (P < 0.05)

Body Weight Gain

Table 3 shows that broilers in T2 (20% inclusion) achieved the highest weight gain (138.9 ± 11g), followed by T1 (136.7 ± 12 g) and the Control group (111.8 ± 10 g). In contrast, T3 (30% inclusion) recorded the lowest gain (87.5 ± 9 g).

These findings indicate that moderate inclusion levels can enhance growth performance by providing a better balance of digestible protein, amino acids, and fiber, which improves nutrient absorption and utilization. However, higher inclusion levels, such as in T3, may reduce growth due to excess fiber and lower metabolizable energy intake, which can limit feed efficiency (El-Ghany, 2020). Overall, a 20% inclusion level appears to be the most effective for promoting broiler growth during the starter phase.

Table 3
Average weight gain (g) of broiler chickens under different dietary treatments

Group of Animals	Average Weight Gain (g)
C	111.8 ± 10^{b}
T1	136.7 ± 12^{a}
T2	138.9 ± 11^{a}
T3	87.5 ± 9^{c}

Note. Values are means ± SE. Different superscript letters within the column indicate significant differences ($P < 0.05$)

Table 4
Average daily weight gain (g) of broiler chickens under different dietary treatments

Group of Animals	Average Daily Weight Gain (g)
C	55.9 ± 5^{b}
T1	68.4 ± 6^{a}
T2	69.5 ± 6^{a}
T3	47.3 ± 5^{c}

Note. Values are means ± SE. Different superscript letters within the column indicate significant differences ($P < 0.05$)

Average Daily Gain (ADG)

Table 4 shows that T2 had the highest average daily gain (ADG) at 69.5 ± 6 g/day, followed by T1 (68.4 ± 6 g/day) and the Control group (55.9 ± 5 g/day). The lowest ADG was seen in T3 (47.3 ± 5 g/day). These results indicate that including 10–20% *A. microphylla* and *T. gigantea* in starter broiler diets can enhance growth performance. The good performance of T2 shows that the mix of protein from Azolla and nutrients from *T. gigantea* improved feed use and growth. However, growth declined in T3, likely due to high fiber and tannin content at 30%, which may have reduced nutrient absorption (El-Ghany, 2020). Overall, 20% inclusion produced the best growth performance, providing an optimal balance between nutrient intake and feed efficiency

Feed Conversion Ratio (FCR)

Table 5 shows that T2 (FCR 1.85 ± 0.2) achieved the best overall growth (ADG 69.5 ± 6 g/day; total gain 138.9± 11 g), reflecting a balanced feed intake and performance. The combination of digestible protein from Azolla and fiber and minerals from Trichanthera likely enhanced nutrient absorption and metabolic efficiency. Although the Control diet

had the lowest FCR (0.88±0.1), growth was limited, indicating that feed efficiency alone does not ensure optimal performance. In contrast, T3 (30% inclusion) showed the poorest performance, with an ADG of 47.3 ± 5 g/day and FCR of 2.30 ± 0.2, indicating that excessive supplementation reduced feed palatability and disrupted nutrient balance (Libatique et al., 2021; Samad et al., 2020).

Table 5
Average feed conversion ratio of broiler chickens under different dietary treatments

Group of Animals	Feed Conversion Ratio
C	0.88 ± 0.1^{b}
T1	2.00 ± 0.2^{a}
T2	1.85 ± 0.2^{a}
T3	2.30 ± 0.2^{c}

Note. Values are means ± SE. Different superscript letters within the column indicate significant differences ($P < 0.05$)

CONCLUSION

This study suggest include *Azolla–Trichanthera* mixtures up to 20% in starter broiler diets, as this level provides the best balance of growth and feed conversion. Inclusion levels beyond 20% (e.g., 30%) should be avoided, as efficiency and palatability decline, reducing overall performance.

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Determination of Antimicrobial Activity of *Pseudomonas aeruginosa* Isolated from Dorper Sheep Milk with Sub-clinical Mastitis Infection

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ABSTRACT

Multidrug-resistant (MDR) *Pseudomonas aeruginosa* has emerged as a major health issue to the world's small ruminant dairy industry. Antimicrobial susceptibility testing (AST) is crucial for identifying resistant *P. aeruginosa* strains linked to milk-borne mastitis infections in Dorper sheep at the farm level in order to create efficient management measures. AST tests of Dorper sheep isolates revealed that isolate 46-1 was resistant to all tested antibiotics, with intermediate resistance to doripenem and resistance to norfloxacin and ciprofloxacin. ATCC BAA-2108 was resistant to all eleven agents, while isolates 66-1 and 00-1 were the most susceptible. Four isolates (46-1, 10-R, 67-1, and 13-1) showed intermediate or resistant responses to oxacillin, penicillin, norfloxacin, and kanamycin. These findings underscore the importance of detecting carbapenem resistance in ***P. aeruginosa*** to guide effective treatment, enhance milk safety, and reduce public health risks from MDR strains.

Keywords: Multidrug resistance, *Pseudomonas aeruginosa*, mastitis, antimicrobial susceptibility test

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INTRODUCTION

Pseudomonas aeruginosa is a pathogenic bacterium that caused mastitis in dairy animals, leading to reduced milk yield, poor quality, and impaired herd development (Abdalhamed et al., 2018). The emergence of multidrug-resistant (MDR) strains has

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increased treatment costs, selective pressure, and risks to both animal and human health (Al-Khazay & Kshash, 2014). Recognized by the World Health Organisation (WHO), *P. aeruginosa* has recently shown resistance to carbapenems. This study aims to investigate carbapenem resistance in *P. aeruginosa* isolated from mastitis-infected milk of Dorper sheeps using the antimicrobial susceptibility disc diffusion test.

MATERIALS AND METHODS

Milk Sampling and Bacterial Isolation

Mastitis-contaminated milk was collected from Agropolitan Besut-Setiu Farm in 5.45° N, 102.88° W in August 2018. The liquid California Mastitis Test (CMT) was used to confirm mastitis (Wan-Azemin et al., 2021, 2022) and 16S rRNA sequencing was performed to identify and validate the *P. aeruginosa* isolates (46-1, 10-R, 67-1, 13-1, 66-1, and 00-1). *P. aeruginosa* strains ATCC 27853 and ATCC BAA-2108 were used as carbapenem control strains.

Antimicrobial Susceptibility

The disc diffusion assay was used to perform the susceptibility antimicrobial test for each isolated strain. 11 antibiotics were assayed on Mueller Hinton Agar (MHA) as listed in Table 1. The CLSI breakpoints determined whether the isolates were sensitive (s), intermediate (i), or resistant (r) to antipseudomonal medicines (CLSI, 2018). Assay was performed in triplicate, and the mean±SD was calculated.

RESULTS AND DISCUSSION

Susceptibility against Conventional Antibiotics

Based on CLSI, (2018) breakpoints, the six *Pseudomonas aeruginosa* isolates showed variable antibiotic susceptibility. Isolate 46-1 was resistant to all agents except doripenem (intermediate). Vancomycin resistance was observed in all isolates, consistent with Swetha et al. (2017). Four isolates (13-1, 46-1, 10-R, and 67-1) also showed intermediate/resistance to penicillin and oxacillin, in line with Swetha et al. (2017). Ciprofloxacin remained effective against most isolates except 46-1 and 67-1, supporting Meng et al. (2020). The variability in resistance may be linked to environmental adaptation and selective exposure (Swetha et al., 2017), while high susceptibility to ciprofloxacin and carbapenems reflects their limited use in dairy mastitis (Aghazadeh et al., 2014).

CONCLUSION

Out of the six isolates, four (46-1, 10-R, 67-1, and 13-1) exhibited a strong MDR phenotype and comparable intermediate/resistance responses to most antibiotics tested. In contrast,

Table 1
Diameter of antibiotics inhibition zone of P. aeruginosa *isolates and ATCC strains (Mean±SD)*

Drugs[a]	Inhibition zone diameter of sample (mm)							
	46-1	**10-R**	**67-1**	**13-1**	**66-1**	**00-1**	**ATCC BAA-2108**	**ATCC 27853**
DOR (10 µg)	17.0±0.0	22.3±0.5	17.0±2.4	31.0±0.8	45.0±0.8	39.0± 0.8	3.3±0.5	44.0± 0.0
MEM (10 µg)	14.3±0.5	20.0±0.0	14.0±1.4	30.3±0.9	43.3±0.5	35.0± 1.6	3.0±0.0	31.3±0.5
FOX (30 µg)	12.3±0.5	22.0±0.0	13.3±0.5	21.3±0.5	34.0±1.4	30.0± 0.5	0.0±0.0	0.0±0.0
OXA (1 µg)	5.0±0.0	17.0±0.0	8.7±0.5	20.0±1.4	34.0±1.4	25.3± 2.5	0.0±0.0	0.0±0.0
PEN (10U)	12.3±0.5	15.3±2.1	18.3±0.9	13.0±1.4	42.3±1.7	37.0± 0.8	0.0±0.0	0.0±0.0
NOR (10 µg)	3.0±0.0	8.0±0.0	10.3±0.9	16.7±0.5	32.0±0.8	24.0± 2.9	5.0±0.8	27.3±2.1
CIP (10 µg)	1.0±0.0	22.3±0.9	14.7±0.5	27.3±2.6	36.3±0.5	27.0±0.5	0.0±0.0	36.3±1.2
KAN (30 µg)	5.3±0.5	6.7±0.5	14.0±0.8	16.0±1.4	29.0±2.9	17.0±1.4	0.0±0.0	0.0±0.0
VAN (5 µg)	5.7±0.5	4.7±0.5	3.3±0.5	12.3±0.5	11.7±0.5	7.3±0.5	0.0±0.0	0.0±0.0
ERY (30 µg)	12.3±0.5	15.7±0.5	17.3±0.5	29.0±1.4	33.3±0.5	23.0±1.6	0.0±0.0	0.0±0.0
LZD (30 µg)	11.7±0.5	20.0±3.6	16.0±1.4	18.0±0.8	37.0±1.6	26.0±2.9	0.0±0.0	0.0±0.0
MHB	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0	0.04±0.0

[a]DOR: doripenem; MEM: meropenem; FOX: cefoxitin; OXA: oxacillin; PEN: penicillin G; NOR: norfloxacin; CIP: ciprofloxacin; KAN: kanamycin; VAN: vancomycin; ERY: erythromycin; LZD: linezolid; MHB: Mueller Hinton Broth. Each point is the mean ± SD of three experiments. [b]Color coded; Red: resistant; Yellow: intermediate; Green: susceptible; Grey: not detected

isolates 66-1 and 00-1 were highly susceptible to the same antibiotics. Further research is needed to elucidate the MDR mechanisms of these isolates including genome sequencing which could confirm whether they belong to known *P. aeruginosa* strains or represent novel variants.

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Effects of Dietary Tryptophan Manipulation on Growth and Survival of African Catfish (*Clarias gariepinus*) Larvae

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ABSTRACT

The African catfish (*Clarias gariepinus*) is a popular aquaculture species owing to its rapid growth, high fecundity, and tolerance to low-oxygen conditions. However, its tendency toward cannibalism during early development often leads to inconsistent larval production. Despite its well-documented biological and environmental adaptability, certain nutritional factors, such as tryptophan, that influence early development remain unexplored. Tryptophan, as an essential amino acid involved in growth and behavioural regulation, is still not fully understood in the context of fish larvae. This study aimed to investigate the effects of dietary tryptophan on the larval growth and survival of *C. gariepinus*. A total of 1000 larvae were produced from artificial stripping induced with hormone Ovaprim (0.5–1.0 ml/kg). Fertilized eggs were incubated in hatching tanks before transfer to aquaria. Larvae were distributed into 20 aquaria under five dietary treatments with four replicates: control (T_0), 2.5 g/kg ($T_{2.5}$), 5.0 g/kg ($T_{5.0}$), 7.5 g/kg ($T_{7.5}$), and 10.0 g/kg ($T_{10.0}$). Growth, survival, and behavioural interactions were monitored. Results indicated that larvae fed $T_{2.5}$ diets exhibited significantly higher survival and improved growth compared to the control and other treatments. While higher tryptophan levels ($T_{7.5}$ and $T_{10.0}$) did not enhance performance, $T_{2.5}$ supplementation reduced cannibalistic behaviour and promoted stable interactions among larvae. This study shows the moderate dietary tryptophan supplementation (2.5 g/kg) optimizes larval growth and survival. These findings highlight the potential of dietary tryptophan manipulation as a nutritional strategy to enhance larval rearing technique and sustainability in aquaculture.

Keywords: African catfish, *Clarias Gariepinus*, growth, survival, tryptophan

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INTRODUCTION

Aquaculture contributes significantly to global food security, especially in developing countries, by supplying affordable protein (Pradeepkiran, 2019). In Malaysia, African catfish (*Clarias gariepinus*) is a major species due to its rapid growth and resilience (Ali et al., 2022). However, seed production is constrained by high larval mortality from early cannibalism (Wubie & Dagne, 2022). While environmental and genetic factors have been studied, the nutritional role of amino acids remains less explored. Tryptophan, a precursor of serotonin that regulates growth, stress, and behaviour may improve larval survival (Umanah & David, 2025). This study examines the effect of dietary tryptophan on enhancing hatchery performance in *C. gariepinus* larvae.

Problem Statement

Current knowledge of tryptophan's role in *C. gariepinus* larvae is limited, particularly regarding growth, survival, and stress. Undefined dietary levels and scarce behavioural research hinder feeding strategies and aquaculture success.

Research Question

Based on the identified gaps, this study seeks to answer the following questions: What are the effects of different dietary tryptophan concentrations on the growth performance of larvae? What is the optimal dietary concentration of tryptophan required to enhance growth and improve overall larval survival?

MATERIALS AND METHODS

The study was conducted at the Hatchery and Aquatic Laboratory, Faculty of Bioresources and Food Industry (FBIM), Universiti Sultan Zainal Abidin (UniSZA), Malaysia (5.1234° N, 103.4567° E), with ethical approval from UniSZA's Animal Ethics Committee (UAPREC/008/025).

Experimental Setup

Two broodfish pairs were acclimatized and underwent a breeding phase to produce fish larvae. These were evenly distributed into 20 aquariums (7 L capacity), each containing 5 L of freshwater and 50 larvae (Wenzel et al., 2022). Each tank was aerated and equipped with a thermometer. Larvae were fed 10 g of pellets mixed with L-tryptophan at five concentrations: 0 (T_C), 2.5 ($T_{2.5}$), 5.0 ($T_{5.0}$), 7.5 ($T_{7.5}$), and 10.0 g/kg ($T_{10.0}$).

Larval Assessment and Data Analysis

Larvae were monitored daily for growth, survival, and behaviour, including feeding, movement, and resting. Size changes were examined microscopically, and key parameters were calculated using the method described by Wenzel et al. (2022). Statistical analysis was performed using one-way ANOVA with post hoc tests at a 95% significance level (Minitab® Statistical Software, Version 19.1).

RESULTS AND DISCUSSION

Larval Growth

Figure 1 shows larval growth over 14 days under different tryptophan treatments. Growth increased after Day 3, with $T_{2.5}$ performing close to the control (T_C), while higher doses ($T_{5.0}$-$T_{2.5}$) suppressed growth, especially $T_{10.0}$. Similar findings were reported in Asian seabass, where 1.0% L-tryptophan improved growth and survival (Khan et al., 2023), and in crucian carp, where supplementation enhanced growth and immunity (Fu et al., 2021). These results highlight that tryptophan benefits larval growth only at optimal levels, while excess reduces performance.

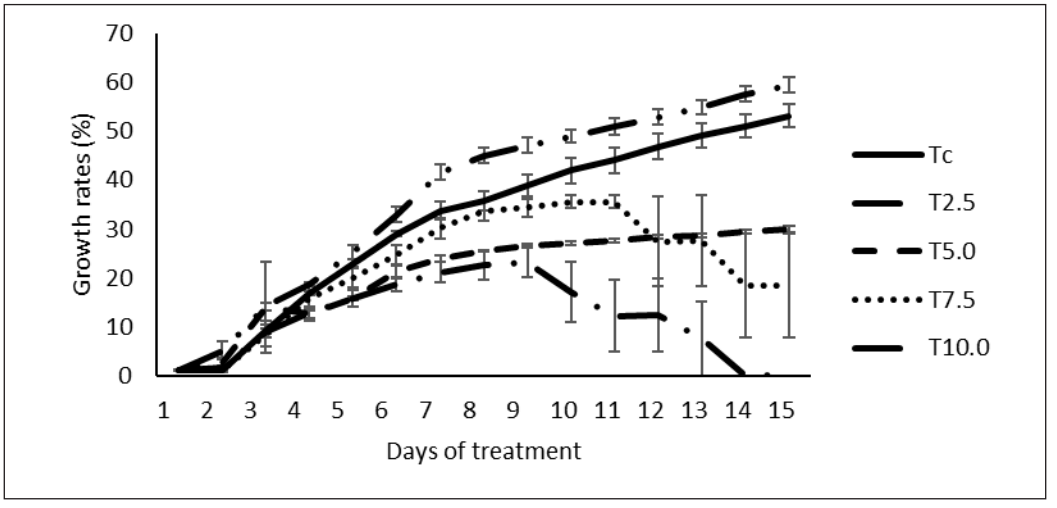

Figure 1. Growth rate of the larvae in a 14-day experiment

Larval Survival Rates

Figure 2 shows larval survival of *C. gariepinus* improved from 28.57±7.71% (control) to 45.36±6.61% at $T_{2.5}$ but declined sharply at higher levels (7.86±1.89% at $T_{5.0}$, 1.79±1.07% at $T_{7.5}$, and 0% at $T_{10.0}$). This indicates a narrow optimal range, with $T_{2.5}$ as the most effective level, while excess tryptophan proved detrimental.

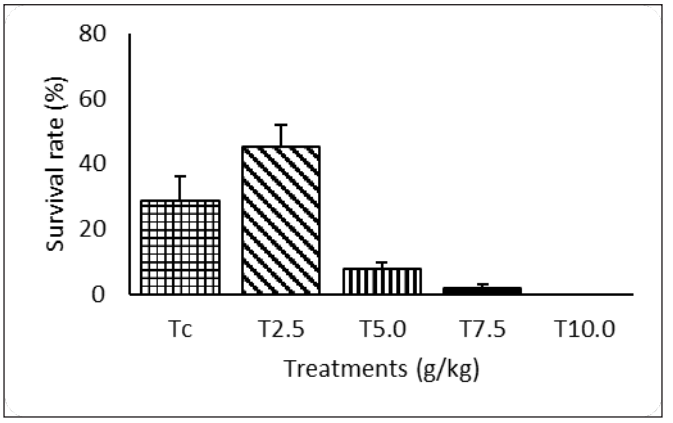

Figure 2. Survival rate of the larvae in a 14-day experiment

Previous studies confirm the threshold effect of dietary tryptophan, with optimal requirements reported in *Channa argus* (0.45-0.46%), blunt snout bream (0.33-0.36%), and silver catfish (2.5-3.4 g/kg) (Ji et al., 2019; Miao et al., 2021; Pianesso et al., 2015). Tryptophan has also been linked to reduced cannibalism, stress, and improved survival in Asian seabass (Khan et al., 2022). In this study, survival of *C. gariepinus* larvae peaked at $T_{2.5}$, confirming that moderate supplementation is beneficial, while excess levels are detrimental.

CONCLUSION

This study shows that dietary tryptophan improved *C. gariepinus* larval performance, with the highest survival (45.36±6.61%) and growth $T_{2.5}$ compared to control (28.57±7.71%). Higher levels reduced performance, with total mortality at $T_{10.0}$, confirming $T_{2.5}$ as optimal.

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Influence of Seasonal Changes on Physicochemical, Nutritional, and Sensory Characteristics of Coconut Sap (Neera)

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ABSTRACT

Coconut sap (neera), a naturally sweet, watery liquid that flows from the cut inflorescence (flower bud) of the coconut palm tree (*Cocos nucifera*), is praised for its economic value. However, in Malaysia, there is limited research on the properties affected by seasonal factors. Hence, the objectives of this study were to determine the physicochemical properties, nutritional content, and sensory attributes of the coconut sap samples collected during wet (NW) and dry (ND) seasons. The results revealed that NW had significantly higher moisture content (86.2 ± 1.41%), while ND contained significantly higher crude protein (0.32 ± 0.01%) and carbohydrate content (13.51 ± 0.02%). Meanwhile, the ND had a darker colour (37.21 ± 0.72), and NW had a higher TSS value (14.68 ± 1.05). The vitamin analysis indicated vitamin C levels of ND (15.40 mg/100 g) were significantly higher than the NW. The sensory evaluation showed NW samples were preferred mainly in terms of colour, sweetness, and aftertaste.

Keywords: Coconut sap, nutritional content, proximate composition, physicochemical properties, sensory attributes, vitamins

INTRODUCTION

Cocos nucifera L. known as the coconut palm, is widely cultivated in tropical regions and belongs to the Arecaceae family. The coconut sap, locally known as neera, typically distinguished by its oyster-white color. In Malaysia, coconut sap holds significant economic value and cultural significance, both as a non-fermented and fermented beverage, vinegar, and palm

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sugar, providing year-round income for farmers (Ghosh et al., 2018). It is rich in natural sugars, essential vitamins, and minerals, and has a low glycemic index (GI) (Asghar et al., 2020). It is considered a healthier alternative option, with a lower GI value as compared to palm and sugarcane sugars (Saputro et al., 2017).

Problem Statement

In Malaysia, research has primarily focused on neera from Negeri Sembilan, West Peninsular Malaysia (Asghar et al., 2020), while comprehensive scientific data from other factors, such as location, season, and tapping times, all of which influence the composition and quality of coconut sap, remain scarce. Most research offering some valuable insights has come from Indonesia (Somawiharja et al., 2018) and India (Ghosh et al., 2018).

Research Questions

Nutritional value may increase during the dry season (May to September) due to tree stress and reduced moisture, while the wet season (November to March) could cause changes in nutrient composition due to higher hydration levels and altered physiological conditions. These inconsistencies may affect consumer preferences.

MATERIALS AND METHODS

Sample Collection

Fresh coconut sap was sourced by a local estate farmer, ABEADI Group Sdn. Bhd. from Besut, Terengganu, Malaysia, during the Southwest Monsoon (dry season) from May to September, and the Northeast Monsoon (wet season) from November to March.

Proximate, Physicochemical, and Nutritional Analyses

Coconut sap was subjected to analysis, including colour determination, pH value, and total soluble solids (TSS). Proximate and mineral compositions analyses were conducted using the AOAC Method (AOAC, 2011). Vitamin analysis was carried out according to Asghar et al. (2020) with slight modifications using a Shimadzu liquid chromatograph LC-10vp fitted with a UV-VIS detector (SPD-10A) set at 260 nm.

Sensory Analysis

This sensory analysis utilised a 9-point hedonic scale involving 50 untrained panelists, who evaluated the samples based on the attributes: color, aroma, consistency, sweetness, aftertaste, and overall acceptance (Kemp et al., 2009).

RESULTS AND DISCUSSION

The results showed that the NW sap contained higher moisture (86.2 ± 1.41%) than ND. In contrast, the ND sample contained a significantly higher crude protein (0.32 ± 0.01%), ash (0.33 ± 0.05%), food energy (55.59 ± 5.01 kCal), TSS value (14.68 ± 1.05%), and vitamin C (15.40 mg/100 g), as compared to NW sample (Table 1). Meanwhile, the sensory evaluation revealed that NW sample was more favored, particularly for its colour, sweetness, and

Table 1
Proximate composition, food energy, vitamins, and physicochemical properties of coconut sap collected during different seasons

Composition	Sample	
	ND	NW
Proximate Composition		
Moisture (%)	85.81 ± 0.73[b]	86.2 ± 1.41[a]
Ash (%)	0.33 ± 0.05[a]	0.29 ± 0.17[a]
Fat (%)	0.03 ± 0.01[a]	0.02 ± 0.01[a]
Crude Protein (%)	0.32 ± 0.01[b]	0.21 ± 0.10[a]
Carbohydrate (%)	13.51 ± 0.02[a]	13.28 ± 1.29[a]
Food Energy (kCal)	55.59 ± 5.01[b]	54.14± 3.01[b]
Mineral (mg/100g)		
Potassium	804.10[a]	844.20[a]
Sodium	116.90[a]	144.60[b]
Phosphorus	88.52[a]	67.24[b]
Ferum	1.68[a]	1.31[a]
Zinc	0.35[a]	0.29[a]
Calcium	0.61[a]	0.76[b]
Magnesium	0.47[a]	0.57[b]
Mangan	0.08[a]	0.08[a]
Cuprum	0.08[a]	0.08[a]
Vitamin (mg/100g)		
C	15.40[a]	13.10[b]
B1	<0.10[a]	<0.10[a]
B2	<0.10[a]	<0.10[a]
Physicochemical		
pH	6.13 ± 0.43[Aa]	6.13 ± 0.52[Aa]
TSS (˚Brix)	14.14 ± 3.23[Aa]	14.68 ± 1.05[Aa]
Colour		
Lightness (L)*	37.21 ± 0.72[Ba]	16.48 ± 0.88[Aa]
*Redness (*a)*	0.59 ± 0.35[Aa]	2.82 ± 0.43[Ba]
*Yellowness (*b)*	0.69 ± 0.55[Aa]	3.35 ± 0.28[Ba]

aftertaste (Table 2). Thus, the findings indicated that seasonal variation had influenced the nutritional profile of coconut sap (neera), with the dry season exhibiting higher nutrient levels, possibly due to physiological stress and reduced moisture. In contrast, the wet season showed nutrient dilution, possibly due to the increased water uptake (Sri et al., 2025). These differences had also affected nutritional quality, sensory traits, and consumer acceptability.

Table 2
Sensory attributes of neera collected at different seasons

Attributes	Sample	
	ND	NW
Colour	5.72^{Bb}	5.54^{Aa}
Aroma	3.96^{Aa}	5.90^{Aa}
Consistency	5.30^{Aa}	5.98^{Ba}
Sweetness	4.86^{Aa}	5.94^{Ba}
Aftertaste	3.68^{Aa}	5.26^{Aa}
Overall Acceptance	4.12^{Aa}	5.58^{Aab}

CONCLUSION

In conclusion, the findings highlight the health-promoting potential of coconut sap, with its nutritional quality being influenced by seasonal factors.

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Polyclonal Antibodies against Zearalenone: Production, Characterisation, and Application in Food Safety Biosensors

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ABSTRACT

Zearalenone (ZEA), a mycotoxin with estrogenic effects, poses significant risks to food and feed safety. This study reports the production and characterisation of polyclonal antibodies (pAb) against ZEA for potential biosensor applications. New Zealand White rabbits were immunized with a ZEA–protein conjugate emulsified in Freund's adjuvant, following approved animal ethics guidelines. Serum antibodies were purified using ammonium sulfate precipitation, dialysis and Protein A affinity chromatography on an ÄKTA Prime system. Antibody titers, determined by indirect ELISA, showed strong immune responses in both rabbits, with the second bleed demonstrating optimal concentration and stability. Cross-reactivity was evaluated via competitive ELISA against aflatoxins, ochratoxins, and fumonisins at 20, 50, and 100 ppb. The antibodies exhibited high specificity for ZEA (100% reactivity) with minimal cross-reactivity (13–19%) toward other mycotoxins. Preimmune serum showed negligible reactivity, confirming the specificity of the immune response. These findings highlight the potential of the generated pAb as reliable bioreceptor for electrochemical biosensors, offering sensitive and selective detection of ZEA in food safety monitoring. Future work will integrate these antibodies into sensor platforms to improve detection limits and extend applicability across diverse agricultural commodities.

Keywords: Biosensor, grain corn, mycotoxins, polyclonal antibody, zearalenone

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INTRODUCTION

Zearalenone (ZEA), a *Fusarium*-produced mycotoxin, contaminates grains such as corn, wheat, barley, rice and oats. Grain corn, a key component in animal feed, is particularly vulnerable. A Department of Veterinary Services report detected *Fusarium* mycotoxins—fumonisins (FUMs), zearalenone (ZEA), and deoxynivalenol (DON)—in all grain corn samples from Peninsular Malaysia (Syahidah et al., 2021). Similarly, a study conducted in grain corn plantations in Terengganu, Malaysia highlighted *Fusarium* species as major contributors to ZEA contamination, with tassels exhibiting the highest fungal loads due to airborne spores during the cropping season (Yazid et al., 2021).

Although less toxic than aflatoxins, ZEA's estrogenic effects pose serious risks to livestock, particularly swine- leading to reproductive disorders, infertility and hyperestrogenism, which can cause endometrial hyperplasia and malignancy. Reliable detection is crucial for feed safety. While gold-standard methods like HPLC, LC-MS and GC offer high sensitivity, they require extensive preparation, skilled personnel and lab-bound equipment, limiting field use. Enzyme-linked immunosorbent assay (ELISA), though widely used, is susceptible to light interference and remains laboratory-dependent.

Immunosensors offer a promising alternative, enabling rapid, on-site mycotoxin detection through antibody-based transduction. This study focuses on developing polyclonal antibodies against ZEA for biosensor applications in grain corn, with potential for broader food safety monitoring. Future innovationsincluding nanomaterials and IoT-based platformscould further enhance biosensor performance, improving accessibility and reliability in agricultural mycotoxin detection.

MATERIALS AND METHODS

Production and Purification of Polyclonal Antibodies against ZEA

Polyclonal antibodies against ZEA were produced by immunizing New Zealand White rabbits, following the guidelines outlined by Leenaars and Hendriksen (2007). Two rabbits (Z1 and Z2) were immunized with a ZEA-protein carrier conjugate mixed with Freund's adjuvant, following a protocol approved by MARDI's Animal Ethics Committee (Approval Number: 20230622/R/MAEC00139). Immunizations and blood collections were conducted fortnightly until the fifth booster. Post-immunization, rabbits were euthanized and incinerated per ethical guidelines. Serum purification involved ammonium sulfate precipitation, dialysis and protein A column chromatography using an AKTA-Prime system. IgG fractions were pooled, neutralized with Tris-HCl and dialyzed, yielding purified antibodies for further use (Kent, 1999).

Characterisation of Polyclonal Antibodies against ZEA

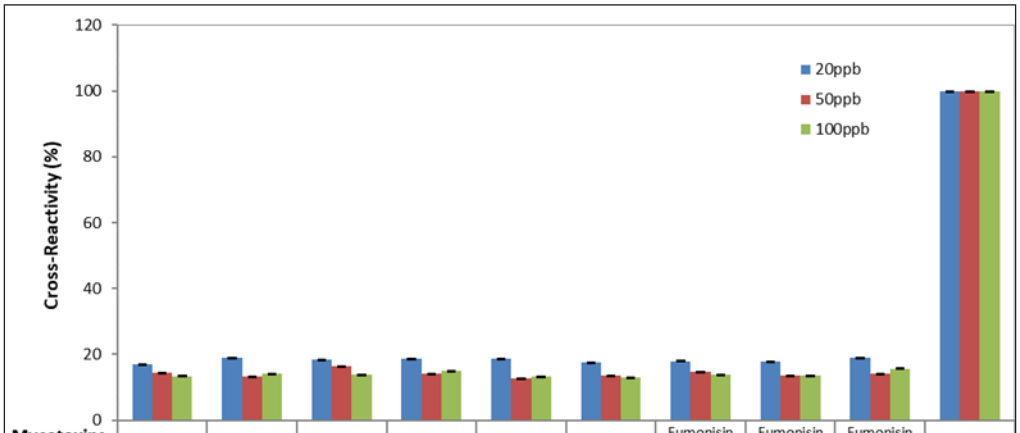
The antibody titer was determined using an indirect ELISA (Liu et al., 1985). Microtiter wells were coated with ZEA-KLH antigen, blocked with dry milk, and incubated with anti-ZEA antibody at varying concentrations. Anti-rabbit Alkaline Phosphatase (AP)-conjugated secondary antibody and p-Nitrophenyl Phosphate (pNPP) substrate were used, with absorbance measured at 405 nm.

Cross-reactivity of the anti-ZEA antibody was assessed via competitive ELISA against mycotoxins (Garg et al., 2022), including aflatoxins, ochratoxins, fumonisins and zearalenone, at concentrations of 20, 50, and 100 ppb.

RESULTS AND DISCUSSION

The antibody titer analysis showed a sigmoidal decrease in absorbance with increasing antibody dilution for both Rabbit 1 (Z1) and Rabbit 2 (Z2), while preimmune serum controls exhibited negligible absorbance, confirming successful immunization and robust antibody production (Liu et al., 1985). The second bleed from both rabbits demonstrated the highest antibody titer, with Z2 showing slightly higher absorbance values at lower dilutions, suggesting a stronger immune response.

Cross-reactivity analysis of the anti-ZEA antibody from the third bleed of Rabbit Z2 demonstrated high specificity for ZEA (100% reactivity) with minimal cross-reactivity (13–19%) to other mycotoxins, such as aflatoxins, ochratoxins, and fumonisins (Figure 1). Although cross-reactivity with ZEA analogues (e.g., α-zearalenol, β-zearalenol) was



Mycotoxins	Aflatoxin B1	Aflatoxin B2	Aflatoxin G1	Aflatoxin G2	Ochratoxin A	Ochratoxin B	Fumonisin B1	Fumonisin B2	Fumonisin B3	Zearalenone
20ppb	17.07	18.97	18.47	18.67	18.62	17.52	17.95	17.77	18.93	100.00
50ppb	14.30	13.33	16.29	14.23	12.76	13.50	14.63	13.67	14.07	100.00
100ppb	13.42	14.08	13.73	14.91	13.39	12.90	13.75	13.48	15.68	100.00

Figure 1. Cross-reactivity analysis of anti-ZEA antibody (third bleed, Z2, 0.1 mg/mL) against various mycotoxins at different concentrations (20 ppb, 50 ppb and 100 ppb)

not assessed in this study, previous research suggest that polyclonal antibodies against ZEA can detect related compounds with up to 60% cross-reactivity (Thongrussamee et al., 2008; Wang et al., 2021).

Overall, the results confirm the high specificity and strong binding affinity of this antibody batch, making it a promising candidate for use in the development of a ZEA biosensor. Its minimal cross-reactivity with other mycotoxins ensures reliable detection, reinforcing its suitability for biosensor applications in food safety monitoring.

CONCLUSION

This study successfully generated and characterized a high-affinity polyclonal antibody against ZEA. The antibody from the third bleed of Rabbit Z2 exhibited exceptional specificity for ZEA, with minimal cross-reactivity against other mycotoxins. These findings highlight its potential as a biorecognition element in ZEA biosensors for food and feed safety, particularly in grain corn monitoring. Its high specificity and strong binding affinity enable accurate and sensitive detection with minimal interference. Future research should focus on optimizing biosensor integration and evaluating performance in real-world food matrices to enhance practical applications in food safety systems.

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The Evaluation of Two Oil Palm Clones Response to Nutrient Deficiency Treatment

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ABSTRACT

Advancements in plant physiology, molecular biology, and genetics have enhanced understanding of oil palm responses to nutrient stress. This study evaluated two clones (CPS1 and CPS2) under nutrient-deficiency treatments (N0 P0 K0, N0 P1 K1, N1 P1 K1 [Control], N1 P0 K1, N1 P1 K0). Nutrient deprivation reduced growth, chlorophyll content, and physiological efficiency in both clones. CPS1 showed superior growth and photosynthetic performance, with a higher net photosynthetic rate (11.72 vs. 9.96 μmol CO_2 m^{-2} s^{-1}), greater intercellular CO_2 exchange (0.0396 vs. 0.0346 mol m^{-2} s^{-1}), and higher Ci/Ca ratio (0.348 vs. 0.326). In contrast, CPS2 exhibited greater water use efficiency (3.54 vs. 3.43 μmol m^{-2} s^{-1}) and intrinsic WUE (51.76 vs. 45.03 μmol mol^{-1}), reflecting stronger stomatal adjustment to nutrient stress. CPS1 maintained higher transpiration (3.48 vs. 2.92 mmol H_2O m^{-2} s^{-1}), stomatal conductance (0.28 vs. 0.23 mol H_2O m^{-2} s^{-1}), and leaf water potential (6.51 vs. 5.63 ψ), while both clones shared similar F_v/F_m values (0.76), indicating consistent photochemical efficiency. Morphologically, CPS1 developed more fronds (13 vs. 10) and greater height (117.7 vs. 94.7 cm), while CPS2 recorded slightly higher chlorophyll content (49.9 vs. 49.5). Nitrogen deficiency most strongly limited photosynthesis in both clones. Despite reduced performance under nutrient stress, both clones displayed physiological and morphological adjustments that highlight their capacity to tolerate nutrient-poor environments, with CPS1 excelling in growth and photosynthesis and CPS2 in water use efficiency.

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Keywords: Morphophysiological responses, nutrient stress, oil palm clones, photosynthesis, physiological characteristics

INTRODUCTION

Originating from West Africa, the oil palm (*Elaeis guineensis*) has become a key contributor to Malaysia's GDP (Kushairi et

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al., 2017), with recent gains in yield despite challenges such as declining export demand and lower CPO prices (Parveez et al., 2024). However, productivity is constrained by low soil fertility, nutrient losses, and stress from pests, weeds, and climate factors (Murdi et al., 2023). Optimising nutrient use efficiency is thus critical for sustainable agriculture and food security (Hawkesford & Barraclough, 2011). Oil palms require balanced NPK fertilisation for growth, stress tolerance, and physiological regulation (Adileksana et al., 2020). Seedlings adapt to stress via physiological adjustments, and seed modification offers potential to improve nutrient uptake, drought tolerance, and yield (Rasid et al., 2020). To further enhance productivity, MPOB developed clonal palms such as CPS1, yielding >30 t FFB ha^{-1} (Tarmizi et al., 2017), and CPS2, capable of up to 35.7 t FFB and 10.8 t oil ha^{-1} at high density (Zamzuri, 2011). This study examines clonal responses to nutrient deficiency by assessing physiological, biochemical, and adaptive mechanisms to identify resilient genotypes for sustainable oil palm production.

MATERIALS AND METHODS

Study Design

The study was conducted at MPOB Kluang research station, located in the northern region of Johor (N 2°27'10.0" E 102°45'25.0"). The experiment was performed in an open nursery. Five levels of fertilizer were used in the treatment: N0 P0 K0, N0 P1 K1, N1 P1 K1 (Control), N1 P0 K1 and N1 P1 K0.

Planting Materials

The experiment used two oil palm clones, P164 (CPS1) and P126 (CPS2), generated from tissue culture for genetic homogeneity. Rooted plantlets were cultivated in jiffy pots for three months in the nursery before being transported to 6 × 9 polybags at the study site in the fourth month.

Physiological and Growth Measurements

Leaf gas exchange was measured on fully expanded leaf two using a LI-6400XT system (Li-COR Inc., USA) between 9:00–11:00 a.m., recording net photosynthesis (A), stomatal conductance (g_s), transpiration rate (E), and water use efficiency (WUE = A/E). The cuvette was set at 30 °C, 60% RH, 400 μmol mol^{-1} CO_2, 500 cm^3 min^{-1} flow, and 800 μmol m^{-2} s^{-1} PPFD. Instantaneous carboxylation efficiency (ICE = A/Ci) was calculated, with Ci/Ca ratio used to assess gas exchange regulation, while intrinsic WUE was determined as A/g_s. Plant height was measured from soil to the tip of the longest leaf, fronds were counted manually, and biomass determined after oven-drying at 70 °C for 48 h; chlorophyll content was recorded using a SPAD-502 meter. Leaf water potential (LWP) was measured on leaf

two using a pressure chamber (Model 615), where pressurisation continued until the first water droplet appeared. Chlorophyll fluorescence parameter, F_v/F_m were recorded on leaf two between 8:00–10:00 a.m. using a Handy PEA (Hansatech, UK) after 10 min dark adaptation with clips.

RESULTS AND DISCUSSION

Leaf Gas Exchanges

As shown in Figure 1a–g, CPS1 recorded higher photosynthetic rate (11.72 µmol CO_2 m^{-2} s^{-1}), stomatal conductance (0.28 mol H_2O m^{-2} s^{-1}), transpiration (3.48 mmol H_2O m^{-2} s^{-1}), ICE (0.04 mol m^{-2} s^{-1}), and Ci/Ca (0.75) compared to CPS2, while CPS2 performed slightly better in WUE (3.54 vs. 3.42 µmol m^{-2} s^{-1}) and iWUE (51.76 vs. 45.03 µmol CO_2 mol^{-1} H_2O). The lowest values were observed under nitrogen-deficient treatment (N0 P1 K1), reflecting nitrogen's essential role in photosynthesis through the synthesis of key enzymes such as Rubisco, PEPC, and PPDK. Nitrogen deficiency reduces photosynthetic efficiency, leaf area, and green leaf longevity, ultimately constraining plant productivity (Ober & Parry, 2011). Similar patterns were reported in other crops, where nitrogen fertilisation enhanced WUE and photosynthetic efficiency (Akkamis & Caliskan, 2023), and increased Ci/Ca due to reduced mesophyll metabolic activity rather than stomatal limitations (Kumagai et al., 2009).

Vegetative Measurements

Overall, the number of fronds (Figure 2a) and height (Figure 2b) of CPS1 (13 fronds and 117.7 cm) is greater than CPS2 (10 fronds and 94.7 cm) except for chlorophyl content (Figure 2c). In this study, the nitrogen nutrient had the greatest influence on total frond, height and chlorophyl content for both clonal ramets. Applying nitrogen to oil palm seedlings enhances plant vegetative growth and nitrogen is more available in leaves of many plants than other parts (Ashraf et al. 2017). Nitrogen (N) is crucial for oil palm seedlings as it is a building block for tissue growth and essential components like chlorophyll and nucleic acids. Optimal nitrogen levels enhance growth and development by improving the partitioning of nitrogen in seedlings, leading to better overall growth (Manurung et al., 2024).

Leaf Water Potential (LWP)

Figure 3 shows LWP of CPS1 (6.51 ψ) is higher than CPS2 (5.63 ψ). Leaf water potential is crucial for plant functioning, regulated optimally in drylands to balance water supply and stress tolerance, impacting growth and photosynthesis (Ratzmann et al. 2019).

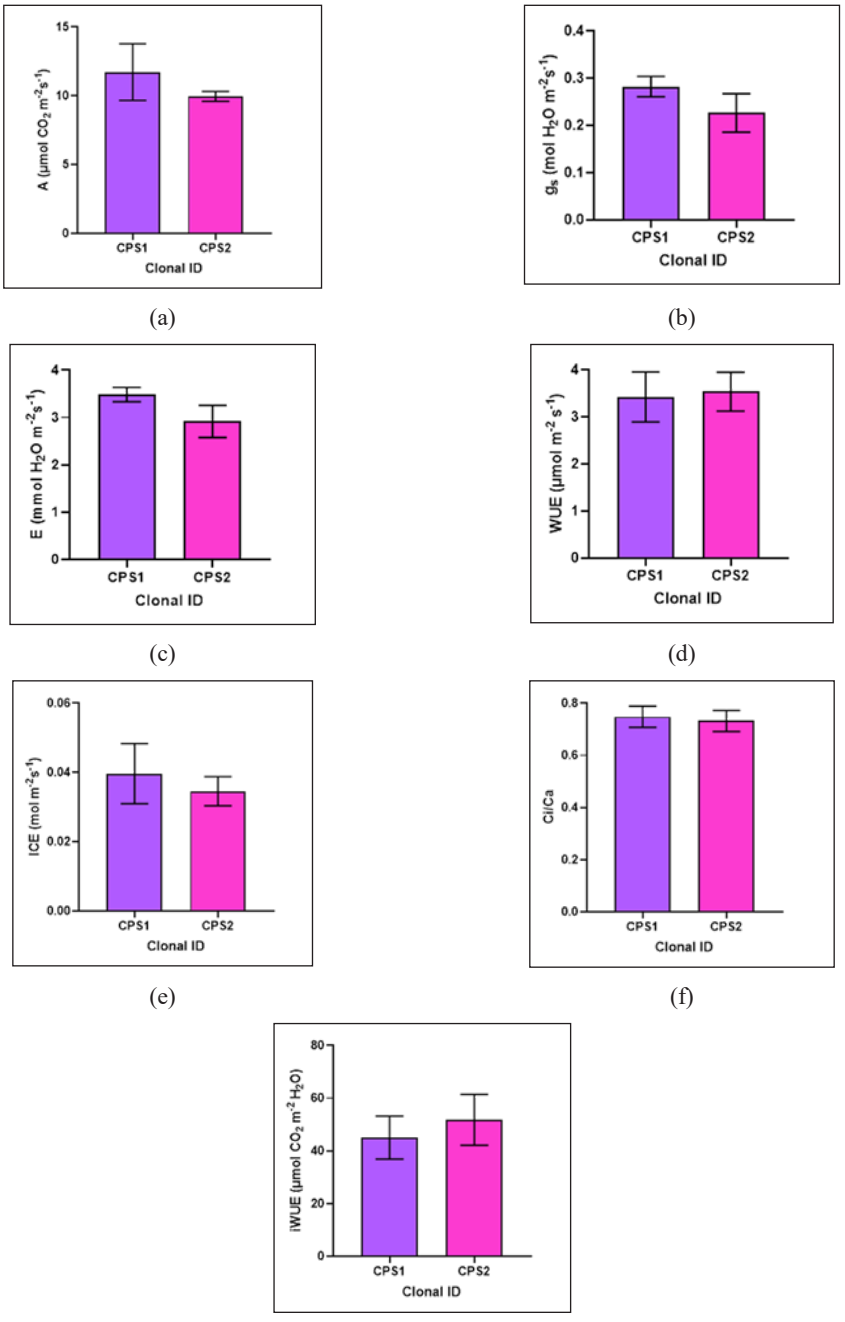

Figure 1. The overall effects of different nutrient stress treatments on the leaf gas exchange of the two clonal oil palm ramets. a) net photosynthetic rate, b) stomatal conductance, c) transpiration rate, d) instantaneous water use efficiency, e) instantaneous carboxylation efficiency, f) ratio of intercellular to ambient CO_2 concentrations, and g) intrinsic water-use efficiency. The bars represent the standard error of mean (SEM) (n =150)

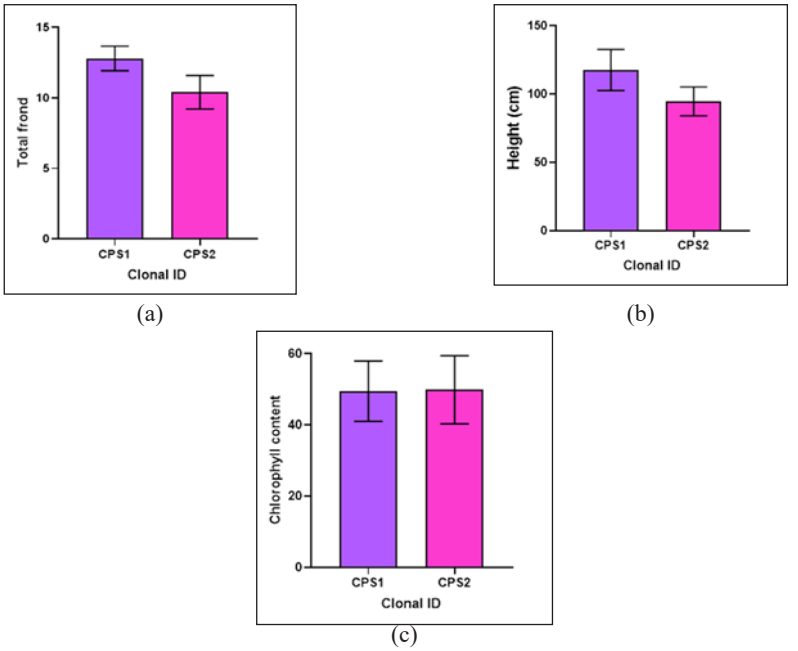

Figure 2. The overall effects of different nutrient stress treatments on vegetative measurement of the two clonal oil palm ramets. a) total frond, b) height of ramets, and c) chlorophyl content. The bars represent the standard error of mean (SEM) (n =150)

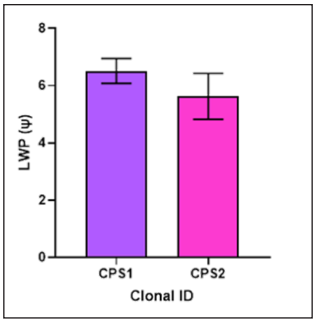

Figure 3. The overall effects of different nutrient stress treatments on leaf water potential of the two clonal oil palm ramets. The bars represent the standard error of mean (SEM) (n =150)

Maximum Efficiency of Photosystem II (F_v/F_m)

Based on Figure 4, both CPS2 and CPS1 demonstrate similar levels of F_v/F_m. The optimum F_v/F_m ratio for many plant species is around 0.79 to 0.84, lower values imply higher plant stress (Wu et al. 2023). The highest mean value result aligns with the research done by Xing & Wu (2014), in which they found that *Pharbitis nil*, a species of climber plant, exhibited a remarkable tolerance to P deficiency. This plant species was able to maintain

higher F_v/F_m values even under 0 mM P concentration, indicating that it was able to continue photosynthetic activity in the face of nutrient stress. This suggests that this species can successfully control its photosynthetic system during extended P stress.

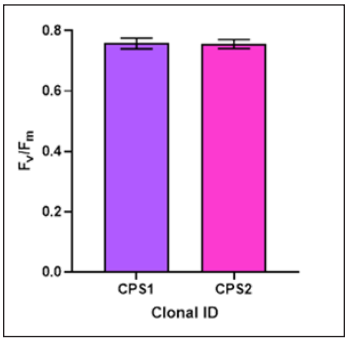

Figure 4. The overall effects of different nutrient stress treatments on the maximum efficiency of photosystem II of the two clonal oil palm ramets. The bars represent the standard error of mean (SEM) (n =150)

CONCLUSION

Overall, this study demonstrates the critical role of nutrient availability in shaping the physiological and morphological responses of oil palm clones, with notable differences between CPS1 and CPS2 under nutrient-deficient conditions. Both clones exhibited tolerance to severe stress through adjustments in growth and physiology; however, CPS1 demonstrated superior growth and photosynthetic performance, whereas CPS2 showed enhanced water use efficiency, indicating divergent adaptive strategies. Among the tested treatments, nitrogen fertiliser had the most pronounced positive effect. These findings provide a foundation for breeding programmes to enhance nutrient use efficiency and resilience, while future research should explore molecular mechanisms and long-term studies to guide sustainable, nutrient-efficient, high-performing oil palm varieties.

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Evaluation of *Pisifera* Male Parents for Producing High-yielding and Sustainable Oil Palm Planting Material

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ABSTRACT

During 2023-2024, oil palm (*Elaeis guineensis* Jacq.) accounted for more than 36% of global vegetable oil supply. The commercial cultivation of oil palm is based on crosses between *dura* (D) and *pisifera* (P) types, which require selection of superior parental lines. This study aimed to identify elite *pisifera* male parents for developing high-yielding D×P planting materials. Eleven *pisifera* male parents were evaluated based on their 38 D×P progenies established at one of the MPOB research stations located in Hulu Paka, Terengganu, Malaysia. Analysis of variance was used to analyse data on bunch quality components (year 2011–2018), bunch yield (year 2014–2017), and vegetative measurements (year 2015), where mean comparison was carried out thereafter. Most of the traits exhibited highly significant differences ($p<0.01$), indicating wide genetic variability among the AVROS, MPOB-Nigeria, and MPOB-Nigeria × United Plantations (UP) *pisifera* male parents. Among them, P6 (*pisifera* 0.337/552) recorded the highest fresh fruit bunch yield (FFB = 206.29 kg palm^{-1} year^{-1}) and oil yield (OY = 63.54 kg palm^{-1} year^{-1}), surpassing the D×P standard cross by 15% and 25%. Its progenies also exhibited a trunk height (HT) of 2.09 m, which was 26% lower than the standard cross. Molecular analysis confirmed P6 as homozygous *virescens*, ensuring a clear fruit colour change at ripening that enhances harvesting efficiency. In conclusion, P6 is a promising male parent for developing new commercial D×P planting materials, maximising productivity and harvesting efficiency.

Keywords: oil yield, *pisifera* male parent, pollen source, *virescens*

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INTRODUCTION

Among global oilseed crops, oil palm or *Elaeis guineensis* Jacq., ranks as the highest-yielding oil crop, consistently exceeding yields from soybean, rapeseed,

sunflower, cottonseed, and peanut (United States Department of Agriculture, 2024). It was estimated that global palm oil output would surpass 79.3 million metric tons in 2023-2024, accounting for over 36% of total vegetable oil production (OFI, 2023). Oil palm commercial seed production relies on the use of *dura* × *pisifera* (D×P) hybrids, with breeding programmes focus on development and improvement of both populations. Most oil palm breeding programmes employ Deli *dura* as the female parent (Rajanaidu et al., 2000). Meanwhile, Kushairi et al. (2011) reported that AVROS *pisifera* has been widely utilised for its strong combining ability with Deli *dura*.

Problem Statement

In Malaysia, oil palm populations exhibit limited genetic diversity due to the extensive use of Deli *dura* and AVROS *pisifera*, both of which originated from a few ancestral palms (Fadila et al., 2024). The limited genetic diversity within these populations has become the limiting factor in achieving further gains in yield potential. This study evaluated 11 *pisifera* male parents from three genetic backgrounds (AVROS, MPOB-Nigeria, and MPOB-Nigeria × UP), based on their 38 D×P progenies, in accordance with the selection criteria outline in the Malaysian Standard (MS 157:2017), titled Oil Palm Seeds for Commercial Planting (Fourth revision), to identify promising *pisifera* male parents as pollen sources for future commercial planting materials.

Research Questions

Table 1 shows the *pisifera* male parents' performance. MPOB-Nigeria *pisifera* P6 (0.337/552) recorded the highest FFB, primarily attributed to its significantly greater bunch number (BNO) compared to other *pisifera* male parents. Meanwhile, MPOB-Nigeria *pisifera* P4 (0.337/147) showed notable performance by recording the highest value for mesocarp to fruit ratio (M/F), as well as oil components such as oil to bunch (O/B) and oil to dry mesocarp (O/DM) ratios. While P6 recorded the highest total economic product (TEP) and oil yield (OY), it did not differ significantly from P4 for these two traits. Based on the Malaysian Standard MS 157: 2017, the *tenera* palms in the progeny test must produce at least 170 kg palm^{-1} year^{-1} of FFB, with O/B and kernel to bunch (K/B) ratios of 25% and 3%, respectively, and OY of 42.5 kg palm^{-1} year^{-1}. Both P4 and P6 meet these minimum requirements, with the exception of K/B. Molecular verification of P6 using the *SureSawit*™ *VIR* kit was conducted in 2024. The results confirmed that P6 is homozygous *virescens*, which ensure a clear fruit colour change at ripening, an important trait for harvesting accuracy and oil yield.

For vegetative traits, P6 also recorded the highest frond production (FP), significantly outperforming other *pisifera* male parents. None of the *pisifera* male parents fulfilled the compactness criteria (rachis length, RL < 5 m and HT < 1.80 m) for high-density planting

Table 1
Means of dura × pisifera *progenies based on* pisifera *male parents*

***Pisifera* male parent code**	**FFB (kg palm^{-1} year^{-1})**	**BNO (no. palm^{-1} year^{-1})**	**Average Bunch Weight (kg)**	**M/F (%)**	**Shell to Fruit (%)**	**O/DM (%)**	**Fruit to Bunch (%)**
P1 (0.292/1218)	199.66^{ab}	13.49^{c}	15.07^{c}	76.86^{f}	15.72^{a}	78.00^{f}	61.72^{e}
P2 (0.292/1250)	172.04^{ef}	13.09^{c}	13.39^{e}	80.28^{d}	13.48^{c}	79.93^{ab}	65.58^{bc}
P3 (0.292/353)	184.63^{cd}	13.04^{c}	14.50^{cd}	78.75^{e}	14.69^{b}	79.14^{cd}	62.15^{e}
P4 (0.337/147)	201.12^{ab}	14.65^{b}	13.93^{de}	86.43^{a}	8.55^{e}	80.41^{a}	65.70^{ab}
P5 (0.337/535)	165.39^{f}	10.44^{f}	16.23^{b}	81.69^{c}	11.67^{d}	75.02^{h}	64.03^{d}
P6 (0.337/552)	206.29^{a}	16.71^{a}	12.67^{f}	83.57^{b}	11.88^{d}	79.32^{bc}	65.93^{ab}
P7 (0.337/94)	189.51^{cd}	11.58^{de}	16.73^{b}	78.67^{e}	13.66^{c}	77.26^{g}	61.25^{e}
P8 (0.394/222)	191.61^{bc}	11.85^{d}	16.46^{b}	77.91^{ef}	13.32^{c}	78.56^{def}	65.19^{bcd}
P9 (0.394/234)	183.12^{cd}	10.23^{fg}	18.35^{a}	81.01^{cd}	11.42^{d}	79.82^{ab}	65.52^{bc}
P10 (0.394/24)	181.26^{de}	11.17^{e}	16.67^{b}	78.36^{e}	12.95^{c}	78.84^{cde}	66.95^{a}
P11 (0.394/456)	167.72^{f}	9.63^{g}	17.95^{a}	77.59^{ef}	13.13^{c}	78.40^{ef}	64.33^{cd}
D×P SC	179.23	9.12	20.01	77.03	13.08	80.68	69.48
Mean	188.78	12.69	15.44	80.20	12.77	78.76	64.56
Standard Error	1.09	0.09	0.09	0.18	0.12	0.08	0.16

***Pisifera* male parent code**	**O/B (%)**	**OY (kg palm^{-1} year^{-1})**	**TEP (kg palm^{-1} year^{-1})**	**FP (no. palm^{-1} year^{-1})**	**RL (m)**	**HT (m)**	**Leaf Area (m^{2})**
P1 (0.292/1218)	23.75^{f}	48.73^{cd}	54.06^{bc}	24.51^{de}	5.30^{e}	2.14^{d}	9.00^{bcd}
P2 (0.292/1250)	28.03^{c}	52.84^{b}	57.26^{b}	24.07^{ef}	5.62^{ab}	2.14^{d}	9.33^{ab}
P3 (0.292/353)	25.51^{e}	51.37^{bc}	56.05^{b}	25.64^{b}	5.47^{cd}	1.90^{e}	8.88^{cd}
P4 (0.337/147)	30.42^{a}	60.87^{a}	64.54^{a}	25.03^{cd}	5.61^{ab}	2.28^{c}	9.11^{bc}
P5 (0.337/535)	22.71^{g}	38.28^{f}	42.24^{e}	21.76^{g}	5.57^{bc}	1.98^{e}	9.67^{a}
P6 (0.337/552)	29.42^{b}	63.54^{a}	67.15^{a}	27.62^{a}	5.55^{bc}	2.09^{d}	8.23^{f}
P7 (0.337/94)	23.99^{f}	45.65^{de}	50.73^{cd}	23.62^{f}	5.68^{a}	2.33^{bc}	9.57^{a}
P8 (0.394/222)	25.34^{e}	50.99^{bc}	57.17^{b}	24.75^{d}	5.25^{e}	2.25^{c}	8.85^{cd}
P9 (0.394/234)	26.76^{d}	51.83^{bc}	57.20^{b}	23.84^{f}	5.47^{cd}	2.47^{a}	8.74^{de}
P10 (0.394/24)	26.67^{d}	49.62^{bc}	55.48^{b}	24.65^{d}	5.44^{d}	2.41^{ab}	9.34^{ab}
P11 (0.394/456)	24.98^{e}	43.46^{e}	49.00^{d}	25.51^{bc}	5.34^{e}	2.40^{ab}	8.39^{ef}
D×P SC	28.35	50.67	57.33	26.32	5.68	2.81	9.71
Mean	26.41	52.03	56.95	24.79	5.46	2.21	8.98
Standard Error	0.13	0.44	0.44	0.07	0.01	0.01	0.04

P1-P3: MPOB-Nigeria × United Plantations, P4-P7: MPOB-Nigeria, P8-P11: AVROS. Mean values sharing the same letter do not differ significantly at $p \leq 0.05$, as determined by Fisher's Least Significant Difference test

(Norziha et al., 2020). Nevertheless, all *pisifera* male parents exhibited lower HT than the D×P standard cross, which favourable for ease of harvesting.

CONCLUSION

Pisifera P6 has shown potential as a male parent for developing new commercial D×P planting materials due to its superior yield, low trunk height, and *virescens* fruit colour, traits that contribute to maximising oil yield per unit land area and efficient harvesting, thereby supporting the goals of sustainable oil palm production.

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The Characterisation of Colloidal Gas Aphrons Generated with Whey Protein Isolate Solution

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ABSTRACT

Colloidal gas aphrons (CGAs) represent a low-cost separation approach utilizing microbubbles covered by surfactant multilayers. This study aimed to determine the characteristics of CGA generated using 1, 3, and 5% whey protein isolate (WPI) solutions including gas hold-up, half-life, and aphron size. CGAs were generated by intensely stirring WPI solutions using a high-speed homogenizer at 14,000 RPM for 5 min. The results revealed that CGA generated with 5% WPI exhibited significantly higher gas hold-up (51.17%) and stability (half-life = 2192 s) compared to those generated with 3% WPI and 1% WPI, which achieved gas hold-up values of 48.75% and 39.31%, and half-lives of 1454 s and 1063 s, respectively. These findings highlight the superior stability of CGA produced with 5% WPI, attributed to its ability to maintain gas within the bubbles over time, preventing rapid coalescence or collapse. Additionally, CGA generated with 5% WPI also had the smallest aphron size (12.56 μm), compared to CGA generated with 3% WPI (15.49 μm), and 1% WPI (19.09 μm). Considering the stability characteristics observed for CGA generated with 5% WPI, this approach offers a promising natural alternative to reduce reliance on synthetic surfactants in CGA production.

Keywords: Colloidal gas aphrons, gas hold-up, half-life, stability, whey protein isolate

INTRODUCTION

Colloidal gas aphrons (CGAs) are microbubbles with increased interface area that can adsorb charged and/or hydrophobic molecules. The efficiency of target compounds separation or recovery in CGA systems significantly depends on the type of surfactant used. Whey protein isolate (WPI) is a natural surfactant that

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can be used to encapsulate perishable compounds. According to Cao and Xiong (2017), WPI is widely employed in protein-polyphenol conjugate formation due to its solubility, functionality, and diverse applications in the food industry.

MATERIALS AND METHODS

CGA Generation

Whey protein isolate (WPI) was purchased from LushProtein Ltd (Selangor, Malaysia). Whey protein isolate (WPI) solutions (1%, 3%, and 5%) were prepared by dispersing the powder in distilled water at 25°C for 2 h, then stored overnight at 4°C. The next day, 500 mL of each solution was stirred at 14,000 rpm for 5 min using a high-speed homogenizer before being pumped into the flotation column via a peristaltic pump.

Determination of Gas Hold-up

Colloidal gas aphrons (CGAs) were poured into a 1000 mL measuring cylinder. After one min, the volume of clear surfactant below the CGAs dispersion was recorded. Once the CGAs were fully collapsed, the liquid volume was measured as the initial surfactant volume ($V_{surfactant}$). The method for determining gas hold-up was adapted from Maidin et al. (2018) and calculated using Equation 1, where V_{CGA} is the CGA volume after 5 mins of intense stirring, and $V_{surfactant}$ is the surfactant volume before CGA generation.

$$\text{Gas hold-up, } \varepsilon = \frac{V_{CGA} - V_{Surfactant}}{V_{CGA}} \times 100\ (\%) \qquad [1]$$

Determination of CGAs Stability

Colloidal gas aphron (CGA) stability was assessed by measuring its half-life (τ), defined as the time required for half of the initial surfactant volume to drain. CGA was first generated by stirring a known surfactant volume at 14,000 rpm for 5 min. Then, the time was recorded immediately after the CGA was transferred into a measuring cylinder (Fuda et al., 2005).

Determination of Aphron Size (in diameter)

The aphron of CGA was analyzed using a light microscope (Jauregi & Varley, 1999).

Statistical Analysis

All analyses were conducted in triplicate (n = 3). Data were analysed using one-way ANOVA in Minitab Version 20, and results are presented as mean ± standard deviation. Different letters indicate statistically significant differences between values ($P < 0.05$).

RESULTS AND DISCUSSION

CGA Characterisation

Overall, it was observed that CGAs generated with 5% WPI solution exhibited higher gas hold-up and smaller aphron size (in diameter), which could maximize the interfacial area, and consequently, enhance their efficiency in the recovery process of target compounds. Moreover, their half-life was longer than that of CGAs generated with 1% and 3% WPI solutions (Table 1), as well as those produced using Tween 20 (10 mM), which had a half-life of 628.07 secs, as reported by Aimara et al. (2025). These results demonstrate that surfactant concentration appeared to significantly influence the characteristics and stability of the generated CGAs.

Table 1
The characteristics of colloidal gas aphrons generated using different concentration of whey protein isolates solution

WPI concentration	Gas hold-up (%)	Half-life (s)	Aphron size (μm)
WPI 1%	39.31 ± 2.85^{a}	1062.67 ± 96.69^{a}	19.09 ± 1.42^{a}
WPI 3%	48.75 ± 2.97^{a}	1453.67 ± 163.57^{b}	15.49 ± 0.37^{b}
WPI 5%	51.19 ± 2.52^{b}	2192.00 ± 49.51^{c}	12.56 ± 0.55^{c}

*WPI: Whey protein isolate. Values are expressed as mean ± SD (n = 3). Different letters in the same column denote significant difference $P < 0.05$.

CONCLUSION

This study confirmed that high concentration of 5% WPI improved gas retention, enhanced overall stability, and led to small aphron sizes, preventing rapid bubble collapse. These results suggest that WPI-based CGAs could serve as an effective and sustainable alternative to conventional synthetic surfactants in separation processes.

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Nutritional Composition and Protein Quality of the *Keropok Lekor* By-products

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ABSTRACT

The main by-products of processing *keropok lekor* include the fillet, frame, tail, head, and viscera. These materials, which are abundant and contain beneficial components like lipids, proteins, enzymes, and peptides, are frequently regarded as industrial waste. This study aims to ascertain the proximate nutritional content and essential amino acid (EAAs) profiles of four *keropok lekor* by-products in order to assess their potential for the production of value-added bio-products. *Keropok lekor* by-products, particularly the head, viscera, and frame, are high in both lipid and protein, ranging from 9.1 to 21.2% and 23.8 to 42.4%, respectively. Carbohydrate content can vary significantly, from 3.1% to 16.2%, depending on the specific by-product and processing method and the amount of ash and moisture contents did not differ significantly ($p > 0.05$). High quality of fish by-products protein based on EAAs content and the results showed that FBP4 (56.0%) and FBP3 (51.7%) predominantly consisted of EAAs. *Keropok lekor* by-products' high protein quality and nutritional composition indicate possibilities for biowaste-driven value-added products that illustrate the circular bio-economy ideas for sustainable development.

Keywords: Amino acid composition, *keropok lekor* by-products, nutritional composition, protein quality

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INTRODUCTION

Fish by-products are valuable raw materials that contain high-value components like collagen and derivates, enzymes, proteins, lipids, bioactive peptides, hydroxyapatite, vitamins, and minerals, that have numerous potential uses in a wide range of industries

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(Ideia et al., 2019). A significant amount of these fish by-products offers a rich and natural resource for the production or recovery of many valuable components, that can be valorised to create high-value products.

Problem Statement

Keropok lekor processing generates an enormous amount of solid fish by-products. The exploitation of fish by-products is consistent with the waste-to-wealth idea when waste is managed effectively.

Research Questions

What are the distinguishing attributes of fish by-products generated during the processing of *keropok lekor*?

In order to evaluate *keropok lekor* by-products' the suitability for the production of value-added bioproducts, this study attempts to determine their proximate nutritional content and essential amino acid (EAA) profiles.

MATERIALS AND METHODS

Collection and Processing of Fish By-products

Fish by-products were sourced from *keropok lekor* production sites located in Tumpat, Kelantan, and Kuala Terengganu, Terengganu. The obtained by-products were first thoroughly washed and then dried in an oven (UF450, Memmert, Germany) at 50°C for 48 hours. After drying, the materials were pulverised into a fine powder using a laboratory blender (model 7011HS, Waring, Japan).

Nutritional Composition

The proximate nutritional content of the various *keropok lekor* by-product samples was determined following the standards established by the Association of Official Analytical Chemists standards (Association of Official Analytical Chemists, 2005).

Determination of Essential Amino Acid Profile

Essential amino acids (EAAs) present in the fish by-products were identified and quantified using ultra-performance liquid chromatography (UPLC) with the ACQUITY UPLC system (Waters), following the method described by Danial et al. (2015). The results were expressed as grammes of amino acids per 100 grammes of the sample's dry weight.

RESULTS AND DISCUSSION

Types and Nutrient Composition of By-products from *Keropok Lekor* Production

We discovered that the ratio and composition of fish by-products produced by *keropok lekor* processing vary greatly based on the type of fish and the filleting technique used (Table 1). Low-value pelagic fish including round scad (*Decapterus macrosoma*) and sardine (*Sardinelle fimbriata*) are often utilised in the production of *keropok lekor.* The production of *keropok lekor* frequently uses low-value pelagic fishes because of their low bone content and tasty flavour (Rosidi et al., 2021).

Table 1
Types of fish, component parts, proportions, and nutritional composition of keropok lekor *fish by-products (FBPs)*

	Fish by-products			
FBPs	**FBP1**	**FBP2**	**FBP3**	**FBP4**
Species	*S. fimbriata*	*Decapterus species*	*D. macrosoma*	*D. macrosoma*
FBPs composition	Tail Fillet Frame Scales	Tail Fillet Frame	Tail Fillet Frame Fin Head Visceral	Head Viscera
Ratio	(2:2:4:2)	(2:2:6)	(1:1:2:1:2:2)	(5:5)
Nutritional composition (%)				
Protein	39.6 ± 0.5^{a}	40.4 ± 0.8^{a}	42.4 ± 0.18^{a}	23.8 ± 0.07^{b}
Lipid	9.1 ± 0.2^{b}	10.6 ± 0.2^{b}	19.3 ± 0.4^{a}	21.2 ± 0.4^{a}
Ash	33.4 ± 0.0^{a}	28.8 ± 0.0^{a}	26.9 ± 0.1^{a}	28.5 ± 0.0^{a}
Fibre	0.4 ± 0.0^{a}	0.8 ± 0.0^{a}	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}
Carbohydrate	4.2^{b}	5.4^{b}	3.1^{b}	16.2^{a}
Moisture content	13.3 ± 0.6^{a}	14.0 ± 0.5^{a}	8.0 ± 0.4^{a}	10.0 ± 0.3^{a}

Values are given in percentage [%] on a dry matter basis. Each value is presented as mean ± SD, and within a row, means that do not share the same superscript letter are significantly different at $p < 0.05$ as determined by one-way ANOVA.

Proteins derived from fish frames, tails, scales, heads, viscera, fins, and fillets (often containing some backbone remnants post-filleting) constituted between 23.8% and 42.4% of the total fish by-products. The highest percentage of protein content is observed in FBP3 (42.4%), followed by FBP2 (40.4%) and FBP1 (39.6%). Lipids range from 9.1 to 21.2%, mainly from the heads, frame, and viscera. As predicted, due to the high lipid content of heads and visceral, FBP3 (19.3%) and FBP4 (21.2%), which are made up of these parts, had higher lipid contents than other fish by-products ($p < 0.05$). All of the fish by-products

had ash and moisture concentrations that were not statistically different from one another ($p > 0.05$), and their carbohydrate content varied from 3.1% to 16.2% of the composition.

The protein and lipid contents of the *keropok lekor* by-products used in this study were comparable to those of earlier research on fish by-products of gilthead sea bream, meagre, and scad shortfin conducted by Ishak and Sarbon (2018) and Kandyliari et al. (2020). Generally, each part of fish by-products exhibits a different nutritional composition. According to Marti-Quijal et al. (2020), depending on the species and size, the frame and head normally have protein contents between 32.5 and 58.5% and fat contents between 15.2 and 40.7%, which makes them suitable for possible use as fish oil and fish protein hydrolysate (FPH).

Evaluation of Protein Characteristics in Fish Processing By-product

The excellent quality of protein contained in fish by-products is determined by the total EAA According to Table 2, the findings indicated that FBP1 (48.1%) and FBP2 (35.1%) were primarily non-essential amino acids (NEAAs), whereas FBP4 (56.0%) and FBP3 (51.7%) were primarily composed of EAAs.

For FBP1, FBP2, FBP3, and FBP4, Lys, Val, and Leu accounted for approximately 87.0%, 70.9%, 54.2%, and 60.1% of all fish by-products, respectively. The FBP1 and FBP2, however, lacked His and Trp and Trp was the least amount of EAA found in FBP3 and FBP4. Furthermore, in terms of NEAA composition, the most common NEAAs in FBP2 and FBP3 were Ala and Glu, which accounted for approximately 67.8% and 41.9% of all NEAAs, respectively. For FBP1 and FBP4, the most common NEAAs were Asp and Glu (54.5%) and Ala and Gly (57.8%), respectively. The presence of the head part, which has a higher concentration of connective tissue, is associated with the high Gly content in FBP3 (0.7 g/100 g) and FBP4 (1.1 g/100 g). Ser and Tyr were also the least abundant NEAAs in all fish by-products.

The nutritional components of EAAs demonstrated the existence of high-quality protein and were crucial in controlling metabolic processes to provide positive outcomes (Corsetti et al., 2024). A prior investigation of fish by-products from *D. maruadsi* revealed that Leu (10.1%), Val (6.7%), and Lys (13.9%) were the most prevalent EAAs, which was consistent with the findings of this work (Thiansilakul et al., 2007). Leu, Lys, and Val have been linked with human health improvement and disease prevention by regulating the immune system (Luo et al., 2014). Variations in the nutritional content and amino acid profiles of *keropok lekor* by-products can be attributed to differences in the fish species utilised, the specific composition of the by-products, and their proportional use. These factors collectively affect the types of proteins extracted, including myofibrillar, sarcoplasmic, and stromal proteins.

Table 2
Amino acid profiles of keropok lekor *fish by-products (FBPs)*

Amino acid (g/100 g)	FBP1	FBP2	FBP3	FBP4
EAA				
Histidine	0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	1.56 ± 0.2^{a}	1.33 ± 0.2^{a}
Threonine	0.14 ± 0.0^{b}	0.60 ± 0.4^{b}	2.76 ± 0.4^{a}	0.55 ± 0.2^{b}
Lysine	0.51 ± 0.1^{b}	1.15 ± 0.1^{b}	5.98 ± 0.5^{a}	6.76 ± 1.6^{a}
Methionine	0.05 ± 0.0^{b}	0.51 ± 0.3^{ab}	1.24 ± 0.1^{a}	1.06 ± 0.7^{a}
Valine	4.65 ± 0.5^{a}	3.27 ± 1.1^{a}	4.09 ± 0.1^{a}	5.11 ± 0.8^{b}
Isoleucine	0.51 ± 0.0^{b}	0.78 ± 0.5^{b}	3.17 ± 0.6^{a}	4.00 ± 0.3^{a}
Leucine	1.01 ± 0.3^{b}	2.44 ± 0.3^{b}	6.49 ± 2.4^{a}	4.69 ± 0.4^{a}
Phenylalanine	0.23 ± 0.1^{b}	0.87 ± 0.2^{b}	4.46 ± 0.6^{a}	3.17 ± 0.4^{a}
Tryptophan	0.00 ± 0.0^{b}	0.00 ± 0.0^{b}	0.64 ± 0.3^{a}	0.37 ± 0.1^{a}
Total EAA	7.08	9.66	30.50	27.09
NEAA				
Serine	0.00 ± 0.0^{b}	0.69 ± 0.4^{b}	1.43 ± 0.1^{a}	0.28 ± 0.0^{b}
Arginine	1.20 ± 0.3^{a}	0.87 ± 0.4^{b}	2.94 ± 0.2^{a}	0.69 ± 0.1^{b}
Glycine	0.64 ± 0.6^{b}	2.25 ± 0.3^{a}	3.27 ± 0.4^{a}	5.06 ± 0.6^{a}
Aspartic acid	2.39 ± 0.5^{ab}	1.01 ± 0.0^{ab}	3.31 ± 0.1^{a}	1.79 ± 0.1^{b}
Glutamic acid	1.75 ± 0.3^{b}	7.13 ± 2.2^{a}	6.26 ± 0.7^{a}	3.40 ± 0.2^{ab}
Alanine	1.20 ± 0.6^{b}	4.97 ± 0.2^{ab}	5.70 ± 0.3^{a}	7.22 ± 2.6^{a}
Proline	0.18 ± 0.2^{b}	0.64 ± 0.2^{b}	1.33 ± 0.1^{a}	2.39 ± 0.5^{a}
Tyrosine	0.14 ± 0.0^{b}	0.18 ± 0.0^{b}	4.19 ± 1.7^{a}	0.74 ± 0.1^{b}
Total NEAA	7.59	17.85	28.52	21.25
TAA	14.72	27.51	59.02	48.39

EAA: Essential amino acids; NEAA: Non-essential amino acids; TAA: Total amino acid. Each value is expressed as mean ± SD, and within each row, means that do not share the same superscript letter are significantly different at $p < 0.05$, as determined by one-way ANOVA

CONCLUSION

The nutritional makeup and quality of proteins in *keropok lekor* by-products show significant variation depending on the specific parts used, the composition of fish by-product blends, and the fish species involved. To identify the best substrate, several fish by-products (FBP1, FBP2, FBP3, and FBP4) were characterised and evaluated. FBP3 had greater nutritional composition and protein quality than the other FBPs. Therefore, further study on the FBP3 as the substrate for producing FPH is warranted.

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Unmasking Mycotoxins: Effects of Sodium Hypochlorite Sterilisation and Autoclaving on Zearalenone Contamination in Grain Corn

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ABSTRACT

This study investigates the effects of sodium hypochlorite (NaOCl) and autoclaving on zearalenone (ZEA), a *Fusarium*-derived mycotoxin in grain corn, to optimize sample preparation for biosensor analysis. ZEA often exists in bound forms, complicating detection. Three grain corn samples—freshly harvested (IC3924), stored for one month (C1M), and two months (C2M) were treated with 30% NaOCl alone or followed by autoclaving. A phosphate-buffered saline (0.01 M) served as a control, resulting in 10 samples. ZEA levels were measured using a commercial enzyme-linked immunosorbent assay (ELISA) kit. Untreated C1M and IC3924 showed no detectable ZEA, while untreated C2M contained 4.89 ppb. Sodium hypochlorite (NaOCl) alone eliminated detectable ZEA, but autoclaving increased its levels, likely due to bound toxin release under heat and pressure. This suggests autoclaving may artificially elevate ZEA detection, potentially affecting food safety assessments. In contrast, NaOCl treatment alone effectively removes detectable ZEA. These findings underscore the importance of optimizing sample preparation for accurate mycotoxin detection in grain corn, particularly for biosensor applications.

Keywords: Autoclaving, grain corn, masked mycotoxins, sterilisation, zearalenone

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INTRODUCTION

Mycotoxin contamination in agricultural commodities poses significant challenges to food safety, public health, and economic stability. Zearalenone (ZEA), a *Fusarium*-derived mycotoxin, is particularly

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concerning due to its estrogenic properties and associated health risks in humans and livestock. Grain corn, a staple in food and feed, is highly vulnerable to *Fusarium* contamination under warm, humid conditions (Qu et al., 2024). ZEA mimics estrogen, causing reproductive disorders in livestock and raising concerns about endocrine disruption in humans, necessitating strict regulatory limits (Berthiller et al., 2009). Additionally, ZEA can exist in free or bound (masked) forms, complicating detection and potentially underestimating contamination levels.

Sterilisation methods like autoclaving and chemical treatments may alter ZEA's structure, degrade it, or release bound forms, affecting quantification (Ibáñez-Vea et al., 2012). However, systematic studies on their impact on ZEA derivatives are limited. This study evaluates the effects of autoclaving and sodium hypochlorite (NaOCl) on ZEA in grain corn, aims to establish an optimized sample preparation method for electrochemical biosensor-based detection. By analyzing ZEA levels in freshly harvested and stored corn under different treatments, this research provides insights into pre-analytical processing for mycotoxin detection. The findings will advance biosensing platforms, improving food safety monitoring and regulatory compliance in agriculture.

MATERIALS AND METHODS

Harvested grain corn samples were obtained from the Industrial Crop Research Centre, MARDI, Malaysia. The samples used in this study were of the P4546 variety from DuPont, Thailand, cultivated at MARDI Seberang Perai, Penang, Malaysia. Three sample batches were tested: freshly harvested grain corn (IC3924), grain corn stored for one month (C1M), and grain corn stored for two months (C2M). Each batch was subjected to either 30% sodium hypochlorite (NaOCl) treatment alone or NaOCl treatment followed by autoclaving at 121°C for 15 min. In total, 10 samples were analyzed, including a phosphate-buffered saline (0.01 M) solution as a negative control. A commercial RIDASCREEN® FAST Zearalenone kit (R5502) was utilized in this study to quantify the amount of zearalenone mycotoxin in the samples.

RESULTS AND DISCUSSION

As presented in Table 1, results indicate that untreated C1M and IC3924 samples had no detectable zearalenone, while untreated C2M contained 4.89 ppb, suggesting prolonged storage may increase contamination risk. Autoclaved samples exhibited higher zearalenone levels, with IC3924-CA (1.29 ppb), C1M-CA (2.86 ppb), and C2M-CA (6.42 ppb), This rise is due to the release of bound or masked zearalenone during autoclaving, a phenomenon documented in mycotoxin research (Gratz, 2017). Under condition of high heat and pressure, bound mycotoxinsconjugated with grain cellular components may be liberated resulting in increasing measured concentrations. Autoclaving may also enhance extraction efficiency, potentially resulting in artificially elevated detection levels.

Table 1
Zearalenone concentration (ppb) in grain corn samples under different sterilisation treatments

Samples	Treatment	Zearalenone (ppb)
0.01 M PBS	(negative control)	Not detected
IC3924	Untreated	Not detected
C1M	Untreated	Not detected
C2M	Untreated	4.89
IC3924-C	Sterile with 30% NaOCl	Not detected
C1M-C	Sterile with 30% NaOCl	Not detected
C2M-C	Sterile with 30% NaOCl	Not detected
IC3924-CA	30% NaOCl and autoclaved	1.29
C1M-CA	30% NaOCl and autoclaved	2.86
C2M-CA	30% NaOCl and autoclaved	6.42

While heat treatment can degrade certain mycotoxins, *Fusarium*-derived toxins like zearalenone and deoxynivalenol (DON) may form new derivatives during thermal processing, potentially altering toxicity or enhancing detectability (Karlovsky et al., 2016). This underscores the complex effect of autoclaving on mycotoxin quantification and food safety assessments. In contrast, samples treated with NaOCl alone showed no detectable zearalenone, indicating that sodium hypochlorite effectively neutralizes or removes the mycotoxin before further processing. This finding aligns with previous studies on the efficacy of chlorine-based disinfectants in reducing mycotoxin contamination in food matrices (Nešić et al., 2023).

These findings highlight the importance of selecting appropriate matrix treatment methods for preparing sterile materials in zearalenone standard curve development, ensuring accuracy in biosensor applications.

CONCLUSION

The study emphasizes the need to consider the effects of storage, chemical treatment, and autoclaving on mycotoxin detection to avoid misinterpretation of analytical results. These findings indicate that autoclaving releases bound zearalenone, increasing detection levels, while sodium hypochlorite (NaOCl) treatment effectively eliminates detectable contamination. For future zearalenone biosensor development, NaOCl treatment will be employed, with C1M selected as the primary sample type to ensure controlled and accurate mycotoxin assessment.

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Effect of Indoor Hydroponic Technique on the Growth and Development of High-quality Tissue Culture Plantlets of *Labisia pumila* Clone FaFaF01

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ABSTRACT

Labisia pumila, known as Kacip Fatimah, is a valuable medicinal herb traditionally used to support women's health and explored for urban cultivation. This study evaluates the hydroponic performance of an elite clone of *L. pumila* (FaFaF01), developed through over a decade of research and development by the Forest Research Institute Malaysia (FRIM). This clone cultivated for the production of raw materials and commercial herbal products. The experiment was conducted at the Department of Physics, Faculty of Science, University of Malaya (UM), as part of a collaborative research project between FRIM and UM. The study aimed to assess the growth performance and phytochemical yield of *L. pumila* under controlled indoor hydroponic which beyond conventional field planting. Plantlets were grown in a hydroponic system under low-temperature, controlled humidity, and LED lighting conditions, promoting energy efficiency. Vegetative growth was monitored over 48 weeks, and both biomass and bioactive compounds were evaluated. The elite clone showed robust growth performance in both front and rear planting positions, with total phenolic content (TPC) recorded at 3335 ± 23.1 mg GAE/100g dry weight (front) and 2258 ± 34.0 mg GAE/100g dry weight (rear), indicating very high and high phenolic levels, respectively. The findings demonstrate that *L. pumila* performs well in indoor hydroponic systems while retaining its medicinal value. Therefore, it is recommended

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as a versatile plant for vertical landscaping, indoor decoration, and green interior applications. Its integration into indoor environments may contribute to lowering ambient temperatures and enhancing environmental quality in institutional settings.

Keywords: Growth performance, indoor cultivation, medicinal herb, phenolic content, versatile plant

INTRODUCTION

Hydroponics enables rapid growth without soil, using substrates such as coco peat, jiffy pellets, rockwool, perlite, or clay pellets. Zig-zag vertical systems improve nutrient delivery, reduce water use, and limit pests. *L. pumila*, a Malaysian medicinal herb for women's health, shows antioxidant, antimicrobial, anticancer, anti-inflammatory, and phytoestrogenic properties (Calapai et al., 2007; Karimi et al., 2011), but is sensitive to light, moisture, and temperature. To meet raw material demand, the FRIM developed the elite clone FaFaF01 with stable growth and high phytochemical yield. In this study, FaFaF01 was cultivated indoors (28 °C, 975 lux, 85% humidity) for 48 weeks, confirming strong vegetative growth, biomass stability, and high total phenolic content (TPC) under controlled hydroponics (Resh, 2012).

Problem Statement

Few studies have applied hydroponics to *L. pumila* indoors. Prior research focussed on in vitro propagation (Rasdi et al., 2013), CO_2 enrichment (Ibrahim et al., 2011), and nutrient manipulation (Ismail et al., 2011). Effects on growth and metabolite retention under indoor hydroponics remain limited.

Research Questions

Few studies have applied hydroponics to *L. pumila* indoors. Prior research focussed on in vitro propagation (Rasdi et al., 2013), CO_2 enrichment (Ibrahim et al., 2011), and nutrient manipulation (Ismail et al., 2011). Effects on growth and metabolite retention under indoor hydroponics remain limited.

MATERIALS AND METHODS

Construction Hydroponic System

The hydroponic system was constructed using the Nutrient Film Technique (NFT), consisting of five stacked layers for planting as illustrated in Figure 1. Each layer contained eight individual planting pots arranged in a row.

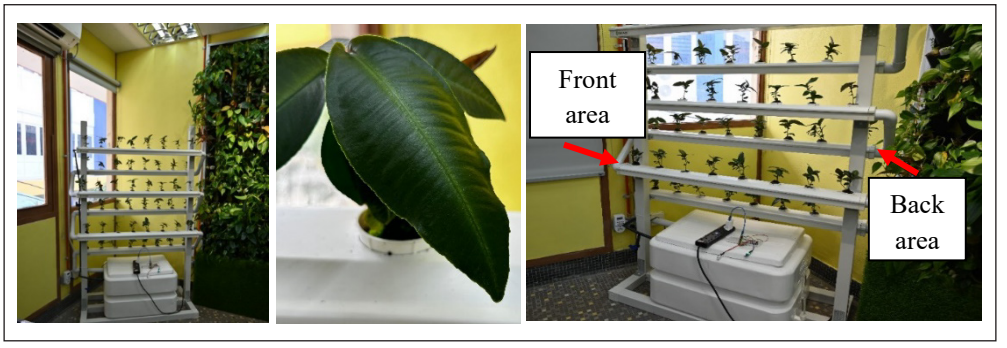

Figure 1. Experimental design of hydroponic system was arranged into two distinct position which front and back within the indoor grow area

RESULTS AND DISCUSSION

Growth of *L. pumila* showed no significant variation across planting positions as shown in Table 1, with only slight leaf size trends likely due to microclimatic effects common in vertical systems (Resh, 2012).

Table 1
One-way ANOVA assessing growth across four time points (Month 3, 6, 9, and 12) indicated no significant variation over time ($p > 0.05$)

Growth Parameter	F-statistic	p-value	Significance
Height	1.81	0.305	Not significant
Number of Leaves	1.25	0.403	Not significant
Leaf Length	1.88	0.296	Not significant
Leaf Width	5.52	0.099	Approaching significance
Collar Diameter	1.43	0.367	Not significant

The stable growth of clone FaFaF01 underscores its versatility as an indoor plant. *L. pumila*, a rainforest understorey herb adapted to low light combines compact form with high medicinal value, making it ideal for vertical gardens and interior landscaping. Indoor plants also reduce temperature and improve air quality (Lohr et al., 1996) positioning FaFaF01 as both a functional medicinal resource and sustainable green interior species.

As shown in Figure 2, the dried biomass of *Labisia pumila* clone showed no significant difference between front and back positions under indoor hydroponics ($p > 0.05$), indicating uniform growth conditions. Similar findings in controlled-environment and IoT-based hydroponic systems (Blunk et al., 2023; Gao et al., 2025; Sowmya et al., 2024) support the species' reliability for compact hydroponic, greening, and ornamental applications.

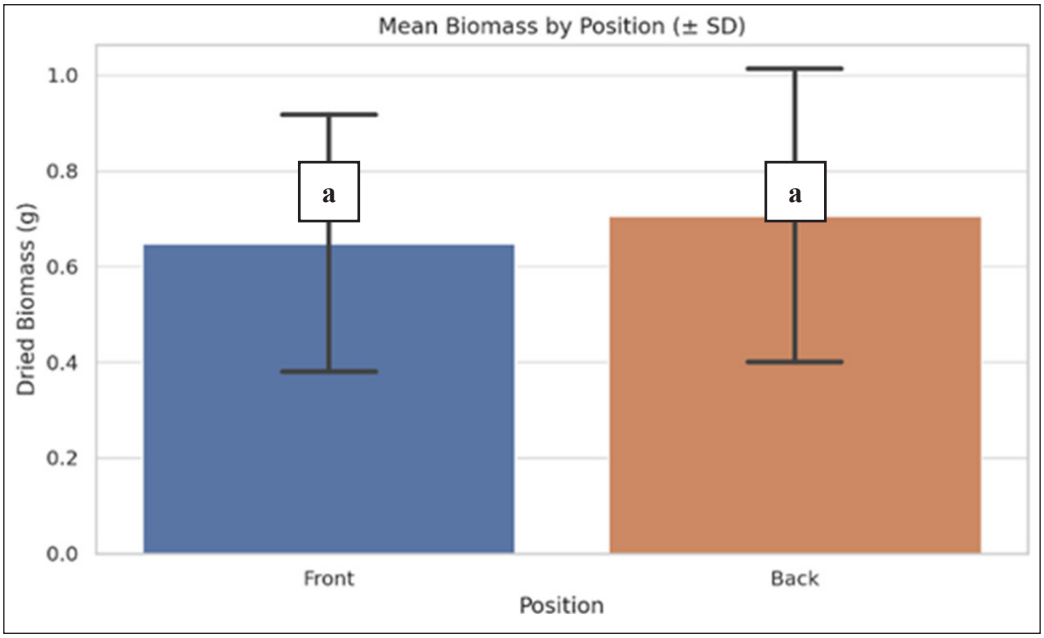

Figure 2. Dried biomass of *Labisia pumila* planted in an indoor hydroponic system with two planting positions

As shown in Figure 3, *Labisia pumila* clone FaFaF01 showed strong bioactive potential which high total phenolic content of 3335 ± 23.1 mg GAE/100 g DW. Growth media strongly influenced phenolic accumulation (Farah Fazwa et al., 2020), while controlled environments enhanced secondary metabolite production (Ismail et al., 2011). Overall, FaFaF01 is valuable for ornamental use, antioxidant-rich applications and its commercial value for green interiors.

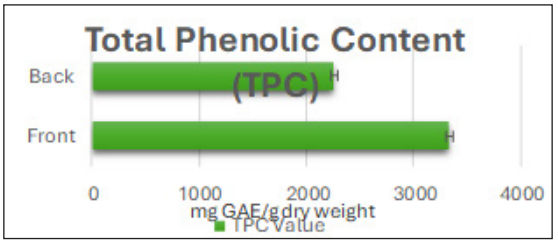

Figure 3. Total phenolic compound of *Labisia pumila* planted in an indoor hydroponic system with two planting positions

CONCLUSION

This study found that *L. pumila* clone FaFaF01 maintained stable growth, biomass, and high TPC under indoor hydroponics. Plant traits were uniform, with only minor leaf width

variation. High TPC confirmed strong medicinal value, while compact form and low-light tolerance highlight potential for vertical gardens, green walls, and indoor use. FaFaF01 is thus a promising dual-purpose plant for ornamental greening and sustainable bioactive production.

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Malaysia's First National Survey of Neonicotinoids in Farmed Shrimp: A Risk Assessment for Food Safety and Trade

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ABSTRACT

Neonicotinoid insecticides, widely used in agriculture, may infiltrate aquaculture systems, raising food safety and trade concerns. This study presents the first nationwide surveillance of eight neonicotinoids in Pacific white shrimp (*Litopenaeus vannamei*) from Malaysian farms. A total of 174 samples from 13 states were analysed using an optimised QuEChERS (quick, easy, cheap, effective, rugged and safe) extraction coupled with liquid chromatography-tandem mass spectrometry (LC-MS/MS). Residues were detected in 22% of samples, mainly flupyradifurone (6.3%), thiamethoxam (5.2%), and imidacloprid (4.0%), all below the limit of quantification (LOQ). Detection was higher in East Malaysia (25.0%) than Peninsular Malaysia (19.8%), with 60.5% of positives occurring during the dry season (Southwest monsoon). Seasonal influence was evident, as most detections of clothianidin (100%), thiacloprid (100%), thiamethoxam (78%), sulfoxaflor (60%), and imidacloprid (57%) were recorded during this period, likely due to runoff and reduced dilution. Dietary exposure estimates indicated a maximum intake of 1.48×10^{-6} mg/kg bw/day (<0.01% of Acceptable Daily Intakes, ADIs), demonstrating negligible risk even under worst-case assumptions. These findings suggest minimal consumer health risks but highlight low-level contamination from agricultural sources. Regular seasonal monitoring is recommended to safeguard food safety, the environment, and compliance with international trade standards.

Keywords: Neonicotinoids, Malaysia, pesticide residues, risk assessment, shrimp

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INTRODUCTION

Shrimp aquaculture is an important sector in Malaysia, with Pacific white shrimp (*Litopenaeus vannamei*) contributing ~50,000 tonnes annually and MYR 1.3 billion in exports (Department of Fisheries Malaysia, 2020). Processed shrimp are exported to markets including China, Taiwan, and Singapore, while raw shrimp serve as a local protein source. Data on neonicotinoid contamination remain limited, hindering dietary exposure assessment (Waras et al., 2024). These systemic insecticides are widely used in Asia (Food and Agriculture Organisation, 2024), and up to 80% may enter ecosystems via runoff or leaching, contaminating aquaculture (El-Nahhal & El-Nahhal, 2021). In Malaysia, maximum residue limits (MRLs) are enforced for plant-based foods, but animal products rely on a default limit of 0.01 mg/kg under the Food Act 1983 and Food Regulations 1985 (https://hq.moh.gov.my/fsq). Although the tropical climate and intensive agriculture increase contamination risks, surveillance data remain scarce, limiting effective food safety management.

PROBLEM STATEMENT

Malaysia's shrimp aquaculture sector lacks data on neonicotinoid contamination in *L.vannamei*, a major species for consumption and exports. With only a default residue limit and risks from intensive pesticide use and monsoon runoff, this study provides the first nationwide surveillance of eight neonicotinoids in farmed shrimp and assesses dietary exposure in Malaysian consumers.

RESEARCH QUESTIONS

This study examined; (i) the occurrence and concentrations of neonicotinoid residues in farmed *L.vannamei* across Malaysia's aquaculture regions, (ii) the influence of Southwest and Northeast monsoons on residue detection, (iii) the potential dietary exposure risk to Malaysian adults.

MATERIALS AND METHODS

A total of 174 Pacific white shrimp *(Litopenaeus vannamei)* samples were collected from farms across 13 Malaysian states during 2023 – 2024, covering both Northeast (October to March, rainy season) and Southwest (April to September, dry season) monsoon. Samples were analysed by LC-MS/MS after extraction with a modified QuEChERS method using certified standards (Omar et al., 2025). For health risk assessment, chronic dietary exposure was estimated using shrimp consumption and body weight data from Ahmad et al. (2016). Concentrations below the LOQ were replaced with half-LOQ values (World Health Organization, 2009), based on LOQs from Omar et al. (2025). Estimated Daily

Intakes (EDIs, mg/kg bw/day) were compared with the Codex Acceptable Daily Intake (ADI) (Codex Alimentarius Commission, 2025).

RESULTS AND DISCUSSION

Residues were detected in 38 of 174 shrimp samples (22.0%), mainly flupyradifurone (11/174, 6.3%), thiamethoxam (9/174, 5.2%), and imidacloprid (7/174, 4.0%), with all concentrations below the limit of quantification (LOQ). Flupyradifurone (11/38) was the most frequently detected, consistent with its widespread agricultural use (Food and Agriculture Organisation, 2024). Detection was higher in East Malaysia (17/68, 25.0%) than in Peninsular Malaysia (21/106, 19.8%), with most positives (23/38, 60.5%) during the Southwest monsoon. Several compounds, including clothianidin, thiacloprid, thiamethoxam, sulfoxaflor, and imidacloprid, were predominantly found in the dry season, reflecting monsoon-driven runoff and hydrological influence (El-Nahhal & El-Nahhal, 2021).

Dietary exposure assessment showed that Estimated Daily Intakes (EDIs) for all neonicotinoids were far below their Acceptable Daily Intake (ADI). Values ranged between 7.10×10^{-7} (thiacloprid) and 1.48×10^{-6} (sulfoxaflor) mg/kg bw/day, all <0.01% of ADI (Table 1). Clothianidin (36%) and thiacloprid (26%) contributed the most due to stricter ADIs, yet cumulative exposure remained well under 100%. These results confirm negligible dietary risk while underscoring the need to prioritise compounds with higher toxicological relevance in monitoring (Waras et al., 2024).

Table 1
Estimated Daily Intake (EDI) and chronic dietary exposure risk (%ADI) of neonicotinoid residues in Pacific white shrimp (worst-case scenario)

Compound	EDI (mg/kg bw/day)	%ADI	Contribution (%)
Thiamethoxam	7.74×10^{-7}	9.67×10^{-4}	3%
Flonicamid	1.23×10^{-6}	1.75×10^{-3}	6%
Clothianidin	9.68×10^{-7}	9.68×10^{-3}	36%
Imidacloprid	1.10×10^{-6}	1.83×10^{-3}	7%
Acetamiprid	1.10×10^{-6}	1.57×10^{-3}	6%
Flupyradifurone	1.03×10^{-6}	1.29×10^{-3}	5%
Thiacloprid	7.10×10^{-7}	7.10×10^{-3}	26%
Sulfoxaflor	1.48×10^{-6}	2.97×10^{-3}	11%

CONCLUSION

Detection of neonicotinoids in *L. vannamei* indicates agricultural runoff, with regional and seasonal patterns reflecting environmental influence. Although overall dietary exposure was negligible, higher %ADI contributions from clothianidin and thiacloprid highlight

the need for stricter monitoring and updated regulations to safeguard aquaculture food safety in Malaysia.

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Effect of Different Types and Concentration of Rooting Hormones on High Yielding Genotype of *Strobilanthes crispus* Stem Cuttings

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ABSTRACT

Strobilanthes crispus is a medicinal shrub recognised for its rich composition of bioactive compounds, particularly phenolics and flavonoids, which are responsible for its antioxidant, anti-inflammatory, and antidiabetic properties. The demand for consistent, high-quality planting materials necessitates the development of an efficient propagation method, particularly through stem cuttings. The propagation efficiency of high yielding *S. crispus* genotypes through stem cuttings was evaluated using three auxin types namely Indole -3-Acetic Acid (IAA), Indole -3- Butyric Acid (IBA), and 1-Naphthalene Acetic Acid (NAA) at concentrations of 0, 0.5, and 2.0 mg/L each. A significant interaction between hormone type and concentration was observed for the number of primary roots, indicating that these traits are highly responsive to specific hormone-concentration combinations. Indole-3-butyric acid (IBA) at 0.5 mg/L resulted in the greatest root number and length, confirming its effectiveness in promoting adventitious root formation. No significant interaction effects were found for survival rate, mean root length, sprouting percentage and number of shoots, suggesting these parameters were influenced more by individual hormone or concentration effects. The results emphasize the importance of selecting appropriate auxins and concentrations based on propagation objectives. This hormone-specific strategy offers a practical and accessible approach to improving vegetative

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propagation protocols for *S. crispu*s, with implications for both conservation and commercial cultivation.

Keywords: Auxin, medicinal plant, plant propagation, rooting performance, stem cuttings

INTRODUCTION

Strobilanthes crispus is a perennial shrub belonging the Acanthaceae family that has long played an important role in traditional medicine across Malaysia and nearby regions. In Malaysia, *S. crispus* is locally known as 'pecah beling' or 'pecah kaca' in Malay, and 'Hei Mian Jiang Jun', translated as "black-faced general" in Chinese dialects, reflecting its recognition across different cultural traditions. This plant naturally grows along riverbanks and in abandoned fields, reaching heights of up to 1.5 metres. It's easy to identify with its dark green, slightly rough leaves and bright yellow flowers that form in dense, spike-like clusters. Originally found in Madagascar and the Malay Archipelago (Burkill & Birtwistle, 2002), *S. crispus* continues to attract attention for its medicinal uses, especially as both local communities and global industries turn back to nature for remedies and wellness solutions.

Traditionally, people consume *S. crispus* as a tea or water decoction, believing it to help with kidney stones (Ernawati et al., 2018), diabetes, and general health maintenance (Permatasari et al., 2025). These traditional beliefs are increasingly supported by modern science. Over the years, researchers have discovered a wealth of phytochemicals in the plant that explain its healing potential. Scientific studies have shown it to possess powerful antioxidant (Ismail et al., 2000; Qader et al., 2011), antimicrobial (Hanafiah et al., 2023; Mahyantika et al., 2025), antidiabetic (Fadzelly et al., 2006; Norfarizan et al., 2009; Sukendi et al., 2025), anti-ulcer (Al-Henhena et al., 2011), and even wound-healing effects (Norfarizan et al., 2009). One of the most promising discoveries about *S. crispus* is its potential role in cancer therapy. A review by Ng et al. (2021) highlighted that extracts from this plant demonstrated significant anticancer effects in both laboratory experiments and animal models, particularly showing strong activity against breast, liver, and colon cancer cells (Hakimi et al., 2025).

Given its remarkable medicinal potential, *S. crispus* has become increasingly sought after, not only within traditional medicine but also in the herbal and pharmaceutical industries. However, the majority of raw material currently available is still sourced through wild collection or imported from unverified origins. This reliance on inconsistent sources raises major concerns regarding product quality, genetic uniformity, and long-term sustainability (Zurinawati, 2004). To address these challenges and ensure a stable, high-quality supply chain, there is a demanding need to establish a reliable system for producing high quality planting material. In line with this, the Forest Research Institute Malaysia (FRIM) has initiated a structured breeding programme focused on identifying, selecting,

and propagating *S. crispus* superior genotypes were collected from diverse populations across the Peninsular Malaysia.

One of the most efficient ways to multiply selected plants while keeping their valuable traits intact is through stem cuttings. However, success with this method can vary depending on the plant's genetic makeup and whether or not rooting hormones are used. Compounds like IBA, NAA, and IAA are known to stimulate root formation and improve survival rates of cuttings (Saini & Anmol, 2024), but their effectiveness often depends on both dosage and the plant genotype. The present study aims to evaluate the effects of different types and concentrations of auxins on the propagation efficiency of high yielding *S. crispus* genotypes through stem cuttings.

MATERIALS AND METHODS

Study Site and Materials

The experiment of stem cutting conducted at the nursery of the Tree and Herb Improvement Branch, Forest Research Institute Malaysia (FRIM). The propagation area was maintained under a high-humidity environment (70–80% RH) and 50% shade, with an intermittent misting system operating for 10 minutes, daily three times at 8.00 am, 12.00 pm and 4.00 pm. A high-yielding *S. crispus* genotype, BPB23, known for its high verbascoside content, was used as the planting material. Stem cuttings approximately 10 cm long, each containing two nodes, were prepared. Leaves were removed to minimize transpiration and prevent desiccation prior to root initiation. The cuttings were then immersed in hormone solution for one minute and immediately planted in 100% coarse river sand as the rooting medium.

Experimental Design

A Randomised Complete Block Design (RCBD) with three replications was used to carry out the experiment. Treatments consisted of three auxin types, i) IAA, ii) IBA and iii) NAA, each applied at three concentrations (0, 0.5, and 2 mg/L) on stem cuttings of the high yielding *S. crispus* genotype. The concentrations of auxins selected for this study were chosen based on effective ranges reported in previous studies on vegetative propagation of medicinal and woody plants. The 0 mg/L treatment served as a control to assess the baseline rooting capacity without hormonal influence.

Data Collection

Data were collected after eight weeks to ensure adequate root development and evaluate the influence of various treatments on the rooting performance, following the procedure described by Ghimire et al. (2022). Parameters recorded included survival rate (%), number of primary roots, mean root length (cm), sprouting percentage, and number of shoots. To

analyse the data two-way ANOVA (analysis of variance) was used, and DMRT (Duncan's Multiple Range Test) was used to evaluate the statistically significant (at $p < 0.05$) among the treatments using the SPSS 25.0 software.

RESULTS AND DISCUSSION

A significant interaction was observed between the type of plant growth regulator (IAA, IBA, and NAA) and their respective concentrations (0, 0.5, and 2.0 mg/L) on the number of primary roots in stem cuttings of the high-yielding *S. crispus* genotype ($p \leq 0.05$; Table 1). In contrast, no significant interaction effects were observed between auxin type and the concentration for survival rate, mean root length, sprouting percentage and number of shoots (Table 1). This suggests that these parameters were more likely influenced by the main effects of either hormone or concentration alone, rather than a combination of the two. Therefore, the discussion will focus primarily on the significant interaction effects observed for the number of roots as this variable is more responsive to the combined influence of hormone treatment and concentration in high yielding *S. crispus* genotype.

Table 1
The main and interaction effects of different hormone types and concentrations on the survival rate, number of roots, means root length, sprouting percentage and number of shoots of high yielding genotype Strobilanthes cripsus *stem cuttings*

Factor	Survival rate (%)	Number of primary roots	Means root length (cm)	Sprouting percentage (%)	Number of shoots
Hormone (H)					
IAA	86.53a	7.23b	5.16b	86.15a	9.96a
IBA	86.53a	8.52a	5.79a	86.53a	9.68a
NAA	88.07a	7.18b	5.27b	88.07a	9.98a
Concentration (mg/L) (C)					
0	83.84a	8.08a	5.21a	83.07b	8.92c
0.5	86.15a	7.43b	5.24a	85.90ab	9.86b
2.0	87.95a	7.61ab	5.57a	87.95a	10.59a
Significance					
Hormone (H)	ns	*	*	ns	ns
Concentration (C)	ns	*	ns	*	*
H x C	ns	*	ns	ns	ns

*significant at $p \leq 0.05$

The effect of auxin concentration on root induction varied significantly depending on the hormone type used, as demonstrated by the hormone × concentration interaction (Figure 1). Among all treatment combinations, 0.5 mg/L IBA produced the highest number

of primary roots, significantly outperforming other hormone *x* concentration combinations. Notably, IBA consistently induced superior rooting performance at all tested concentrations, with 0.5 mg/L yielding the highest root number, followed closely by 2.0 mg/L. This trend is consistent with previous findings by Nabilah et al. (2023), who stated that generic *S. cripus*s genotype stem cuttings produced the most roots when treated with 0.5 mg/L IBA.

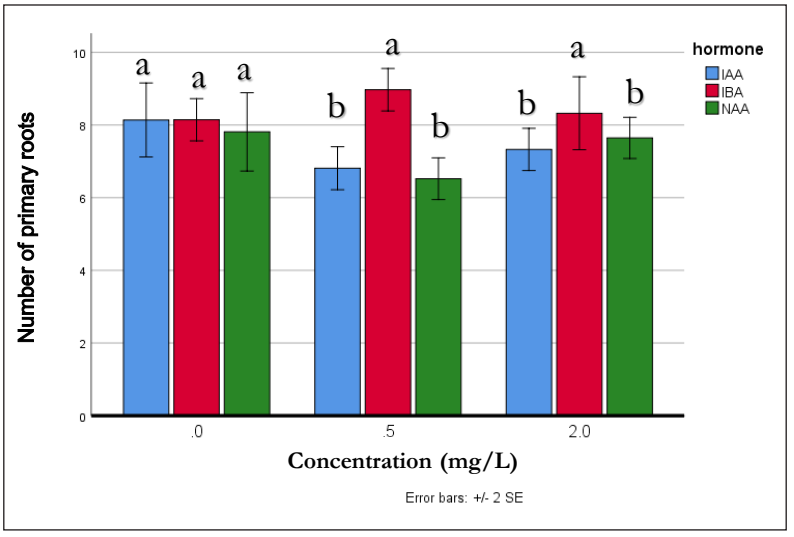

Figure 1. Interaction effect of hormone type (IAA, IBA, and NAA) and concentration (0, 0.5, and 2.0 mg/L) on the number of primary roots in high-yielding Strobilanthes crispus stem cuttings. Bars with different letters indicate statistical significant

Indole-3-butyric Acid (IBA) is one of the most important effective auxins type plant growth regulators which can stimulate adventitious root development from the stem cuttings of various plants, including *Piper betle* (Muttaleb et al., 2017*), Solanum procumbens* (Tien et al., 2020), and *Andrographis paniculata* (Hossain et al., 2021). Its superior efficacy is often associated with its higher chemical stability and lower susceptibility to enzymatic breakdown, which allows it to remain active for a longer duration at the application site (Klerk et al., 1999; Păcurar et al., 2014). These properties likely explain the improved rooting response observed in *S. crispus* stem cuttings treated with IBA, particularly at 0.5 and 2.0 mg/L concentrations in this study.

In contrast, IAA and NAA produced more moderate and inconsistent rooting responses across the tested concentrations. As reported by Hartmann et al. (2011) and Pacurar et al. (2014), IAA is known to degrade rapidly within plant tissues, reducing its effectiveness over time, whereas NAA, though more chemically stable, may exhibit limited uptake and translocation efficiency in stem. Additionally, it is possible that *S. crispus* cuttings possess sufficient endogenous auxin to initiate rooting without the need for exogenous application.

The introduction of external auxins such as IAA and NAA at 0.5 or 2.0 mg/L may have disrupted the internal hormonal equilibrium, leading to suboptimal rooting or physiological stress (Hartmann et al., 2011). These factors may explain the observed variability and overall lower performance of IAA and NAA treatments compared to the more consistently effective IBA formulations in this study.

While this study offers valued understandings into the interactive effects of auxin type and concentration on root growth and development in. *crispus* stem cuttings, several areas need further exploration. The investigation was limited to a single high yielding genotype; therefore, future studies should include multiple genotypes to evaluate genotypic variability in hormonal responsiveness, especially since auxin sensitivity can differ among genetic backgrounds. Moreover, exploring a broader concentration gradient (e.g., 0.25, 1.0, 1.5 mg/L) could help identify more precise threshold levels for optimal growth responses and minimize the risk of hormonal overdose or under treatment. Future studies may also benefit from incorporating endogenous hormone profiling or gene expression analysis of root and shoot related genes to understand the underlying molecular mechanisms driving differential responses to exogenous auxins.

CONCLUSION

This study highlights the importance of hormone type and concentration in optimizing root induction of high yielding *S. crispus* stem cuttings, with IBA at 0.5 mg/L producing the highest number of primary roots. The significant hormone × concentration interaction indicates that rooting response is highly dependent on the specific auxin treatment used, with IBA demonstrating greater effectiveness compared to IAA and NAA. These findings provide a reliable protocol for vegetative propagation, supporting the large scale production of uniform and genetically stable planting materials for commercial cultivation.

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Palm Kernel Meal as Sustainable Ingredient for Tilapia (*Oreochromis* sp.) Feed: Effects on Growth Performance, Body Colour, and Carcass Composition

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ABSTRACT

The inconsistent availability of imported feed ingredients like fishmeal and soybean meal has driven the aquaculture industry to explore sustainable protein alternatives. This study assessed the effects of varying inclusion levels of palm kernel meal (PKM) and its premium variant, PURAFEX on the growth performance, body colour, and carcass composition of tilapia fish. Six isonitrogenic and isocaloric diets were tested: T1 (control, without PKM or PURAFEX), T2 (10% PKM), T3 (20% PKM), T4 (10% PURAFEX), T5 (20% PURAFEX), and T6 (30% PURAFEX). After a 16-week feeding trial, fish fed T3 had the highest ($P<0.05$) body weight, feed intake and the best feed conversion ratio among treatments. The body colour analysis showed no significant differences ($P>0.05$) in lightness (L*), but the values for redness (a*) and yellowness (b*) varied significantly ($P<0.05$) with increasing inclusion levels of PKM and PURAFEX. Carcass analysis revealed similar moisture levels across groups, but diets with PKM or PURAFEX had lower ash content (13%) compared to the control (15.93%). Crude protein (CP) was highest in T3 but decreased with higher PURAFEX levels, while gross energy increased with PKM or PURAFEX inclusion levels (4.4–5.2 kcal/g). The results showed that incorporating PKM and PURAFEX into tilapia diets enhanced growth performance, improved colour intensity, and boosted the fish's appearance and nutritional compositions. The findings suggest that PKM and PURAFEX can serve as nutritionally valuable alternatives to conventional imported feed ingredients for tilapia feed.

Keywords: Carcass composition, colour analysis, growth performance, palm kernel meal, tilapia

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INTRODUCTION

The rapid growth of aquaculture has increased the demand for high-quality protein sources such as fishmeal and soybean meal. However, reliance on these imported ingredients raises concerns regarding cost, sustainability, and environmental impact. Overfishing has reduced fish stocks, driving up prices (Ritchie & Roser, 2025), while the production of soybean meal competes with food production and contributes to deforestation soybean meal competes with food production and causes deforestation (Caro et al., 2018).

Palm Kernel Meal (PKM) and its premium variant, PURAFEX, are by-products of palm oil production that offer a sustainable alternative for fish feed. Studies show that PKM can replace fishmeal without affecting fish growth (Ng et al., 2020), while also supporting a circular economy by reducing waste. Since feed accounts for 70% of aquaculture costs (Zhao et al., 2020), identifying cost-effective alternative ingredients is crucial. Therefore, this study examines the effects of PKM and PURAFEX in tilapia diets, focussing on growth performance, body colour, and carcass composition, to assess their potential as sustainable feed options.

Problem Statement

Although palm-kernel hold potential as cost-effective, sustainable feed ingredients, their role as partial replacements in tilapia feed and the consequent effects on growth performance, body colour and carcass composition of tilapia, remain under-investigated. Thus, further research is required to evaluate and optimise the inclusion levels of PKM and PURAFEX in tilapia diets to enhance feed sustainability while maintaining or improving production outcomes.

Research Questions

How do PKM and PURAFEX affect tilapia growth and carcass composition?

MATERIALS AND METHOD

Six experimental diets were formulated to be isonitrogenous and isocaloric, with different inclusion levels of PKM or PURAFEX as partial replacements of conventional feed ingredients. The diets included: T1 (control, without PKM or PURAFEX); T2 (10% PKM); T3 (20% PKM); T4 (10% PURAFEX); T5 (20% PURAFEX); and T6 (30% PURAFEX). In this study, the percentages refer to % w/w of the total feed (i.e., 10 % or 20 % of the complete diet by weight was comprised of PKM or PURAFEX).

A total of 900 juvenile Nile tilapia was evenly distributed across eighteen pond partitions (50 fish per partition; three replicates per treatment), each equipped with continuous aeration and a water-recycling system. After a two-week acclimatisation period,

the fish were fed their respective diets five times daily (Fava et al., 2022) for 16 weeks. Body weight was measured every two weeks, and at the end of the trial, growth performance parameters were calculated. Body colour was assessed using a chromameter and carcass composition was determined in accordance with the procedures of the Association of Official Analytical Chemists (AOAC 2005). Data were statistically analysed using one-way analysis of variance (ANOVA) and Duncan's multiple-range test ($P<0.05$) to identify significant differences among treatments.

RESULTS AND DISCUSSION

Growth Performance

The results in Table 1 show that the inclusion of PKM and PURAFEX significantly influenced ($P<0.05$) the growth performance parameters of tilapia, except for the survival rate. Fish in the T3 group exhibited the highest ($P<0.05$) final body weight. Additionally, fish in this group had the highest feed intake (160.51 g) and the most efficient FCR (1.53) compared to other treatment groups.

Table 1
Growth performance of tilapia fed PKM and PURAFEX at different inclusion levels

Growth performance	Experimental diet						SEM	P-value
	T1	T2	T3	T4	T5	T6		
Final weight (g)	85.36^{ab}	80.28^{b}	121.63^{a}	64.74^{c}	98.29^{ab}	75.25^{b}	2.09	<0.05
SGR (% per day)	0.93^{ab}	0.83^{ab}	1.15^{a}	0.61^{b}	1.01^{a}	0.78^{b}	0.28	<0.05
Survival rate (%)	96.67^{a}	94.67^{a}	98.67^{a}	100.00^{a}	94.00^{a}	93.33^{a}	1.25	0.18
Feed intake (g)	125.57^{b}	105.93^{c}	160.51^{a}	101.65^{c}	126.34^{b}	118.99^{b}	2.58	<0.05
FCR	1.81^{ab}	1.67^{b}	1.53^{b}	2.16^{a}	1.54^{b}	2.03^{a}	0.34	<0.05

Note. T1 – Control (0% PKM); T2 – 10% PKM; T3 – 20% PKM; T4 – 10% PURAFEX; T5 - 20% PURAFEX; T6 – 30% PURAFEX; ADG – average daily gain; SGR – specific growth rate; FCR – feed conversion ratio

Body Colour and Carcass Composition

Table 2 presents the body colour and carcass composition of tilapia fed diets with varying levels of PKM or PURAFEX. The analysis of body colour showed that a* and b* values were significantly influenced ($P<0.05$) by the inclusion levels of PKM and PURAFEX. The increased levels of PKM and PURAFEX appeared to enhance the a* and b* values, which potentially improved the market appeal of farmed tilapia. The impact of dietary ingredients on fish pigmentation has been well-documented, with studies indicating that carotenoid-rich feeds can intensify skin colouration, thus influencing consumer preference (Micah et al., 2022).

Carcass analysis data on the tilapia whole-body revealed that total moisture levels were consistent across all groups. However, diets containing PKM or PURAFEX resulted in a significantly lower ($P<0.05$) total ash content (13.33 – 13.77%) compared to the control (15.93%). Crude protein content was highest in T3 but exhibited a decreasing trend as PURAFEX inclusion levels increased. Gross energy levels increased with the inclusion of PKM and PURAFEX (4.4–5.2 kcal/g), indicating enhanced energy availability in these diets. Similar findings have been reported in studies on alternative feed ingredients, where carcass composition was positively influenced by PKM inclusion (Sangavia et al., 2020).

Table 2
Body colour analysis and carcass composition of tilapia fed PKM or PURAFEX at different inclusion levels

Parameter	Experimental diet						SEM	P-value
	T1	T2	T3	T4	T5	T6		
Colour analysis								
Lightness (L*)	59.68^{a}	56.92^{a}	55.41^{a}	57.54^{a}	55.73^{a}	55.01^{a}	1.98	0.24
Redness (a*)	3.95^{ab}	4.98^{a}	5.46^{a}	2.99^{b}	4.34^{ab}	5.29^{a}	0.85	<0.05
Yellowness (b*)	5.03^{b}	7.24^{ab}	9.43^{a}	7.05^{b}	7.96^{ab}	9.14^{a}	0.29	<0.05
Carcass composition								
Total Moisture (%)	9.80^{a}	9.50^{a}	9.21^{a}	10.08^{a}	10.09^{a}	9.99^{a}	0.21	0.57
Total Ash (%)	15.93^{a}	13.33^{b}	13.77^{b}	13.59^{b}	13.47^{b}	13.31^{b}	0.24	<0.05
Crude fat (%)	16.77^{b}	25.07^{a}	23.55^{a}	13.43^{c}	17.35^{b}	24.65^{a}	0.52	<0.05
Crude protein (%)	12.27^{a}	12.03^{a}	12.90^{a}	12.51^{a}	11.18^{ab}	10.69^{b}	0.38	<0.05
Crude fibre (%)	2.18^{a}	2.08^{a}	2.09^{a}	2.12^{a}	2.07^{a}	2.25^{a}	0.14	0.16
Gross energy (kcal/g)	4.45^{b}	5.24^{a}	5.10^{a}	4.73^{b}	4.69^{b}	5.09^{a}	0.04	<0.05

Note. T1 – Control; T2 – 20% PKM; T3 – 30% PKM; T4 – 10% PURAFEX; T5 - 20% PURAFEX; T6 – 30% PURAFE

CONCLUSION

The findings of this study highlight the potential of PKM and PURAFEX as sustainable feed ingredients for tilapia. The inclusion of 20 % PKM (T3) yielded the highest growth performance, optimal carcass composition and improved colour intensity. While both ingredients show promise, the PKM treatments out-performed the PURAFEX treatments under the conditions tested, suggesting that PKM may be the more effective alternative to conventional imported feed ingredients. These results support the use of PKM as a viable alternative ingredient, promoting sustainability in aquaculture feed production.

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