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REVIEW ARTICLES

- Oil Palm Economic Performance in Malaysia and R&D Progress in 2024
- Spent Bleaching Earth as Value-added Material to Material Construction Applications: A Review



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OIL PALM ECONOMIC PERFORMANCE IN MALAYSIA AND R&D PROGRESS IN 2024

GHULAM KADIR AHMAD PARVEEZ^{1*}; RAZMAH GHAZALI¹; NUR AIN MOHD HASSAN¹; AHMAD ZAIRUN MADIHAH¹; ABRIZAH OTHMAN¹; PARTHIBAN KANNAN¹; TEH SOEK SIN¹; VOON PHOOI TEE¹; ZAINAB IDRIS¹ and RAMLE MOSLIM¹

ABSTRACT

Malaysia's palm oil sector experienced significant growth in production, exports, and prices in 2024, despite a slight decrease in total planted area due to replanting initiatives. The crude palm oil (CPO) production rose in early 2024 due to improved fresh fruit bunches (FFB) yield and labour availability, but fell below 2023 levels in the last quarter due to unfavourable weather patterns. The total exports of oil palm products increased compared to 2023, primarily driven by higher demand from major importing countries, with India leading the way. Research and development (R&D) across the industry have advanced significantly, adopting innovative and interdisciplinary approaches. In the upstream sector, efforts focused on enhancing soil and plant health, conserving biodiversity, and developing high-yielding, resilient oil palm varieties. There were efforts to use mechanisation in plantations and Industrial Revolution 4.0 (IR4.0) in mills, such as Artificial Intelligence of Things (AIoT)-based fruit sorting, targeted spraying systems and mechanised harvesting in the midstream industry. The adaptability of palm oil allows for the investigation of its nutritional values for application in dietary supplements and functional meals as well as value addition in oleochemical derivatives to secure a greater share of the value chain in the downstream sector.

Keywords: biotechnology, mechanisation and automation, nutrition and value addition, smallholders, yield performance.

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INTRODUCTION

The economic performance of the oil palm industry in Malaysia has shown remarkable resilience over the past years, driven by both domestic demand and international markets. The oil palm industry continued to demonstrate its economic prowess, solidifying Malaysia's position as one of the global leaders in palm oil production and export. The oil palm sector is a vital pillar of Malaysia's economy, generating RM109.39 billion in export revenue in 2024 [Malaysian Palm Oil Board (MPOB), 2025]. With approximately 5.6 million hectares of cultivation, the industry is striving harder than ever to address issues related to sustainability and its safety put forward by global concern.

In recent years, Malaysia has focussed on improving productivity and sustainability within the oil palm sector. Research and development (R&D) initiatives have been enhanced to address challenges such as climate change, pest management, and land-use efficiency. Innovations in agricultural techniques, pest management, and soil health are gaining traction. Enhanced genetic research is also helping to cultivate more resilient palm varieties that can withstand climate challenges and disease pressures. Simultaneously, advancements in agronomic practices and the adoption of precision agriculture technologies are expected to further boost yields while minimising environmental impact. As we envisage, integrating technology in the oil palm industry through practices like data analytics and automated harvesting systems is anticipated to transform operations. This evolution is essential for maintaining competitiveness in the global market and to cater to the increasing demand for sustainable palm oil. This shift has prompted

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producers to adopt more eco-friendly practices, focussing on reducing greenhouse gas (GHG) emissions and improving land use efficiency, thus enhancing market competitiveness. Collaborative initiatives between government agencies, industry stakeholders, and research institutions are vital in promoting these sustainable practices.

Meanwhile, investments in state-of-the-art milling facilities and refining processes allowed for greater efficiency, quality control, and diversification of palm-based goods, from edible oils and biofuels to oleochemicals and specialty food ingredients. This has translated to soaring export figures and remunerative returns for Malaysian palm oil stakeholders, from smallholder farmers to major conglomerates. Moreover, the Malaysian government's proactive policies to promote downstream processing and value-added palm-based products have further diversified the industry, opening new revenue streams and cementing Malaysia's status as a comprehensive palm oil powerhouse. Additionally, Malaysia remains committed to meeting international sustainability standards, which is crucial for accessing key markets. Collaborations with international bodies and adherence to certifications have bolstered Malaysia's reputation as a responsible producer.

This review provides insight into the performance of the oil palm industry as well as the R&D progress and achievements for the year 2024. The performance of the Malaysian oil palm industry will be specifically examined, followed by global R&D advancements in the upstream, midstream, and downstream segments of the oil palm industry, and finally, key areas for development and future paths for the industry's sustained expansion in the coming years.

PERFORMANCE OF THE MALAYSIAN OIL PALM INDUSTRY

The year 2024 saw the Malaysian palm oil sector as resilient and adaptive, achieving notable growth in production, exports, and prices. The total planted area decreased marginally below the

2023 level due to strategic replanting initiatives to ensure long-term productivity. The crude palm oil (CPO) production in 2024 outperformed that in 2023, supported by improved fresh fruit bunches (FFB) yield and labour availability. However, due to unfavourable weather, CPO production dropped in the last quarter of 2024 to lower levels than in the last quarter of 2023. Exports of palm oil products increased by 8.9% amidst strong demand from major markets, particularly India and China, resulting in increased revenue. Although the closing stocks of palm oil dropped by 25.3%, this helped to support the upward trend of CPO prices, which rose 9.7% year on year, especially during the last quarter of the year. The combined effect of strategic changes and robust export demand has placed Malaysia in a good position in the global palm oil market.

Oil Palm Planted Area

As of December 2024, the oil palm planted in Malaysia underwent a marginal decline of 0.70%, dropping from 5.65 million hectares in December 2023 to 5.61 million hectares (MPOB, 2025). This reduction is partly due to continuous national efforts in the replanting programme to replace ageing, less productive oil palm trees with high-yielding oil palm planting material, which is fundamental to the long-term sustainable production of Malaysia's palm oil.

At the regional level, Peninsular Malaysia and Sabah experienced a drop in planted area of 0.56% and 1.74%, respectively, while Sarawak saw an increase of 0.04%. Sarawak remains the largest oil palm planted area in Malaysia (*Table 1*). Sarawak presently accounts for 1.62 million hectares, or 28.94%, of the nation's total planted area, followed by Sabah with 1.48 million hectares (26.43%) and Pahang with 0.74 million hectares (13.20%). The industry's commitment to replanting is further demonstrated by a 1.24% reduction in the mature oil palm area, from 5.13 million hectares in 2023 to 5.07 million hectares in 2024, reflecting strategic efforts to boost yield through replanting and modernisation (McGill, 2024).

TABLE 1. MALAYSIAN OIL PALM PLANTED AREA IN DECEMBER 2024 AND 2023

Region	Planted area (ha)			Mature area (ha)		
	2024	2023	Difference (%)	2024	2023	Difference (%)
Peninsular Malaysia	2,504,786	2,518,883	-0.56	2,268,705	2,307,546	-1.68
Sabah	1,483,699	1,510,025	-1.74	1,291,591	1,316,356	-1.88
Sarawak	1,624,366	1,623,661	0.04	1,506,228	1,506,271	-0.00
Malaysia	5,612,852	5,652,569	-0.70	5,066,524	5,130,172	-1.24

Source: MPOB (2025).

Regarding ownership, private and government-owned estates covered 4.13 million hectares, or 73.6% of the total planted area (*Figure 1*). This was followed by independent smallholders with 0.82 million hectares (14.6%) and the remaining 11.8% owned by organised smallholders. Compared to 2023, the oil palm planted area declined across all ownership categories in 2024, with the largest reduction seen in organised smallholders (1.49%), followed by private and government-owned estates (0.72%) and independent smallholders (0.00% or no change).

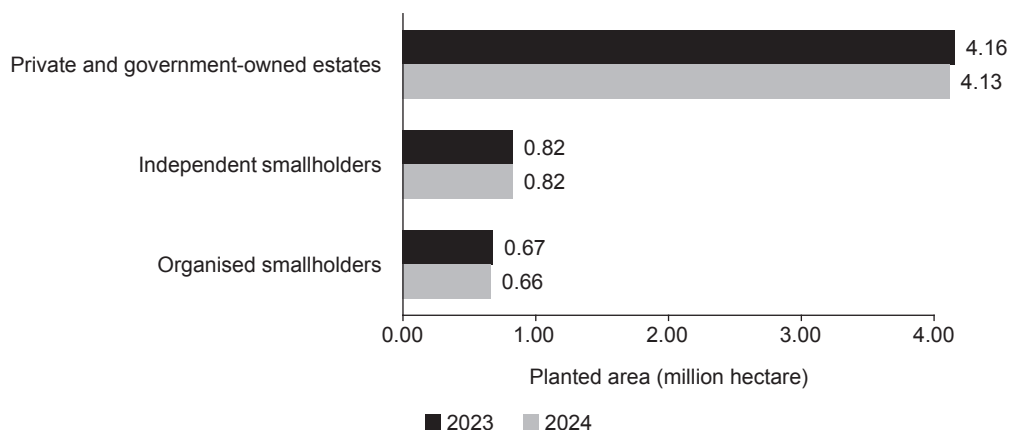
Status of Mills and Plants

Table 2 highlights the regional disparities of Malaysia's palm oil processing facilities in 2024. Malaysia has a total of 453 palm oil mills with a combined processing capacity of 123.46 million tonnes of FFB yr⁻¹, 53 refineries [26.39 million tonnes of CPO and crude palm kernel oil (CPKO) yr⁻¹], and 43 kernel crushers (7.36 million tonnes yr⁻¹).

The milling capacity utilisation rate also rose by 2.56% in 2024, reaching 80.15% compared to 78.15% in the previous year due to a higher volume of FFB processed in 2024 as against 2023 (MPOB, 2025). Oleochemical plants are exclusively located in Peninsular Malaysia, while biodiesel plants are relatively limited nationwide, with 18 facilities having a total capacity of 2.53 million tonnes yr⁻¹. In conclusion, Peninsular Malaysia dominates in all categories, housing the highest number and capacities of palm oil mills, refineries, kernel crushers, oleochemical plants, and biodiesel plants.

CPO Production

CPO production in 2024 saw an increase of 4.2% to 19.34 million tonnes compared to 18.55 million tonnes in 2023, driven by a higher volume of FFB processed as a result of improved FFB yield. Enhanced labour availability in oil palm plantations also helped increase production levels back to a



Note: In the Malaysian context, independent smallholders are individuals who manage oil palm on their land, typically less than 40.46 ha (100 acres), without being part of any government or organised schemes.

Source: MPOB (2025).

Figure 1. Ownership distribution of oil palm planted area in 2024 vs. 2023.

TABLE 2. NUMBER AND CAPACITIES OF MALAYSIAN PALM OIL MILLS, REFINERIES, PALM KERNEL CRUSHERS, OLEOCHEMICAL PLANTS AND BIODIESEL PLANTS IN 2024

Facility type	Peninsular Malaysia		Sabah		Sarawak		Malaysia	
	No.	Capacity (Mn t yr ⁻¹)	No.	Capacity (Mn t yr ⁻¹)	No.	Capacity (Mn t yr ⁻¹)	No.	Capacity (Mn t yr ⁻¹)
Palm oil mill	240	64.05	129	34.99	84	26.35	453	125.40
Palm oil refinery	37	15.42	10	7.88	6	3.10	53	26.39
Palm kernel crusher	27	4.61	10	1.99	6	0.76	43	7.36
Oleochemical plant	19	2.68	-	-	-	-	19	2.68
Biodiesel plant	15	2.01	2	0.30	1	0.22	18	2.53

Note: Mn t yr⁻¹ - million tonnes yr⁻¹.

Source: MPOB (2025).

typical trajectory in early 2024, following only marginal production growth in 2023 (Parveez *et al.*, 2024). However, from September 2024 onwards, production performance fell below the 2023 level, largely due to unfavourable weather patterns from 2023 impacting yield in the fourth quarter of 2024. Peak CPO production for 2024 was recorded in August at 1.89 million tonnes, while the lowest was in February at 1.26 million tonnes. In Peninsular Malaysia, CPO production rose by 10.9% to 10.89 million tonnes, while CPO production in Sabah and Sarawak declined by 5.2% and 1.1%, reaching 4.27 million tonnes and 4.17 million tonnes, respectively (Table 3).

In 2024, FFB yield increased slightly by 5.8% to 16.70 t ha⁻¹ as against 15.79 t ha⁻¹ in 2023, mainly driven by better yield performance in the first eight months of the year, which was largely supported by an improved labour situation in the oil palm plantation sector (Table 4). Regionally, FFB yield in Peninsular Malaysia increased by 14.5% to 18.42 t ha⁻¹. Sabah's FFB yield registered a decline of 4.0% to 15.74 t ha⁻¹ from 16.39 t ha⁻¹, while that of Sarawak was higher by 0.9% to 14.89 t ha⁻¹ from 14.75 t ha⁻¹ in 2023.

Despite the increase in FFB yield, the oil extraction rate (OER) in 2024 experienced a slight decline of 1.00% to 19.67% compared to 19.86% recorded in 2023. This reduction was primarily attributed to the lower quality of FFB processed in palm oil mills, which was adversely affected by prolonged rainfall and flooding in oil palm areas, especially during the northeast monsoon season in the second half of 2024. Also, the washing of CPO to reduce contamination and lower the levels of

3-monochloropropane-1,2-diol esters (3-MCPDE) further contributed to this decline.

Table 4 also suggests a negative relationship between FFB yield and OER across regions. For instance, Peninsular Malaysia recorded a 14.5% increase in FFB yield, but OER fell by 0.9%. Similarly, Sarawak recorded a slight increase in yield accompanied by a 2.3% reduction in OER. This inverse relationship may be due to the trade-off between higher fruit yields and oil content, which means an increase in fruit production may lead to the processing of lower-quality fruits, thus, reducing oil extraction efficiency. Balancing efforts to enhance yield while maintaining OER remains a critical challenge for the industry.

OER performance in 2024 generally fell across most regions, apart from Sabah, compared to the previous year. Specifically, the OER in Peninsular Malaysia and Sarawak dropped by 0.90% to 19.46% and 2.30% to 19.37%, respectively (Table 4).

Trade Performances of Oil Palm Products

Total exports of oil palm products in 2024 reached 26.66 million tonnes, representing an 8.9% increase from 24.49 million tonnes recorded in 2023 (Table 5). This growth was driven by a rise in exports across various oil palm products, including palm oil itself and its by-products. Palm oil exports alone rose notably by 11.7%, amounting to 16.90 million tonnes, compared to 15.14 million tonnes in the previous year, driven by a substantial 15.0% increase in processed palm oil (PPO) exports, while CPO exports recorded a modest rise of 0.9%. This surge in exports was largely due to higher demand from

TABLE 3. MALAYSIAN CRUDE PALM OIL PRODUCTION

Region	2024 (t)	2023 (t)	Difference	
			Volume (t)	%
Peninsular Malaysia	10,891,417	9,825,140	1,066,277	10.9
Sabah	4,274,440	4,507,460	-233,020	-5.2
Sarawak	4,172,409	4,219,350	-46,941	-1.1
Total	19,338,266	18,551,950	786,316	4.2

Source: MPOB (2025).

TABLE 4. MALAYSIAN FRESH FRUIT BUNCH PRODUCTIVITY AND OIL EXTRACTION RATE

Region	FFB productivity (t ha ⁻¹)				OER (%)			
	2024	2023	Difference		2024	2023	Difference	
			Volume	%			Volume	%
Peninsular Malaysia	18.42	16.09	2.33	14.5	19.46	19.64	-0.18	-0.9
Sabah	15.74	16.39	-0.65	-4.0	20.53	20.40	0.13	0.6
Sarawak	14.89	14.75	0.14	0.9	19.37	19.83	-0.46	-2.3
Total	16.70	15.79	0.91	5.8	19.67	19.86	-0.19	-1.0

Source: MPOB (2025).

major importing countries, reflecting the resilience of global demand despite fluctuating commodity markets.

Besides, Malaysia's palm kernel oil (PKO) exports recorded the highest growth rate at 17.2% to 1.15 million tonnes, up from 0.98 million tonnes in 2023. The major demand for Malaysian PKO came from the European Union (EU), China, and India, where it is used extensively in both food and non-food industries. Meanwhile, Malaysia's exports of palm kernel cake rose modestly by 3.7%. Palm-based oleochemicals and biodiesel exports grew by 4.5% and 4.4%, respectively. Finished products showed an 11.1% increase, while exports of other oil palm products experienced a slight decline of 2.0%. These results reflect a strong demand for palm oil and its derivatives in the global market, with significant contributions from value-added products.

In addition to the impressive export performance, the export revenue of Malaysian palm oil and oil palm products in 2024 saw significant growth of 15.1% from RM94.95 billion in 2023 to RM109.39 billion in 2024. The largest contributor to this growth was palm oil. Palm oil export revenue rose 16.4% to RM72.95 billion, compared to RM62.60 billion in 2023. This increase reflects higher export volume and improved prices, driven by strong global demand for palm oil. Furthermore, the revenue from PKO showed remarkable performance, with a 35.9% increase in export revenue to RM6.79 billion, reflecting strong global demand. Meanwhile, palm-based oleochemicals grew by 8.8%, while finished products recorded an impressive 19.7% increase, reaching RM4.31 billion. Biodiesel exports experienced a declining growth of 4.4%, while other products increased by 8.4%. This higher growth in export revenue compared to volume indicates improved prices together with strong demand for premium and value-added products in the global market.

Malaysian palm oil exports in 2024 were predominantly directed to traditional markets. India

led the way with an increase of 185.46 thousand tonnes, followed by the EU with 227.00 thousand tonnes and Kenya with 345.27 thousand tonnes (Figure 2). Other key markets included China, which remained one of Malaysia's top importers despite a decline of 77.95 thousand tonnes, as well as Iran (252.51 thousand tonnes), the Philippines (245.21 thousand tonnes), South Korea (42.78 thousand tonnes), Japan (53.71 thousand tonnes), Pakistan (83.65 thousand tonnes) and Türkiye (21.02 thousand tonnes). Together, these top 10 importing countries accounted for 63.7% of Malaysia's total palm oil exports in 2024 with a combined year-on-year increase of 1.46 million tonnes.

India strengthened its position as the largest Malaysian palm oil importer in 2024, with a notable 6.5% increase in imports, reaching 3.03 million tonnes, up from 2.84 million tonnes in 2023. This shift was due to a 12.4% decline in Indonesian palm oil imports, which fell from 5.10 million tonnes in 2023 to 4.47 million tonnes in 2024. The reduction in Indonesia's exports was primarily due to increased domestic consumption driven by a higher biodiesel blending mandate and a slight decrease in production. Meanwhile, China's imports fell by 5.3% due to increased soybean imports from Brazil by 4.2% to 75.45 million tonnes (ISTA Mielke GmbH, 2025).

The EU saw a significant 21.3% increase in Malaysian palm oil imports to 1.29 million tonnes in 2024, driven by a sharp drop in Indonesian supply by 48.4% to 99,000 t in January-November 2024. Moreover, Kenya also boosted imports by 37.7% to 1.26 million tonnes in 2024, as it reduced imports of Indonesian palm oil by 41.9% in January-November 2024.

Palm Oil Closing Stocks and Prices

The palm oil closing stocks in December 2024 declined by 25.3% reaching 1.71 million tonnes *vis-à-vis* 2.29 million tonnes recorded in December 2023. The lower closing stocks were mainly

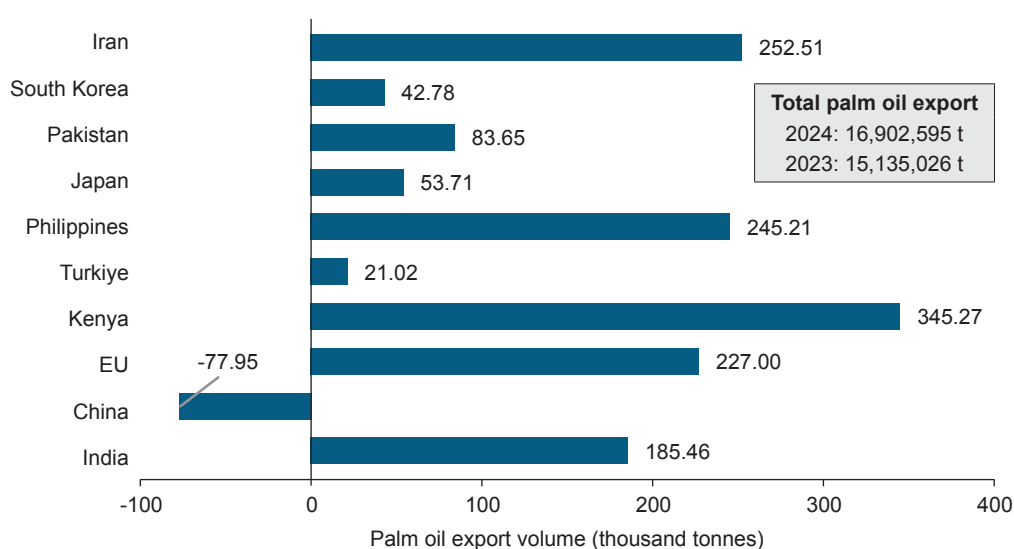
TABLE 5. MALAYSIAN EXPORTS OF PALM OIL AND OIL PALM PRODUCTS

Item	Volume (t)			Value (RM million)		
	2024	2023	Difference (%)	2024	2023	Difference (%)
Palm oil	16,902,595	15,135,026	11.7	72,952	62,601	16.4
Palm kernel oil	1,150,105	981,800	17.2	6,789	4,993	35.9
Palm kernel cake	2,396,517	2,311,506	3.7	1,497	1,667	-10.2
Palm-based oleochemicals	2,993,592	2,864,004	4.5	17,410	15,999	8.8
Biodiesel	255,669	244,793	4.4	1,253	1,310	-4.4
Finished products	585,764	527,013	11.1	4,313	3,602	19.7
Other oil palm products	2,375,206	2,422,905	-2.0	5,179	4,776	8.4
Total	26,660,448	24,487,045	8.9	109,391	94,949	15.1

Source: MPOB (2025).

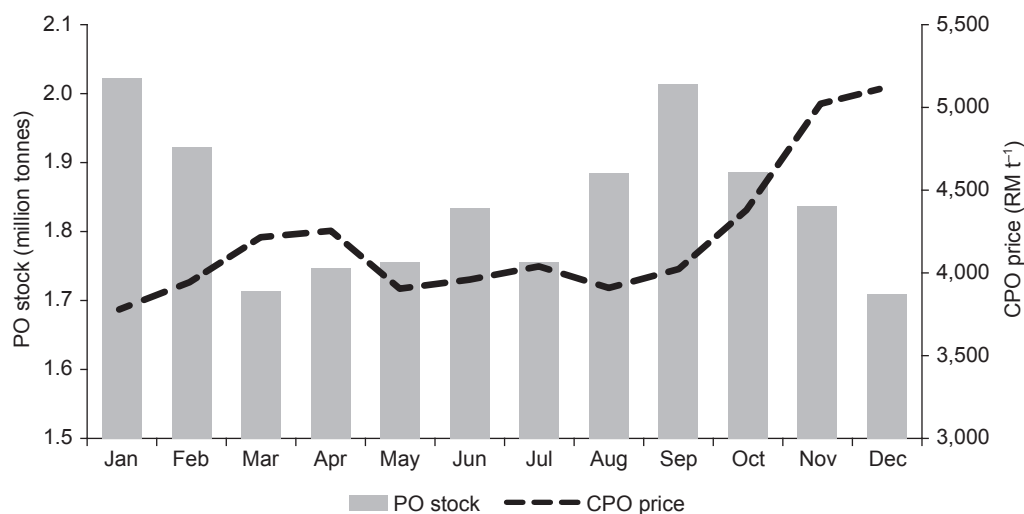
caused by 71.8% lower imports, equivalent to 0.65 million tonnes, and 11.7% increase in palm oil exports. The higher export volume was driven by stockpiling activity among importing countries coupled with lower CPO production, particularly in the fourth quarter of 2024. The palm oil closing stocks comprised 52.0% CPO and 47.4% PPO. All regions recorded declines in palm oil stocks ranging between 21.0% and 30.9%. The significant low stocks compared to the preceding year had a stronger effect on CPO prices throughout 2024, with the average price increasing by 9.7% to RM4,179.50 t⁻¹, in contrast to the recorded RM3,809.50 t⁻¹ in 2023 (Figure 3).

The highest traded CPO price in 2024 occurred in December at RM5,119.50 t⁻¹, while the lowest was in January at RM3,783.50 t⁻¹. Despite starting the year lower, CPO prices generally trended upward, particularly in the last quarter of 2024. The average CPO price in the first half of 2024 rose marginally by 2.3% to RM4,010.50 t⁻¹ compared to the same period in 2023. However, in the second half of 2024, the average CPO price continued to increase, rising remarkably by 18.1% to RM4,414.00 t⁻¹ compared to the same period in 2023, mainly due to the sentiment factor of Indonesia's plans to implement B40 biodiesel programme in January 2025 as well as stronger demand from major importing countries.



Source: MPOB (2025).

Figure 2. Year-on-year change in Malaysia's palm oil export volume to major destinations (2024 vs. 2023).



Note: PO - palm oil; CPO - crude palm oil.

Source: MPOB (2025).

Figure 3. Palm oil stocks and crude palm oil prices, 2024.

In the domestic market, alongside the increase in CPO prices, other oil palm products also traded higher in 2024 as against 2023. The highest percentage rise was observed in CPKO (40.5%), followed by palm kernel (PK) (31.2%) and RBD palm stearin (14.9%) (Table 6).

The surge in CPKO prices in 2024 aligned with the rise in global PKO and coconut oil prices, which increased by at least 40.0% compared to 2023. Additionally, the average FFB price in 2024 was up by 13.0% to RM875.00 t⁻¹, reflecting the higher CPO and PK prices.

Figure 4 shows the difference between CPO prices and other vegetable oil prices traded in the international market. CPO price was traded higher by 12.4% to USD1,084.00 t⁻¹ in 2024 as against USD964.00 t⁻¹ in 2023. Meanwhile, sunflower oil (SFO) was traded higher at USD1,061.00 t⁻¹, up by 5.4%, while soybean oil (SBO) price was

traded lower at USD1,045.00 t⁻¹ in 2024 as against USD1,124.00 t⁻¹ in 2023. Although CPO, SBO and SFO prices continued to move in tandem, the CPO price premium against SBO was at USD39.00 t⁻¹ in 2024, compared to a wide discount of USD160.00 t⁻¹ in 2023. In the case of SFO, the CPO price premium against the former was at USD23.00 t⁻¹ *vis-à-vis* a discount of USD43.00 t⁻¹ in 2023. The price gap between CPO and SBO, as well as SFO, narrowed in 2024. The premium in CPO price against other vegetable oil prices was mainly attributed to the supply disruption in the global market, which resulted in less competitiveness of CPO price as against SBO and SFO (McGill, 2025).

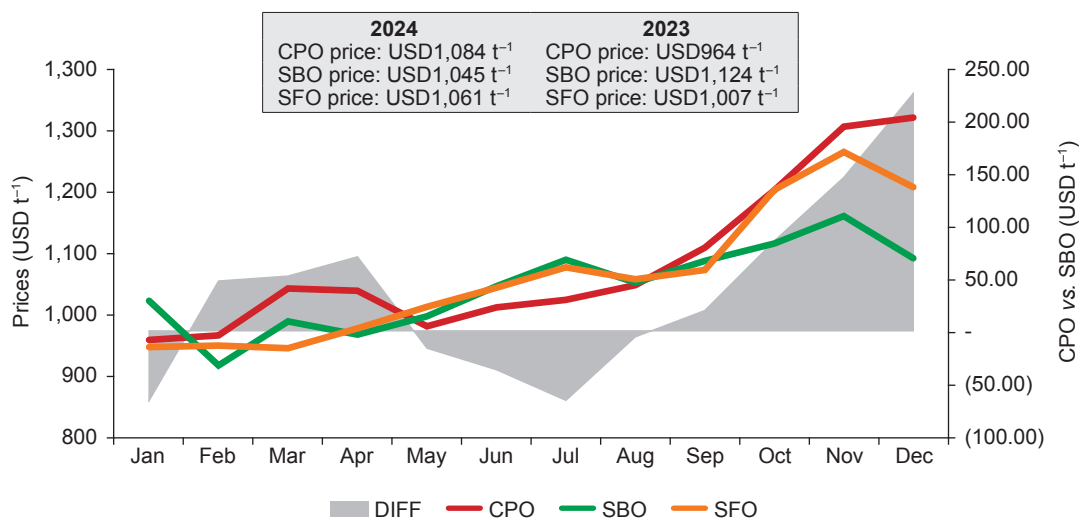
Moving forward to 2025, the CPO price is expected to strengthen, averaging between RM4,000.00 t⁻¹ and RM4,300.00 t⁻¹ compared to RM4,179.50 t⁻¹ in 2024, primarily driven by a tightening global palm oil supply. The

TABLE 6. MALAYSIAN PRICES OF OIL PALM PRODUCTS

Item	2024 (RM t ⁻¹)	2023 (RM t ⁻¹)	Difference	
			RM t ⁻¹	%
CPO	4,179.50	3,809.50	370.00	9.7
RBD palm oil	4,296.50	3,989.00	307.50	7.7
RBD palm olein	4,359.50	4,055.00	304.50	7.5
RBD palm stearin	4,402.50	3,833.00	569.50	14.9
Palm kernel	2,645.50	2,016.00	629.50	31.2
CPKO	5,475.50	3,896.00	1,579.50	40.5
FFB	875.00	774.00	101.00	13.0

Note: CPO - crude palm oil; RBD - refined, bleached and deodorised; CPKO - crude palm kernel oil; FFB - fresh fruit bunches.

Source: MPOB (2025).



Note: SBO - soybean oil; SFO - sunflower oil; CPO - crude palm oil; DIFF - difference between CPO and SBO prices.

Source: MPOB (2025).

Figure 4. Prices of palm oil vs. other vegetable oils in 2024.

implementation of the B40 biodiesel (a blend of 40% palm oil and 60% regular diesel) mandate in Indonesia is expected to further reduce the availability of global palm oil for export, as a significant portion of production will be allocated for domestic use. Additionally, biodiesel demand in the USA is anticipated to rise and Malaysia's palm oil stocks are projected to remain below 2.00 million tonnes, contributing to price stabilisation and further tightening the global supply of palm oil (Parveez, 2025).

R&D FOCUS AREAS IN 2024

Climate Change Mitigation and Carbon Sequestration in Oil Palm Plantations

Climate change significantly impacts oil palm cultivation in terms of soil moisture, unpredictable weather changes, and monsoon patterns, thus causing heat stress (Tey & Brindal, 2024). According to the 2018 Intergovernmental Panel on Climate Change (IPCC) report, global average temperatures will increase by 1.5°C to 2.0°C between 2030 and 2050, which will reduce yields and increase costs, threatening the oil palm's productivity and subsequently its sustainability (Abubakar *et al.*, 2023). Tey and Brindal (2024) emphasised that accelerating climate change adaptation for oil palm necessitates effective dissemination of mechanisation advancements, focussing on plant breeding and bio-fertiliser technologies, accentuating the need to enhance resilience and yield stability. It is therefore recommended that a model should be developed to mitigate emerging adverse effects, including optimising agronomic practices such as planting density, irrigation, fertilisation, pruning, pest, and disease control; developing resilient palm varieties that are drought-tolerant, disease-resistant, and pest-resistant; improving land use strategies; utilising hyperspectral imaging drones for early disease detection; promoting intercropping and agroforestry systems; and implementing an early weather monitoring system.

The global carbon cycle balance has deteriorated over the last century, as carbon sequestration has failed to keep pace with anthropogenic CO₂ emissions, causing climate instability and jeopardising future food security. Rising CO₂ levels may promote photosynthesis, but biogeochemical considerations, notably nitrogen (N) availability, may impede fruit development (Beringer *et al.*, 2023). Rebalancing the carbon flux and minimising GHG emissions requires lowering net emissions and increasing carbon sequestration activities. As such, the development of high-sequestration systems, such as natural forests, coastal wetlands, and tropical croplands, should be prioritised (Murphy, 2024).

The study recommended that recovering degraded lands, producing high-yield agricultural genotypes, and attaining net-zero carbon objectives should be adopted. The African oil palm (*Elaeis guineensis*) is well recognised for its high carbon absorption capability, especially in agroforestry systems where intercropping increases carbon uptake. A carbon sequestration study in India discovered that the overall carbon storage capacity across eight planting sites with 1,015 standing palms was 345.1 t yr⁻¹, underscoring a significant contribution to carbon capture (Lalawmpuia *et al.*, 2023). However, the planters need to be guided on sustainable management practices, such as how to treat degraded lands for intercropping activities, to reduce negative environmental effects and maximise oil palm carbon sequestration potential while balancing the need for climate change mitigation with forest preservation.

Good Practices and Biodiversity in Oil Palm Cultivation

Good oil palm planting materials are essential for maximising yield and palm oil production under optimised planting density. Afandi *et al.* (2024) demonstrated that the PS1 trait provided characteristics that are ideal for planting at higher densities of 160 palms ha⁻¹ through shorter rachis length and lower height increment. However, long-term evaluations revealed that planting at 140 palms ha⁻¹ density provided yields with the highest cumulative and average FFB at 427.0 and 24.2 t ha⁻¹ yr⁻¹, respectively. No significant interactions were observed among planting density, N, and progeny lineage. Furthermore, significant N₂O flows and N losses have been discovered in several locations, especially following fertiliser application (Zawawi *et al.*, 2024). A study by Chen *et al.* (2024) proposed that precision agriculture through controlled-release fertilisers to manage soil may reduce N₂O flux. However, oscillations in N₂O emissions between fertilisations reflect changes in N dynamics throughout the year, suggesting a need for further research using stable isotope tracers to monitor N fertiliser and comprehensive sampling to estimate its total emissions (Zawawi *et al.*, 2024).

Additionally, fertiliser created from biochar generated from oil palm biomass (OPB), such as mesocarp fibre and empty fruit bunches (EFB), enhances fertiliser efficiency, seedling development, and soil health, establishing it as a sustainable solution for oil palm cultivation in Malaysia (Tugiman *et al.*, 2024). In some cultivation areas, high aluminium toxicity levels cause acidic soil, impeding root growth and nutrient uptake by oil palm seedlings (Husein *et al.*, 2024). Among the four varieties evaluated, Ulu Remis Deli *dura* x Ulu

Remis tenera seedlings appeared more tolerant to aluminium toxicity with no significant effects on bole width, chlorophyll content, or biomass. Mobile apps may be used to optimise oil palm fertilisation practices, as demonstrated in this study on the Oil Palm Fertiliser Infographic Mobile Application, also known as *Baja Sawit* (Ishak *et al.*, 2024). The app encourages efficient fertilisation techniques among smallholders with a simple, user-friendly design based on Mayer's 12 Principles of Multimedia Learning, as well as straightforward infographics, real-time crop monitoring, and artificial intelligence integration to increase oil palm production and sustainability.

Biodiversity in oil palm plantations is crucial for preserving ecosystem balance, boosting pollination, encouraging pest and disease control, and ensuring long-term agricultural sustainability while minimising negative environmental consequences (Dalheimer *et al.*, 2024). Biodiversity flourishes in oil palm smallholdings because smaller field sizes and staggered replanting practices generate ecosystems that promote ecological equilibrium. Smallholders can contribute to local biodiversity conservation by fostering spatial heterogeneity and crop diversification while large plantations can adjust replanting strategies (Azhar *et al.*, 2023).

Sustainable Development for Smallholders

Malaysia has introduced the Malaysian Sustainable Palm Oil (MSPO) certification to encourage sustainable practices in the palm oil sector. One critical factor for enhancing smallholders' participation in MSPO involves addressing their attitudes and perceived behavioural control (Majid *et al.*, 2023). Abidin *et al.* (2024) further reported that awareness and knowledge gaps among smallholders regarding certification benefits and requirements need to be addressed. Besides addressing social and environmental concerns, the MSPO standards provide smallholders with guidance on Good Agricultural Practices (GAP), including waste management (Hamid *et al.*, 2024).

The adoption of GAP is important for the sustainable management of oil palm cultivation among smallholders. MPOB, through its extension services, provides training to smallholders on various GAP designed to optimise oil palm management and yields. Adoption of GAP, such as improved harvesting and nutrient management, leads to an increase in yields (Lim *et al.*, 2024b; Ramachandran *et al.*, 2024). Sham *et al.* (2024) emphasised the importance of reducing nutrient leaching from fertilisers and promoting sustainable agricultural practices, encouraging smallholders to adopt strategies that minimise nutrient losses and mitigate environmental impacts. Another study by Ahmad *et al.* (2024a) recommended selecting

suitable soil types to increase oil palm production without compromising high-value tropical forests. This approach promotes sustainable practices while maintaining productivity in oil palm cultivation.

There is a greater need to accentuate the crucial role of Information and Communication Technology (ICT) in empowering oil palm smallholders to adapt to climate change and improve their livelihoods (Peng *et al.*, 2024). As stated earlier, Ishak *et al.* (2024) have developed a user-friendly mobile application platform (*Baja Sawit*) for farmers to access current information, thereby enhancing productivity and supporting informed decision-making. MPOB has embarked on developing ICT tools to enhance the monitoring of oil palm product transactions, ensuring greater transparency and traceability throughout the supply chain [MPOB, 2023; MSPO & the European Forest Institute (EFI), 2024].

In addition to the challenges smallholders face in managing oil palm cultivation, they also struggle with global regulations, such as the European Union Deforestation Regulation (EUDR). The EUDR is the latest regulation from the EU aimed at addressing deforestation-related concerns. The EU's focus on ensuring palm oil traceability to its specific plantation origin poses challenges, especially for smallholders, who may find it difficult to comply with these requirements [Malaysian Palm Oil Council (MPOC), 2024]. Smallholder producers may face challenges in meeting traceability requirements, which could exclude them from global supply chains (Nadras *et al.*, 2024). Solidaridad Network (2023) suggests acknowledging the initiatives that promote the sustainable development of palm oil in producing countries. These include mandatory sustainability frameworks like the MSPO and the Indonesian Sustainable Palm Oil (ISPO), which are vital tools for promoting adherence to national regulations and laws. Therefore, continuous communication of reliable and accurate information about Malaysia's efforts in sustainable practices is crucial for addressing and clarifying any misconceptions about the palm oil industry (Naidu *et al.*, 2024).

The use of technologies, especially machinery, in oil palm cultivation is often limited for smallholders due to economies of scale. This limitation suggests the need for forming groups of smallholders to leverage collective resources and benefits (Ahmad *et al.*, 2023). To address this, the government, through the MPOB, introduced a cooperative structure to empower smallholders to better manage their resources collectively. Various forms of financial and management assistance have been provided to cooperatives to engage in business ventures, such as establishing FFB collection centres. This enables smallholders to sell FFB directly to mills, thereby enhancing economic development. In addition, the establishment

of cooperatives among indigenous oil palm smallholders could provide substantial benefits to the indigenous community (Chin *et al.*, 2023).

Effective Pest and Disease Management Strategies

Several vertebrate and invertebrate pests were found to be potential threats to oil palm plantations. The bagworm *Metisa plana* (Lepidoptera: Psychidae) causes significant leaf defoliation, especially in Peninsular Malaysia (Ahmad *et al.*, 2024b). Management strategies utilising palm-based sticky glue, neem oil (*Azadirachta indica* A. Juss) and Quad trap have been successfully implemented to reduce bagworm incidence in plantations (Ahmad *et al.*, 2024b; Kamarudin *et al.*, 2024; Sattar *et al.*, 2024). Another pest, the mealybug, has had a substantial impact on oil palm plantations in Sabah, particularly in Tawau and Lahad Datu, by spreading the sooty mould disease (Miwil, 2024). This disease obstructs the photosynthesis of oil palm fronds, resulting in stunted growth and a 5.6% annual decline in FFB production between 2018 and 2023. Treatments such as trunk injections and chemical sprays have the potential to manage outbreaks effectively. Meanwhile, to address rodent infestation, artificial hunting perch spots strategically positioned within oil palm plantations are ideal in attracting predatory birds, in this case barn owls (*Tyto alba*), as a natural solution for pest control and thus reducing dependency on chemical pesticides (Tohiran *et al.*, 2024).

Aside from pests, oil palm disease is a serious threat to Malaysian oil palm plantations, with the most prevalent being basal stem rot (BSR) caused by *Ganoderma boninense*, which may cause economic losses of up to 80.0% (Zakaria, 2023). During the 37-month observation period, BSR progressed significantly faster in oil palms planted in peat soil, with 93.8% reaching critical infection (Stage 4) compared to 62.5% in mineral soil, most likely due to higher organic matter and nutrient deficiencies, such as copper and zinc, in peat soil (Virdiana *et al.*, 2024). A genomic study of *G. boninense* isolates from three Malaysian oil palms revealed seven haplotypes with modest genetic variation (0.1%-0.9%), indicating low genetic recombination and strong population similarity (Roslan *et al.*, 2024). In addition, Syarif *et al.* (2025) identified 53 arthropod species (Coleoptera) and *Eumorphus* sp. beetle associated with *Ganoderma* basidiocarps in oil palm plantations as probable vectors of BSR disease.

Detection strategies, such as deep learning techniques and dedicated drone cameras through hyperspectral image processing, have significantly improved *Ganoderma* infection detection in oil palm canopies, with accuracy rates ranging from 70.0% to 92.5% (Izzuddin *et al.*, 2024). Simultaneously, employing nanotechnology to enhance azole-based fungicide delivery provides a game-changing

solution for managing BSR disease in oil palm plantations, increasing fungicide efficacy by up to 75% compared to earlier techniques (Asmawi *et al.*, 2024). The incorporation of fungal biocontrol agents (BCA) into integrated disease management (IDM), especially BSR, represents a significant advancement in sustainable agriculture by utilising natural microbial communities for safe disease control, reducing synthetic chemical impacts, improving plant health and yield, and supporting United Nations Sustainable Development Goals (UNSDG) (Sundram *et al.*, 2023). Recent advances in early detection and resistance breeding highlight the need for integrated approaches that combine cultural practices, biological management, and resistant varieties to promote plant health and sustainability in oil palm plantations (Karunarathna *et al.*, 2024).

In Malaysia, the integrated pest management (IPM) and IDM are designed to provide a sustainable approach to pest and disease control. However, concerns persist regarding pesticide, herbicide, and fungicide residues in oil palm and its extracted oils, such as CPO and PKO. Fortunately, most pesticides used in oil palm plantations degrade quickly, minimising the risk of groundwater contamination. Analysing pesticides in palm oil matrices presents significant challenges, particularly in multi-component analyses. Ping and Maurad (2024) provided an overview of recent advancements in analytical methods for detecting pesticides in CPO and PKO, ensuring food safety and regulatory compliance. The high lipid content and diverse fatty acid profiles of palm oil and PKO can create substantial matrix effects, complicating pesticide extraction and detection.

Malaysia has addressed the challenges facing the industry by establishing long-term solutions balancing economic growth, environmental preservation, biodiversity conservation, and social welfare (Ali *et al.*, 2024). The adoption of modern technologies in management practices, harvesting, and productivity activities, as well as infrastructure improvements and the implementation of supportive government funding and enforcement policies, such as primary forest conservation for local inhabitants and biodiversity, have all contributed to the industry's positive growth. Shehu and Salleh (2024) agreed that Malaysia serves as an example for other countries, including Nigeria, which might benefit from its approach to revitalising the sustainable palm oil industry.

Biotechnology: Key Solutions for Sustainable Future

The MPOB-Angola oil palm germplasm provided a high level of genetic variability within the selected *dura* and *pisifera* parental lines, which could effectively broaden the genetic base of the

current parental lines used for commercial seed production. The potential of the MPOB-Angola germplasm to further improve oil yield, in addition to its other useful secondary characteristics, provides breeders with a good source of new genetic material for breeding and selection (Norziha *et al.*, 2024). An exotic germplasm from Nigeria that demonstrated a high level of genetic variability for all yield-related traits has also been introduced into existing breeding populations to develop new high-yielding planting materials (Malike *et al.*, 2024).

One of the main challenges facing the oil palm industry is the abnormal fruits observed in clonal palms, including the mantled somaclonal variant, which can lead to a reduced oil yield. Karma-EgDEF1 methylation was shown to alter upon clonal propagation, resulting in high mantling rates upon recloning (Ooi *et al.*, 2024). The application of the single nucleotide polymorphism (SNP) array demonstrated in a quantitative trait locus (QTL) study involving a selfed Nigerian oil palm family (T128) allowed the identification of the genomic region associated with the compactness trait, including candidate genes with the QTL regions (Ting *et al.*, 2023).

The selection of ideal planting materials that are resistant or tolerant to BSR could also be expedited with the availability of genomics and molecular tools, including an RNAi-mediated gene silencing approach. A study involving the manipulation of the *lanosterol 14 α -demethylase* (*GbERG11*) gene of *G. boninense* via RNAi-mediated gene silencing showed a reduction in both fungal growth and gene expression, as well as a loss of ergosterol production, an important cell membrane sterol in fungal pathogenicity (Lim *et al.*, 2024a).

Secondary metabolism in different oil palm fruit tissues at the ripening stage was also explored using the proteomics and metabolomics approaches (Hassan *et al.*, 2024). Several enzymes and metabolites associated with stilbenoid biosynthesis were determined through profiling of the proteomes and metabolomes of the tissues. The presence of stilbenoids, along with other proteins and metabolites in oil palm fruit, indicates their potential as candidate compounds for improving oil palm yield and quality. Selecting and breeding oil palm varieties rich in natural antioxidants will enhance the safety of oil palm products and improve the health benefits associated with palm oil consumption.

Efforts to improve oil palm planting materials through *Agrobacterium*-mediated genetic engineering were pursued by constructing new and efficient transformation vectors carrying different genes, promoters, and backbones and testing them in oil palm tissues (Hanin *et al.*, 2024). Recently, Norfaezah *et al.* (2024) reported the first breeding innovations through the DNA-free clustered regularly interspaced short palindromic repeats/

associated protein 9-ribonucleoproteins (CRISPR/Cas9-RNPs) genome editing in oil palm protoplasts. Oil palm breeding advancements using this platform could accelerate the targeted development of desirable traits in the oil palm. In addition, genetic manipulation of multiple transgenes for the production of high oleic palm oil using the MSP-C6 promoter deployment strategy may also accelerate the development of planting material with improved nutritional quality (Badai *et al.*, 2023).

Mechanisation and Automation

Industry Revolution 4.0 (IR4.0) enhances efficiency, productivity, and precision while reducing costs and environmental impact. However, its implementation requires significant financial and technical support. Key initial steps include centralising data through big data and cloud computing and integrating automation and the Internet of Things (IoT), which improve decision-making with real-time insights and boost productivity by enabling early problem detection and optimising equipment efficiency.

There have been several advancements in R&D focused on mechanisation and automation. Hexa IoT developed an AIoT solution, Luxio-AI, to aid workers in determining palm fruit harvest readiness with ease. The solution can automatically sort palm fruit harvest readiness by visual means. IoT solutions also include Pandora software that aids in various stages of the plantation process, *i.e.*, to accurately determine upcoming weather conditions and detect soil quality (Wong & Koo, 2024). Furthermore, a LiDAR sensor-based spraying system has the potential to reduce chemical usage by 25% through targeted spraying, subsequently leading to an overall reduction in the amount of chemicals used. This system integrates traceability via IoT, which enhances productivity and reduces the need for labour (Bakri *et al.*, 2023).

While robotic solutions for harvesting show promise, their high cost and complexity currently limit widespread adoption. Therefore, an innovative lifting device has been developed to aid in loading FFB in the palm oil industry. This device utilises a simple hydraulic tipping system and can handle 400 kg in just 40 s, resulting in a 35% reduction in loading time. A comparative study of various FFB harvesting methods revealed that the manual method is the least efficient, with a labour-to-land ratio of 1:21 ha, even though it has the lowest capital and operational expenses. The mechanised cutters, such as CANTAS (RZTech Resources Sdn. Bhd., Malaysia), are more efficient, with a labour-to-land ratio of 1:35 ha, albeit with higher initial costs. Despite their high upfront costs, mid-size harvesting machines cover the most land for each worker involved, with a labour-to-land ratio of 1:86 ha,

ultimately reducing operational expenses due to increased efficiency (Ramli *et al.*, 2023). Despite these challenges, these advancements represent significant strides towards increasing productivity and sustainability in Malaysia's palm oil industry.

Biomass, Bioenergy and Biorefinery

The country's palm bioenergy potential could reach 63.5 million tonnes of oil equivalent, fulfilling 64% of the primary energy demand. Optimising second-generation feedstocks could unlock an additional 31.7 million tonnes, increasing the renewable energy share to 38% and reducing GHG emissions by up to 57% from 2005 levels (Gourich *et al.*, 2023).

At the heart of this transformative shift is a cutting-edge technology: The Novel-Lignoripper Snipper (Hur Far Engineering Works Sdn. Bhd., Malaysia) press machine, which efficiently transforms OPB into short fibres suitable for renewable energy feedstock. Biopellets made from OPB using this machine in combination with a pelletising unit meet international quality standards (Fazliana *et al.*, 2024).

Palm-pressed mesocarp fibre (PPMF), having approximately 11% residual oil, could be diversified to produce hydrogels from its holocellulose component using citric and acetic acids as cross-linkers (Teh *et al.*, 2024). Additionally, ferulic acid, a low-toxicity compound with various health benefits, can be extracted from EFB using a deep eutectic solvent made from choline chloride and acetic acid, with the process allowing for recycling of the solvent to enhance yields with each extraction (Ng *et al.*, 2024).

Biodiesel blends, typically composed of 5% to 35% biodiesel mixed with conventional diesel, have uncertain impacts on emissions and fuel consumption. A study on six VOLVO FM400 I-Shift trucks using B10 and B20 revealed that engine oil quality met manufacturer standards within the 30,000 km service interval without significant maintenance differences (Daryl Jay *et al.*, 2024). The palm oil industry could potentially contribute to the reduction of carbon emissions and enhance sustainability, encouraging other sectors to adopt biodiesel by using higher blends like B20 or B100, which can also help to meet local demand and reduce import dependency (Yung *et al.*, 2024). While palm biodiesel is well-established in the lowlands, its performance in the highlands is uncertain. A trial in a highland, *i.e.*, Cameron Highlands, showed that Euro 2M blends work well at 15°C for B20 and 20°C for B30, while Euro 5 blends up to B30 have no startability issues. On-road tests confirmed that diesel vehicles using the three blends up to B30 are suitable for Malaysia's highlands with no technical problems encountered (Nursyairah *et al.*, 2024).

A renewable diesel with 73% C16 hydrocarbon content that can meet Ultra Low Sulphur Diesel (ULSD) specifications, making it compatible with existing engines, was successfully prepared (Nur Azreena *et al.*, 2024). In addition to biodiesel, there is a growing effort to diversify palm biodiesel into bio-lubricants by utilising high-oleic palm methyl ester (HO-PME) and trimethylolpropane (TMP) as substrates for enzymatic transesterification. Recent research has identified TMP and HO-PME as competitive inhibitors of the lipase enzyme (Novozyme 435) used in this process, providing critical insights for large-scale production of biodegradable lubricants that offer improved biodegradability and lower toxicity compared to conventional lubricants (Wafti *et al.*, 2023). Furthermore, OPB is being explored as a feedstock for furfural production, a versatile biorefinery platform chemical for biofuels and fuel additives. A collaborative effort between MPOB and Tsinghua University, China has developed a non-isothermal kinetic model that predicted a 71% yield of furfural from EFB through intermittent steam discharge. This integrated process not only enhances sustainability and green chemistry but also promotes responsible waste management, supporting a circular economy by incentivising millers to improve milling efficiency (Ouyang *et al.*, 2023).

Additionally, certifying residual oils from palm oil mills offers significant environmental and economic benefits for sustainable fuel production. The International Sustainability and Carbon Certification (ISCC) includes feedstocks like EFB, palm oil mill effluent (POME), and PPMF. Malaysia could potentially recover 0.7 million tonnes of waste oil annually for biofuel under ISCC. By utilising biomass and residual oils, mills can diversify their product portfolios, minimise environmental impact, and support the transition to a low-carbon economy. This globally recognised certification ensures that such oils are sustainably sourced and processed into biodiesel and other biofuels that meet stringent standards (Lau *et al.*, 2024).

Palm Oil and Its Nutritional Benefits

Palm oil is known for its distinctive reddish-orange colour due to its high carotenoid content. One characteristic of palm oil is its comparatively balanced fatty acid composition. Saturated and unsaturated fatty acids have the same compositional makeup (Arias *et al.*, 2023), making them suitable for various food product applications. The relatively low linoleic acid (C18:2, 10%-11%) and higher palmitic acid (C16:0, 44%-45%) content in palm oil increases its heat stability and makes it more resistant to oxidative deterioration during food and food product preparation (Loganathan

et al., 2022) compared to other vegetable oils with high polyunsaturated fats. Polyunsaturated oils such as SFO, SBO and corn oil are particularly susceptible to oxidation and are therefore less suitable for prolonged cooking. Palm oil or its blends are preferable for prolonged cooking as they help to maintain oil quality and delay lipid oxidation. It was found that the presence of antioxidants can increase the oxidative stability of the oil, but their effectiveness decreases with improper storage and prolonged exposure to heat (Loganathan *et al.*, 2022).

The World Health Organization (WHO) recently conducted an overview of systematic reviews highlighting the significant impact of palm oil intake on cardiovascular health, particularly concerning cardiovascular disease (CVD), a major cause of mortality worldwide, and it is particularly prevalent in low- and middle-income countries (WHO, 2024). CVD is often characterised by dyslipidaemia, an imbalance of blood lipids such as cholesterol, with elevated levels of non high-density lipoprotein (HDL) and low-density lipoprotein (LDL) cholesterol associated with an increased risk of CVD. Keeping these cholesterol levels low has been shown to be associated with a reduction in CVD risk. Dietary factors also play a crucial role in determining CVD risk. The health effects of palm oil consumption have attracted considerable attention worldwide due to its saturated fat content. The literature search was performed in March 2021 and was conducted again in January 2022 in various databases. The overview provides pooled analyses of the data from the individual clinical trials by evaluating the results of these studies based on eligibility criteria and statistical analyses. The authors extracted data for comparisons between the consumption of palm oil and other vegetable oils, including a subgroup analysis of PUFA-rich and MUFA-rich oils. The results of the systematic reviews that used a pairwise meta-analysis were reported. The risk of bias in each clinical trial included in the systematic reviews was also determined using validated instruments. The results showed varying effects on lipid profiles, with palm oil intake showing trivial to minimal effects on total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides.

Further to the above, a recent review has updated and summarised the health effects of consumption of various vegetable oils, including rapeseed, peanut, olive, flaxseed, sesame, rice bran, coconut, and palm oils (Voon *et al.*, 2024). The study synthesised evidence from systematic reviews and meta-analyses to evaluate the health outcomes associated with different types of vegetable oils. The study was conducted across 12 databases until 31 July 2023, and identified studies examining the

association of various vegetable oils with health outcomes in adults. The review article included 48 studies, and 206 meta-analyses reported that oils rich in monounsaturated and polyunsaturated fatty acids, such as canola, virgin olive, and rice bran oils, moderately reduce serum total cholesterol (TC) and LDL cholesterol concentrations, while palm oil and coconut oil positively influence lipid profiles by raising the good cholesterol (HDL) levels even though they are also found to raise TC and LDL cholesterol levels (Voon *et al.*, 2024). In addition, palm oil was found to have a neutral effect on i) lipid profile (total/HDL cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B), ii) glycemic outcomes (fasting glucose and insulin), and iii) body weight changes. The review suggested that all vegetable oils included in the study, including palm oil, offer different health benefits, particularly lipid parameters. Therefore, the recommendation for palm oil intake must be carefully examined, particularly when it is used in a balanced diet in tropical populations.

A healthy diet could provide a favourable environment that allows essential bacteria to grow and ferment beneficial metabolites, short-chain fatty acids (SCFA), through various metabolic pathways. SCFA, including linear and branched-chain SCFA (BSCFA), is important for regulating energy, haemostasis, inflammation and appetite. Malaysian dietary guidelines are in line with global recommendations for a healthy diet and emphasise the consumption of fruits and vegetables. However, there is limited research on the gut microbiota and SCFA profiles associated with the Malaysian diet, particularly in relation to palm oil consumption. Acetate, propionate and butyrate are the most abundant SCFAs, with Firmicutes and Bacteroidetes being the predominant phyla and *Faecalibacterium* and *Prevotella* being the major SCFA-producing genera (Yap *et al.*, 2024). Given the uniqueness of Malaysian cuisine prepared with palm oil, there is potential to create a favourable environment for the gut microbiota and improve SCFA production to promote gut health. Therefore, the incorporation of palm oil into the diet may also provide holistic benefits for digestive health. These findings highlight palm oil's potential in functional foods targeting gut health and suggest a need for further research to confirm these effects across different populations in dietary contexts.

Red palm olein contains a high level of carotenoids (rich in beta-carotene, a precursor to vitamin A) in the oil, which plays a role in vision and immune function. Tan *et al.* (2024) studied red palm olein biscuit supplementation to help control vitamin A deficiency (VAD), aiming to prevent childhood morbidity and mortality in developing countries. A significant improvement in retinol and retinol-binding protein-4 levels was observed.

The blood alpha- and beta-carotenes, iron levels, and haematological parameters were increased and thus contributed to a reduction in the prevalence of microcytic anaemia and inflammation. In addition, the control group (palm olein biscuits) was also found to have higher improvements in retinol levels among those with marginal VAD.

A study by Teng *et al.* (2024) on 143 adults with central obesity who consumed red palm olein, virgin olive oil, and virgin coconut oil for 12 weeks revealed that the red palm olein group had higher levels of alpha- and beta-carotenes ($P < 0.05$) compared to the extra virgin olive oil and extra virgin coconut oil groups, which corresponds to the high carotenoid content in the red palm olein. The three diets were found to have comparable effects on cardiometabolic biomarkers, lipid profile, and bone mineral density. Therefore, similar to the well-accepted virgin olive oil and virgin coconut oil, it was suggested that red palm olein is suitable for use in food preparation, especially as a salad dressing, preserving its antioxidant effects.

In addition to carotenoids, palm oil also contains a variety of phytonutrients, notably tocotrienols and tocopherols. These compounds have antioxidant properties that help protect cells from oxidative stress, a factor linked to aging and chronic diseases (Fatah & Tee, 2024).

New Developments in Palm Oleochemical Applications

Grand View Research (2024) projects a 7.0% compound annual growth rate (CAGR) in revenue growth for the global oleochemicals market between 2024 and 2030. Demand growth can be attributed to a shift toward sustainable chemicals. Traditional applications of oleochemicals, such as surfactants, personal care products, and food additives, have been extended to new applications in segments such as biosurfactants, lubricants, and polymers in recent years, which offer substantial prospects for companies in the long run.

A noteworthy advancement has been in the circular economy and waste-to-wealth concept for the development of sustainable building materials by utilising OPB and polyurethanes (PU) (Poopalam *et al.*, 2024). Conventional building materials are manufactured using energy-intensive materials that degrade the environment and contribute to increased GHG emissions. By prioritising eco-friendly building materials and practices, sustainable construction helps to preserve ecosystems, conserve natural resources, and reduce energy consumption. Consequently, this leads to a decrease in GHG emissions and a lower carbon footprint for the construction industry. In addition, Ismail *et al.* (2024) revealed that the incorporation of palm-based dihydroxystearic acid (DHSA) as a

co-polyol to synthesise rigid PU foams significantly affects the PU cell matrix. Open cell content notably increased from 6.83% to 90.99% when the DHSA content was increased from 0.00% to 50.00%. This opens up the potential applications of rigid PU foams as sound absorption materials. This not only fosters environmentally sustainable materials but also contributes to long-lasting solutions for the sustainable construction sector.

Similarly, the lubricant and grease sector has embraced the unique qualities of palm oleochemicals. Fajar *et al.* (2024) have begun tapping into the lubricating power of palm-based bio-lubricant formulations for diesel engines using machine learning and experimental techniques to enhance thermal-oxidative stability and lubricity. The bio-lubricant formulation combines various modified base stocks to meet SAE-30 and SAE-40 diesel lubricant specifications. This study underscores the effectiveness of integrating chemical modifications, machine learning predictions, and blending palm-based stocks to optimise bio-lubricant formulations without the need for prior synthesis or modification of palm olein and palm methyl ester.

Polyglyceryl polyricinoleate (PGPR), an emulsifier for food, cosmetics, and personal care products, is commercially produced by the esterification of polyricinoleic acid and polyglycerol. Since polyricinoleic acid is expensive compared to other polymerised forms of fatty acids, Hoong *et al.* (2024) attempted to produce an analogue made from less costly fatty acids by converting oleic acid to polyhydroxy estolide (Figure 5) and using it as a substitute for polyricinoleic acid to produce PGPR analogues. By tailoring the synthesis parameters and using specific fatty acids, a spectrum of emulsifiers can be produced, each with different properties suitable for diverse emulsion formulations.

Triglycerides from plant oils provide a sustainable alternative for ultraviolet (UV)-curable coatings, which can be used in electronics, inks, coatings, and adhesives. However, the alkene group of unsaturated fatty acids in the triglyceride structure is insufficiently reactive for various polymerisation processes. A bio-based resin was obtained by replacing part of a petroleum-based resin with natural plant oil via UV curing technology, which has key attributes such as low temperature, fast curing, and solvent-less processes (Cheong *et al.*, 2024a).

The synthesis of sulphonated carbon heterogeneous acid catalyst from glycerol pitch is an emerging field exploring the utilisation of this waste resource of the oleochemical industry. A study by Armylisas *et al.* (2024) highlights the synthesis of a sulphonated carbon catalyst from glycerine pitch. Glycerine pitch is a feasible carbon source due to its high organic content, being renewable

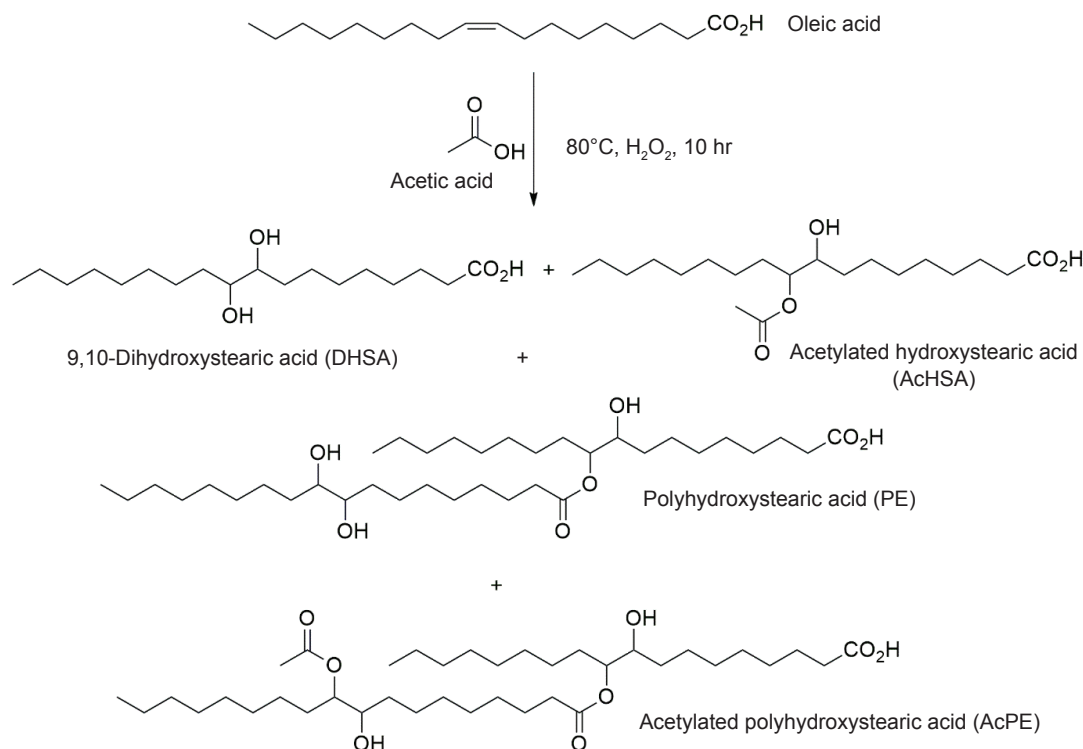


Figure 5. Synthesis of polyhydroxy estolide mixture from oleic acid.

and cost-effectiveness. The catalysts can be used in applications such as hydrolysis, esterification, and transesterification of triglycerides, glycerol acetylation and acetalisation, hydrolysis, and dehydration of biomass into value-added chemicals. This approach simplifies the production process, making it greener by minimising wastewater generation, decreasing energy consumption in biodiesel purification, and reducing production costs.

Aziz *et al.* (2024) investigated the heterogeneous transesterification of palm methyl ester and N-methyldiethanolamine, an alkanolamine feedstock, to produce biobased esterquats. The esterquats provide high di-ester content and resolve all limitations related to the hydrophobicity effect. They offer good biodegradability, with similar chemical and physical properties to commercial esterquats, which make them greener alternatives to conventional petrochemical- and tallow-based surfactants.

Levulinic acid is a promising renewable building block chemical that can be produced from biomass, such as oil palm fronds, oil palm EFB, and oil palm mesocarp fibre, with various applications in pharmaceuticals and cosmetics. The partition and distribution behaviour of the compound are key parameters used for assessing environmental fate, risk assessment, and toxicity in these applications. Understanding the partitioning processes of a compound is important for numerous applications, such as the

development of drug delivery system design and the extraction of the compound in a separation process. The octanol-water system is commonly used to determine the partition coefficient, which is defined as the concentration of a compound in the octanol and water phases after distribution and equilibration. Ramli *et al.* (2024) established a simple procedure for the determination of the distribution coefficient of levulinic acid in octanol based on the OECD 107 method, which reflected the preference of levulinic acid for an aqueous phase (hydrophilic environment) rather than the hydrophobic environment, which may enhance the understanding of its use in product formulation and design as well as assessing the environmental fate.

Meanwhile, the hydrophilic-lipophilic deviation (HLD) concept, which was initially applied in enhanced oil recovery to produce ultra-low interfacial tensions in surfactant/ester oil/water (SOW) systems, is now being used to prepare microemulsions for topical application (Cheong *et al.*, 2024b). The HLD method provides a practical guide for general emulsion formulation of most cosmetic and personal care products. The SOW system exhibits many useful properties and is universally used in various consumer goods, industrial formulas, and crude oil extraction for enhanced oil recovery.

Life cycle assessment (LCA) has long been used as a tool for environmental evaluation of products or services to promote sustainable production.

Several LCA studies have addressed sustainability and environmental issues in the oil palm industry. A cradle-to-gate LCA for the production of palm-based fatty acids in Malaysia was performed (Zolkarnain *et al.*, 2024). The environmental hotspots identified can be used to establish baseline information on the environmental profile, especially the carbon footprint, of fatty acids and assist manufacturers in taking steps to mitigate the environmental impacts. They may explore process improvement using alternative energy sources, energy efficiency technology, biogas, and cogeneration and heat integration systems to reduce dependency on fossil fuels.

CONCLUSION

The Malaysian oil palm industry has been a significant contributor to Malaysia's GDP and export earnings. Market conditions in 2024 indicate a favourable outlook, with rising global demand for palm oil. Malaysia's oil palm sector continues its upward trajectory, benefiting from advancements in agricultural practices and sustainable techniques and capitalising on technological advancements and strategic investments in plantation management and infrastructure. Sustainability remains a priority, with increasing pressures from consumers and regulatory bodies. The increasing demand for sustainable palm oil has prompted producers to improve productivity and sustainability by adopting eco-friendly practices, reducing GHG emissions, and improving land use efficiency, thereby enhancing their market competitiveness.

Meanwhile, the research community is exploring new frontiers, leveraging cutting-edge technologies and interdisciplinary approaches to address evolving needs. Novel cultivation practices, breeding strategies to produce higher-yielding and disease-resistant varieties, IR4.0, biodiesel strategy, product development, and value addition are the main strategies for the industry to remain competitive. The oil palm sector is expected to attain self-sufficiency despite ongoing challenges related to environmental, economic, and welfare issues. It is evident, time and again, that by immersing ourselves in new technologies and employing them routinely, both in research and field practices, we will expedite the transformation of the oil palm industry into a more resilient industry when facing the challenges of the future. As Malaysia navigates the complexities of international trade and competition, continued investment in R&D is crucial in ensuring the industry's competitiveness and long-term viability. Although significant efforts are still needed to remain viable and sustainable, the industry remains committed to face the challenges ahead and thrive for excellence.

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SPENT BLEACHING EARTH AS VALUE-ADDED MATERIAL TO MATERIAL CONSTRUCTION APPLICATIONS: A REVIEW

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ABSTRACT

Crude palm oil (CPO) serves as a significant export commodity for Indonesia, contributing positively to the country's economic growth and generating income for communities. Nonetheless, the production of CPO results in an environmental drawback in the form of refined, bleached and deodorised (RBD) palm oil, which poses environmental concerns. One pivotal material used in the purification process to transform the reddish-orange CPO into a lighter yellow hue is known as bleaching earth. As a consequence, a substantial amount of waste, referred to as spent bleaching earth (SBE), is generated and recovered to prevent harm to the environment. Remarkably, research has revealed that SBE exhibits pozzolanic properties, rendering it suitable for incorporation into construction materials. The primary objective of this study is to emphasise the advantages of integrating SBE into the construction material manufacturing process. Mixtures containing eco-process pozzolan (EPP), the product derived from SBE, have exhibited superior strength characteristics when compared to mixtures using ordinary portland cement (OPC). Furthermore, concrete incorporating pozzolan mixtures has displayed increased strength values when subjected to high temperatures, outperforming concrete made exclusively with OPC. However, further investigation is required to ascertain the thermal resistance capabilities of EPP as a component in mortar or concrete.

Keywords: concrete, crude palm oil, eco process pozzolan, mortar, spent bleaching earth.

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INTRODUCTION

The economies of Indonesia and Malaysia are significantly supported by the palm oil industry. It has not only created income opportunities for individuals and generated employment within the palm oil sector but has also served as a catalyst for economic activity, tax revenue generation, and the augmentation of foreign exchange reserves (Begum *et al.*, 2019; Dey *et al.*, 2020; Maksum *et al.*, 2021; Mareeh *et al.*, 2022). Notably, the area dedicated to oil palm plantations in Indonesia experienced significant growth from 2018 to 2022, with an expansion of land totalling 1.01 million hectares.

Additionally, crude palm oil (CPO) production surged to 3.94 million tonnes from 2018 to 2022. Table 1 presents data on CPO production and oil palm plantations in Indonesia from 2018 to 2022.

TABLE 1. INDONESIA CPO PRODUCTION AND OIL PALM PLANTATION FROM 2018-2022

Year	CPO production (million tonnes)	Oil palm plantation (million hectares)
2018	42.88	14.33
2019	47.12	14.46
2020	45.74	14.59
2021	45.12	14.62
2022	46.82	15.34

Source: Badan Pusat Statistik Indonesia (2023).

Malaysia, like Indonesia, is one of the greatest palm oil producers in the world, with 5.74 million hectares of oil palm plantations and approximately 18 million tonnes of CPO produced in 2021 (Parveez *et al.*, 2022).

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The process of refining CPO into refined, bleached and deodorised (RBD) palm oil requires bleaching earth as a colour-removing agent. After use, bleaching earth becomes a by-product of this process. The use of bleaching earth is proportional to the amount of RBD palm oil produced. The larger the volume of RBD palm oil produced, the greater the amount of bleaching earth needed, consequently, this affects the amount of SBE produced, resulting in an escalation.

The eco process prozzolan (EPP), or solid extraction from spent bleaching earth (SBE), has pozzolanic characteristics. Recent developments have highlighted the growing utilisation of EPP as a component in blended cement production. Although research on EPP as a pozzolanic material is limited, waste products with pozzolanic characteristics are being used in concrete as cement substitutes. This approach aims to minimise the use of traditional cement, thereby reducing the release of CO₂ associated with cement production.

Cement plants were responsible for a significant contribution of 2.9 billion tonnes of CO₂ emissions all over the world in 2021, according to the data that is currently available (Kaptan *et al.*, 2024). However, in 1990, the level of CO₂ emissions that was documented was 0.57 billion tonnes (Benhelal *et al.*, 2021). The emission of CO₂ during the manufacturing of 1 t of Portland cement is predicted to range from 0.73 to 0.99 t throughout various geographical regions (Zhu, 2011). It can be asserted that the manufacturing of 1 kg of Portland cement results in the emission of about 1 kg of CO₂ into the atmosphere (Adesina, 2020).

By incorporating pozzolanic materials like EPP in concrete, the reliance on cement can be reduced, resulting in lower CO₂ emissions. The use of EPP as a pozzolanic material offers an environmentally friendly solution to decrease the carbon footprint associated with the cement industry. By utilising waste products with pozzolanic characteristics, the goal is to create more environmental-friendly concrete mixtures and contribute to the reduction of CO₂ emissions in the cement manufacturing process.

CPO as Raw Material for RBD Palm Oil

CPO is a vegetable oil that contains solid triacylglycerides or triacylglycerols, as well as various other components including lipases, free fatty acids (FFA), pigments, phosphatides, partial glycerides, colouring agents and carotene. The yellow and red colour in CPO is caused by the intensity of carotenoid pigments. These pigments not only affect the colour of the CPO but can also impact the clarity level of the CPO (Ifa *et al.*, 2021).

CPO is produced through a series of procedures within the palm oil production process. Palm oil can

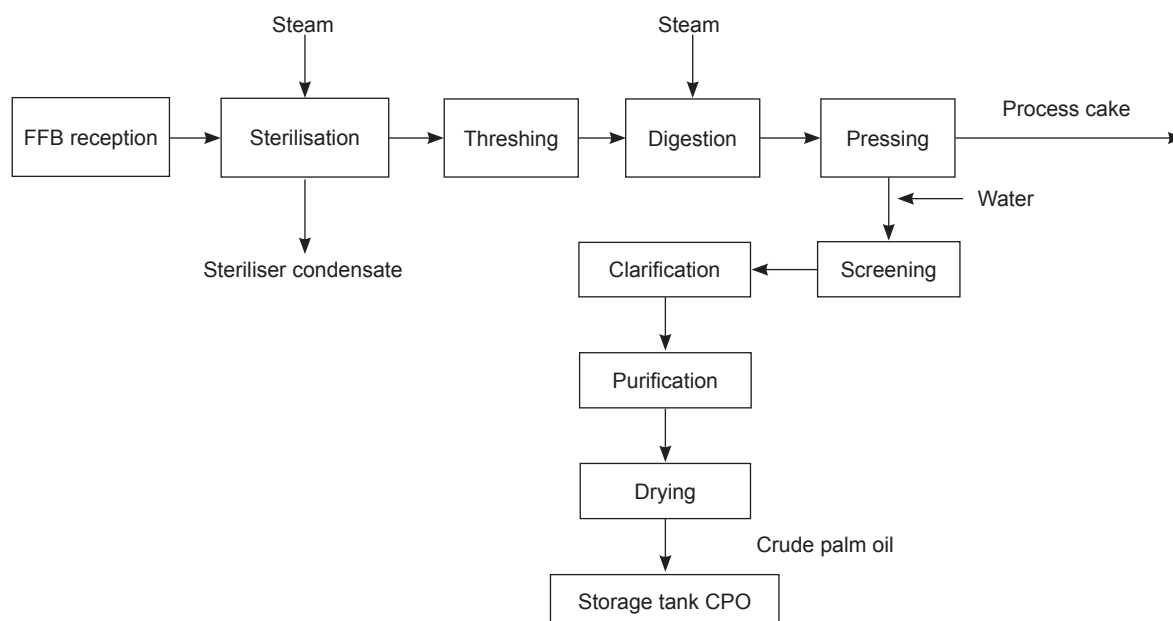
also undergo fractionation, a process that separates it into two distinct forms: Olein (liquid) and stearin (solid). Cooking oil and frying oil typically use olein as their base ingredient, while stearin is commonly employed in food processing and solid fat compositions (Hong, 2023).

In *Figure 1*, the production of palm oil begins with the processing of oil palm crops. Upon arrival at the palm oil mill, fresh fruit bunches (FFB) go through several stages, including reception, sterilisation, threshing, digestion, pressing, clarification, purification and kernel recovery. Before being placed into the hopper and cage to start the process of FFB into CPO, FFB are first examined for their level of ripeness and characteristics. The quality of FFB can be distinguished by observing the ripeness degree of the FFB, in which a ripe FFB should have an OER of at least 21% and FFA less than 5% (Sharif *et al.*, 2017). FFB are then placed into a horizontal steriliser using cages and subjected to a specific temperature and pressure for several minutes, following the standards set by the factory (Hong, 2023). This sterilisation process halts the enzymatic process, preventing an increase in the FFA content and conditions the palm kernel to reduce cracking during subsequent processing.

Following sterilisation, the threshing process is employed to separate the steamed fruit from the bunch, preparing it for further processing. Digestion comes next, breaking down the oil cells before the extraction and separation of the mesocarp from the kernel. The pressing process is then utilised to extract oil from the digested palm fruit. A screw press is used to squeeze the mashed and chopped fruit fibre from the digester, yielding crude oil. This oil product consists of a blend of solids and a cake comprising kernels and fibre.

Subsequently, the clarification stage takes place. At this point, approximately 60% of the extracted crude oil is oil, 24% is water and 10% is non-oil solids (NOS). The coarse contaminants in crude oil are separated by diluting the oil with hot water and passing it through a vibrating screen. Following this step, the purified oil undergoes clarification in a vertical settling tank or clarifier. Within this tank, the different phases naturally segregate due to gravity. The oil rises to the top and is skimmed off before undergoing purification through a high-speed centrifuge to eliminate any remaining impurities. Finally, CPO is transferred into a storage tank before it is dispatched to refineries for further processing.

One effective strategy for enhancing the value of palm oil products is to transform them into oleochemical products. Currently, the oleochemical industry offers substantial market opportunities, with global demand for oleochemicals exceeding supply by approximately 290,000 t (Fajarika *et al.*, 2023).



Source: Hong (2023).

Figure 1. Process production of CPO.

Beyond its conventional applications as a vegetable oil in the food industry and for biodiesel production, CPO boasts properties that are particularly beneficial to the health sector, notably the pharmaceutical industry. Notably, tocotrienol, a potent antioxidant found in palm oil, strengthens the immune system and complements the components of vitamin A and E. Palm oil comprises various components that promote human health, including alpha-carotene, beta-carotene, gamma-carotene, sources of vitamin E (tocopherol, tocotrienol), lutein, sterols, unsaturated fatty acids and ubiquinone (Rada-Bula *et al.*, 2023).

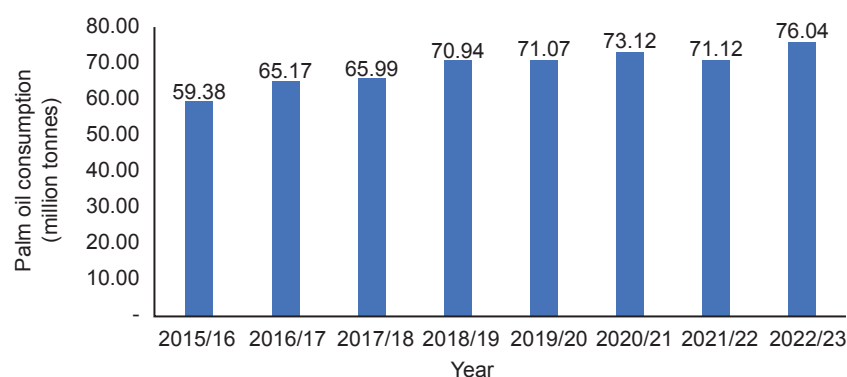
Alpha-carotene reduces the risk of liver, lung, pancreas and stomach cancers, while beta-carotene helps in lowering the risk of heart disease and maintaining eye health. Lutein contributes to a reduced risk of epithelial cancer and cataracts, while ubiquinone aids in enhancing the immune system, preventing heart disease and hypertension, and safeguarding red blood cells from oxidative damage. All of these components underline the multitude of benefits that palm oil offers to the pharmaceutical industry (Alfin, 2021).

In addition to the pharmaceutical sector, palm oil finds extensive use in the cosmetics industry due to its vitamin E content. Palm oil assists in maintaining smooth skin, eliminating acne and promoting healthy hair. It is a suitable remedy for dry and dandruff-prone hair. Utilising natural substances like palm oil in cosmetics is considered a safer and more organic alternative to synthetic skincare products. While CPO, obtained through palm fruit processing, contains several health-beneficial

components, additional processing is required to fully harness its benefits (Alfin, 2021).

Approximately 60% of the global vegetable oil trade and 25% of worldwide consumption are attributed to palm oil, making it one of the most widely produced vegetable oils globally. Palm oil used globally 24% to serve industrial purposes, while 74% is employed in food products. Figure 2 illustrates the demand for palm oil over the past few years (Shahbandeh, 2023).

Nonetheless, the production of CPO often faces scrutiny due to its emission of greenhouse gases (GHG) and other sustainability concerns. The primary greenhouse gases in earth's atmosphere include carbon dioxide, methane, nitrous oxide and hydrofluorocarbons (Matthew, 2023). Recognising the importance of addressing global climate change, Indonesia and Malaysia, the world's top two palm oil producers, have made substantial efforts to adopt sustainable practices in palm oil production and improve their carbon efficiency. To achieve this objective, both countries have initiated programmes to repurpose waste generated from palm oil processing. Various measures have been implemented, including the utilisation of waste as biomass derived from palm kernel shells, empty fruit bunches, and fibre. The palm oil industry produces substantial quantities of these by-products, presenting opportunities for their conversion into biodiesel, bioenergy, industrial materials, composting, animal feed production and the efficient utilisation of palm oil waste residues (Cheah *et al.*, 2023; Gani *et al.*, 2023). Life cycle assessments have also been conducted to evaluate the environmental



Source: Shahbandeh (2023).

Figure 2. Palm oil consumption worldwide.

impact of CPO production (Chuen & Yusoff, 2012; Nijaya *et al.*, 2008). Substantial endeavours have been undertaken to enhance the sustainability of the oil palm industry, focusing on environmental, social and economic aspects.

Bleaching Earth as Bleaching Material

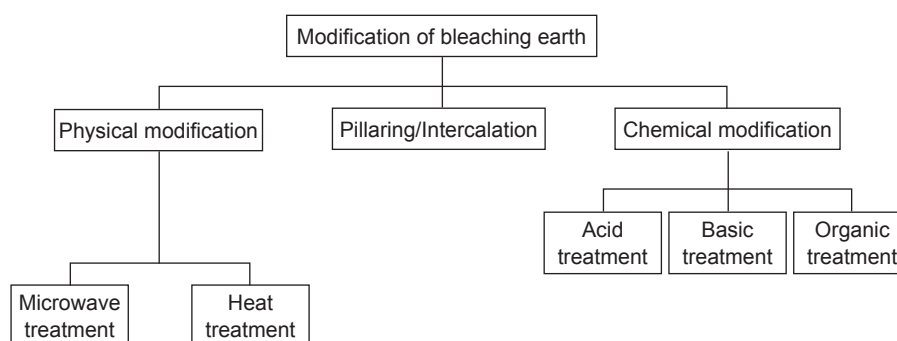
Bleaching earths typically comprise up to three distinct types of clay minerals with varying compositions: Bentonite, attapulgite, and sepiolite. These clay minerals consist of aluminium silicate crystals (SiO_2 , Al_2O_3), bound water, alkali metals [such as calcium oxide (CaO) and magnesium oxide (MgO)] and other transition metals [e.g., iron oxide (Fe_2O_3)], represented by the formula $\text{Al}_2\text{O}_3 \cdot 4\text{SiO}_2 \cdot n\text{H}_2\text{O}$ (Ashari *et al.*, 2017).

The remarkable capacity of bleaching earth to adsorb colourants and other undesirable components during the production of edible oil is due to this composition (Jumaah *et al.*, 2019). This feature makes it an invaluable resource in the edible oil production process, whether it is natural or activated bleaching earth. The goal is to enhance various aspects of the final products, including their overall quality, flavour, aroma and consistency. Factors such as particle size distribution, surface

area, porosity and surface activity, along with mineralogical structure and characteristics, influence the minerals' ability to adsorb unwanted particles.

The performance of bleaching earth in adsorbing colourant particles is primarily attributed to the aluminium ions present on the adsorbent's surface. Bentonite clay can be processed into activated bleaching earth but has a higher concentration of montmorillonite. Activation refers to the application of chemical or physical treatments to specific clay types to enhance their ability to absorb colouring matter and other impurities in oils and solutions (Babaki *et al.*, 2008; Diaz & De Souza Santos, 2001). These treatments or modifications can be categorised into three main groups as shown in Figure 3: (1) Physical modification, involving changes in chemical composition and crystalline structure through high-temperature or microwave treatment, (2) chemical modification, typically altering the structure and surface functional groups, and (3) pillaring treatment, a combination of chemical and physical processes that enhance the clay mineral structure's adsorption capacity or create spaces for the adsorption of specific ions (Hussin *et al.*, 2011).

Acid treatment stands as a widely employed chemical modification of bleaching earth (Komadel



Source: Husin *et al.* (2011).

Figure 3. Modification of bleaching earth.

et al., 2006). In this process, mineral acids like hydrochloric acid (HCl) or sulphuric acid (H_2SO_4) are utilised to activate the adsorbent. The acid treatment increases the adsorbent's surface area by dissolving or reacting with materials such as tar, calcium, and magnesium salts that coat its pores. This enhancement significantly improves the adsorbent's bleaching capacity (Tanjaya *et al.*, 2006). The presence of silanol (Si-OH) groups on the adsorbent's surface, in proportion to the amount of silica dioxide (SiO_2) present, allows for the absorption of free fatty acids, organic materials and other polar chemicals like peroxides (Yang, 2003).

The activation process begins with the exchange of H^+ ions from the acid with cations from mineral salts (Ca^{2+} and Mg^{2+}) in the bentonite interlayer. Subsequently, Al^{3+} ions and other metal ions, such as Fe^{3+} , are dissolved from the lattice layers of bentonite. This dissolution imparts a negative charge to the bentonite, thereby enhancing its capacity to absorb acid, resulting in activated clay. Furthermore, the dissolution of ions during acid activation contributes to an increase in the clay's surface area (Hussin *et al.*, 2011).

Volcanic ash that undergoes natural transformation over the years can serve as a raw material for the formation of bleaching earth. These bleaching earths can be found in regions with volcanoes, such as in Europe, North and South America, the Middle East, and Asia, including Indonesia. The extensive utilisation of activated bleaching earth in the global market is estimated to reach around USD4.00 billion by the year 2032, with a projected Compound Annual Growth Rate (CAGR) of 5% from 2022 to 2032. In 2022, the estimated market value for activated bleaching earth is expected to be USD2.46 billion (Nikhil,

2022). The increasing demand for edible oils due to the world's expanding population is a primary driver of the demand for activated bleaching earth.

Additionally, the growing interest in biodiesel production has further fueled the demand for activated bleaching earth (Oils & Fats International [OFI], 2016). Moreover, developed countries are witnessing a rise in the adoption of healthier eating habits and low-fat oils, which is contributing to the demand for activated bleaching earth in North America and Europe (Nikhil, 2022).

SBE By-product from Refinery RBD Palm Oil Plant

The refining process of CPO into RBD Palm oil typically involves three phases: Degumming, bleaching and deodorisation (Ahmed *et al.*, 2019; Ifa *et al.*, 2021; Nidzam *et al.*, 2022; Tan *et al.*, 2021). Throughout the process of refining at the plant, the bleaching earth becomes a by-product referred to as SBE. SBE is a solid oil waste generated during the refining of CPO and serves as an industrial residue resulting from the bleaching earth's utilisation in the production of edible oils.

The colour of the refined CPO serves as a crucial quality indicator and increasing the quantity of bleaching earth used can result in a lighter-coloured product. However, it is important to note that bleaching earth eventually becomes deactivated after its use in the purification process. This deactivation occurs as the surface of the bleaching earth becomes coated with impurities during the refining of CPO. These impurities encompass phosphatides, gums, metals, fatty acids and dyes present in CPO. Figure 4 illustrates the production process of SBE.

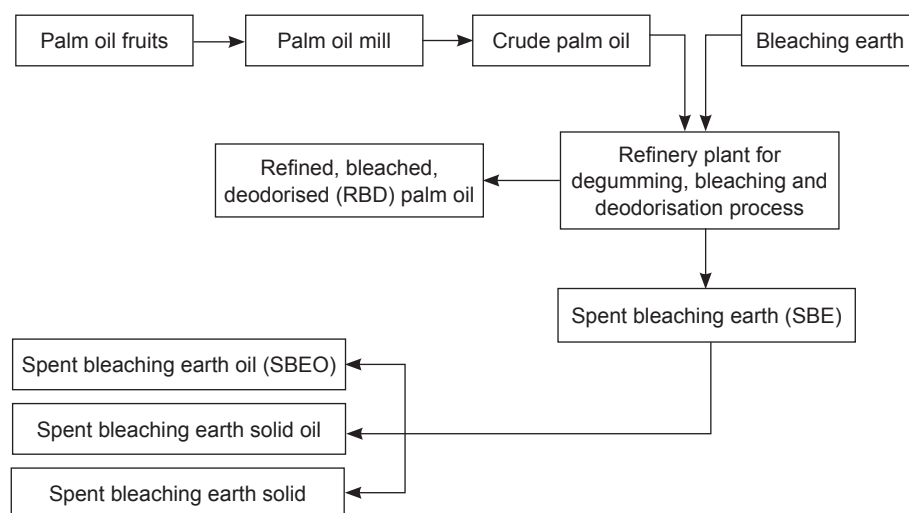


Figure 4. Production of spent bleaching earth (SBE).

The composition of material contained (by %) in SBE is shown in Table 2.

TABLE 2. CHEMICAL COMPOSITION IN SPENT BLEACHING EARTH

No.	Component	%
1	SiO ₂	83.05
2	Al ₂ O ₃	3.93
3	Fe ₂ O ₃	3.57
4	CaO	0.41

Source: Ashari *et al.* (2017).

In accordance with the Republic of Indonesia Law Number 101 of 2014, SBE was classified as hazardous waste under category number 2 (Indonesian Government Regulation, 2014). Consequently, mills were required to designate specific areas for the storage of this waste and ensure its proper disposal by entrusting it to authorised companies for recovery. However, in line with the Republic of Indonesia Law Number 22 of 2021, SBE has been reclassified as non-hazardous waste, thereby allowing private entities to engage in its utilisation and recovery (Indonesian Government Regulation, 2021).

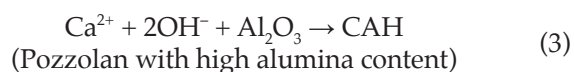
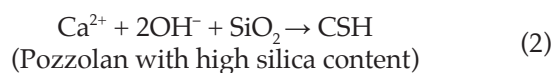
A portion of the SBE waste can be recovered and transformed into sustainable materials, including recovered oil (R-Oil), de-oiled bleaching earth (De-Obe), and EPP. Meanwhile, other waste is appropriately disposed of in landfills or other designated facilities, with waste management practices adhering to national regulations (Research Team PASPI, 2020). SBE comprises several significant components, such as silica oxide, alumina oxide, ferro oxide and oil residues. The oil residues constitute approximately 20%-30% of the waste, alongside solid components like ash. These constituents can be separated to yield two distinct products: The liquid phase, referred to as SBE oil or recovered oil and the solid phase known as SBE Solid-Oil or De-Obe, which contains an oil content of less than 3%. The extraction process responsible for this separation is known as solvent extraction (SE) technology, and it has been implemented for the extraction of SBE (Research Team PASPI, 2020).

The liquid phase recovered oil from SBE has various applications including its use as feedstock for biodiesel and bio-lubricants (Abdulbari *et al.*, 2011; Aladetuyi *et al.*, 2014; Kheang *et al.*, 2007; Suryani *et al.*, 2017; Widyawati & Ufidian, 2017). On the other hand, the solid phase, De-Obe which contains a minimal amount of CPO, can be utilised as a fuel in boilers to generate steam. The produced steam can be employed within the refinery, including in the solvent extraction process or other processes to heat the CPO. After combustion, De-Obe is transformed into either spent bleaching earth solid or EPP (Abdelbasir *et al.*, 2023).

The chemical composition and physical properties of EPP are contingent on the specific bleaching earth utilised. EPP's chemical composition is as follows: Silica dioxide 47.60%, aluminium oxide 11.60%, iron oxide 9.80%, calcium oxide 12.55%, magnesium oxide 6.20% and loss on ignition (LOI) 5.78%. In terms of particle size distribution, EPP exhibits the following characteristics: Particle size $d=10$ (μm) 7.04%, mean particle size $d=50$ (μm) 29.30%, particle size $d=90$ (μm) 80.42% and a specific gravity of 1.93 (Raihana *et al.*, 2019).

Type of Pozzolan

Pozzolan is a substance containing silica or silica-alumina and alumina oxide. Unlike cement, it does not possess inherent binding properties. However, when it takes the form of fine granules and reacts with water, these compounds undergo a reaction with calcium hydroxide at room temperature. This reaction leads to the creation of hydraulic calcium hydrates characterised by relatively low solubility rates (Becerra-Duitama & Rojas-Avellaneda, 2022; Sanchez De Rojas & Frias, 2013). The definition of pozzolan relies not on the material's source but on its capacity to react with lime and water. When pozzolan encounters lime in the presence of water, it releases OH⁻ ions, resulting in a pH increase (typically around 12.4). This pH rise triggers pozzolanic reactions, wherein silicon and aluminium combine with available calcium, culminating in the formation of cementitious compounds known as calcium aluminate hydrates (CAH) and calcium silicate hydrates (CSH). These reactions can be summarised as follows (Pourakbar & Huat, 2016).



The presence of sulphates (SO₄²⁻) in pozzolans has a detrimental effect on pozzolanic reactions because it results in the formation of a highly hydrated mineral known as ettringite [Ca₆Al₂(SO₄)₃(OH)₁₂·26H₂O]. The formation of ettringite is contingent upon the availability of soluble aluminium, calcium and sulphates, elevated pH levels, and the presence of water. High temperatures can expedite the formation

of ettringite and its occurrence can be rapid depending on environmental conditions. Ettringite is characterised by its expansive nature, indicated by the presence of 26 water molecules and this expansion has the potential to harm cementitious materials produced through pozzolanic reactions (Seco *et al.*, 2012).

Furthermore, pozzolanic materials contain significant quantities of silicon, iron and aluminium oxides. To qualify as a pozzolan, the combined weight percentage of these three oxides must be at least 70%. These chemical compounds play a crucial role in the formation of a cementitious gel known as C-S-H. However, the extent of paste formation within the mixture is influenced by several factors, including the specific surface area of the pozzolan, its intrinsic properties, the chemical compounds present, the pozzolan's extraction method and the content of reactive silica.

Physical and Chemical Properties Pozzolan

The physical and chemical properties of pozzolan can be described according to the ASTM Standard C618-12a (2019). The chemical properties of pozzolan in classes N, F and C depend on the composition Al_2O_3 , SiO_2 , SO_3 , Fe_2O_3 , and moisture content. On the other hand, the physical properties are determined by factors such as fineness, strength activity index, water requirement and soundness. The minimum composition of SiO_2 combined with Fe_2O_3 and Al_2O_3 for Class N is 70%, for Class F is 70%, and for Class C is 50%.

The Classification of Pozzolan

According to ASTM C618-92a (1994), the quality standards for pozzolan are classified into three classes based on their physical properties and chemical composition. The quality of pozzolan is considered good if the levels of $\text{Fe}_2\text{O}_3 + \text{SiO}_2 + \text{Al}_2\text{O}_3$ are high and if it exhibits high reactivity with lime. The three classes of pozzolan are as follows: Class N, Class C and Class F.

Natural pozzolan can vary in terms of quality, shape, and colour depending on the specific deposit. For example, the quality of pozzolan in one area like Kalibagor, may differ from that in other areas like Wingi or Blitar. Due to these variations, it is important to standardise the quality of pozzolan using ASTM standards to ensure consistent and controlled quality.

Ordinary Portland Cement Specification

Cement, in general, is a material known for its ability to bind different substances through various reactions, often facilitated by the

presence of water (Bahhou *et al.*, 2021; Paul *et al.*, 2018). Cement is a crucial industrial commodity produced commercially in more than 120 countries, primarily used in the construction of buildings, roads, bridges and other structures. Cement is indispensable in the production of concrete, its utilisation surpassing more than twice that of other building materials involved in the concrete-making process. Ordinary Portland Cement (OPC) stands as the most extensively utilised type of cement, well-suited for a wide range of general concrete construction applications.

The performance of concrete made with OPC strongly hinges on the characteristics of the cementitious binder and the environmental conditions to which it is exposed. Cement production primarily involves grinding raw materials, blending them in specific proportions, and then subjecting them to high-temperature sintering in a large rotating kiln, resulting in small gray clinker pellets. After cooling, these clinkers are mixed with a minor quantity of gypsum to regulate the setting time and are further finely ground in ball mills before packaging. The composition of raw materials utilised in cement production is meticulously controlled within defined parameters. Table 3 (Ali *et al.*, 2008) illustrates the elements in Portland Cement, while Table 4 (Vidican *et al.*, 2008) presents the bogue compounds formed because of chemical reactions during the burning and fusion of raw materials. Table 5 (Sutar *et al.*, 2021) provides detailed information about the composition of these bogue compounds.

TABLE 3. CHEMICAL CONSTITUTIONS OF OPC

Components	Percentage (%)
Lime (CaO)	60.0 to 67.0
Silica (SiO_2)	17.0 to 25.0
Alumina (Al_2O_3)	3.0 to 8.0
Iron oxide (Fe_2O_3)	0.5 to 6.0
Magnesium oxide (MgO)	0.1 to 4.0
Sulphur trioxide (SO_3)	1.0 to 3.0
Soda and/ or Potash ($\text{Na}_2\text{O}+\text{K}_2\text{O}$)	0.5 to 1.3

Source: Ali *et al.* (2008).

TABLE 4. BOGUE COMPOUNDS

Compound abbreviated designation	Abbreviated designation
Tricalcium silicate ($3\text{CaO}.\text{SiO}_2$)	C3S
Dicalcium silicate ($2\text{CaO}.\text{SiO}_2$)	C2S
Triacalcium aluminate ($3\text{CaO}.\text{Al}_2\text{O}_3$)	C3A
Tetra calcium alumina ferrite ($4\text{CaO}.\text{Al}_2\text{O}_3.\text{Fe}_2\text{O}_3$)	C4AF

Source: Vidican *et al.* (2008).

TABLE 5. COMPOSITION AND COMPOUND OF PORTLAND CEMENT

Portland cement	Normal	Rapid hardening	Low heat
(a) Composition (%)			
Lime (CaO)	63.1	64.5	60.0
Silica (SiO ₂)	20.6	20.7	22.5
Alumina (Al ₂ O ₃)	6.3	5.2	5.2
Iron oxide (Fe ₂ O ₃)	3.6	2.9	4.6
(b) Compound (%)			
C3S	40.0	25.0	25.0
C2S	30.0	21.0	35.0
C3A	11.0	9.0	6.0

Source: Sutar *et al.* (2021).

The manufacturing of Portland Cement involves four distinct phases. (1) Raw materials are ground and crushed, (2) blending the materials in the correct proportions, (3) subjecting the created mixture to high temperatures in a kiln, and (4) pulverising the resulting product, referred to as clinker, along with around 5% of gypsum (to regulate the setting time of the cement). The three manufacturing processes are known as the wet, dry, and semidry processes. These terms characterise how raw materials are readied and introduced into the kiln. During the wet process, the raw materials are milled in a wet state and subsequently conveyed as a slurry. In the dry process, before being introduced into the kiln, the raw materials will be pulverised. In the semidry process, before being fed into the kiln, the raw materials are initially ground dry and then moistened to create modules (Fasi, 2018; Sutar *et al.*, 2021).

Types of Portland Cement

The differentiation of OPC cement is based on the different codes in different countries.

Bureau of Indian Standards

Three distinct classes of OPC cements have been classified by the Bureau of Indian Standards (BIS) (2004; 2013; 2015). These grades are as follows: OPC 33 Grade, OPC 43 Grade and OPC 53 Grade cements in Table 6.

The minimum compressive strength that the cement must achieve is indicated by the grade number. For example, the minimum compressive strength of 53 Grade OPC that must be achieved in the 28 days must not be less than 53 MPa, which is equivalent to 530 kg/cm². At this point, it is important to point out that the chemical composition of OPC 33, 43 and 53 grades is identical. The only difference lies in the fact that the higher-grade cement undergoes a more thorough grinding process during the final stage, resulting in a much finer consistency. This results in a product that is far more robust and long-lasting due to its superior strength and durability (Sutar *et al.*, 2021).

OPC 33-Grade Cement

The compressive strength of OPC 33-grade cement is 33 N/mm² referred to Indian standard IS 269:2015, after 28 days. However, this grade of cement has become obsolete in recent years and is no longer in use. Furthermore, cement with a grade of 33 is not suitable for use in the production of concrete with a grade of M20 or higher. It is typically employed for general construction purposes under normal environmental conditions. Nevertheless, the utilisation of OPC 33 has declined due to its lower compressive strength and the ready availability of higher-grade cement.

TABLE 6. OPC COMPRESSIVE STRENGTH

Period	Compressive strength		
	OPC 33-grade ¹	OPC 43-grade ²	OPC 53-grade ³
72 +/- 1 hr	Not less than 16 N/mm ²	Not less than 23 N/mm ²	Not less than 27 N/mm ²
168 +/- 2 hr	Not less than 22 N/mm ²	Not less than 33 N/mm ²	Not less than 37 N/mm ²
672 +/- 4 hr	Not less than 33 N/mm ²	Not less than 43 N/mm ²	Not less than 53 N/mm ²

Note: ¹ IS Code - IS 269: 1989 for OPC, 33 Grade; ² IS Code - IS 8112: 1989 for 43 Grade OPC; ³ IS Code - IS 12269: 1987 for Specification for 53-grade OPC.

Source: Sutar *et al.* (2021).

OPC 43-Grade Cement

According to the Indian standards outlined in IS 8112:2013, the compressive strength of cement is 43 N/mm². This cement is familiar with OPC 43-grade cement, which is well-known. This grade of cement produce concrete with grades as high as M30. Additionally, it finds applications in various areas, including brickwork mortar, precast products like tiles and cement pipes and even plain cement concrete, commonly referred to as PCC.

OPC 53-Grade Cement

This cement is of superior quality and readily available for purchase in the market. Cement with a compressive strength of 53 N/mm² is referred to as OPC 53-grade cement. The cement's curing time is faster in comparison to the OPC 33 and 43-grade cement. Using OPC 53-grade cement makes it easily attainable to achieve any grade higher than M25. This cement variant is specifically employed in the construction of precast walls, concrete roadways, bridges, dams and reinforced concrete structures.

American Standard OPC ASTM C150-07

Portland cement is classified into eight types based on specific specifications, these types include Type I, IA, II, IIA, III, IIIA, IV and V. The cement outlined in this specification shall exclusively comprise the following constituents: Limestone, an air-entraining addition for air-entraining Portland cement, Portland cement clinker, processing additions and water or calcium sulfate or a combination of both (Fasi, 2018).

European Standard OPC EN 197

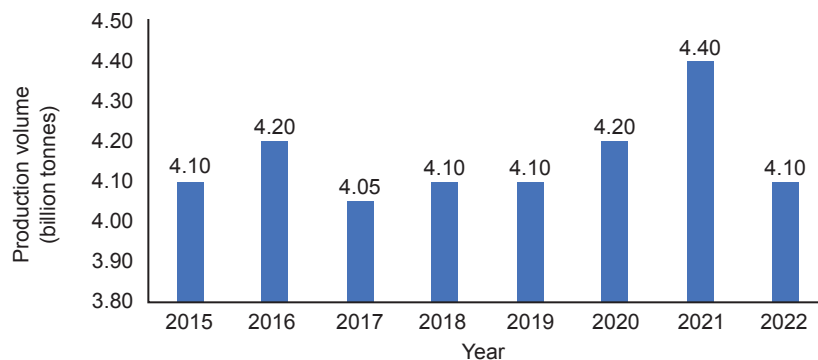
The OPC standard for Europe, as per EN 197, has five types including CEM I, CEM II, CEM III, CEM IV, and CEM V based on composition, specifications and conformity criteria for common cement (Fasi, 2018).

In 2022, the global cement output reached an approximate volume of 4.10 billion tonnes (Figure 5). In 1995, the worldwide production of cement was just 1.39 billion tonnes, demonstrating the significant expansion of the building sector since that time (Statista Research Department, 2023).

The Strength Analysis of the Use of Processed Spent Bleaching Earth (PSBE) in Concrete

Various studies have explored the potential utilisation of EPP as a sustainable alternative to cement in different construction applications. Kusaimi *et al.* (2020) conducted a study concentrated on integrating EPP as a cement substitute in pavement blocks. The research aimed to evaluate the performance of pavement blocks formulated with EPP, considering properties such as water absorption and compressive strength. Two variations of paving blocks were formulated: Set A, where EPP partially replaced cement in pavement formulation (ranging from 20% to 90% substitution) and Set B, which integrated both EPP and FA as some cement replacements. The research indicated that adding FA resulted in pavement blocks achieving a maximum compressive strength of 36 MPa when EPP was added at a rate of 15%-20%. It was concluded that the maximum EPP addition to the pavement formulation should not exceed 20%.

Yunus *et al.* (2019) investigated the compressive strength of EPP concrete under chloride and sulphate exposure conditions. The study found that concrete containing EPP exhibited higher compressive strength compared to concrete without EPP when exposed to water for 28 days. This suggested the occurrence of chemical interactions involving the silica in EPP and the calcium hydroxide led to pozzolanic reactions released during OPC hydration, contributing to concrete strength development. However, exposure to a 3.5% sodium chloride solution led to a decrease in compressive strength, highlighting the need to consider specific exposure conditions for optimal EPP concrete performance.



Source: Statista Research Department (2023).

Figure 5. Global cement production volume from 2015 to 2022.

Raihana *et al.* (2019) focused on investigating EPP properties and their potential as a cement substitute in mortar and concrete. The study concluded that EPP exhibited pozzolanic properties due to its high SiO₂ content, making it suitable as a partial cement replacement. The research highlighted that up to 20% EPP may serve as a substitute for cement in mortar and concrete, contributing to reduced environmental impact associated with cement production.

Kho (2021) explored partial cement replacement by EPP and superplasticisers in concrete. The study found that the addition of EPP led to changes in workability, making the concrete easier to compact. However, the use of superplasticisers improved workability and strength, achieving comparable compressive strengths to 100% OPC concrete.

Jones and McCarthy (2005) investigated foamed concrete using EPP as a cement substitute. The research attained a compressive strength of 28 MPa at a 30% EPP replacement of cement in foamed concrete, suggesting its viability for structural applications.

Rokiah *et al.* (2019) examined the influence of PSBE on foamed concrete's compressive strength and observed that replacing 30% of the cement with PSBE enhanced compressive strength and other properties of the foamed concrete mixture.

Othman *et al.* (2021) explored the use of PSBE as a partial cement substitute in foamed concrete, achieving the desired density, stability, workability, and significantly increased compressive strength.

Ahmat *et al.* (2023) in geopolymers concrete, substituting FA with EPP within the range of 10% to 30% yields compressive strengths above 28 but does not reach 40 MPa.

Those studies collectively demonstrate the potential of EPP in enhancing concrete performance while reducing reliance on traditional cement, contributing to sustainable construction practices. For a detailed comparison of compressive strength from various experiments, refer to Table 7.

Fire Resistance Properties in Concrete with Additional Material

Concrete has inherent fire-resistant properties, which make it suitable for applications where fire protection is important (Korytchenko *et al.*, 2021). Low thermal conductivity is a factor in the fire resistance of concrete, along with a high thermal inertia factor. The low thermal conductivity of concrete slows down the transfer of heat during a fire, preventing the rapid spread of flames and limiting damage to the structure. This property

TABLE 7. COMPARISON OF COMPRESSIVE STRENGTH OPC WITH EPP IN MIXTURE CONCRETE

No.	Material/ Condition used	Outcome	References
1	EPP as some cement substitute in pavement blocks	The highest compressive strength of the pavement block is observed when FA and EPP are added, reaching a compressive strength of 36 MPa, with EPP added at levels between 15% to 20%. It's important to note that the limitation for adding EPP is not to exceed 20%.	Kusaimi <i>et al.</i> (2020)
2	Concrete mix cement with EPP	The compressive strength of E20 (Concrete mix with 20% EPP) was 34.2 MPa after 28 days of curing, which was higher than E0 (100% OPC) 30.13 MPa, E10 (Concrete mix with 10% EPP) 32.10 MPa and E30 (Concrete mix with 30% EPP) 26.72 MPa.	Yunus <i>et al.</i> (2019)
3	Mortar mix cement with EPP	In comparison to a control specimen consisting of 100% OPC in mortar, a mortar incorporating a 20% replacement of EPP as a substitute for cement demonstrates varying compressive strengths. After 7 days of curing, the strengths are recorded as 13.50 MPa for OPC and 15.26 MPa for the 20% EPP substitution. Subsequently, after 28 days of curing, there is a further increase in compressive strength, with values reaching 25.53 MPa for OPC and 26.61 MPa for the 20% EPP substitution. Additionally, it's noteworthy that the strength activity index of EPP for both the 7 day and 28 day periods is reported to be above 101%.	Raihana <i>et al.</i> (2019)
4	Combining fine aggregate FA and EPP as cement replacement in foamed concrete	Compressive strength 28 MPa is achieved by combining fine aggregate FA and EPP as a cement replacement rate of 30% in foamed concrete with a density of 1,600 kg/m ³ .	Jones and McCarthy (2005)
5	Utilising PSBE in the production of foamed concrete	Utilising 30% and 40% PSBE in the production of foamed concrete, the highest flexural strength 6 MPa is obtained with a curing time of 28 days. In comparison, the control foamed concrete (100% OPC) achieved flexural strength of 2 MPa.	Rokiah <i>et al.</i> (2019)
6	EPP as a substitute for fly ash in geopolymers concrete	EPP as a substitute for fly ash in geopolymers concrete found that using 10%-30% EPP as a replacement for fly ash resulted in compressive strengths ranging from 28-39 MPa.	Ahmat <i>et al.</i> (2023)

helps to compartmentalise the building and prevent the fire from spreading to other areas, providing additional time for evacuation and firefighting efforts (Smedberg *et al.*, 2023).

Concrete walls and partitions act as effective barriers in containing fire within a specific area, helping to control its spread and minimising property damage. This is particularly important in commercial and industrial settings where warehouses and storage buildings contain valuable goods and materials. Furthermore, the fire-resistant nature of concrete can result in reduced fire insurance rates for buildings constructed with concrete. Insurance companies recognise the fire-resistant qualities of concrete structures and often offer lower premiums as a result.

It is important to note that while concrete provides fire resistance, it is not entirely immune to fire. High temperatures and prolonged exposure to fire can cause concrete to undergo thermal spalling, which is the breaking off of surface layers due to the rapid expansion of moisture trapped within the concrete. However, concrete's ability to withstand fire and its contribution to overall fire safety make it a preferred construction material in many applications (Templeton *et al.*, 2023).

When concrete made of OPC is subjected to heat, the water within its pores and capillaries evaporates as the temperature rises. The temperature reaches 100°C, and both water and hydrated calcium silicate in the cement paste begin to evaporate, resulting in a reduction in strength. The increased vapour pressure within the concrete pores can cause explosive spalling, where certain portions detach from the surface, typically occurring at temperatures exceeding 300°C. Exposed to temperatures surpassing 600°C, a crack manifested on the surface. Subsequent temperature increments to 720°C and 950°C resulted in the generation of additional cracks, leads to significant weakening and brittleness of the concrete, resulting in a loss of its structural integrity (Li *et al.*, 2022; Sulistyawati, 2005).

Lubloy *et al.* (2017) examined the fire resistance of concrete, involving different types of specimens such as cement paste and concrete. It compared the performance of blended and OPC, considering various curing durations. The findings demonstrated that the concrete specimens prepared with pozzolans and fly ash-containing Portland-composite cement exhibited higher or at least equal relative residual compressive strength compared to the concrete specimens prepared with OPC.

Umasabor and Okovido (2018) conducted research that centred on assessing the fire-resistant properties of concrete samples incorporating rice husk ash (RHA). In the experimental method utilised in the study, concrete specimens were prepared by incorporating varying percentages of RHA (5%, 10% and 15% by weight) as some

substitute for OPC. Following the curing process, the samples underwent testing at various intervals, spanning from seven to 200 days. Subsequently, these specimens were exposed to temperatures ranging between 100°C-700°C for 2 hr in a muffle electric furnace. The study's findings indicate that OPC can be successfully substituted with RHA pozzolans, with a weight replacement of up to 15%, in grade 20 binary blended cement. This replacement, when subjected to curing for up to 200 days, does not result in a reduction of its compressive strength.

Farzadnia *et al.* (2013), conducted research on mortar specimens with the replacement of cement by 1% to 3% nano-alumina. These specimens were then exposed to temperatures ranging from 100°C to 1,000°C. The research results concluded that by adding 1% nano-alumina at room temperature, the compressive strength of the mortar can increase up to 16%. After exposure to high temperatures, a decrease in strength occurred in all test samples. However, the residual compressive strength remained higher than the original levels when 1% nano-alumina was added, even at temperatures up to 800°C.

Bendary *et al.* (2023) study evaluated the impact of firing temperature on the properties of pozzolanic cement. Specimens were prepared by OPC with different mass ratios of imported granulated blast furnace slag (IGBFS): A1 (30% OPC, 70% slag), A2 (40% OPC, 60% slag), A3 (50% OPC, 50% slag) and A4 (60% OPC, 40% slag). The compressive strength initially rises with temperature up to 250°C, but beyond this point and up to 800°C, it starts to decrease. At 800°C, the highest compressive strength is observed in sample A1, A2, A3 and A4, respectively.

CONCLUSION

In conclusion, these findings support EPP as viable substitute for OPC in blended concrete applications. Most of EPP research focused on compressive strength analysis but less on heat transfer analysis. Therefore, there is a need to explore the heat transfer on EPP. The suitability of different pozzolans for cement substitution may vary due to their unique characteristics. Determining the optimal percentage of cement replacement depends on several factors, including the specific type of pozzolan used, the curing duration, and the composition of aggregates in the concrete mixture. These findings highlight the potential of EPP and similar pozzolanic materials as sustainable alternatives to reduce the reliance on OPC in concrete production. However, it is essential to consider various factors when determining the most suitable pozzolan and the percentage of substitution for a given concrete application.

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APPLICATIONS OF ORGANIC FUNGICIDES, ENDOPHYTIC BACTERIA, BIOFUNGICIDES AND BIOSTIMULANTS IN CURATIVE CONTROL OF *Ganoderma*

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ABSTRACT

Basal stem rot (BSR) caused by *Ganoderma boninense* is a major oil palm (OP) disease. However, the effective treatment that is practicable in the field as a recommendation remains elusive. This study aimed to assess the effectiveness of the combinations that consisted of organic fungicide (OF), biofungicide (BF), endophytic bacteria (EN) and biostimulant (BS) applied to 20-year old (TM 20) and 14-year old (TM 14) OP with a mild, moderate, and severe level of infection. Results revealed that TM 20 palm recovery response is slower for all infection levels. The effective treatments for mild infection of TM 20 were BF, EN and BS, meanwhile, for moderate infection, the treatments included OF, BF, EN and BS. The effective treatment for both mild and moderate infections for TM 14 was the treatment which comprises BF, EN and BS. It seems that there is no need for BS in this mature palm for both mild and moderate *Ganoderma* sp. infection since it has rapid recovery. However, the most effective treatment for severe *Ganoderma* infection in both OP groups was a complete treatment consisting of OF, BF, EN and BS. We found that severe BSR symptoms could be reduced by providing the complete treatment several times, both holistically and progressively.

Keywords: basal stem rot, biofungicide, biostimulant, endophytic bacteria, organic fungicide.

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INTRODUCTION

Basal stem rot (BSR) is one of the major diseases that affect oil palm (OP) which leads to economic losses, estimated at USD256 million per year for each 1% of its infection (Alexander *et al.*, 2021). Thus far, BSR control management has been investigated in several studies (Ariffin *et al.*, 2002); however, an effective treatment that is practical in the field remains elusive (Bharudin *et al.*, 2022). The current

BSR disease control includes the improvement of the cultural control methods, both mechanically and chemically. The alternative control method is the use of biological agents. However, the control methods have not been found practical in field applications. Furthermore, many experiments on BSR disease control were performed *in vitro* or limited to nurseries, with very few studies conducted in the field (Siddiqui *et al.*, 2021).

Observations in the nursery using scanning electron microscopy showed that the *Ganoderma*'s mode of infection at the early stage was indicated by the penetration of *Ganoderma* mycelium into the epidermal cells on the root surface. To penetrate the epidermal cells, the mycelium secretes lignolytic enzymes to break down the lignin in the root epidermal cells, which affects the xylem, and consequently, the transport of nutrients and water (Ho *et al.*, 2016).

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Symptoms of BSR include rotting at the base of the stem, that eventually spread to the fruiting bodies and the roots. This condition limits the uptake of water and nutrients to the leaves which finally causes leaf chlorosis (Breton *et al.* 2009; Turner & Gillbanks, 1974). Other symptoms observed are stunted crown growth, including unopened spear leaves. In more severe cases, the trunk breaks and falls (Rees *et al.*, 2012). The infection is also visible through the appearance of the *Ganoderma* fruiting body at the base of OP and the canopy becomes yellowish (Breton *et al.*, 2009).

The results of testing organic fungicide (OF) on TM 15 plants that were attacked by *Ganoderma* sp. showed that the plants were able to recover, as indicated by root growth and began to open their spear leaves and start flowering and produce fruits (Widiastuti *et al.*, 2018). OFs play a role in suppressing the activity of *Ganoderma* sp. through the toxicity of the active ingredient contained in it, namely, allicin. Biostimulants (BS) function in optimising photosynthesis. Plants that are able to suppress the attack of *Ganoderma* sp. will carry out photosynthesis again, so it is necessary to give BS to optimise their photosynthesis.

Ganoderma sp. is a soil-borne pathogen and hence the soil characteristics can affect plant responses to *Ganoderma* sp. Goh *et al.* (2020) showed an example of *Ganoderma* sp. infection in two plots named Bernam and Blenheim, at an OP estate in Perak, Malaysia. The *Ganoderma* attack was higher in Bernam compared to Blenheim. Blenheim contains prokaryotic and eukaryotic diversity with high pH and calcium (Ca) content. In addition, Blenheim contains suppressive bacteria such as *Calditrichaeota*, *Zixibacteria*, *Omnitrophicaeota*, *Rokubacteria*, *Planctomycetes* strain AKYG587, *Defferibacteres* strain JdFR-76, and *Rubrobacter* (Actinobacteria). Meanwhile, in Bernam, there is a high proportion of *Uronema* (Ciliophora), mammals and *Chloroflexi* and *Acidothermus* (Actinobacteria) bacteria (Goh *et al.*, 2020). The antagonistic microorganisms of *Ganoderma* were added to the soil to manipulate the diversity in the rhizosphere, which was then expected to inhibit the pathogen infection. *Trichoderma* is a biological agent that has been widely used to control *Ganoderma* sp. (Badalyan *et al.*, 2004; Munthe & Dahang, 2018). The mechanism of *Trichoderma* sp. involves microparasites, antibiotics, and nutrient competition and induces a defence response or systemic resistance response in plants. *Trichoderma*'s hypermechanism is by penetrating *Ganoderma*'s cell wall, entering the cell and absorbing the nutrients (Ismail, 2007). *Trichoderma* sp. also produces β -1,3-glucanase and chitinase that can hydrolyse chitin from the cell wall of pathogens that cause hyphae lysis (Hazabar & Yaharwandi, 2006).

Another treatment is by using endophytes and their mechanism of producing several bioactive antimicrobial and antiviral metabolites along with producing various antioxidants to suppress pathogens (Gouda *et al.*, 2016). Endophytes protect plants against pathogen attack by triggering the host to induce resistance through several molecular events. Upon pathogen attack, the interaction between plant endophytic associations leads to an alteration in the second messenger such as Ca^{2+} in the cytosol (Vadassery & Oelmüller, 2009). Jasmonic acid (JA), ethylene (ET), and salicylic acid (SA) trigger induced resistance. JA and ET pathways are known to encourage resistance toward necrotrophic pathogens; however, the SA pathway activates resistance toward biotrophic and hemibiotrophic pathogens (Ding *et al.*, 2011). Moreover, biocontrol practices through endophytes may be achieved through direct inhibition of pathogens or indirectly by establishing the plant's systemic resistance (Chaudhary *et al.*, 2022; Pathak *et al.*, 2022; Santoyo *et al.*, 2016).

This research aimed to assess the effectiveness of some BSR control through environment-friendly techniques. Moreover, this study addresses the question about the different responses of the two age groups of OP, firstly, for plants aged over 20 years as the maximum production period (then mentioned as TM 20) and secondly, the group of plants at peak production, aged around 14 years (in this study mentioned as TM 14).

MATERIALS AND METHODS

The research was conducted in Afdeling 1, Block 11, Cislak Baru Plantations, the Banten Province, with the planting year of 1997 for TM 20. Furthermore, for TM 14, the research was conducted in Afdeling 1, Block 24, with the planting year of 2003. The planted area used was approximately 0.3 ha (45 palms) for both TM 20 and TM 14.

Plant Treatment

This study used four treatments based on the combination of OF, BS, biofungicide (BF), and endophytic bacteria (EN), as detailed in Table 1. Each treatment was tested in three categories of BSR disease's attack levels, namely mild, moderate and severe attack, each of which was represented by three biological replicates.

The OF used was developed from the main active compound, namely, allicin, which was tested at laboratory scale as an effective inhibitor for *Ganoderma*'s growth. Furthermore, the BS utilised in this study was derived from seaweed. This compound has been currently widely developed



Figure 1. Some examples of OP affected by *Ganoderma*, from left to right: TM 20 mild infection with treatment B; TM 20 moderate infection with treatment B; TM 14 severe infection with treatment A; TM 14 severe infection with treatment B.

as commercial products. OF and BS were applied through stem injection (approximately 20 cm in depth) in opposite directions (Figure 2). Both treatments were applied through a 25 cm long and 2 cm in diameter polyvinyl chloride (PVC) pipe planted in OP stem at 40° to 45° slope.

BF used in this research was selected as *Trichoderma* sp., which has been effective in controlling *Ganoderma* sp. While endophytic (EN) bacteria consist of two bacterial types, *i.e.*, SM-58, which is a photosynthetic bacteria and EDF 64, which is *Serratia* sp. Both species were obtained from Indonesian Oil Palm Research Institute (IOPRI)'s collection, as reported by Eris *et al.* (2017). Either BF or EN was applied at 1.0-1.5 m horizontally around the trunk.

The application of treatment for the TM 20 subjects for mild infection was at every two weeks (four times in total), while that for the moderate and severe categories was once a week (eight times in total), for two months. Application frequency for all categories in TM 14 was once a week for three months (12 times in total). This application frequency refers to the risk of falling or death of

trees within 6-24 months for young palm, from their initial symptoms; meanwhile, the adult trees continue their metabolism function for 2-3 years after the first infection (Rees *et al.*, 2007).

Observations were also made on five parameters, namely, the number of *Ganoderma* fruiting bodies, spear leaves that do not open (twisted), occurrence of leaves chlorosis, the number of fresh fruit bunches (FFB), and both the number and weight of the harvested FFB. Observations on TM 20 trees were carried out up to the 51st week after treatment application. Meanwhile, TM 14 trees were monitored every month until the 24th week after the treatment. Observations of harvested fruit bunches were carried out for 7 and 5 months for TM 20 and TM 14, respectively.

There were four treatments provided: Treatment A allows *Ganoderma* control by combining a wholesome ingredient, Treatment B acts as a negative control for BF and bacterial endophytes, Treatment C was given to investigate the absence of OF and Treatment D was given to observe the plant's response upon *Ganoderma* attack without BS (Table 1).

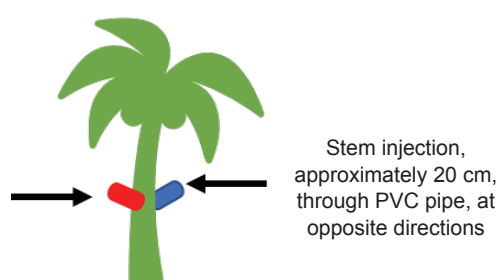


Figure 2. Illustration of OF and BS applications at opposite directions of the oil palm stem.

TABLE 1. THE TREATMENT TESTED IN THIS STUDY

Treatment	Organic fungicide (OF)	Biofungicide (BF) and Endophytic bacteria (EN)		Biostimulant (BS)
		<i>Trichoderma</i> (BF)	Endofit-64 and SM 58 (EN)	
Control	-	-	-	-
A	+	+	+	+
B	+	-	-	+
C	-	+	+	+
D	+	+	+	-

Data Observation and Analysis

To facilitate the discussion, observational data obtained were then quantified by giving weighted scores based on the following provisions as shown in Table 2.

RESULTS AND DISCUSSION

During the observation of treatment A, the TM 20 palm tree for moderate infection, fell at the 23rd week, while another TM 20 palm with severe infection, fell at the 47th week. Further observations could not be made on these palms. Figure 3 presents the number of fruiting bodies of *Ganoderma* sp. on TM 20 until the 51st week's observation. It was observed that *Ganoderma* fruiting bodies appeared only in the control palm, starting from the 47th until the 51st week of observation.

The TM 14 palms in the severe category for the B and C treatments (Figure 3), fell at weeks 24 and 20, respectively, hence no further observations could be made on these trees. Figure 3 also illustrates that *Ganoderma* fruiting bodies could not be found in the mild and moderate categories. The low values observed in the mild and moderate

levels of infection were not definitive indicators. Furthermore, Corley and Tinker (2003) explained that the infection can also occur without any symptoms, such as the absence of *Ganoderma* fruiting body and normal development of spear leaves, although the pathogen was already present in the plant (Widiastuti *et al.*, 2016). These results indicate that all treatments tested can be used as a preventive strategy to avoid the formation or reduction of the number of fruiting bodies of *Ganoderma* sp.

Interesting results were also found in the TM 14 palms of the severe category. Each treatment resulted in lower number of fruiting bodies, relative to the control. Treatments A, B and C showed the presence of fruiting body formation until the 24th week, although the number was much lower relative to control. Moreover, treatment D showed the highest growth repression of *Ganoderma* fruiting bodies. It seems that a mature OP tree at a younger age (TM 14) has better recovery or immunity even without any BS treatment (treatment D), compared to older OP (TM 20).

In the TM 20 palms of the severe infection category, only treatment D showed higher production of fruiting bodies compared to other treatments. Treatments A and B which consisted of

TABLE 2. REFERENCE WEIGHTED SCORE OF PARAMETERS OBSERVED

Parameter	Observed data	Score
Number of <i>Ganoderma</i> fruiting bodies	1	0.3 point per 1 fruiting body
Spear leaves	1-2 spear leaves	0.0
	3 spear leaves	0.3 point
	4 spear leaves	0.6 point
	Absence of chlorosis leaf	1.0
Chlorosis leaves occurrence	1 chlorotic leaf	2.0
	2 chlorotic leaves	3.0
	3 chlorotic leaves	4.0

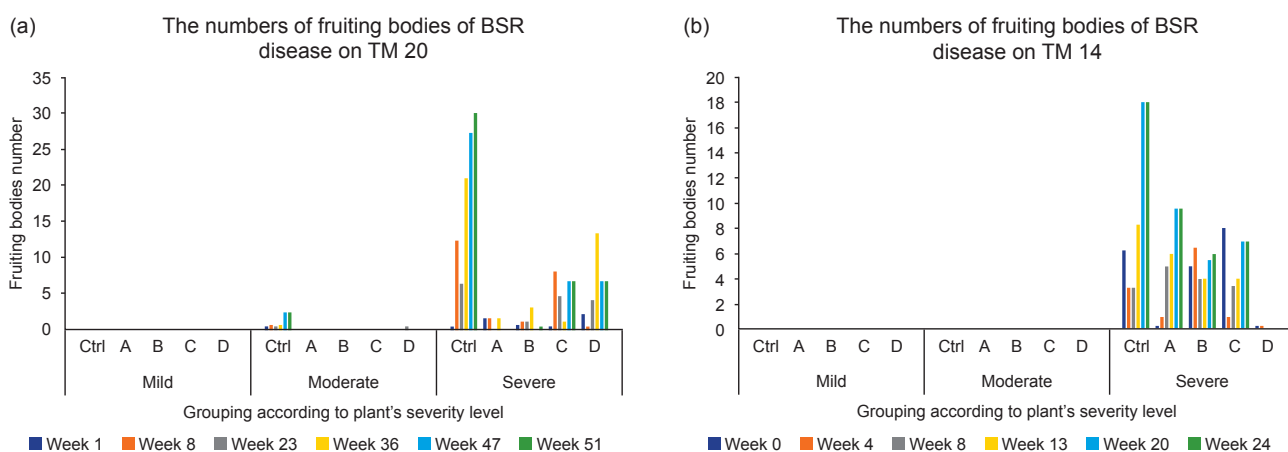


Figure 3. The number of fruiting bodies of oil palm trees in each treatment and category of BSR disease attack [(a) TM 20 and (b) TM 14].

a complete formulation of treatment and OF added with BS had caused a very low number of fruiting bodies, which were indicative of successful control. In this research, the prolific number of fruiting bodies also caused them to become dried naturally and therefore was not counted for the next observation, even if the palms were still standing then. It seems that the number of *Ganoderma* sp. fruiting bodies did not correlate with the severity of BSR disease, because sometimes there were occurrences of fallen palms with the absence of fruiting bodies in the moderate infection category, while up to 80 fruiting bodies were observed in the severely infected palms.

In the infected OP, new spear leaves did not open, due to the malfunction in the roots to absorb water. The TM 20 palms in all the control treatments of the mild infection category, did not produce any unopened spear leaves (Figure 4a). However, in the moderate category, the control treatments showed an increase in unopened spear leaves from the first observation up to the 36th week. A similar response pattern was found in treatment C, which showed the presence of unopened spear leaves until the 47th week of observation. No palms with unopened spear leaves were found in treatments A, B and D, from the beginning to the end of the observation. However, in the severe category, there were quite a lot of unopened spear leaves, reaching 1.6 leaves per palm in the control treatments, in the 51st week of observation. The presence of unopened spear leaves was observed in treatment B, only on the 51st week of observation, while treatments C and D showed unopened spear leaves at the beginning of the observation and were reduced by the 51st week of observation.

In contrast to the earlier findings (Sapak *et al.*, 2008) that suggest an improvement of OP seedling's vegetative growth due to endophytic bacteria treatment, our finding shows that treatment B, as the negative control of endophytic bacteria, has shown more incidence of unopened spear leaves. Further experimental investigation is needed to differentiate

the responses of mature and seedlings to the endophytic bacteria treatment. The interesting result was shown in treatment A, where in the beginning, the spear leaves were unopened, but by the 36th week, there were no more unopened spear leaves up to the last observation, which was at 51 weeks. This could be due to the return of the roots' function to absorb water, enabling the spear leaves to open. The results from treatment A which contain a complete package of all ingredients which include OF, BF, EN, and BS when applied simultaneously, seem to demonstrate a beneficial role for health recovery in aged OP (TM 20).

The results also indicate that for TM 14 in each infection category (Figure 4b), different response patterns were observed to each treatment tested. In the moderate category, treatment A showed the best result in avoiding presence of unopened spear leaves. Specifically, in the severe category, treatment C showed a decrease in the number of unopened spear leaves, contrary to treatments A and D, which have an increase in number of unopened spear leaves at the 24th week of observation. Treatment C showed good results in terms of reducing numbers of unopened spear leaves, in the moderate and severe categories. In contrast, treatment B showed an increase in the number of unopened spear leaves and was the highest compared to other treatments, although in general, unopened spear leaves were no longer found, at the 24th week.

The tree canopy is generally affected by BSR infection, which causes the roots to rot thus disturbing the water and nutrient uptake. The greenness of the leaves elucidates the ability of the plant to absorb nutrients. In the mild attack category, the TM 20 palms without any additional treatment, had increased leaf chlorosis symptoms until the end of the observation (Figure 5). The chlorotic palms were observed in treatments A, B and D. However, treatment C showed the most stable and lowest number of leaf chlorosis symptoms. Response in treatments C and D was evidence to support the

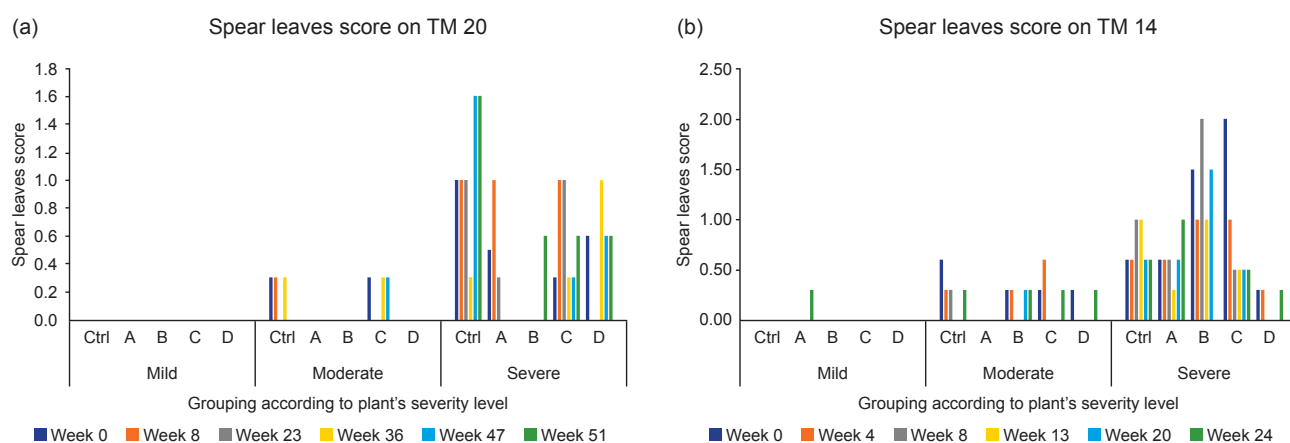


Figure 4. The unopened spear leaves values in each treatment and category of BSR disease attack [(a) TM 20 and (b) TM 14].

hypothesis that the addition of BF and EN could reduce leaf chlorosis in mild infection. Leaf chlorosis has been known as a symptom of *Ganoderma* attack due to the disturbance in nutrition and water transport mechanism in infected plants. To address this concern, some studies have underlined the role of endophytic bacteria in nitrogen uptake recovery (Da Silva *et al.*, 2018).

The response of treatment B in the moderate category of TM 20, explained that the absence of BF and EN inflicts a higher incidence of leaf chlorosis. However, contrary to the severe category, treatment B also gave the lowest in number of leaf chlorosis. For moderate and severe categories, treatment D seems to show the tolerance of TM 20 palms (with the absence of BS in its formulation), which showed a gradual decrease of chlorotic leaves during the long-term observation (51 weeks). In the moderate category, treatment A, having a complete package of ingredients, (consisting of OF, BF, EN and BS), can be recommended as having a beneficial role in decreasing the number of leaf chlorosis, as well as response in reducing unopened spear leaves.

In the mild infection category (Figure 5), there was a decline in leaf chlorosis for the TM 14 palms in treatments A, B and C, at week 20. From the beginning to the end of observation, the

lowest chlorosis number occurred in treatment D. Meanwhile, in the moderate infection category, the TM 14 palms in all treatments showed a decrease in leaf chlorosis, with the fastest decline in treatment D (week 13) and maintained up to week 24. Different results were shown in the severe infection category, where the TM 14 palms only showed a decline in number of leaf chlorosis in treatment A. However, the main positive response to treatment A only appeared about 4 months after its first application.

The TM 20 palms with mild infection showed the highest number of FFB harvested in treatment C, followed by B, D and A, relative to control (Figure 6). In the moderate infection category, the highest number of FFB harvested was in treatment A, followed by treatments C and D. In the severe infection category, palms in the control treatment did not produce any FFB, within 51 weeks of observation. Overall, palms given treatment A produced the highest FFB harvest compared to other treatments.

The TM 14 palms with mild and moderate infection, had produced the highest number of FFB when given treatment D (Figure 6), followed by treatments C and A. Palms given treatment D also showed a better development of FFB. The

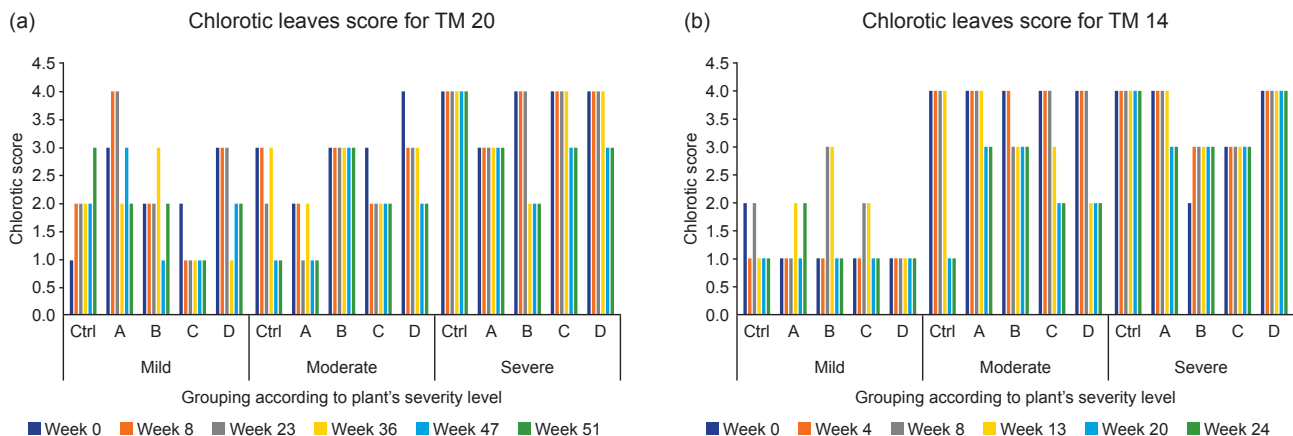


Figure 5. The value of oil palm trees with chlorotic leaves in each treatment and category of BSR disease attack [(a) TM 20 and (b) TM 14].

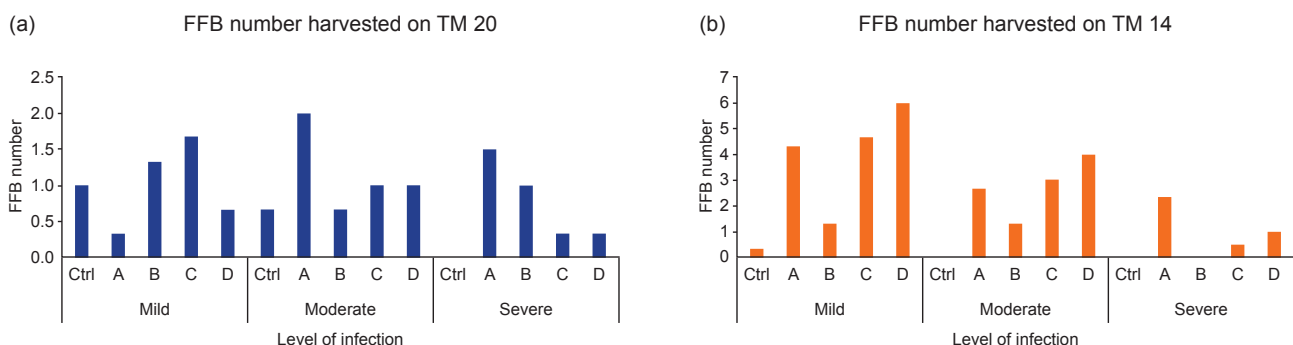


Figure 6. Total number of FFB harvested in each treatment and category of BSR disease attack [(a) TM 20 and (b) TM 14].

formation of FFB started at 24 months; and was observed over 51 weeks or 12 months. In the severe infection category, palms given treatment A produced the highest number of FFB. These results indicated that during a severe infection, the palms need to be supported by the complete formulation of treatments, to enable the palm to restore nutrients to produce the optimal number of FFB. It can be seen in the number of FFB that can still be slowly produced, with sizes not as large as the FFB produced by palms with mild and moderate levels of *Ganoderma* infection.

Fruit formation normally starts between 30 to 160 days after pollination (Tranbarger *et al.*, 2011). Due to the *Ganoderma* infection, the length of time required for the formation of FFB and harvesting was delayed in the TM 20 palms. Furthermore, it was reported that severe infection can linearly affect FFB performance (Evizal & Prasmatiw, 2022).

The weighing results of FFB produced by the TM 20 palms (Figure 7) in the mild and moderate infection categories, showed the same trend with the FFB numbers. Palms with mild infection of *Ganoderma*, when given treatments C and B, had produced the highest FFB weights, while the lowest FFB weights were observed in treatment A. Palms with a moderate infection when given treatment A, had produced the highest FFB weight. However, palms in the severe infection category when given treatment B, had produced much higher FFB weight, compared to treatment A. The smaller weight of FFB in treatment A was apparently due to the disruption of nutrients in palms with *Ganoderma* infection.

In the mild infection category, the TM 14 palms had produced the highest weight of FFB in treatments D, followed by A and C, which was far above the control treatment. At the moderate and mild infection, the palms produced the highest FFB weight in treatment D. However, palms in the severe infection category can still produce high FFB weights, provided they are treated with the complete treatment package of OF, BF and BS.

Comparison of the Response of TM 20 and TM 14 Plants to Treatment

Under normal conditions, the TM 20 palms have passed the peak production phase, and moving towards the old palm age of 25 to 30 years. Meanwhile, the TM 14 palms are still in the peak production stage. Therefore, responses to the four treatments were varied due to the production phase differences. In general, the fresh fruiting bodies of *Ganoderma* were formed in much greater numbers in the control than in treatments A, B, C and D. However, the number of fruiting bodies observed did not always correlate with the level of *Ganoderma* infection since the dead, fallen trees have a varied number of *Ganoderma* fruiting bodies. For example, the fallen TM 20 palms, had no fruiting bodies in the moderate infection category, while there can be up to 80 fruiting bodies in the palms of the severe infection category. On two fallen TM 14 palms with severe infection, the number of fruiting bodies varied between 4 and 87. It is therefore suspected that the colonisation of *Ganoderma* sp. in the stem could better determine the severity of infection compared to the number of fruiting bodies observed.

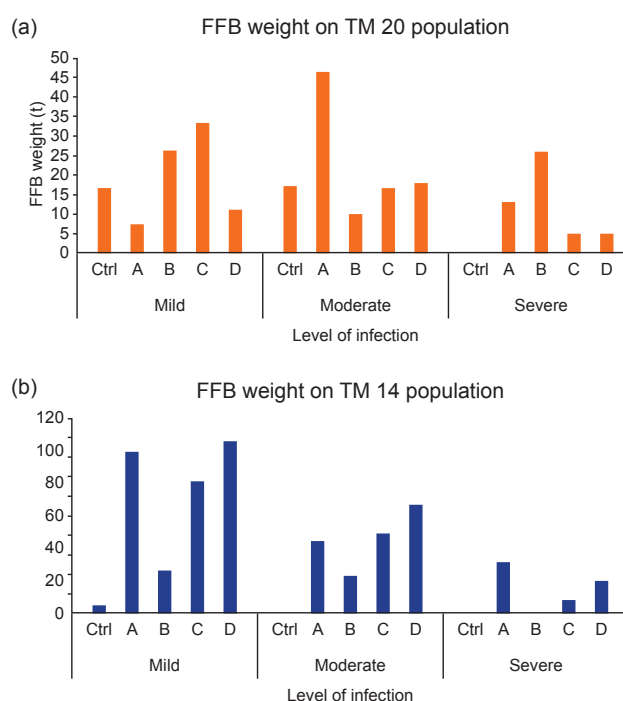


Figure 7. Total weight of FFB harvested in each treatment and category of BSR disease attack [(a) TM 20 and (b) TM 14].

There was also a tendency of high correlation between a number of fruiting bodies and unopened spear leaves (Table 3). In TM 20 palms, the correlation was high in week 8, even though no more treatment was added for the moderate and severe infection categories. The phenotypic response through number of unopened spear leaves and *Ganoderma* fruiting bodies was high. For TM 14 palms, the critical period of infection was visible between week 0 and 13, indicated by the high incidence of *Ganoderma* fruiting bodies that was positively correlated to the high number of unopened spear leaves.

Several potential treatments are summarised in Table 4, based on several main phenotypic parameters indicative of *Ganoderma* infection on TM 14 and TM 20. These treatments succeeded in influencing the dynamics of the presence of *Ganoderma* fruiting bodies, leaf chlorosis, unopened spear leaves, and FFB on the palms. In this study, the emphasis on the best treatment was based on the decrease in the number of fruiting bodies of *Ganoderma* sp., unopened spear leaves and leaf chlorosis, along with an increase or a high number and weight of FFB after treatment. It is assumed that with the treatment, the number of *Ganoderma*

TABLE 3. CORRELATION ANALYSIS BETWEEN PARAMETERS

TM 20					TM 14				
Week 1	Fruiting bodies	Spear leaves	Chlorosis	FFB	Week 0	Fruiting bodies	Spear leaves	Chlorosis	FFB
Fruiting bodies	1.00				Fruiting bodies	1.00			
Spear leaves	0.55	1.00			Spear leaves	0.85	1.00		
Chlorosis	0.40	0.48	1.00		Chlorosis	0.09	0.21	1.00	
FFB	-0.38	-0.46	-0.42	1.00	FFB	-0.72	-0.77	-0.50	1.00
Week 8	Fruiting bodies	Spear leaves	Chlorosis	FFB	Week 47	Fruiting bodies	Spear leaves	Chlorosis	FFB
Fruiting bodies	1.00				Fruiting bodies	1.00			
Spear leaves	0.81	1.00			Spear leaves	0.69	1.00		
Chlorosis	0.48	0.40	1.00		Chlorosis	0.17	0.46	1.00	
FFB	-0.55	-0.28	-0.81	1.00	FFB	-0.50	-0.62	-0.35	1.00
Week 23	Fruiting bodies	Spear leaves	Chlorosis	FFB	Week 8	Fruiting bodies	Spear leaves	Chlorosis	FFB
Fruiting bodies	1.00				Fruiting bodies	1.00			
Spear leaves	0.82	1.00			Spear leaves	0.79	1.00		
Chlorosis	0.60	0.46	1.00		Chlorosis	0.26	0.19	1.00	
FFB	-0.63	-0.51	-0.43	1.00	FFB	-0.78	-0.57	-0.32	1.00
Week 36	Fruiting bodies	Spear leaves	Chlorosis	FFB	Week 13	Fruiting bodies	Spear leaves	Chlorosis	FFB
Fruiting bodies	1.00				Fruiting bodies	1.00			
Spear leaves	0.61	1.00			Spear leaves	0.84	1.00		
Chlorosis	0.58	0.58	1.00		Chlorosis	0.43	0.38	1.00	
FFB	-0.71	-0.60	-0.57	1.00	FFB	-0.73	-0.70	-0.16	1.00
Week 47	Fruiting bodies	Spear leaves	Chlorosis	FFB	Week 20	Fruiting bodies	Spear leaves	Chlorosis	FFB
Fruiting bodies	1.00				Fruiting bodies	1.00			
Spear leaves	0.97	1.00			Spear leaves	0.57	1.00		
Chlorosis	0.60	0.63	1.00		Chlorosis	0.42	0.38	1.00	
FFB	-0.68	-0.71	-0.45	1.00	FFB	-0.59	-0.19	-0.14	1.00
Week 51	Fruiting bodies	Spear leaves	Chlorosis	FFB	Week 24	Fruiting bodies	Spear leaves	Chlorosis	FFB
Fruiting bodies	1.00				Fruiting bodies	1.00			
Spear leaves	0.92	1.00			Spear leaves	0.41	1.00		
Chlorosis	0.61	0.62	1.00		Chlorosis	0.59	0.66	1.00	
FFB	-0.76	-0.75	-0.36	1.00	FFB	-0.67	-0.40	-0.62	1.00

fruiting bodies will decrease, and the spear leaves will open, which decreases the unopened spear leaves number, followed by a decrease in leaf chlorosis; thus, the metabolism of the palm tree recovers and gradually becomes healthy. In very mature TM 20 palms, their responses to the treatments take longer, up to 51 weeks, while for TM 14, it takes only about 20 weeks.

TABLE 4. POTENTIAL TREATMENTS FOR CURATIVE TREATMENTS OF BSR

BSR disease attack rate	Potential treatment	
	TM 20	TM 14
Mild	C	D
Moderate	A	D
Severe	A	A

For TM 20 palms in the mild infection category, the best treatment was C (BF, EN and BS), while for TM 14 palms, it was D (OF, BF and EN). On trees with moderate attacks, the best treatments for TM 20 palms were A (OF, BF, EN and BS) and D (OF, BF and EN), while for TM 14 palms, the best treatments were D (OF, BF and EN) and C (BF, EN and BS). In severely affected palms, recovery was apparent in treatment A (OF, BF, EN, BS) for both TM 20 and TM 14 palms. A seaweed-based BS has been assayed for its antifungal function against *G. boninense* (Aziz *et al.*, 2019), and the efficacy relies on different phytol components, due to seaweed species extracted. The application of the product in each treatment of this study was only conducted in a single period. The use of the chemical fungicide hexaconazole to control BSR, as recommended by the Malaysian Palm Oil Board, is applied three times within six months. The dosage of that fungicide was 4.5 g, dissolved in 3 L of water (The Star, 2021). It is recommended that the treatments need to be carried out at least every six months.

CONCLUSION

Ganoderma BSR disease in OP is identified through signs (part of the pathogen) and symptoms that appear on the palm. Both are the main determinants of the criteria for the development of pathogen colonisation in destroying plant tissue and indicating the existence of malignancy in plants. The treatments given to TM 20 and TM 14 palms exhibited a dynamic effect in reducing the number of fruiting bodies of *Ganoderma* sp., unopened spear leaves and leaf chlorosis occurrences, and an increase in the number and weight of FFB after treatments. The response to cell repair was slower and required more time to recover in the older

TM 20 palms compared to TM 14. It is concluded that applications of OF, BF, EN and BS have the potency to cure the basal stem rot disease caused by *Ganoderma* sp. However, the continuous application of these products is necessary, since *Ganoderma* propagules are supposed to be present in the root area and possibly even in plant tissues.

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MATERIAL CIRCULARITY INDICATOR FOR THAI OIL PALM INDUSTRY

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ABSTRACT

The oil palm industry is vital in food and energy production and significantly contributes to the Thai economy. Nevertheless, the sector faces environmental management challenges, especially waste management. With an awareness of the importance of a circular economy, this study aims to utilise the material circularity indicator (MCI) to measure the material circularity in multiple products of the Thai oil palm industry and plan for sustainable improvement. The circularity measurement result shows that palm oil production is more circular than linear, with a calculated MCI of 0.5372. This shows the importance of recycling, reusing and composting of co-products, residual oil and wastes of oil palms to achieve the sustainable development of Thai agriculture. The results also show that the MCI value may be enhanced by increasing the biomass's oil extraction rate (OER) and calorific value. By improving the OER from 18% to 23%, the MCI value rises to 0.6230, explaining a better circularity of the oil palm industry. The oil palm industry, the government and related authorities may use the study results to plan for the palm oil process improvement to enhance the circularity in the long term. The study has a limitation: The data used in the analysis are from a limited number of palm oil factories. An increasing number of participating factories may enhance the study results.

Keywords: circular economy, material circularity indicator, multiple products, oil extraction rate, palm oil production.

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INTRODUCTION

Oil palm is an essential crop in Thailand in terms of agriculture, economy, and industry, as the country is the world's third-largest oil palm producer (Sowcharoensuk, 2020a). The production value of oil palm in Thailand increased by almost 2.5 times in the past 10 years (Figure 1). It contributes to the stability of food and energy in the country. It is grown nationwide, with dense plantation areas in Southern Thailand (Sowcharoensuk, 2020a) (Figure 2). From 2011 to 2019, the yield area increased by 4.63% annually. The total yield of fresh fruit

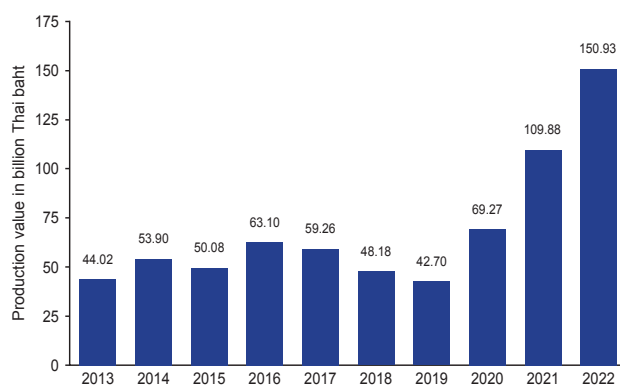
bunches (FFB) increased by 4.30%/yr, with a total output of 16,408,440 t in 2019, representing an average yield of 1.81 kg/m²/yr (Figure 3) (Office of Agricultural Economic [OAE], 2020; Siam Legal, 2023).

The palm oil extraction process involves five steps: 1) Reception, transfer, and temporary storage of FFBs, 2) sterilisation, 3) bunch stripping, 4) digestion, and 5) extraction (Kaewmai, 2012). When FFBs go through the extraction process, they produce approximately 18%-22% of crude palm oil (CPO), which is processed into edible oil, used in energy production, such as biodiesel, and sold to palm oil refineries or biodiesel plants (Sangkharak, 2014). FFBs also produce palm kernels that are processed into palm kernel oil, and dried palm kernels are transported to palm kernel crushers. By-products and wastes, accounting for 78%-82% of FFBs, include empty fruit bunches (EFBs), palm fibres (PFs), palm kernel shells (PKSs), decanter cakes and palm oil mill effluents (POMEs) (Sangkharak, 2014).

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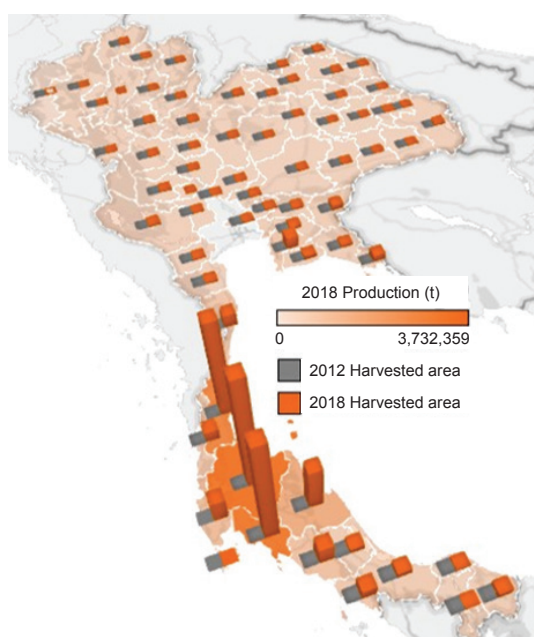
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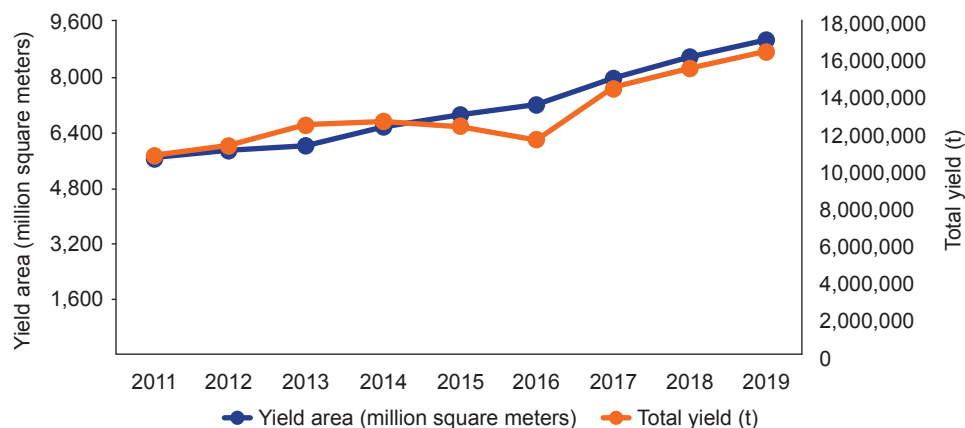
Source: Statista (2023).

Figure 1. Production value of oil palm in Thailand from 2013-2022.



Source: Sowcharoensuk (2020a).

Figure 2. Harvested areas of oil palm in Thailand.



Source: OAE (2020); Siam Legal (2023).

Figure 3. Yield area and total FFB yield of Thailand.

Kaewmai (2012) mentioned that palm oil production processes generate solid waste, wastewater, and air pollution that require proper management to avoid long-term environmental problems. Improper management of these by-products and wastes can severely harm the environment. Palm oils, similar to other production and consumption patterns that use more natural resources and energy, cause a shortage of oil palms and solid waste management problems. Most natural resources and energy used are non-renewable and limited. Efforts to find renewable resources are required to meet the increasing demand. Although some of the waste generated can be recycled, their non-reusable parts must be appropriately disposed of or treated. Therefore, they remain in the environment and may cause deterioration to the ecosystem in the long run (Carbon Market, 2021).

A circular economy concept is a new alternative that can develop the country's economy towards sustainability. It is recognised and driven by governments and businesses worldwide to improve their sustainability levels (Chorolque *et al.*, 2022; De Oliveira Neto *et al.*, 2023). The circular economy is expected to replace the traditional linear economy based on the take-make-use-dispose concept (TGO, 2021). It is considered as a sustainable economy, social and environmental development of the country that creates economic opportunity through innovating ideas, creating jobs, and driving economic growth (World Economic Forum, 2014). In Thailand, the circular economy concept is applied in many agricultural-related studies. For example, Suksaroj *et al.* (2023) investigated the biogas production and circular economy perspective in the Thai oil palm industry through co-digestion of EFB pressing wastewater and POME. The results provide information on the circular economic approach to promote sustainable palm oil processing in Thailand.

Edyvean *et al.* (2023) developed a bio-circular-green (BCG) economic solution to mitigate the effects of CO₂ emissions in the food and agricultural, medical, bioenergy, and tourism sectors in Thailand. Usapein *et al.* (2022) performed strengths, weaknesses, opportunities, and threats (SWOT) analysis of the oil palm industry to help drive the BCG economy model in Thailand.

To effectively plan for the circular economy implementation, its value must be measured and tracked through evaluation tools and indicators to support the move toward a circular economy. Various indicators may be used to assess the circularity, such as the circular economic value (CEV), material reutilisation score (MRS), product-level circularity metric (PLCM), mass recovery index (MRI), quantitative indicators and value assessment (QIVA) and material circularity indicator (MCI) (Syu *et al.*, 2022). Syu *et al.* (2022) stated that the PLCM measures circular inputs into the manufacturing process; thus, production waste and product end-of-life management only affect the score if they are closed-loop recycling pathways. The MRI, in contrast, focuses on the value output and measures the amount of material consumed that is converted into a product. The CEV and MCI can capture differences between all scenarios. Saidani *et al.* (2019) mentioned that the CEV is the indicator used in the meso-level (*i.e.*, businesses and industrial symbiosis), while the MCI is used in the micro-level (*i.e.*, products, components and materials). Kristensen and Mosgaard (2020) stated that the MCI is one of the first circular economy indicators to draw attention from practical and

academic parties. It considers the amount of recycling in the product and the lifetime use of the product.

In this study, the MCI measures the circularity of the oil palm industry as it focuses on the lifetime extension, recycling and waste management perspectives (Matos *et al.*, 2023). Rocchi *et al.* (2021) commented that compared to other indicators, the MCI is one of the few that includes retention in its metric, as it considers how much an element is used in the process in terms of intensity and duration. It covers more aspects of lifespan, recycling rate, and use intensity than the MRI, QIVA, PLCM, and CEV indicators (Syu *et al.*, 2022). The product-level MCI is used to measure the circularity in multiple products of the oil palm industry and the company-level MCI of both small and large palm oil mills to determine the overall MCI value of the country and enhance the sustainability of the oil palm industry. The study results are expected to provide an indicator for the oil palm industry to plan for long-term sustainable and circular operations.

The research flow of this study is in Figure 4. The process of palm oil milling is explained and the by-products and wastes of oil palm are listed based on the related literature. The reuse, recycling, composting, and landfill processes are also reviewed to understand the circularity concept. Data is collected through interviews, on-site observations and secondary data from previous studies. The MCI is calculated, and different scenarios are tested to achieve suitable strategies for the sustainable development of the oil palm industry.

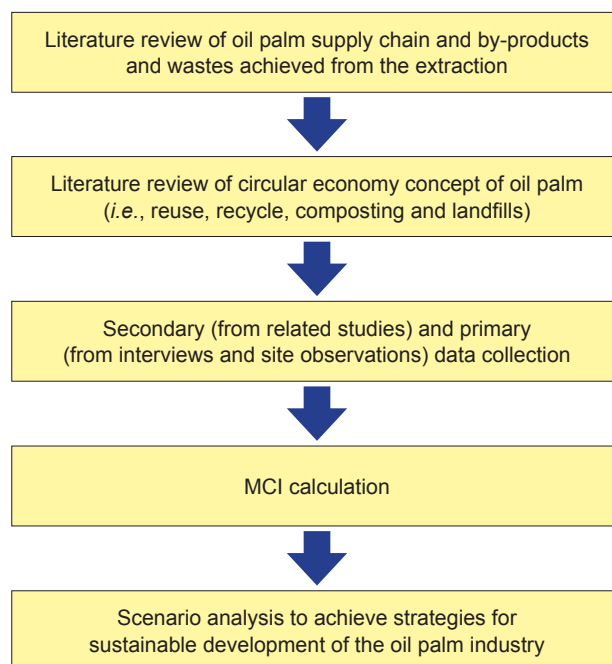


Figure 4. Research flow of the study.

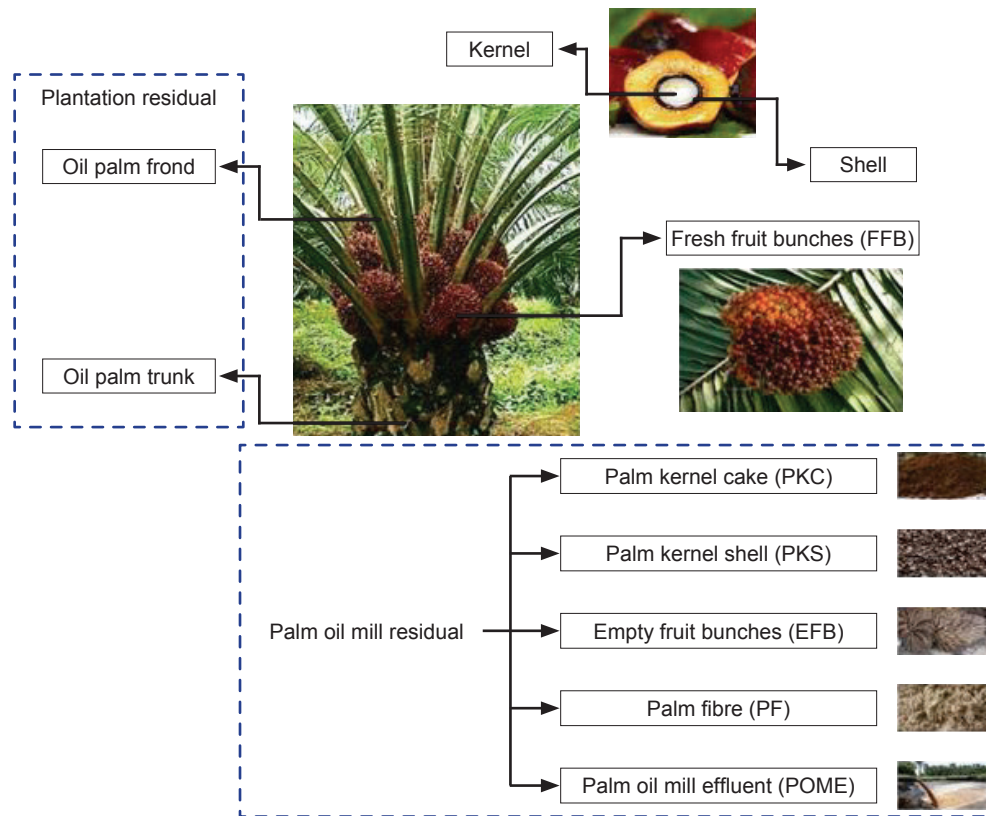


Figure 6. Waste in the oil palm industry.

MATERIALS AND METHODS

Material Circularity Indicator (MCI)

MCI is proposed by the Ellen MacArthur Foundation (EMF) as a tool to assess product and business model performance. It considers product recycling, including waste generated by the manufacturing processes. It considers the product's utility throughout its lifetime and the functional units compared to the same industrial product (Bejarano *et al.*, 2022; Kristensen & Mosgaard, 2020). It covers biomaterials and addresses challenges associated with combining biological and technical materials. It also identifies risk-based complementary metrics for biomaterials and defines energy recovery conditions as part of a circulating strategy. It has been applied in various studies to assess product-level circularity. For example, Gue *et al.* (2018) proposed an MCI framework to determine the circularity of microalgae biodiesel. Due to its similar popularity, they also compared it with *Jatropha* biodiesel, a reference feedstock. In addition, they also used the life cycle assessment (LCA) framework to assess the environmental impact of products. The results showed that *Jatropha* has less environmental impact than microalgae in all three impact categories, including resource depletion, bulk waste and global warming potential. MCI of microalgae

biodiesel is 0.04 higher than that of *Jatropha* biodiesel, meaning that microalgae biodiesel has a better material circularity.

The scale of MCI ranges from 0-1, where 1 indicates a full circular product, and 0 represents a full linear product (*i.e.*, no circularity). A diagram of the parameters used for product-level MCI calculation in this study is in Figure 7. In this study, the mass of virgin feedstock (V), FFBs, is sent to the palm oil mills and refineries to achieve the products (M), such as edible oil, biodiesel and beauty products. Once the products are consumed, they produce unrecoverable waste (W_o) that is disposed of in landfills. The components that can be reused (C_u) are collected and sent back to the plants as the reuse feedstock (F_u) for further processes. Similarly, the recyclable components (C_R) are returned to the plants as recycled feedstock (F_R) for processing. The unrecoverable waste from the recycling process (W_C) and the recycled feedstock (W_F) is sent to landfills.

MCI Calculation

To calculate the MCI, several steps are performed. The first parameter to be calculated is the virgin feedstock (V). This parameter links to recycling, reuse and biological material in the virgin raw material, as shown in Equation (1), where M is the mass of the final product, F_R is the

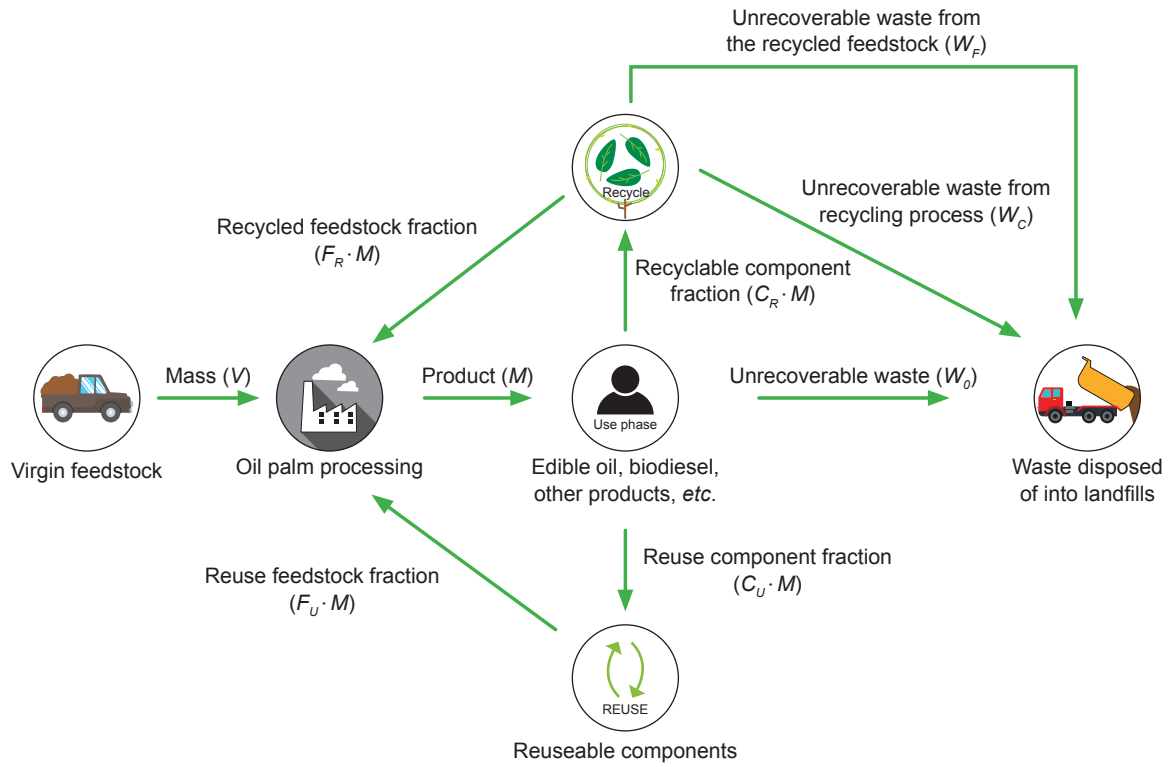


Figure 7. Parameters used to calculate product-level MCI.

fraction of recycled feedstock, F_U is the fraction of reused feedstock, and F_S is the fraction of biological feedstock from sustainable production.

$$V = M(1 - F_R - F_U - F_S) \quad (1)$$

The unrecoverable waste after use to landfill (W_0) is next calculated in Equation (2). C_R is the fraction of the mass of the product collected for a recycling process, which produces the waste from the recycling process (W_C). This waste depends on the efficiency of the recycling process used to recycle the product (E_C) and $C_{R'}$ as shown in Equation (3). C_U is a fraction of the mass of the product collected for reuse. At the same time, C_C is a fraction of the mass of the product collected for the composting process and C_E is a fraction of the product collected for energy recovery.

$$W_0 = M(1 - C_R - C_U - C_C - C_E) \quad (2)$$

$$W_C = M(1 - E_C)C_R \quad (3)$$

There is also waste generated from recycled feedstock (W_F) production. This waste depends on the efficiency of the recycling process used to

produce the recycled feedstock (E_F), as shown in Equation (4). The overall amount of unrecoverable waste (W) can then be obtained from Equation (5).

$$W_F = M \frac{(1 - E_F)F_R}{E_F} \quad (4)$$

$$W = W_0 + \frac{W_F - W_C}{2} \quad (5)$$

Linear flow index (LFI) indicates the linearity of the product, which is calculated by Equation (6). This value ranges from 0-1, where 1 represents full linearity and 0 represents nonlinearity.

$$LFI = \frac{V + W}{2M + \frac{W_F - W_C}{2}} \quad (6)$$

The utility factor, $F(X)$, is calculated based on the lifetime of the product and the functional unit of the product, which are combined to create the utility (X), see Equations (7) and (8), where L represents the actual average lifetime of a product, L_{av} represents the average lifetime of the same industry average product, U is the actual average number of functional units of the product, and U_{av} is the average number of functional units of the same industry average product.

$$X = \left(\frac{L}{L_{av}} \right) \left(\frac{U}{U_{av}} \right) \quad (7)$$

$$F(X) = \left(\frac{0.9}{X} \right) \quad (8)$$

The MCI of the product (MCI_p) is then calculated using Equation (9), and the MCI of the company (MCI_C) is finally computed using Equation (10). It is based on the weighted average of MCI_p , where N_C is the sum of the normalising factor used, N_p is the normalising factor of each product, and MCI_p is the MCI value of each product.

$$MCI_p = 1 - LFI \cdot F(X) \quad (9)$$

$$MCI_C = \frac{1}{N_C} \sum_{\alpha} (N_{p(\alpha)} \cdot MCI_{p(\alpha)}) \quad (10)$$

Many studies modify the Ellen MacArthur Foundation's formula to suit their products (Anastasiades *et al.*, 2023; Moller *et al.*, 2023). For example, Razza *et al.* (2020) modified the MCI calculation formula for bio-based and biodegradable (BB) products and applied it to BB mulch film. The results showed that considering the proportion of 30% bio-based and 70% fossil-based, the MCI of BB mulch film is 0.37, which is less than 0.50, indicating the linearity related to the bio-based feedstock content. Rocchi *et al.* (2021) proposed a modification of the MCI formula to be used with the biological cycle of poultry production by adding the feed conversion rate (FCR), which is the ratio between input (feed) and output (meat) in production. The utility is calculated using the utility conversion factor, which is calculated from the mortality rate. The results showed a modified MCI value of 0.49, representing low circularity. Phimpakarn *et al.* (2021) proposed a modification of MCI to the biological cycle of broiler feed production. The results showed an MCI value of 0.68, representing high circularity. Pavlovic *et al.* (2020) applied a company-level MCI to paper and cardboard packaging and cupcake liners in the Republic of Serbia. The normalising factor is used as the mass of the product, and the results concluded the MCI of 0.47, representing the middle point between linear and circular economies.

For oil palm products, the MCI formula is also required to be modified. When considering CPO as a product, M becomes the mass of CPO. CPO use occurs at a palm oil refinery when it is transformed into refined bleached and deodorised palm oil (RBDPO). A co-product, the palm fatty acid distillate

(PFAD), can be used for soap and oleochemical industries. Bleaching earth, on the other hand, is sent to landfills.

Using the traditional MCI formula, the amount of unrecoverable waste from the oil palm industry is very high, meaning many CPOs are sent to landfills. This is inaccurate as CPOs can be transformed into RBDPOs, which are not considered unrecoverable wastes. Therefore, the formula must be modified to suit oil palm products in the unrecoverable waste section. The unrecoverable waste after use (W_o) is modified [Equation (11)], where M^* is the sum of the mass of the co-products, wastes, and residual oil. C_R is the fraction of the mass of the co-products, wastes, and residual oil collected for the recycling process, which produces the waste from the recycling process (W_c). This waste depends on the efficiency of the recycling process used to recycle the co-products, wastes, and residual oil (E_c) [Equation (12)]. C_U is the fraction of the co-products, wastes, and residual oil collected for reuse. At the same time, C_C is the fraction of the co-products, wastes, and residual oil collected for the composting process. C_E is the fraction of the co-products, wastes and residual oil collected for energy recovery.

$$W_o = M^* (1 - C_R - C_U - C_C - C_E) \quad (11)$$

$$W_c = M^* (1 - E_c) C_R \quad (12)$$

Data Collection

Both secondary and primary data are used in this study. Primary data are obtained from two oil palm plantations (Figure 8) and two palm oil mills in Thailand with different operations and production capacities. The first palm oil mill performs palm oil extraction with a production capacity of 30 t CPO/hr. The second palm oil mill, on the other hand, conducts palm oil extraction and palm oil refining at a production rate of 120 t CPO/hr. Both factories operate 24 hr/day and 330 day/yr. The proportions of multiple products obtained from the two factories are in Table 1. The data used in MCI calculations are collected through direct fieldwork and secondary sources. The circularity measurement in this study considers five oil palm products at palm oil mills, including; 1) CPO, 2) palm kernel, 3) EFB, 4) PF, and 5) PKS that are used for several purposes (Figure 9).

The CPO is refined at a palm oil refinery. CPO should be stored for a maximum of a year. The estimated shelf life of CPO, when kept at 20°C-25°C in the dark, is approximately 6 months (De Almeida *et al.*, 2019). Based on Table 1, the

oil extraction rate (OER) of CPO is 18.00%. This extraction rate is typical for the palm oil mill that operates with the nut-separated palm oil extraction process. Palm oil is extracted from FFBs and loose palm fruits as raw materials, with an extraction rate of about 18 kg of oil per 100 kg of raw materials (Ministry of Industry, 2019). The 18.00% OER of CPO comprise RBDPO 16.90%, the co-product of palm fatty acid distillate (PFAD) 0.92% and the waste bleaching earth 0.18%. PFAD can be considered as the fraction of co-product collected for the recycling process as it is used in the animal feed, soap, and oleochemical industries.

The use of palm kernels takes place at a palm kernel crusher. The 6.00% palm kernel comprises crude palm kernel oil (CPKO) 2.50%, the co-product of palm kernel meal 3.20% and the waste, which is other impurities 0.30%. Palm kernel meal can be considered as the fraction of co-product collected for the recycling process as it is used in the animal feed industry. Dried palm kernels should be stored for up to seven days. Oil palm biomass products, including EFB, PF, and PKS, are used as fuel for electricity generation. The shelf lives of EFB, PF, and PKS are five days, three months, and one year,

respectively (Zafar, 2022). Burning oil palm biomass produces ash that can be used to increase the FFB yield. The average ash contents of EFB, PF, and PKS are 3.97%, 4.89%, and 3.68%, respectively. The heating values of PF, PKS and EFB are 20.24, 18.90, and 18.90 MJ/kg, respectively. The residual oil is a fraction of the residual oil collected for reuse. The average residual oils in EFB and PF are 8.00% and 6.00%, respectively, while there is no residual oil in PKS.



Figure 8. Two oil palm plantations.

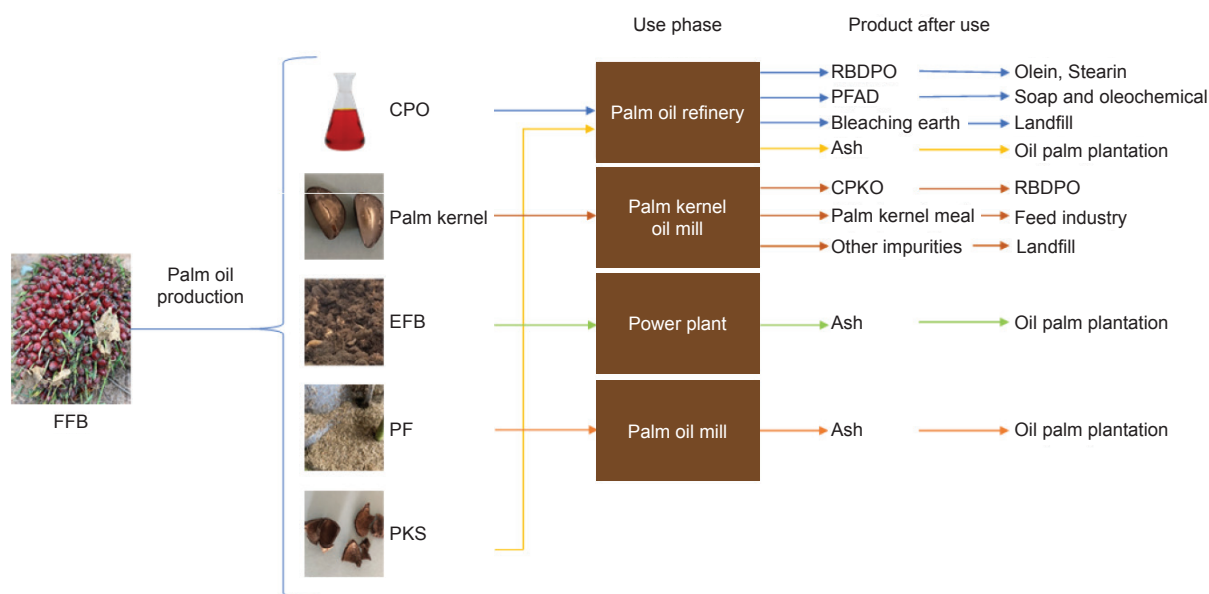


Figure 9. The use of multiple products in the oil palm industry.

TABLE 1. THE PROPORTION OF MULTIPLE PRODUCTS FROM TWO PALM OIL MILLS IN THAILAND

Data	Product	The proportion of multiple products (%)	
		1 st factory (mill)	2 nd factory (mill and refinery)
Input	FFB	100.0	100.0
Output	CPO	18.0	18.0
	Palm kernel	6.0	6.0
	EFB	23.0	20.0
	PF	13.0	14.0
	PKS	6.0	6.0
	Decanter cake	2.5	4.0
	POME	21.5	21.0
	Other impurities	10.0	11.0
	Total	100.0	100.0

RESULTS AND DISCUSSION

Results of Circularity Measurement

To calculate MCI and define the circularity of the oil palm industry, the process begins with FFBs, which are sent to palm oil mills. They produce CPO, palm kernel, EFB, PF, PKS, which are considered multiple products, and decanter cakes, palm oil mill effluent and other impurities, which are considered wastes and are not included in this study. The modified formula assesses the overall circularity of palm oil production. The quantity used in the MCI calculation is in t/yr (Table 2). It is then converted to t/t CPO by dividing the quantity of CPO. As the data are collected from two palm oil mills, the weighted average using the capacity ratio is used to obtain the average quantity of each product.

This study uses the OER as the product's functional unit of CPO and palm kernel, while the heating value is the functional unit of EFBs, PFs, and PKSs. The results of MCI calculation for multiple products are in Table 3. The MCI calculation of the CPO is provided as an example. As palm oil production does not have recycling and reuse processes and has no biological material of virgin feedstock, V is equal to M in all multiple products. M^* of the CPO is the sum of the co-products, wastes, and residual oil mass and is 0.0611 t (based on interviews). About 83.64% of the CPO products can be recycled, leading to the $W_0 = 0.01$ [Equation (13)]. The efficiency of the recycling process of the CPO (E_C) is 90.00%, leading to W_C of 0.0051 [Equation (14)].

$$W_{0,CPO} = M_{CPO} * (1 - C_{R,CPO} - C_{U,CPO} - C_{C,CPO} - C_{E,CPO}) \quad (13)$$

$$= 0.0611(1 - 0.8364 - 0 - 0 - 0) = 0.01$$

$$W_{C,CPO} = M_{CPO} * (1 - E_{C,CPO}) C_{R,CPO} \quad (14)$$

$$= 0.0611(1 - 0.9)(0.8364) = 0.0051$$

There is no fraction of recycled feedstock; W_F is then 0. The overall amount of the CPO's unrecoverable waste (W) is then calculated [Equation (15)]. The LFI of CPO is then calculated [Equation (16)].

$$W_{CPO} = W_{0,CPO} + [(W_{E,CPO} + W_{C,CPO})/2]$$

$$= 0.01 + [(0 + 0.0051)/2]$$

$$= 0.0126 \quad (15)$$

$$LFI_{CPO} = (V_{CPO} + W_{CPO}) / \{2M_{CPO} + [(W_{E,CPO} - W_{C,CPO})/2]\}$$

$$= (1 + 0.0126) / \{2(1) + [(0 - 0.0051)/2]\}$$

$$= 0.5069 \quad (16)$$

The utility of CPO (X_{CPO}) is calculated based on the lifetime and the average lifetime of CPO of half a year (*i.e.*, L and $L_{av} = 0.5$) [Equation (17)]. The functional units of the CPO (U and U_{av}) are represented by an oil extraction rate of 18.00% (based on the interviews). The $F(X)$ and the product-level MCI of CPO are then achieved in Equations (18) and (19).

$$X_{CPO} = (L_{CPO}/L_{av,CPO})(U_{CPO}/U_{av,CPO}) \quad (17)$$

$$= (0.5/0.5)(0.18/0.18) = 1$$

$$F(X)_{CPO} = 0.9/X_{CPO} = 0.9/1 = 0.9 \quad (18)$$

$$MCI_{CPO} = 1 - [(LFI_{CPO})(F(X)_{CPO})]$$

$$= 1 - [(0.5069)(0.9)] = 0.5438 \quad (19)$$

Table 3 shows that PF has the highest MCI value of 0.6159, followed by EFB, CPO, PKS, and palm kernel. The high MCI value of PF relates to its use in biodiesel production. This is consistent with

TABLE 2. THE AVERAGE QUANTITY OF EACH PRODUCT PER TONNES OF CPO

Output	Quantity (t/yr)		Quantity (t/t of CPO)		Average quantity (t/t of CPO)
	1 st factory (mill)	2 nd factory (mill and refinery)	1 st factory (mill)	2 nd factory (mill and refinery)	
FFB	237,600	950,400	5.5556	5.5556	5.5556
CPO	42,768	171,072	1.0000	1.0000	1.0000
Palm kernel	14,256	57,024	0.3333	0.3333	0.3333
EFB	54,648	190,080	1.2778	1.1111	1.1444
PF	30,888	133,056	0.7222	0.7778	0.7667
PKS	14,256	57,024	0.3333	0.3333	0.3333
Decanter cake	5,940	38,016	0.1389	0.2222	0.2056
POME	51,084	199,584	1.1944	1.1667	1.1722
Other impurities	23,760	104,544	0.5556	0.6111	0.6000

Bejarano *et al.* (2022) that palm fibre is favourable for incineration in boilers to obtain energy. Yeo *et al.* (2020) stated that oil palm biomass in Malaysia favours the linear over the circular economies in terms of profitability; however, the reduction in imported resources in electricity production raises the circularity implementation.

To further calculate the company-level MCI, sales revenue is used as the normalising factor, with the product price data obtained from the Department of Alternative Energy Development and Efficiency (DEDE), Ministry of Energy, and the Department of Internal Trade, Ministry of Commerce (DEDE, 2023). The MCIs of multiple products are then summarised and the company-level MCI is achieved as 0.5372, in which the CPO contributes the highest portion, followed by palm kernel, PKS, PF and EFB, respectively (Table 4).

Results of Circularity by Improving OER

The MCI value of 0.5372 in Table 4 comes from the OER of 18.00%. However, the OER may

be increased by 5.00% (or 23.00% OER) based on the highest OER of Indonesia's OER value in 2020 (IJM Corporation Berhad, 2021). Calculating the MCI value based on the higher OER of 23.00% yields a better MCI value, as in Table 5 and 6. Table 5 shows that CPO, PF and EFB have the highest MCI values. It is clear that when the percentage of OER is increased by 5.00%, from 18.00% to 23.00%, the company-level MCI increases by 15.97%, from 0.5372 to 0.6230.

In summary, the product-level circularity measurement at 18.00% OER shows that PF has the highest MCI value among the five oil palm products (Table 4), as PF is utilised in the plant. It also has residual oil collected for reuse and ash collected to be used in oil palm plantations to increase FFB yields. The company-level MCI, when using the sales revenue as the normalising factor, is 0.5372. This is greater than 0.5000, meaning Thailand's current palm oil production is circular rather than linear. Moreover, when the % OER increases to 23.00%, the company-level MCI value rises to 0.6230, indicating that a higher % OER brings a higher MCI value.

TABLE 3. MCI OF MULTIPLE PRODUCTS

Parameter	CPO	Palm kernel	EFB	PF	PKS
M	1.0000	0.3333	1.1444	0.7667	0.3333
V	1.0000	0.3333	1.1444	0.7667	0.3333
$M_{\text{Co-product}}$	0.0511	0.1778	0.1391	0.1711	0.1286
M_{Waste}	0.0100	0.0167	-	-	-
$M_{\text{Residual oil}}$	-	-	0.0806	0.0593	-
M^*	0.0611	0.1944	0.2196	0.2303	0.1286
W_0	0.0100	0.0167	-	-	-
C_R	0.8364	0.9143	-	-	-
C_U	-	-	0.3669	0.2573	-
C_C	-	-	0.6331	0.7427	1.0000
C_E	-	-	-	-	-
W_C	0.0051	0.0178	-	-	-
E_C	0.9000	0.9000	-	-	-
W_F	-	-	-	-	-
E_F	-	-	-	-	-
W	0.0126	0.0256	-	-	-
L	0.5000	0.0194	0.0139	0.2500	1.0000
L_{av}	0.5000	0.0194	0.0139	0.2500	1.0000
U	18.00%	41.67%	18.9000	20.2400	18.9000
U_{av}	18.00%	43.00%	17.4600	17.2800	20.4000
X	1.0000	0.9690	1.0823	1.1714	0.9267
F(X)	0.9000	0.9288	0.8315	0.7683	0.9712
LFI	0.5069	0.5456	0.5000	0.5000	0.5000
MCI	0.5438	0.4932	0.5842	0.6159	0.5144

Note: The efficiency of the co-product recycling process (E_C) is 0.9.

TABLE 4. COMPANY-LEVEL MCI USING SALES REVENUE AS THE NORMALISING FACTOR

Product	Sales revenue (\$)	Sales revenue contribution (%)	Calculated MCI	Company-level MCI with the sales revenue as the normalising factor
CPO	809.6	81.93	0.5438	$0.8193 \times 0.5438 = 0.4455$
Palm kernel	126.6	12.81	0.4932	$0.1281 \times 0.4932 = 0.0632$
PKS	36.9	3.73	0.5144	$0.0373 \times 0.5144 = 0.0192$
PF	12.7	1.29	0.6159	$0.0129 \times 0.6159 = 0.0079$
EFB	2.4	0.24	0.5842	$0.0024 \times 0.5842 = 0.0014$
Total	988.2	100.00	-	0.5372

TABLE 5. MCI OF MULTIPLE PRODUCTS AT 23.00% OER

Parameter	CPO	Palm kernel	EFB	PF	PKS
M	1.0000	0.2609	0.8957	0.6000	0.2609
V	1.0000	0.2609	0.8957	0.6000	0.2609
$M_{\text{Co-product}}$	0.0511	0.1391	0.1391	0.1711	0.1286
M_{Waste}	0.0100	0.0130	-	-	-
$M_{\text{Residual oil}}$	-	-	0.0846	0.0622	-
M^*	0.0611	0.1522	0.2237	0.2333	0.1286
W_0	0.0100	0.0130	-	-	-
C_R	0.8364	0.9143	-	-	-
C_U	-	-	0.3783	0.2667	-
C_C	-	-	0.6217	0.7333	1.0000
C_E	-	-	-	-	-
W_C	0.0051	0.0139	-	-	-
E_C	0.9000	0.9000	-	-	-
W_F	-	-	-	-	-
E_F	-	-	-	-	-
W	0.0126	0.0200	-	-	-
L	0.5000	0.0194	0.0139	0.2500	1.0000
L_{av}	0.5000	0.0194	0.0139	0.2500	1.0000
U	23.00%	41.67%	18.9000	20.2400	18.9000
U_{av}	18.00%	43.00%	17.4600	17.2800	20.4000
X	1.2778	0.9690	1.0823	1.1714	0.9267
F(X)	0.7043	0.9288	0.8315	0.7683	0.9712
LFI	0.5069	0.5456	0.5000	0.5000	0.5000
MCI	0.6429	0.4932	0.5842	0.6159	0.5144

Note: The efficiency of the co-product recycling process (E_C) is 0.9.

TABLE 6. COMPANY-LEVEL MCI USING SALES REVENUE AS THE NORMALISING FACTOR AND 23.00% OER

Product	Sales revenue (\$)	Sales revenue contribution (%)	Calculated MCI	Company-level MCI with the sales revenue as the normalising factor
CPO	809.60	85.28	0.6429	$0.8528 \times 0.6429 = 0.5483$
Palm kernel	99.10	10.44	0.4932	$0.1044 \times 0.4932 = 0.0515$
PKS	28.90	3.04	0.5144	$0.0304 \times 0.5144 = 0.0156$
PF	10.00	1.05	0.6159	$0.0105 \times 0.6159 = 0.0065$
EFB	1.90	0.20	0.5842	$0.0020 \times 0.5842 = 0.0011$
Total	949.50	100.00	-	0.6230

CONCLUSION

This study applies the product-level MCI to multiple products of the oil palm industry and the company-level MCI to obtain the overall MCI that represents the circularity of a country's palm oil production. It is essential to measure the circularity of oil palm products because this industry is related to the food and energy security of the country, with approximately 75% of the CPO being used for domestic consumption. Waste from the oil palm industry requires crucial attention to reduce the environmental impacts. This is consistent with Cheah *et al.* (2023) that full ranges of biowaste management, such as waste-to-energy, waste treatment, and waste valorisation for by-products productions, are required for the oil palm industry in Malaysia to enhance the sustainability in environmental, social, and economics aspects.

This study applies the modified unrecoverable waste formula to calculate the circularity of multiple products in the oil palm industry. Rocchi *et al.* (2021) agreed that the modified MCI should be applied to the agricultural sector to reflect its biological cycles. The overall MCI of palm oil production is greater than 0.5000 (*i.e.*, 0.5372), indicating the high circularity of palm oil production. This is consistent with Falcone *et al.* (2022) that MCI values for agricultural products range between 0.4600–0.6800 due to the reuse of the co-products obtained in both agriculture and extraction phases and lower production of unrecoverable waste.

CPO is found to represent the highest contribution to the company-level MCI. As 75% of CPO is used for domestic consumption, especially in the biodiesel and refined palm oil industries, the circularities of palm oil as biodiesel and for cooking palm oil production should be further studied. The oil palm industry should also focus on improving the quality of palm oil and investing in new technology to enhance the CPO extraction processes, as the results show an improvement of MCI values from 0.5372 to 0.6230 when the extraction rate improves by 5.00%. This is consistent with Anyaoha and Zhang (2023), that a low CPO extraction rate affects greenhouse gas emissions, and that research is required to achieve a greener economy for the oil palm industry in Nigeria. Usapein *et al.* (2022) suggested strategies to enhance the bio-circular-green economy of the oil palm industry in Thailand. They include developing regulations to manage the oil palm industry systematically, evolving smart innovation to improve harvesting technologies, raising palm biomass consumption rate, and increasing CPO value-added. Lim *et al.* (2021) suggested using new technologies, such as spray drones and modified drones with smart spectrometers, to enhance the circular economy of the oil palm industry in Malaysia.

This study contributes to the oil palm industry. The use of by-products and proper waste management of oil palm raise the environmental standard of the industry, making it compatible to compete in the global market. The company-level MCI using the sales revenue as the normalising factor proves the circularity of the reuse and recycling of oil palms. The government and related agencies may provide training on new knowledge and technologies to enhance the OER to increase the circularity. Such training should focus on the CPO and PF to enhance the biodiesel and edible oil production processes.

This study has some limitations. Primary data used in the analysis are from two palm oil mills in Thailand. An increasing number of data may yield more accurate results. Moreover, secondary data are retrieved from the literature not limited to Thai literature. The use of secondary data must be adjusted to suit the working culture.

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BELOWGROUND FUNGAL COMMUNITY COMPOSITION AND DISTRIBUTION IN PEAT FORESTS AND A MANAGED PEAT ECOSYSTEM OF MALAYSIAN TROPICAL PEATLAND

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ABSTRACT

Fungi have a primary role in the decomposition of organic matter in tropical peatlands and contribute to maintaining ecosystem functions. Understanding the belowground fungal diversity and composition is a preliminary step to predicting the stability of ecosystem functions, especially in ecosystems that have been exposed to anthropogenic impacts. This study determined soil fungal diversity and community composition in two types of forest: Peat swamp forest (PSF) and logged-over secondary forest (LOF) and a managed ecosystem (oil palm plantation, OPP) in peatland in Sarawak, Malaysia. Fungi from peat samples were isolated and identified using 18S rDNA region sequencing. Fungal diversity was calculated, and Bray-Curtis dissimilarity was performed to compare fungal communities between the study sites. LOF has the highest diversity, followed by PSF and OPP. In addition, the fungal community composition is more similar between LOF and PSF than between the peat forests and OPP. Aspergillus spp. and Trichoderma spp. contributed to the great dissimilarities between the peat forests and the managed ecosystem due to their dominance. In conclusion, LOF is an important ecosystem that retains relatively high fungal diversity. Different fungal communities were observed, which contained ecologically important fungal groups incorporated into the peat forests and an oil palm plantation.

Keywords: fungal diversity, logged-over forest, primary forest, tropical peatland.

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INTRODUCTION

A growing interest in belowground biodiversity in peatlands has been observed over the last two decades, owing to their functional importance in regulating nutrient cycling, decomposition, primary production, and climate change (Bach *et al.*, 2020; Song *et al.*, 2020). Belowground biodiversity, comprising primarily of microorganisms (bacteria, archaea, and fungi) and larger organisms (earthworms, ants, termites and arachnids), play important ecological roles as symbionts, mutualists, decomposers, and pathogens (Bardgett

& Van Der Putten, 2014; Food and Agriculture Organization, 2020; Tedersoo *et al.*, 2014). Soil microorganisms, particularly bacteria, archaea, and fungi, are key indicators of various soil quality and health attributes, such as nutrient availability, carbon sequestration, incidence of plant diseases and promotion of plant growth (Fierer *et al.*, 2021). Their roles are crucial in terrestrial ecosystems, particularly in agricultural ecosystems, to address challenges and solutions for sustainable farming, food security, and climatic impacts. Understanding the community, structure, and diversity of soil microorganisms is critical for predicting ecosystem functioning in response to environmental changes so as to allow the implementation of conservation and management efforts (Egidi *et al.*, 2019) and maintain ecosystem stability during climate change (Li *et al.*, 2019).

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In peatlands, soil fungi constitute a large fraction of the belowground community (Juan-Ovejero *et al.*, 2020). Most of the dominant soil fungi in peatlands are known as saprotrophs, which play a significant role in the decomposition of organic matter (Thormann, 2006; Thormann & Rice, 2007). Fungi produce a variety of extracellular enzymatic substances to assist in the degradation of simple and complex recalcitrant polymers, such as starch, cellulose, proteins, chitin, keratin and lignin of wood (Eichlerová *et al.*, 2015; Paredes *et al.*, 2014). In addition, other fungi classified in the fungal guilds of pathogens, yeasts, ectomycorrhizal fungi, ericoid mycorrhizal and dark septate endophytic fungi also contribute to the decomposition of organic matter (Andersen *et al.*, 2013; Juan-Ovejero *et al.*, 2020; Robinson *et al.*, 2020). In a recent commentary, symbiotic fungi are considered ecosystem engineers due to their role in regulating the carbon sink in ombrotrophic peatlands via nutrient transfer (Barel & Robroek, 2023). Therefore, soil fungal communities and their diversity are recognised as the main components in regulating carbon dynamics and nutrient turnover in the peatland ecosystem (Juan-Ovejero *et al.*, 2020).

Soil fungal communities have been extensively identified and studied in many ecosystems across the globe (Egidi *et al.*, 2019; Newsham *et al.*, 2016; Shi *et al.*, 2014) including in peatlands. However, studies are limited to bogs (Filippova & Thormann, 2014; Golovchenko *et al.*, 2013; Morrison *et al.*, 2020) and fens (Asemaninejad *et al.*, 2017; Mpamah *et al.*, 2017; Zhang *et al.*, 2017), while tropical peatlands remain overlooked (Juan-Ovejero *et al.*, 2020). As tropical peatlands are one of the largest carbon stock reservoirs (Page *et al.*, 2022), it is important to protect and restore them by studying the diversity and functional role of their inhabiting soil fungi. This is because the carbon balance in peatland ecosystems is largely carried out by microbial activity, besides the interactions between plants and diverse soil communities (Barel & Robroek, 2023; Page *et al.*, 2022). In Malaysia, tropical peatlands are exposed to anthropogenic disturbances, such as agricultural conversion, which led researchers to conduct studies on soil fungal communities. A few studies have emphasised the sensitivity of soil fungal communities towards the conversion of forest to oil palm cultivation (Kerfahi *et al.*, 2014; McGuire *et al.*, 2015; Robinson *et al.*, 2020). These studies showed that soil fungal communities can be an indicator or basis to monitor the impact of land use and response to environmental changes, especially in agricultural ecosystems. Thus, it is imperative to deepen the understanding of microorganisms in these ecosystems. Considering the importance of soil fungi and the limited studies in tropical

peatlands, this study was conducted to determine soil fungal communities and diversity in the tropical peatlands of Sarawak, Malaysia. The objective of this study is to determine soil fungal community, diversity, and structure under two types of forests, *i.e.*, peat swamp forest (PSF) and logged-over secondary forest (LOF), and a managed ecosystem in an oil palm plantation (OPP).

MATERIALS AND METHODS

Site Description and Sample Collection

Study sites were selected in the district of Sri Aman, Sarawak, Malaysia, representing the area of lowland tropical peatland. Peat samples were collected in August 2013 during the dry season (precipitation <200 mm/sampling month) from peat swamp forest (PSF), logged-over secondary forest (LOF) and a managed peat ecosystem represented by an eight years old OPP. PSF is a gazetted mixed tropical peat swamp forest, owned by the Malaysian government, with an extensive area of 43,147 ha (Melling, 2016) and located along a riverbank of the Maludam River. PSF is a habitat for several important high-value timber species, such as *Gonystylus bacanus*, *Dactylocladus stenostachys* and *Shorea* spp. This forest contains diverse faunal species, with a few endemic fauna such as *Presbytis chrysomelas crucige* (Red Banded Langur) and *Nasalis larvatus* (Proboscis monkey) (Melling, 2016). LOF is a secondary forest that was recovered after timber extraction for land preparation years ago. It was covered with shrubs and small woody trees (Kusai *et al.*, 2018). In OPP, routine management practices have been carried out throughout the year, such as the application of fertiliser and herbicides, tilling, compaction and maintaining the water table for palm growth and production. The reduction of the water table within the oil palm plantation was maintained by a drainage system on site (Dhandapani *et al.*, 2020). The total area of LOF was 2,411.5 ha, while the planted OPP area was 4,152.7 ha.

Peat samples were collected at ten sampling points at each study site. In PSF and OPP, the samples were collected in two transects with approximately a distance of 10 to 100 m between each sampling point, while samples from LOF were taken in a line transect at a 100 m interval (Ali *et al.*, 2016; Maidin *et al.*, 2016). The coordinates for each sampling point were recorded (Kusai *et al.*, 2018).

Peat soil cores were taken from peat surfaces up to 60 cm using a peat auger (Eijkelkamp, The Netherlands) with a half-cylindrical shape (diameter: 5.2 cm). A core sample collected in the peat auger was divided into four subsamples according to depths of 0-30, 30-40, 40-50 and

50-60 cm and placed into designated 50 mL tubes before being transported to the laboratory for analysis. All peat samples were stored at 4°C for a short-term period of one month, and biological analyses such as the isolation of fungi were conducted within two weeks of sampling.

Isolation and Identification of Fungi

Peat samples were serially diluted up to a dilution of 10^{-5} for fungal isolation. A volume of 100 μ L of dilutions 10^{-4} and 10^{-5} was spread evenly on potato dextrose agar (PDA) and malt extract agar (MEA) and incubated at $25 \pm 2^\circ\text{C}$ for three days. Colonies grown on both media were subcultured onto a new PDA to obtain pure cultures and used for identification.

The pure cultures were grown on PDA and incubated at $25 \pm 2^\circ\text{C}$ for three and seven days for fast-growing fungi and slow-growing fungi, respectively. The mycelium of the isolate was harvested, and genomic DNA was extracted using Fungi/ Yeast Genomic DNA Isolation Kit (NorgenBiotek Corp., Canada) according to the manufacturer's instructions.

A pair of primer; forward primer, EF4-F (5'-GG AAGGG[G/A]TGTATTTATTAG-3') and reverse primer fung5-R (5'-GTAAAAGTCCTGGTTCC CC-3') were employed to amplify the 18S rDNA region of fungi (Smit *et al.*, 1999). A volume of 25 μ L PCR master mix was used; 1 $\times$ PCR buffer, 1.0 μ M of each primer, 0.2 mM of each dNTP, 0.3 U of GoTaq Flexi DNA polymerase (Promega, Madison, WI, USA), 2.5 mM MgCl_2 , 0.3% of bovine serum albumin and 1.0 μ L DNA template. Amplification of 18S rDNA was carried out in a thermal cycler (Applied Biosystems Veriti™ Thermal Cycler, USA) with the following cycle: Initial denaturation at 94°C for 3 min, 30 cycles of denaturation, annealing and extension at 94°C for 1 min, 48°C for 1 min, 72°C for 1 min, respectively. The final extension was performed at 72°C for 5 min.

The PCR product was electrophoresed in 1% agarose gel, stained with FloroSafe DNA Stain (First BASE Laboratories, Malaysia) and run at 80 V for 30 min. The gel was viewed under UV to observe the amplified band of interest with a size of 550 bp. The amplified bands were excised and purified using a purification kit, EasyPure® Quick Gel Extraction Kit (Transgen Biotech, China). The purified products were sent to First BASE Laboratories (Malaysia) for sequencing according to the Sanger sequencing-based method. Sequences were compared with the nucleotide sequences in GenBank using Basic Local Alignment Search Tool (BLAST) analysis (<http://blast.ncbi.nlm.nih.gov/blast/Blast.cgi>) to verify the similarity of the sequences and classify the fungal isolates into genus and species levels.

Diversity Indices and Data Analysis

Species diversity in the study sites was calculated using the Shannon's diversity index (H') (Shannon & Weaver, 1949) and the Simpson diversity index (D) (Simpsons, 1949). Species richness (S) was determined using the total number of species in each study (Magurran, 2004; Pielou, 1969). Evenness (E) was also calculated to determine the distribution of abundance across the species in a community (Hill *et al.*, 2003). Analysis of similarities (ANOSIM) was run to determine the significant variation in the fungal composition between PSF, LOF and OPP. Prior to analysis, the square root transformation was applied to the fungal composition data. The comparison of fungal composition between study sites was evaluated using pairwise Bray-Curtis distance and displayed using non-metric multidimensional scaling (NMDS). Similarity percentage (SIMPER) was also determined to identify the dominant species contributing to the fungal composition in the study sites. All the analyses were performed using PRIMER version 7 software (PRIMER-E Ltd., Plymouth, United Kingdom).

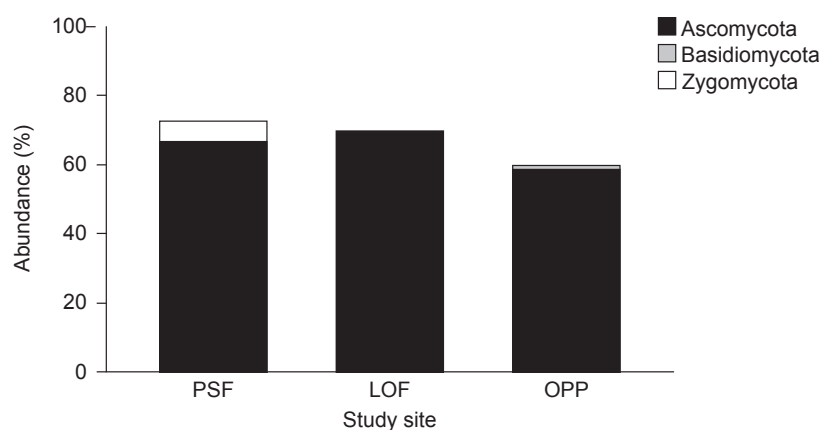
RESULTS AND DISCUSSION

Belowground Fungal Community and Composition

A total of 203 isolates were isolated and identified from all study sites, representing 3 phyla, 16 genera, and 30 species. The percentage of similarity between the isolated fungi and the sequences in the GenBank was high, between 81% and 100%. In total, 73 isolates were obtained from PSF, while 70 and 60 isolates were obtained from LOF and OPP, respectively. Three phyla comprising Ascomycota, Basidiomycota, and Zygomycota were identified using the 18S rDNA region, with Ascomycota being the most frequently isolated from the peat samples (12 genera) (Figure 1). Meanwhile, only Ascomycota was found in the LOF. A small proportion of Basidiomycota was obtained from the OPP, whereas Zygomycota was only found in PSF.

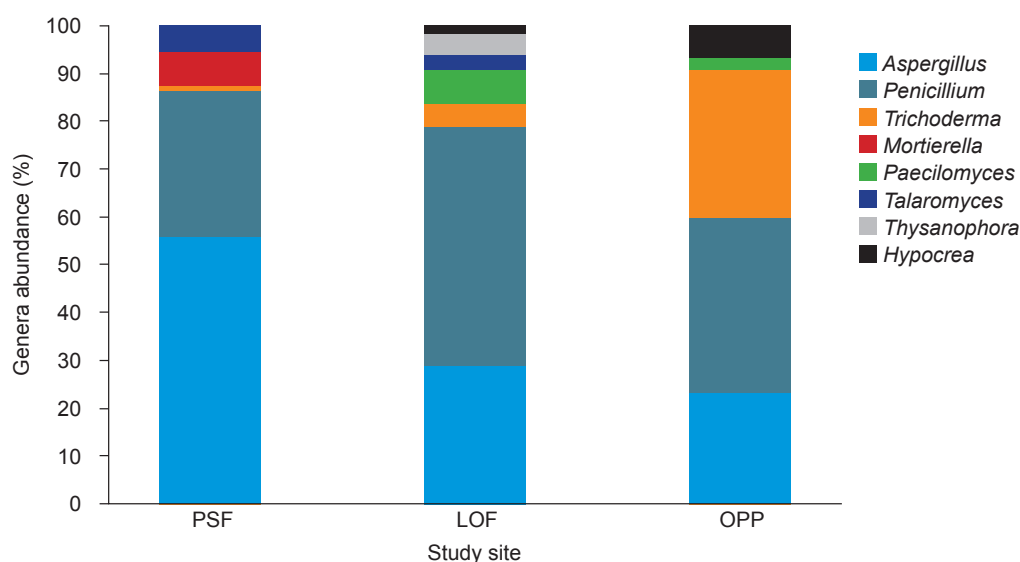
At the genus level, *Aspergillus*, *Penicillium* and *Trichoderma* were commonly found in all study sites (Figure 2). About 54.8% of the fungi found in the PSF were *Aspergillus* and half of this number was from the LOF and OPP. *Penicillium* was highly isolated from the LOF at 47.1%, compared to PSF (30.2%) and OPP (34.9%). Meanwhile, *Trichoderma* was distinctly found in OPP (30%), compared to PSF (1.4%) and LOF (4.3%).

The common species present in all study sites were *Aspergillus fumigatus*, *Penicillium corylophilum*, *P. roqueforti*, *Penicillium* sp. and *Trichoderma* sp.



Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation.

Figure 1. Abundances of soil fungal taxa.



Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation.

Figure 2. Abundance of fungal genera in all study sites. Genera less than 2% were excluded. Genera excluded were *Cordyceps*, *Coniochaetales*, *Lecythophora*, *Meyerozyma*, *Myrothecium*, *Polyporus*, *Purpureocillium* and *Umbelopsis*.

(Table 1). The teleomorph of *Trichoderma*, i.e., *Hypocrea*, was found in the LOF (1.4%) and OPP (6.7%). A species of Basidiomycota, *Polyporus ciliatus*, was isolated at a low frequency (1.7%) in OPP. Meanwhile, *Mortierella chlamydospora* (6.8%) and *Umbelopsis isabellina* (1.4%), which are classified in the phylum Zygomycota, were only found in the PSF.

Five species unique to PSF, *A. fischeri*, *A. flavipes*, *M. chlamydospora*, *P. purpureogenum* and *U. isabellina*, were absent in the LOF and OPP. Likewise, five species from the OPP, i.e., *Coniochaetales* sp., *H. rufa*, *Lecythophora* sp., *P. freii*, and *Polyporus ciliates*, were not found at other study sites. In contrast, nine species were found only in the LOF and not detected in the PSF and OPP. The species were *Cordyceps cylindrica*, *Meyerozyma guilliermondii*,

Myrothecium sp., *P. chrysogenum*, *P. commune*, *P. oxalicum*, *Purpureocillium lilacinum*, *Thysanophora* sp., and *Trichoderma harzianum*.

Fungal Diversity

Based on Shannon's and Simpson's diversity indices, LOF displayed a higher fungal diversity, at 2.321 and 0.864 (Table 2), than the other sites. This was followed by PSF ($H' = 2.166$, $D = 0.852$) and OPP ($H' = 2.049$, $D = 0.832$). Species richness was also highest in the LOF, with 18 species. However, PSF and OPP recorded the same richness, with 14 species. Species evenness ranged between 0.776 and 0.821. The least even distribution was observed in OPP (0.776), while the highest evenness was recorded in PSF (0.821).

The fungal community composition differed significantly between PSF, LOF, and OPP (ANOSIM, $R_{\text{global}} = 0.336$; number of permutations = 999; $P < 0.001$; Table 3). The fungal community in PSF and OPP showed a significant dissimilarity ($R = 0.452$, $P < 0.001$). The ordination plot showed differences in species composition between PSF and LOF, where the composition resembled each other more than OPP (Figure 3).

The SIMPER analysis revealed that three species contributed to more than 80% of the total species identified in PSF (Table 4). *Aspergillus fumigatus* contributed about 50.85% of the fungal assemblages in PSF, while *Penicillium* sp. (37.24%) and *Trichoderma* sp. (55.83%) contributed

greatly to fungal assemblages within the LOF and OPP.

SIMPER analysis for dissimilarity indicated greater dissimilarity between PSF and OPP (average dissimilarity = 74.87%) than LOF and OPP (average dissimilarity = 73.24%) and between PSF and LOF (average dissimilarity = 60.87%, Table 5). The species that contributed to the largest differences were *Trichoderma* sp. and *A. fumigatus*, abundant in OPP and PSF. The taxa that contributed to the dissimilarity between LOF and OPP were *Trichoderma* sp. and *Penicillium* sp. The fungal community in PSF was more similar to LOF, with *Aspergillus* sp., *Penicillium* sp. and *P. roqueforti* as the main contributors.

TABLE 1. PERCENTAGE ABUNDANCE OF FUNGI ISOLATED IN DIFFERENT STUDY SITES

Species	Percentage abundance (%)		
	PSF	LOF	OPP
<i>Aspergillus fischeri</i>	1.4	0	0
<i>Aspergillus flavipes</i>	2.7	0	0
<i>Aspergillus fumigatus</i>	31.5	25.7	16.7
<i>Aspergillus niger</i>	1.4	1.4	0
<i>Aspergillus nomius</i>	5.5	0	1.7
<i>Aspergillus</i> sp.	12.3	0	3.3
<i>Coniochaetales</i> sp.	0	0	1.7
<i>Cordyceps cylindrica</i>	0	1.4	0
<i>Hypocrea atroviridis</i>	0	1.4	5.0
<i>Hypocrea rufa</i>	0	0	1.7
<i>Lecythophora</i> sp.	0	0	1.7
<i>Meyerozyma guilliermondii</i>	0	1.4	0
<i>Mortierella chlamydospora</i>	6.8	0	0
<i>Myrothecium</i> sp.	0	1.4	0
<i>Paecilomyces</i> sp.	0	7.1	1.7
<i>Penicillium chrysogenum</i>	0	1.4	0
<i>Penicillium commune</i>	0	5.7	0
<i>Penicillium corylophilum</i>	5.5	2.9	3.3
<i>Penicillium freii</i>	0	0	3.3
<i>Penicillium oxalicum</i>	0	1.4	0
<i>Penicillium purpurogenum</i>	1.4	0	0
<i>Penicillium roqueforti</i>	8.2	11.4	23.3
<i>Penicillium</i> sp.	15.1	24.3	5.0
<i>Polyporus ciliatus</i>	0	0	1.7
<i>Purpureocillium lilacinum</i>	0	1.4	0
<i>Talaromyces purpureogenus</i>	5.5	2.9	0
<i>Thysanophora</i> sp.	0	4.3	0
<i>Trichoderma harzianum</i>	0	1.4	0
<i>Trichoderma</i> sp.	1.4	2.9	30.0
<i>Umbelopsis isabellina</i>	1.4	0	0

Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation.

TABLE 2. DIVERSITY INDICES OF SOIL FUNGAL COMMUNITIES

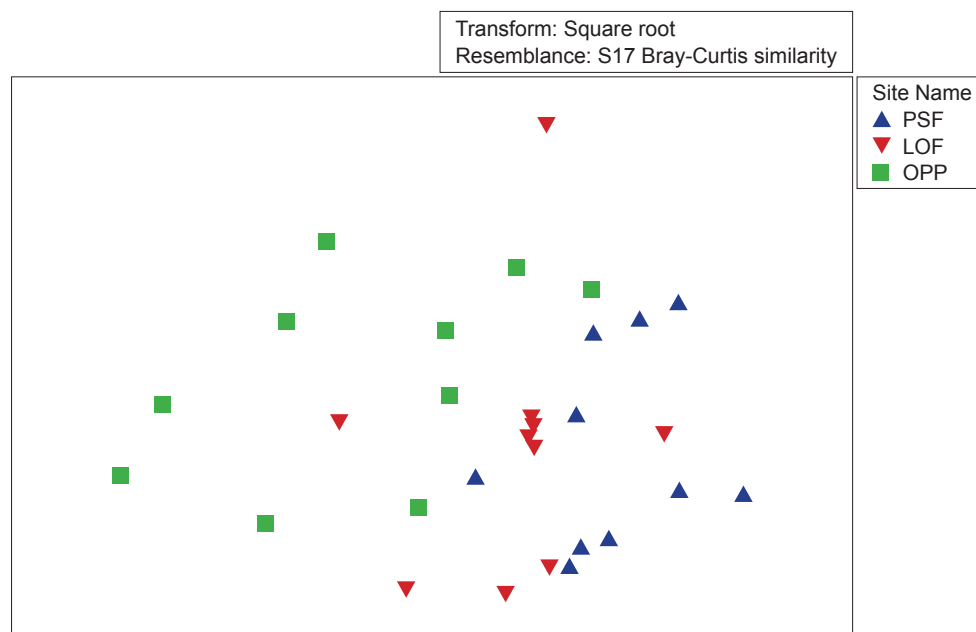
Sites	Total isolates	Shannon's diversity index	Simpson's diversity index	Species richness	Evenness
PSF	73	2.166	0.852	14	0.821
LOF	70	2.321	0.864	18	0.803
OPP	60	2.049	0.832	14	0.776

Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation.

TABLE 3. ANALYSIS OF SIMILARITIES (ANOSIM) IN SPECIES COMPOSITION OF FUNGI

Sites	R value ^a	P-value
PSF vs. LOF	0.2	0.003
PSF vs. OPP	0.452	0.001*
LOF vs. OPP	0.372	0.001*

Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation; vs. - versus; ^a ANOSIM; R global = 0.336; *Number of permutations = 999; P < 0.001.



Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation.

Figure 3. Non-metric multidimensional scaling (N-MDS) ordination comparing the fungal community in different study sites.

TABLE 4. SIMPER ANALYSIS OF THE SIMILARITY OF SPECIES COMPOSITION

Sites	Average similarity	Species	Average abundance	Contribution (%)	Cumulative contribution (%)
PSF	47.90	<i>Aspergillus fumigatus</i>	1.49	50.85	50.85
		<i>Aspergillus</i> sp.	0.78	16.49	67.34
		<i>Penicillium</i> sp.	0.80	15.91	83.24
LOF	44.99	<i>Penicillium</i> sp.	1.21	37.42	37.42
		<i>Aspergillus fumigatus</i>	1.19	34.30	71.72
OPP	34.13	<i>Trichoderma</i> sp.	1.16	55.83	55.83
		<i>Penicillium roqueforti</i>	0.87	23.24	79.07

Note: Study sites: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation.

TABLE 5. SIMPER ANALYSIS OF DISSIMILARITY OF FUNGAL COMMUNITIES

Study site	Average dissimilarity (%)	Species dissimilarity	Average abundance	Contribution (%)
PSF vs. LOF	60.87	<i>Aspergillus</i> sp.	0.78	11.30
		<i>Penicillium</i> sp.	0.80	10.63
		<i>Penicillium roqueforti</i>	0.48	10.02
		<i>Aspergillus fumigatus</i>	1.49	7.97
		<i>Mortierella chlamydospora</i>	0.44	6.80
		<i>Penicillium corylophilum</i>	0.34	5.98
		<i>Talaromyces purpureogenus</i>	0.34	5.63
		<i>Paecilomyces</i> sp.	0.00	5.47
		<i>Penicillium commune</i>	0.00	5.06
		<i>Aspergillus nomius</i>	0.34	4.72
PSF vs. OPP	74.87	<i>Trichoderma</i> sp.	0.10	15.44
		<i>Aspergillus fumigatus</i>	1.49	13.37
		<i>Penicillium roqueforti</i>	0.48	11.17
		<i>Penicillium</i> sp.	0.80	11.11
		<i>Aspergillus</i> sp.	0.78	9.51
		<i>Mortierella chlamydospora</i>	0.44	6.25
		<i>Penicillium corylophilum</i>	0.34	5.44
LOF vs. OPP	73.24	<i>Trichoderma</i> sp.	0.20	15.54
		<i>Penicillium</i> sp.	1.21	15.16
		<i>Aspergillus fumigatus</i>	1.19	12.87
		<i>Penicillium roqueforti</i>	0.74	10.94
		<i>Paecilomyces</i> sp.	0.38	5.70
		<i>Penicillium commune</i>	0.34	4.81
		<i>Penicillium corylophilum</i>	0.20	4.46
		<i>Hypocrea atroviridis</i>	0.10	3.87

Note: PSF - peat swamp forest; LOF - logged-over secondary forest; OPP - oil palm plantation; vs. - versus.

Discussion

Fungi obtained in this study belonged to the Ascomycota, Basidiomycota, and Zygomycota taxa, commonly present across peatlands, such as in China (Zhang *et al.*, 2017), Japan (Yabuki *et al.*, 2014), Canada (Asemaninejad *et al.*, 2017; Zhang *et al.*, 2023), Europe (Elliott *et al.*, 2015; Sun *et al.*, 2016; Vinogradova *et al.*, 2023), Malaysia (Ayob *et al.*, 2018; Kusai *et al.*, 2018), Brunei (Tripathi *et al.*, 2016a), and Australia (Birnbaum *et al.*, 2023). These taxa can degrade organic matter, including cellulose, hemicellulose, pectin, lignin, tannin and polyphenolic compounds (Thormann, 2006). A large proportion of Ascomycota indicated that this taxon is predominant in the peatlands of Sarawak, either in natural or managed ecosystems, due to their physiological characteristics as heavy sporulators and fast-growing microorganisms (Thormann & Rice, 2007). Moreover, ascomycetes have high adaptability to environmental stresses by utilising resources, leading to more generalist strategies

and consequently contributing to their dominance in soil (Egidi *et al.*, 2019). On the other hand, Basidiomycota is primarily a slow-growing fungus and a less prolific sporulator than Ascomycota (Thormann & Rice, 2007), which might explain the low abundance of Basidiomycota in Sarawak's peatland. Less distribution of Zygomycota in peatlands was commonly reported than the other two taxa (Juan-Ovejero *et al.*, 2020; Liu *et al.*, 2020).

The dominance of several genera was observed and identified in all study sites. Species belonging to the genus *Aspergillus* were greater in number and more frequently isolated than other genera in PSF. *Aspergillus fumigatus* dominated the fungal communities in PSF. It is a saprophytic fungus known as a recycler in nature by degrading plant polysaccharides (Benoit *et al.*, 2015; Kwon-Chung & Sugui, 2013). Diverse metabolites enable this species to grow in different environmental conditions (Houbraken *et al.*, 2014). Thus, it was also detected under managed ecosystem like oil palm plantations.

The genus *Penicillium* was more frequently isolated in LOF, with unknown *Penicillium* sp. making up the fungal communities. *Penicillium* is a predominant fungus found in a wide range of environments and substrates, including peat soil (Yabuki *et al.*, 2014). *Penicillium* was previously detected in the peatlands of Argentina (Paredes *et al.*, 2014), Finland (Sun *et al.*, 2016), and Russia (Dobrovolskaya *et al.*, 2012). *Penicillium* produces extracellular enzymes to degrade polysaccharide such as cellulose in plant cells (Vaishnav *et al.*, 2018; Zhang *et al.*, 2014). Therefore, the presence of *Penicillium* is important for plant biomass degradation and carbon cycling in the logged-over secondary forest ecosystem.

Trichoderma spp. were the dominant fungi and contributed the most to the fungal community in OPP. They are root-colonising fungi and important plant-symbiotic microbes, with a high distribution in agricultural soils (Jiang *et al.*, 2016). A sampling conducted in 2012 also found that this group of fungi was more highly distributed in oil palm plantations than in other ecosystems (Kusai *et al.*, 2018). The existence of *Trichoderma* spp. in plantations enhances plant growth and nutrient uptake efficiency, increases photosynthesis, improves root structure, and induces systemic resistance to abiotic and biotic stresses (Harman, 2011; Shores *et al.*, 2010; Tyśkiewicz *et al.*, 2022). Metabolites produced by *Trichoderma* spp. (Mendoza-mendoza *et al.*, 2018) provide a promising biological control for severe oil palm disease, such as basal stem rot caused by *Ganoderma boninense* (Alexander *et al.*, 2017; Supramani *et al.*, 2022). Thus, the presence of *Trichoderma* may serve as a bioindicator for monitoring palm health and productivity besides reducing the use of chemical fungicides to control soil-borne diseases. It is still unknown why *Trichoderma* is exclusively isolated from oil palm plantations. *Trichoderma* may adapt to obtain nutrients from other soil organisms (Chaverri & Samuels, 2013) or enriched nutrients from soil environments that favour their growth in oil palm plantations.

Fewer species of Basidiomycota and Zygomycota were isolated from the sites in comparison to ascomycetes. Only one species of Basidiomycota was found in OPP, *i.e.*, *Polyporus ciliatus*. The species was previously reported in the Russian bog (Grum-Grzhimaylo *et al.*, 2016) and Caucasus regions, such as Iran, Turkey, and Russia (Gjobad-Nehjad, 2011). Two species of zygomycetes isolated from PSF, *Mortierella chlamydospora* and *Umbelopsis isabellina*, were previously isolated by Kusai *et al.* (2018) in 2012 from the same site, PSF. Despite the low isolated numbers of *M. chlamydospora* and *U. isabellina*, these species still reproduce and were detected in PSF in this study. *Mortierella chlamydospora* has a

specialised structure with a thick wall and resistant spores that can survive for an extended period and remain dormant in peat soil (Lin *et al.*, 2014). *Umbelopsis isabellina* has been reported to be highly distributed in boreal forest in Canada (Summerbell, 2005), while *M. chlamydospora* was found in agricultural areas in the United Kingdom (Hunt *et al.*, 2004).

This study showed that primary and secondary logged-over forests and managed ecosystems have different fungal communities and dominant species within the study sites. The overall compositions of the isolate assemblage that make up the fungal communities in PSF and LOF were more similar to one another compared to those in OPP. Notable differences were observed in the fungal community of OPP and PSF compared with LOF, as shown in the NMDS ordination, which can be attributed to the impacts of anthropogenic disturbances in the OPP (Kerfahi *et al.*, 2014). These findings are consistent with Nurulita *et al.* (2016), who reported a distinct microbial community in the peatland forest that was converted into an oil palm plantation and rubber plantation in Indonesia. A similar finding in mineral soil was also reported in Peninsular Malaysia (Mcguire *et al.*, 2015). Both studies demonstrated that fungal communities differ between primary forest and oil palm plantations compared to regenerating forests. Based on a study of fungal mycelium production by Robinson *et al.* (2020), fungal communities of saprophytic, mycorrhizal and pathogenic species were significantly different between oil palm plantations and forests (old-growth and selectively logged). After six months of installation of hyphal-in growth bags, mycelium fungal communities were unaffected by the logging event since both peat forest types showed similar fungal communities (Robinson *et al.*, 2020). Although oil palm plantations showed different fungal communities, they can still support several ecologically important fungal groups like plant-symbionts (*Trichoderma* spp.) and saprotrophs (*Aspergillus* spp. and *Penicillium* spp.) (Sun *et al.*, 2016), which are beneficial to plant development and decomposition processes, respectively.

In this study, LOF showed a higher diversity for both diversity indices when compared with PSF and OPP. This finding is consistent with a study by Mcguire *et al.* (2015), who revealed that the diversity of soil fungal communities is higher in regenerating forests (selectively logged 50 years ago) than in primary forest and oil palm plantations. Several aspects need to be considered when explaining this result, such as soil physical and chemical properties (Juan-Ovejero *et al.*, 2020) at each study site. Besides, the vegetation composition and chemical composition of litter could also contribute to the present results (Artz, 2009). LOF, a regenerating

forest logged years ago, would accumulate wood debris on the upper layer of peat soil, which might attract a more diverse species of fungi for the decaying processes. Logged-over secondary forests can retain the same taxonomy and structure of microbes as in a forest (Tripathi *et al.*, 2016b).

In this study, PSF was expected to have a higher fungal diversity than LOF and OPP due to the absence of anthropogenic activities at the site. Interestingly, lower fungal diversity and richness were recorded in PSF than in LOF. The result might be due to the dominance of *Aspergillus* spp., which may exclude less competitive or slow-growing species during isolation (Thormann, 2006), and subsequently reduce diversity. A similar observation was recorded in OPP for *Trichoderma* spp. A study by Repečkienė *et al.* (2012) reported how the abundance of fast-growing and sporulating species like *Trichoderma* and *Penicillium* suppressed the growth of other fungal species in peatland. However, studies by McGuire *et al.* (2015); Nurulita *et al.* (2016), and Brinkmann *et al.* (2019) showed that the fungal diversity-based Shannon diversity index in OPP was similar to or even slightly higher than the primary forest ecosystem. No significant effect on the fungal diversity due to the conversion of forests to agricultural lands, such as rubber and oil palm plantations, was reported in their studies that were conducted in Malaysia and Indonesia. Nurulita *et al.* (2016) who focused on peatland, showed that fungal diversity in OPP was not significantly affected by oil palm practices compared to rubber practices. The contrasting finding may be due to the method of analysis by Nurulita *et al.* (2016), who used the culture-independent method PCR-DGGE, which identified a higher rate of microbial diversity compared to the culture-dependent method (Ayob & Kusai, 2021).

This study showed that the slightly reduced fungal diversity in OPP compared to the natural ecosystems is likely to be related to the addition of fertiliser (Berkelmann *et al.*, 2020), organic matter stock (Allen *et al.*, 2016), and vegetation richness (Drescher *et al.*, 2016) which can all exert some effects on the fungal diversity in OPP. However, a managed ecosystem like OPP can support considerable fungal diversity and richness. Numerous factors can also influence the structure of the fungal diversity and community in ecosystems, such as soil properties (Birkhofer *et al.*, 2012; Lauber *et al.*, 2008; Zhang *et al.*, 2016), vegetation diversity (Allingham *et al.*, 2023; Dassen *et al.*, 2017), hydrology (Lamit *et al.*, 2021) and tree species (Prescott & Grayston, 2013; Sun *et al.*, 2016; Urbanová *et al.*, 2015) and further investigation is needed to provide more concrete evidence.

CONCLUSION

In summary, the composition of the fungal community and diversity were different between forests (PSF and LOF) and a managed peat ecosystem OPP. However, similar species composition was recorded between PSF and LOF in comparison to OPP. Remarkable results were found in LOF, which recorded the highest fungal diversity and richness compared to PSF and OPP. OPP does not always negatively impact diversity as they still support several fungal species that are important to carry out ecosystem functions, such as the decomposition of organic matter. However, proper and sustainable management within the OPP needs to be applied to preserve its fungal diversity. Natural and managed peat ecosystems have their unique species composition, which is ecologically important in regulating the decomposition of peat and, consequently, for the carbon cycle. The presence and absence status of particular fungal species could be an indicator for monitoring ecosystem disturbances. Nevertheless, further studies using advanced technology, such as next-generation sequencing (NGS), especially for spatio-temporal analysis, are necessary for a more comprehensive understanding of soil fungal diversity in mediating biogeochemical cycles in tropical peatlands.

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ASSESSMENT OF PARASITIC NEMATODES *Elaeolenchus parthenonema* POINAR, ON POLLINATING WEEVIL, *Elaeidobius kamerunicus* AT SELECTED OIL PALM PLANTATIONS IN PAHANG, MALAYSIA

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ABSTRACT

The introduction of the oil palm pollinating weevil, *Elaeidobius kamerunicus* (Coleoptera: Curculionidae) from Africa in 1980s brought substantial benefit to the Malaysian economy. However, it was discovered that the weevil was parasitised by a nematode, *Elaeolenchus parthenonema* Poinar. This raising concern is addressed in this article based on the assessment of the parasitic nematodes *E. parthenonema* on the weevil at selected oil palm plantations in Pahang, Malaysia. This study was conducted at Ladang Sungai Bebar Selatan Jerantut (peat soil), FELCRA Tembeling Tengah Jerantut (mineral soil) and MPOB Jerantut (mineral soil). The anthesis stage of oil palm male spikelets was sampled for weevil and nematode inspection. The weevils were inspected and showed a high number of nematodes from the larval, pupa, to adult stages. The nematode was not detected at the egg stage. The mean and percentage of total infected *E. kamerunicus* per spikelet by nematodes in March 2021 was highest at Ladang Sungai Bebar Selatan Jerantut (peat soil) with 19.19 ± 0.83 , 91.03%, followed by September 2021 at 27.04 ± 1.95 , 84.88% and October 2021 at 23.74 ± 2.24 , 76.45% as compared to FELCRA Tembeling Tengah Jerantut (mineral soil) and MPOB Jerantut (mineral soil). Thus, the finding of high numbers of infected *E. kamerunicus* with nematodes raises significant concerns regarding the negative effects of nematode parasitism on weevils.

Keywords: mineral soil, oil palm, parasitic nematode, peat soil, pollinator weevil.

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INTRODUCTION

Malaysia is one of the most well-known and significant exporters of palm oil in the world, holding a stake of 34.30% in the worldwide palm

oil trade (Parveez *et al.*, 2021). As the second-largest palm oil producer in the world, Malaysia has the opportunity to play a significant role in the food and biofuel market due to its huge and expanding palm oil sector and strong global demand for oil palm (Gan & Li, 2014). In 2021, Malaysia reported 451 palm oil mills were in operation, with a total annual processing capacity of 115.87 million tonnes of fresh fruit bunches (FFB) (Parveez *et al.*, 2022). Moreover, 52.50% of the total processing capacity of 57.50 million tonnes were contributed from Peninsular Malaysia (Parveez *et al.*, 2022). The Arecaceae family includes the oil palm (*Elaeis guineensis* Jacq.), the main established industrial crop in Malaysia (Nurul Fatimah *et al.*, 2019). Malaysia earned RM108.52 billion in total revenue from exporting palm oil and palm oil products in

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2021 (Malaysian Palm Oil Board [MPOB], 2022). Apart from that, the Malaysian palm oil industry employs over 500,000 people and provides for the welfare of an estimated one million people (Ministry of Plantation Industries and Commodities [MPIC], 2018). The most valuable resource in Malaysia's agricultural sector is oil palm (Zulkefli *et al.*, 2020). In the year 2016, 5.74 million hectares were planted with oil palm (Kushairi *et al.*, 2018). The total area of the oil palm planted reached 5.74 million hectares in 2021 as compared to 5.87 million hectares in 2020, indicating a 2.2% decline in total oil palm area due to the COVID-19 outbreak (Parveez *et al.*, 2022). The COVID-19 epidemic had halted replanting operations in oil palm plantations. A significant performance improvement was reported in the Malaysian oil palm sector in 2019 compared to 2018, with notable gains made in exports, palm oil stockpiles, and prices with export revenue of RM67.52 billion in 2018 and RM68.84 billion in 2019 (Parveez *et al.*, 2020). The export revenue climbed year after year as Malaysia generated RM108.52 billion in revenue from the export of palm oil and its products in 2021 compared to 2020 which earned RM73.33 billion (Parveez *et al.*, 2023). This happened due to the outbreak of the coronavirus disease (COVID-19) and the year 2020 faced an unusual challenge for nearly all of the worldwide revenue growth (Parveez *et al.*, 2021).

Oil palm is a monocotyledonous plant due to the prevalence of both male and female inflorescences on the same palm (Adam *et al.*, 2011). The female and male inflorescences bloom at various times and pollinate throughout the year (Gintoron *et al.*, 2023). The oil palm inflorescence is known to be self or cross-pollinated, wind-pollinated (anemophily) and insects-pollinated (entomophily) (Appiah & Dwarko, 2013). Until the late 1970s, oil palm was considered an anemophily instead of an entomophily (Swaray *et al.*, 2023). There are two native insect pollinators for oil palm in Malaysia, *Thrips hawaiiensis* and *Pyroderces* sp., but these pollinators proved to be inefficient (Teo, 2015). The pollination of oil palm is mostly entomophilous and attracts numerous insects mainly the genus *Elaeidobius* which are drawn to the inflorescences (Hala *et al.*, 2012).

During the anthesis stage, the oil palm pollinating weevil, *Elaeidobius kamerunicus* was drawn to the oil palm by the volatile organic compounds (VOCs) emitted from fully developed flowers with a strong anise smell known as estragole (Fahmi *et al.*, 2021; Aisagbonhi *et al.*, 2004). *Elaeidobius kamerunicus* is mostly lured to male inflorescences, but it is misled by a scent shared by both male and female inflorescences, which resulted in pollination and pollen unintentionally being carried from male to female inflorescences

(Yousefi *et al.*, 2020; Sambathkumar & Ranjith, 2011). Estragole compounds are prominent for oil palm pollination activity and their capability to draw *E. kamerunicus* even without food source (Fahmi Halil *et al.*, 2021). In addition to depositing their eggs on the anthesising male flowers, adult weevils consume oil palm pollen. The larvae feed on pollen on the male flowers and become adult weevils within 10 days (Tuo *et al.*, 2011).

Elaeidobius kamerunicus is a small weevil that belongs to the order Coleoptera, family Curculionidae and it was found to reproduce and spread quickly in oil palm plantations across the country (Swaray *et al.*, 2023; Syed, 1981). The pollination assistance is primarily delivered by *E. kamerunicus*. Along with its high reproductive rate and ability to carry more pollen than other *Elaeidobius* species, *E. kamerunicus* was preferred for introduction into Malaysia (Yousefi *et al.*, 2020). *Elaeidobius kamerunicus* was brought into Malaysia in 1981 from Cameroon to Ladang Pamol in Sabah and Kluang, Johor (Nurul Fatimah *et al.*, 2019; Syed, 1979). It further improved pollination and increased oil palm yields (Tuo *et al.*, 2011). The pollinator *E. kamerunicus* effectively resolved the pollination problem in oil palm plantations throughout the period, ultimately resulting in improved oil production in most countries, including Malaysia, Indonesia and India (Caudwell *et al.*, 2003).

In some areas in East Malaysia, low pollination has led to poor fruit sets, as documented by Yousefi *et al.* (2020). Recent findings point out that inadequate pollination due to the lack of weevils causes seasonal low fruit sets. Weevil numbers precipitously declined as a result of the wide nematode infection and unfavourable weather (Vaknin, 2012). The wet season is also one of the factors that influence the emergence of nematode parasitism in the *E. kamerunicus* population. *Elaeolenchus parthenonema* is a weevil parasite that belongs to the superfamily Sphaerulariidea, and was first documented by Lubock in 1981 (Jackson and Bell, 2001). Since it survives and adapts well inside the weevil host, it is assumed that this nematode was associated with the introduced *E. kamerunicus* from Africa. Although every effort has been made to control all relevant organisms before importation into Malaysia, these internal parasites might have missed further inspection (Poinar *et al.*, 2002; Syed *et al.*, 1981). Caudwell *et al.* (2003) and Kang (1999) suggested that a low population of *E. kamerunicus* weevils can be associated with various problems. It may be due to direct or indirect effects of weather or nematode parasitism that can occur because of climate change. Further research by Mohamad *et al.* (2023) indicated that although both sexes of *E. kamerunicus* harbour nematodes, only extensively infected female weevils show the symptoms, which

include a decrease in egg production, sterility, and reduction in size in response to a decrease in body fat. The weevil's ability to pollinate efficiently may be significantly affected by its interaction with this parasitic nematode. Thus, this study was conducted to inspect the percentage infection of parasitic nematode on *E. kamerunicus* in selected locations of study in Pahang.

MATERIALS AND METHODS

Location of the Study

This study was carried out at Site I, Ladang Sungai Bebar Selatan, Jerantut (3°14'55.00"N 103°21'26.00"E) (Year planted: 2010, age: 13 years, tree type: DxP); Site II, FELCRA Tembeling Tengah, Jerantut (4°13'18.12"N 102°23'13.63"E) (Year planted: 2011, age: 12 years, tree type: DxP); and Site III, MPOB Jerantut, Pahang (4°17'14.45"N 102°24'44.12"E) (Year planted: 2013, age: 10 years, tree type: CLONE P456). Site I was picked to represent peat soil, and both Site II and Site III represented mineral soil. Every study location experienced rain in March, October and September with Hourly Relative Humidity (%) of >70% (MET, 2021) (Figure 1). The collection of samples in this study involved field and fruit sampling at all three sites. The fruit samplings were randomly collected at each location.

Sampling Procedures for *E. kamerunicus* and Parasitic *E. parthenonema*

Elaeidobius kamerunicus were collected in oil palm plantations through anthesis male flower cutting. Nine spikelets were randomly selected from each anthesis male flower and three were picked from the top, middle and bottom (Jackson *et al.*, 2003). Samples of anthesis male flowers were brought to the laboratory for examination. The weevils were collected from each spikelet for nematode identification (n = 27).

The weevils (larva, pupa and adult) were crushed and examined under a compound microscope to identify the presence of nematodes in the weevils. The crushed *E. kamerunicus* were taken out, cleaned in sterile water and put in White Traps in accordance with the nematode isolation protocol described by Orozco *et al.* (2014). The samples were inspected under a compound microscope Amscope to determine the presence of nematodes in the weevils. The characteristics of the parasitic nematode, *E. parthenonema* were observed using RaxVision 9.7-inch Touch Screen Tablet XPAD-97. Then, the morphological features of the nematode were referred to Caudwell *et al.* (2003), Zulkefli *et al.* (2011) and

Poinar *et al.* (2002). The emergence of parasitic nematodes inside the haemocoel of *E. kamerunicus* was evaluated to classify the severity of the infections.

Data Analysis

The total number of parasitic nematodes was recorded, and the percentages of infections were evaluated to measure the infestations and their impact on weevil populations. The following Equation (1) by Zulkefli *et al.* (2011) was used to determine the percentages of infected pollinating weevil *E. kamerunicus* by *E. parthenonema*:

$$\frac{\text{Number of infected } E. \text{ kamerunicus by nematode } E. \text{ parthenonema}}{\text{Total number of } E. \text{ kamerunicus}} \times 100 \quad (1)$$

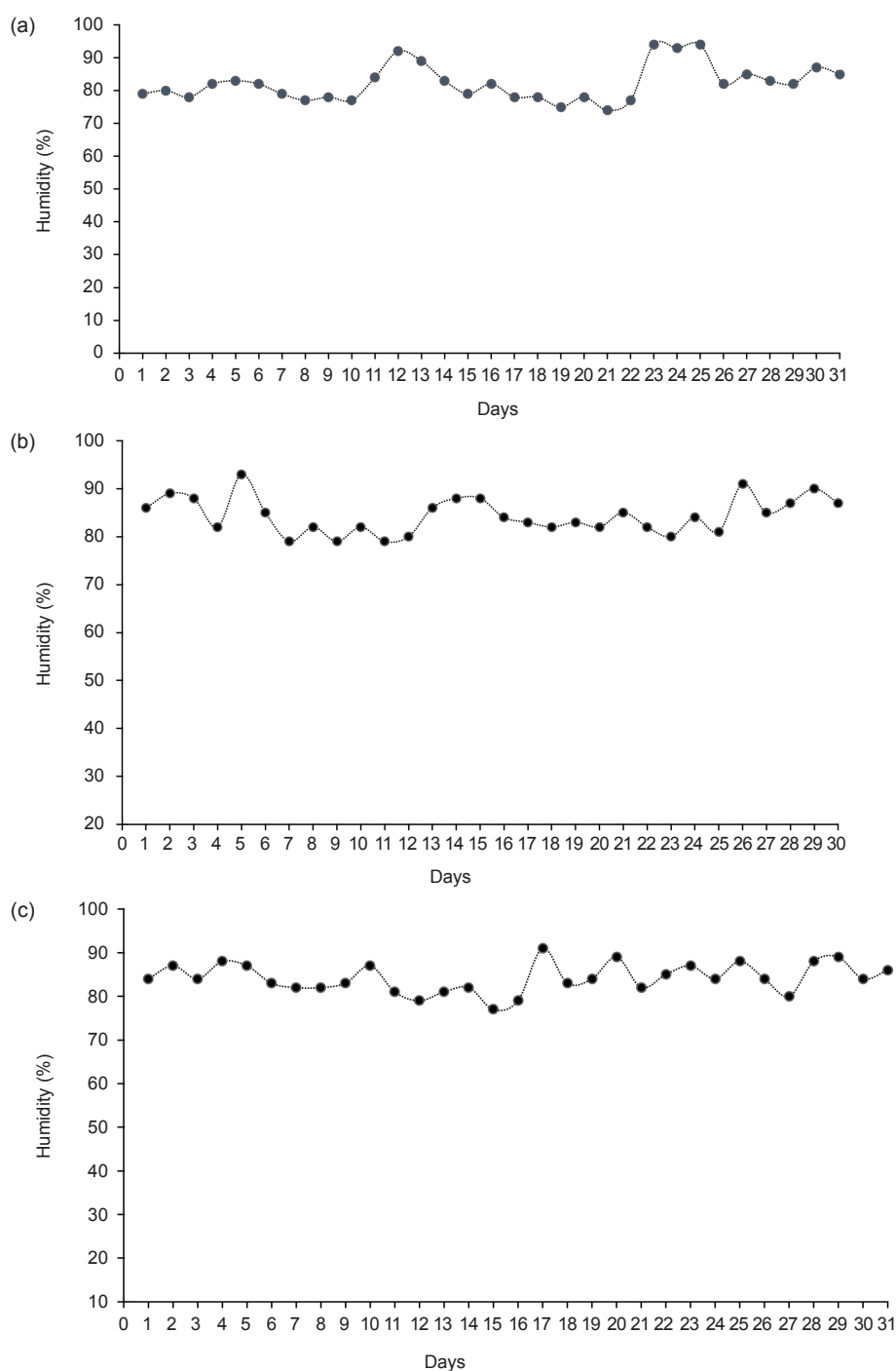
The ANOVA test was performed to analyse the infected *E. kamerunicus* (larvae, pupae, adult) per spikelet by using R studio Version 3.1.3.

RESULTS AND DISCUSSION

The Characteristics of Parasitic Nematodes *E. parthenonema* Isolated from *E. kamerunicus*

During the study, parasitic nematodes were found in the weevil population at all locations of the study. All life stages of *E. kamerunicus* were found with the nematodes, except for the egg. *Elaelenchus parthenonema* that infected the *E. kamerunicus* was identified as an internal parasite to the weevil and belonged to the superfamily Sphaerularioidea Lubbock 1861 (Poinar *et al.*, 2002; Jackson & Bell, 2001). Figure 2 shows an adult stage of *E. parthenonema*. The mature female nematode was mostly found in the haemocoel of late larval instars, pupae and adult stage of *E. kamerunicus* weevil. Capinera (2008) revealed that obligate parasites belonging to the Sphaerulariidae are commonly discovered in the body cavity of their insect host. The shape of a mature female nematode is sausage-shaped (Figure 2).

The entire body of a mature female nematode is filled up with genital system. It has a stylet and an elongated ovary. Adult *E. parthenonema* body is loaded with eggs and has developed stylet (Figure 2). Poinar *et al.* (1993) stated that the foremost features of fertilised female nematode in the Sphaerulariidae family had an elongated uterus and bulb-shaped pharyngeal glands. They also added that the glands lengthened over the intestine and formed a short lobe. The identification of adult nematode is based on the coiled body (Figure 2). Zulkefli *et al.* (2011)



Source: MET (2021).

Figure 1. The percentage of humidity on (a) March 2021, (b) September 2021 and (c) October 2021 in Pahang.

indicated that the coiled body of the parasitic female nematode aided in the identification. In this study, it can be found at a minimum number of one to 20 nematodes per weevil. Poinar *et al.* (2002) found one weevil could host up to 30 mature female and juveniles. Besides, no male nematodes were found in this study, similar to the previous study reported by Poinar *et al.* (2002).

The juvenile stage four (J4) is a pre-adult nematode stage (Figure 3a-3b). This nematode moults to a female adult. The body of juvenile

stage four nematode has a nearly curved form and rounded body in contrast to juvenile stage three which has a slim body (Figure 3b). Furthermore, it possesses a circled head and short stylet as compared to juvenile stage 3, which has a pointy stylet. The infective stage (J3) has a similar stylet as the mature female nematode (Poinar *et al.*, 2002). It has a slender formed body and is swift in swimming (Figure 3c). This nematode stage is extended and thin. The tail is pointed and 70% of the nematode body is filled with granular material.

They are highly active and can swim in the water by undulating their bodies like snakes or S shape movements. The infective stage (J3) nematodes can be found mostly in the digestive system of the weevil or within the weevil haemocoel. It can be observed in larvae, pupae, live weevils dead weevils. These nematodes are isolated by squashing the insects and maintained in White Trap for a certain period only. These nematodes can only be maintained for a certain period of time, because they are highly adapted to the insect host, *E. kamerunicus* weevil. Furthermore, juvenile stage three can be found to clump together (Figure 3c), with juvenile stage four

(Figure 3b) other stages of nematodes. Aisagbonhi *et al.* (2004) found that the abdomen of a single adult weevil has 20 to 30 nematodes that fit the *E. parthenonema* infective stage (J3) description. Juvenile stage two (J2) nematodes are small and slender with very poor locomotion (Figure 3d). It is difficult to distinguish the nematodes' features. Juvenile stage two (J2) nematodes are found in the White Traps that keep adult female nematodes. They are mostly found in ruptured adult female nematodes haemocoel. Aisagbonhi *et al.* (2004) found juvenile stage two (J2) nematodes in the haemocoel of one weevil.

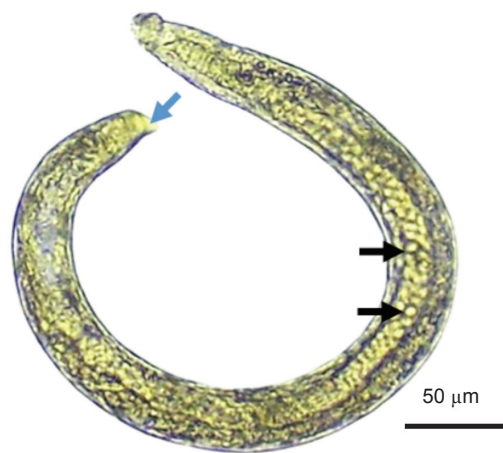


Figure 2. Adult *E. parthenonema* with a developed stylet (blue arrow) and eggs (black arrows).



Figure 3. *Elaeolenchus parthenonema*, (a) juvenile stage 4, (b) juvenile stage 3 (infective stage) together with juvenile stage 4, (c) juvenile stage 3 (infective stage) and (d) juvenile stage 2.

Severity of Infections Parasitic Nematodes, *E. parthenonema* on *E. kamerunicus*

The occurrence of parasitic nematodes indicates the severity of infection in *E. kamerunicus*. Camino *et al.* (2016) and Poinar (1975) stated that the insect's body cavities can host nematodes from the superfamilies Sphaerulariidae and the consequence of these nematode infection to insects has resulted to infertility and mortality. The uninfected weevil showed no nematode infection in *E. kamerunicus* (Figure 4a). Meanwhile, mild nematode infection on weevil can indicate a low infection of nematodes. There were only a few nematodes found inside the weevil (Figure 4b). Nematodes can take up half of the haemocoel and elytra space and are classified as moderate nematode infections. The infection is considered high when nematodes from various stages fully occupy the weevil (Figure 4c). In heavily infected *E. kamerunicus*, nematodes can critically impact the weevil as they parasitised all space in the haemocoel (Figure 4d). It is a severe nematode infection on the weevil. Nematode parasitism can be worse as they develop fast and multiply in the host.

Assessment of *E. kamerunicus* Infected by Parasitic Nematodes per Spikelets

Parasitic nematode infections were positively identified at all three locations of the study. Based on the statistical analysis, it was observed that the

mean of larvae that were infected by nematodes at Sungai Bebar Selatan (peat soil) was significantly greater than other locations, which was 24.74 ± 0.96 followed by MPOB Jerantut (mineral soil) 23.33 ± 1.63 and FELCRA Tembeling Tengah Jerantut (mineral soil) 11.26 ± 0.96 ($p < 0.05$, $p = 0.001$) (Table 1). There was a significant difference between the mean number of pupae infected by the nematodes during the study period on samples collected in peat soil and mineral soil in March ($p < 0.05$, $p = 0.0216$). The mean number of infected pupae in Sungai Bebar Selatan (peat soil) was higher at 15.48 ± 1.37 as compared to infected pupae in FELCRA Tembeling Tengah Jerantut (mineral soil) at 12.74 ± 1.03 and the mean number of infected pupae in MPOB Jerantut (mineral soil) was 10.59 ± 1.03 . Meanwhile, samples collected in March showed no significant differences detected in all locations of the study in terms of the mean number of infected adult weevils ($p > 0.05$, $p = 0.4691$). FELCRA Tembeling Tengah Jerantut (mineral soil) was 34.07 ± 1.47 , followed by Sungai Bebar Selatan (peat soil) was 17.33 ± 1.32 and MPOB Jerantut (mineral soil) with 12.37 ± 0.91 .

It was observed that the mean of the larvae infected by the nematodes at Sungai Bebar Selatan (peat soil) was significantly greater than other locations, which was 44.07 ± 3.55 , followed by FELCRA Tembeling Tengah Jerantut (mineral soil) with 38.00 ± 4.25 and MPOB Jerantut (mineral soil) with 37.56 ± 3.05 ($p < 0.05$, $p = 0.000$) (Table 2). Meanwhile, the mean number of pupae infected by the nematodes showed no significant differences

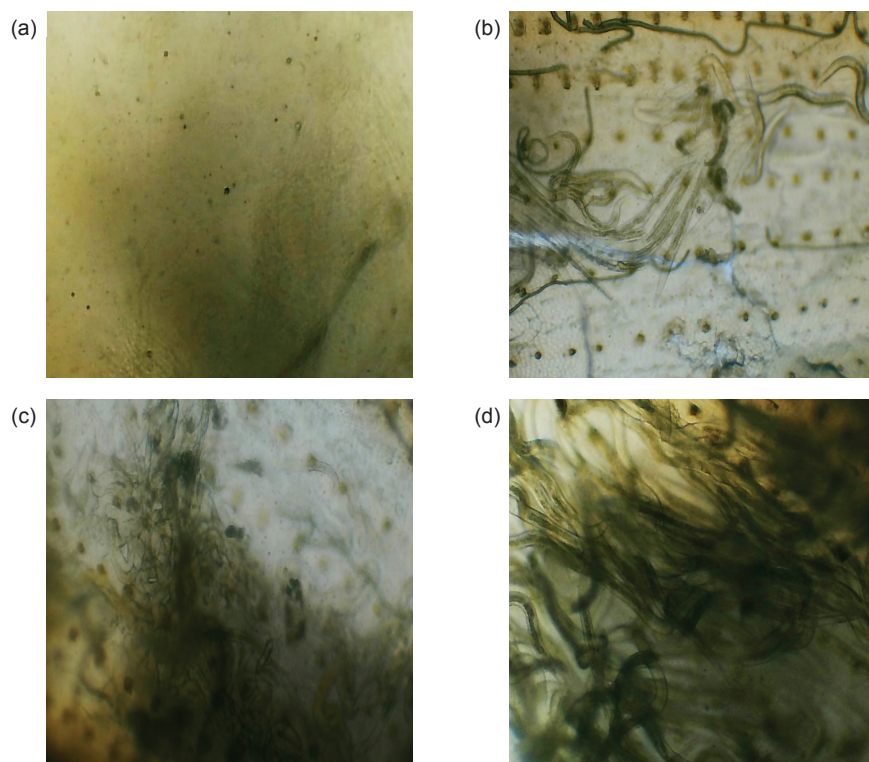


Figure 4. Classification of infection severity in haemocoel *E. kamerunicus*. (a) No nematode infection in *E. kamerunicus*, (b) mild nematode infection in *E. kamerunicus*, (c) moderate nematode infection in *E. kamerunicus* and (d) severe nematode infection in *E. kamerunicus*.

in all the study locations. The mean number of infected pupae in MPOB Jerantut (mineral soil) was 21.41 ± 1.23 , FELCRA Tembeling Tengah Jerantut (mineral soil) was 20.93 ± 1.59 , and Sungai Bebar Selatan (peat soil) was 17.41 ± 1.61 ($p > 0.05$, $p = 0.122$). Generally, there was a significant difference between the mean number of adult weevils that were infected by nematodes in all three locations, within FELCRA Tembeling Tengah Jerantut (mineral soil) at 34.63 ± 3.00 , Sungai Bebar Selatan (peat soil) 19.63 ± 1.76 and MPOB Jerantut (mineral soil) 18.81 ± 1.80 ($p < 0.05$, $p = 0.000$).

A comparison between the mean number of *E. kamerunicus* infected with nematodes per spikelet in October is presented in Table 3. The data based on October sampling showed that the study location in Sungai Bebar Selatan (peat soil) had higher infection of the nematodes compared to other locations. The statistical analysis showed that there were no significant differences ($p > 0.05$, $p = 0.082$) between the mean of larvae in all the study locations. The mean of the larvae that were infected by the nematodes at Sungai Bebar Selatan (peat soil) was 43.72 ± 2.59 , followed by FELCRA Tembeling Tengah Jerantut (mineral soil) 36.00 ± 4.96 and MPOB Jerantut (mineral soil) 32.89 ± 2.07 . Meanwhile, there was a significant difference ($p < 0.05$, $p = 0.000$) between the mean number of pupae infected by the nematodes in all three locations. The number of pupae that were infected in mineral soil was greater than in peat soil. FELCRA Tembeling Tengah Jerantut (mineral soil) was 23.78 ± 2.31 , MPOB Jerantut (mineral soil) was 18.00 ± 1.48 and Sungai Bebar Selatan (peat soil) was 10.22 ± 1.22 . The number of infected adults *E. kamerunicus* in FELCRA Tembeling Tengah Jerantut (mineral soil) was significantly greater (31.28 ± 2.93) than the number of infected adults in MPOB Jerantut (mineral soil), which was 24.98 ± 1.40 followed by Sungai Bebar Selatan (peat soil) with 23.74 ± 2.24 .

Rainfall with hourly relative humidity percentage of greater than 70% were reported in every study location in Pahang on March, October September 2021 (MET, 2021) (Figure 1a-1c). The results showed the weather patterns correlated to the parasitic nematode infection because the higher the moisture contents on the spikelets, the higher the infection by the parasitic nematodes. The moisture content of the spikelets might be influenced by the rainy season. Jackson *et al.* (2003) stated that the spikelets with the highest percentage of nematode infection on the larvae and pupae were three-hourly sprayed with water and had the tip of the spikelet soaked in water. They also suggested in comparison to spikelets that were sprayed water hourly, the daily water sprayers showed greater nematode infection on the larvae and pupae. This indicated that as the moisture level of the spikelet increased, the infection by the nematodes increased. Nonetheless, as the moisture increased, most nematodes would have been rinsed off from the spikelets and decreased the infections on the weevils. Furthermore, Zulkefli *et al.* (2011) stated that moderate rainfall showed higher infection of female nematodes on adult weevils compared to low and high rainfall in Pamol Sandakan Plantation in Sabah and MPOB Lahad Datu. They also added that excessive rainfall is associated with low fruit set as it inhibits weevil pollination efficiency. Low weevil populations had been identified as the root cause of occasionally observed poor pollination in Riau, Sumatra, Indonesia. The dramatic drop in the population numbers has been attributed to two factors, namely fewer male inflorescences (weevil breeding sites) during the rainy season and greater nematode parasitism (which reduces both weevil fecundity and longevity) before the conclusion of the rainy season (Donough & Law 1987; Jackson *et al.*, 2003; Zulkefli *et al.*, 2011).

TABLE 1. MEAN AND PERCENTAGE OF INFECTED *Elaeiodobius kamerunicus* WITH NEMATODES PER SPIKELET (MARCH 2021)

Location	Larvae		Pupae		Adult		Total	
	Mean	Nematode infection (%)	Mean	Nematode infection (%)	Mean	Nematode infection (%)	Mean	Nematode infection (%)
Sungai Bebar Selatan (peat soil)	24.74 ± 0.96^a n=27 spikelets total larvae = 732	91.26	15.48 ± 1.37^a n=27 spikelets total pupae = 455	91.87	17.33 ± 1.32^{ab} n=27 spikelets total adult = 520	90.00	19.19 ± 0.83	91.03
FELCRA Tembeling Tengah Jerantut (mineral soil)	11.26 ± 0.96^b n=27 spikelets total larvae = 476	63.87	12.74 ± 1.03^b n=27 spikelets total pupae = 516	66.67	34.07 ± 1.47^{ab} n=27 spikelets total adult = 1,252	73.96	19.35 ± 1.34	69.86
MPOB Jerantut (mineral soil)	23.33 ± 1.63^b n=27 spikelets total larvae = 963	65.42	10.59 ± 1.03^b n=27 spikelets total pupae = 511	55.97	12.37 ± 0.91^{ab} n=27 spikelets total adult = 498	67.07	15.34 ± 0.94	63.39

TABLE 2. MEAN AND PERCENTAGE OF INFECTED *Elaeidobius kamerunicus* WITH NEMATODES PER SPIKELET (SEPTEMBER 2021)

Location	Larvae		Pupae		Adult		Total	
	Mean	Nematode infection (%)	Mean	Nematode infection (%)	Mean	Nematode infection (%)	Mean	Nematode infection (%)
Sungai Bebar Selatan (peat soil)	44.07 ± 3.55 ^a n=27 spikelets total larvae = 1,399	85.06	17.41 ± 1.61 ^{ab} n=27 spikelets total pupae = 587	80.07	19.63 ± 1.76 ^a n=27 spikelets total adulte = 594	89.23	27.04 ± 1.95	84.88
FELCRA Tembeling Tengah Jerantut (mineral soil)	38.00 ± 4.25 ^b n=27 spikelets total larvae = 1,449	70.81	20.93 ± 1.59 ^{ab} n=27 spikelets total pupae = 744	75.94	33.93 ± 2.90 ^b n=27 spikelets total adult = 1,194	76.72	30.95 ± 1.95	74.02
MPOB Jerantut (mineral soil)	37.56 ± 3.05 ^b n=27 spikelets total larvae = 1,526	66.45	21.41 ± 1.23 ^{ab} n=27 spikelets total pupae = 818	70.66	18.81 ± 1.18 ^b n=27 spikelets total adult = 901	56.38	25.93 ± 1.48	64.71

TABLE 3. MEAN AND PERCENTAGE OF INFECTED *Elaeidobius kamerunicus* WITH NEMATODES PER SPIKELET (OCTOBER 2021)

Locations	Larvae		Pupae		Adult		Total	
	Mean	Nematode infection (%)	Mean	Nematode infection (%)	Mean	Nematode infection (%)	Mean	Nematode infection (%)
Sungai Bebar Selatan (peat soil)	43.72 ± 2.59 ^{ab} n=27 spikelets total larvae = 1,005	78.31	10.22 ± 1.22 ^a n=27 spikelets total pupae = 247	74.49	17.29 ± 1.40 ^a n=27 spikelets total adult = 425	73.18	23.74 ± 2.24	76.45
FELCRA Tembeling Tengah Jerantut (mineral soil)	36.00 ± 4.96 ^{ab} n=27 spikelets total larvae = 1,040	62.31	23.78 ± 2.31 ^b n=27 spikelets total pupae = 649	64.56	31.28 ± 2.93 ^b n=27 spikelets total adult = 841	66.94	30.19 ± 2.15	64.43
MPOB Jerantut (mineral soil)	32.89 ± 2.07 ^{ab} n=27 spikelets total larvae = 1,044	56.71	18.00 ± 1.48 ^b n=27 spikelets total pupae = 579	55.99	24.05 ± 2.22 ^b n=27 spikelets total adult = 673	64.34	24.98 ± 1.40	58.75

CONCLUSION

In conclusion, parasitic nematode infection was detected in all three study locations. The parasitic nematode infection was influenced by the weather. The higher the humidity, the higher the moisture content on spikelets. This indicates that when the moisture is increased, the parasitic nematode infection on *E. kamerunicus* is increased. There is also insufficient research on entomoparasitic nematode related to the type of oil palm soil and fruit set production in Peninsular Malaysia. It is vital to conduct extensive research on the ecology, biology, and distribution of the entomoparasitic nematode *E. parthenonema* to examine the parasite's actual and potential effects on the host, as well as the cascading effect on pollination and, ultimately the palm oil output. The fruit set in all of the experimental locations was excellent

for the time being. However, it is still unclear whether the nematodes can significantly influence the main pollinator of oil palm, *E. kamerunicus*'s longevity over a long period. Indeed, this matter has to be reinforced as reported in various published studies that this entomoparasitic nematode, *E. parthenonema* could give a detrimental effect on insects.

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ASSESSING THE PRODUCTIVITY, ECONOMIC AND SUSTAINABILITY IMPACTS OF *SHELL* DNA TESTING IN INDONESIAN OIL PALM SMALLHOLDINGS IN MUSI BANYUASIN AND PELALAWAN REGENCIES

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ABSTRACT

To assess the potential industrial vegetable oil (IVO) increase made possible by SHELL DNA supply chain testing, we conducted a field survey to quantify the presence of low-yielding contaminant oil palms in smallholder plantations. In the survey, 9,341 palms in 81 smallholdings in Musi Banyuasin (representing 88.8% of the total planted area) and 74 smallholdings in Pelalawan (representing 85.5% of the total planted area) were sampled and tested (planting year 2001 to 2020). Musi Banyuasin and Pelalawan regencies contained only 26.2% or 27.4% legitimate high-yielding hybrid tenera palms, respectively. The remaining 73.8% and 72.6% of palms were one of four undesirable low-yielding contaminant types. We determined that if SHELL DNA testing had been conducted in the past, smallholder FFB production (i.e., smallholder income) would have increased by 31.0% and 28.0%, PKO production would have increased by 31.0% and 28.0%, and CPO production would have increased by 49.0% and 46.0% in Musi Banyuasin and Pelalawan, respectively. We demonstrate that a ‘screen-then-plant’ paradigm for oil palm cultivation in Indonesia has the potential to improve the income of millions of Indonesian smallholders, increase the productivity of CPO and PKO mills, provide significantly increased feedstocks for biofuel conversion and further enhance the sustainability of the Indonesian palm oil industry.

Keywords: non-*tenera* contamination, oil yield, *SHELL* gene.

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INTRODUCTION

Indonesia has become the main driver for increasing vegetable oil usage for renewable biofuel production. At present, a small fraction of national transportation fuel is renewable under a Biodiesel B30 program which blends 30% of palm oil-based fuel into petroleum-based diesel fuel, primarily produced by large palm oil producers who supply first generation biofuel conversion plants with vegetable oil produced in their vertically integrated plantations and mills. Today, even though smallholders manage 41.2% of oil palm planted area nationally, smallholder production suffers from the cultivation of low-yielding palm types, little oil produced by smallholders is used as a green biofuel feedstock. Furthermore, smallholders have long been associated with deforestation through continuous expansion.

The government of Indonesia established the Smallholder Plantation Scheme (PIR) at the beginning of the 1980s to improve the economic welfare of smallholders. The scheme is of critical importance as smallholders have been, by far, the fastest increasing segment of the oil palm planted area even though large plantations were the cornerstone of early industry expansion. Increasing smallholder yield is an important strategy for enhancing smallholder welfare. While comprising less than 1% of the total input costs borne by smallholders, the purchase of high-purity oil palm seeds and seedlings remains the most important cost component of the replanting program, and to a great extent, the purity of the planting material determines fresh fruit bunches (FFB) yield over the life of the plantation. FFB yield and quality, in turn, determine smallholders' income as they sell fruit to supply chain intermediaries or directly to independent oil mills. To ensure that the oil palm industry, especially oil palm smallholders, benefit from high purity seeds, in 2015 the Government of Indonesia established the national standard for oil palm seeds (SNI 8211:2015) requiring that germinated seeds and the resulting nursery seedlings are the product of commercial DxP crosses by registered seed sources and that they have at least a 98% *tenera* purity. However, prior to the discovery of the *SHELL* gene (Singh *et al.*, 2013), collecting accurate measurements of *tenera* purity on seed, nursery seedlings and production palms in plantations has been difficult, if not impossible, to obtain.

Recently, breakthrough technologies provided a path for Indonesia to power vehicles with green biofuels based on the conversion of smallholder-produced industrial vegetable oil (IVO). First, *SHELL* DNA testing technology (hereafter referred to as "*SHELL* testing") has demonstrated the ability to identify low-yielding palms, enabling their removal

from the smallholder seedling supply chain prior to field planting (Ooi *et al.*, 2023; Singh *et al.*, 2013; 2022). With a test-then-plant paradigm, *SHELL* testing can protect the smallholder seed and seedling supply chain, while enabling the exclusive cultivation of elite planting material nationally. *SHELL* testing promises to dramatically increase smallholder production while simultaneously driving the sustainable supply of biofuel feedstock originating from the smallholder sector. Second, a next-generation biofuel conversion technology, developed in Indonesia, has demonstrated an alternative conversion of IVO to green biofuel in small-scale conversion mills, enabling the construction of small next-generation mills throughout the country adjacent to smallholder cooperatives which can supply the mills with higher quantity and quality fruit. Combined, these advances will forge a renewable green fuel future for Indonesia while benefitting smallholders.

The National Research and Innovation Agency (BRIN) through the National Strategic Project (PSN) has entered the pilot feasibility stage through the construction of two green biofuel conversion mills in Musi Banyuasin and Pelalawan regencies. The National Research and Innovation Agency through the National Research Project (PRN) funded the Estate Crop Research Center under the former Indonesian Agency for Agricultural Research and Development (IAARD) to achieve the following aims: Validate the accuracy of *SHELL* testing, conduct a contamination survey with *SHELL* testing to quantify the productivity of smallholdings in the two regencies, determine the economic cost/benefit of *SHELL* testing as a means to protect the smallholder seedling supply chain, and demonstrate whether *SHELL* testing operations can cost-effectively scale to support the national PSN/PRN smallholder green biofuel initiative.

Previous surveys of smallholder non-*tenera* contamination rates were limited to fruit-bearing palms and fruit form prediction by visual inspection of bisected fruits for shell thickness and the presence of fibres on the shell, a labour-intensive method with inherent ambiguity. For example, in a survey of 231 smallholdings in Rokan Hulu regency, Riau province, *dura* contamination was estimated to range from 53.6% in areas managed by large investment farmers to 83.0% in areas managed by small migrant farmers (Jelsma *et al.*, 2017). Here, we report a pilot study evaluating *SHELL* testing utility to guard the smallholder seed and seedling supply chain, the first genetic assessment of non-*tenera* contamination in Indonesia. We assess the accuracy of *SHELL* testing and report a contamination survey conducted in the Pelalawan and Musi Banyuasin Regencies. Finally, we report the value and economic impact of CPO and PKO production gains realised from the incorporation of *SHELL* testing into the smallholder seedling supply chain.

MATERIALS AND METHODS

Sampling Sites

Smallholdings from Musi Banyuasin and Pelalawan regencies were selected for *SHELL* testing. In these regencies, construction of the first IVO conversion plant to be supplied by local smallholdings FFB is already completed and is in production, while construction of a second is underway. Pelalawan is located in Sumatra's Riau province which provides the largest oil palm area nationally. Since smallholders manage 61.6% of Riau's oil palm planted area, Riau has the largest area cultivated by smallholders nationally. Pelalawan manages 164,000 ha (9.30%) of the total 1.76 million hectares smallholder managed area of Riau, 5.73% of the 2.86 million hectares total planted area of Riau, 2.70% of the 6.00 million hectares national smallholder-managed area and 1.10% of the 14.60 million hectares total national planted area (Direktorat Jenderal Perkebunan [DJP], 2023). Musi Banyuasin, South Sumatra province, has the third largest area cultivated by smallholders (122,000 ha, representing 23.40% of the total 522,000 ha smallholder managed area of Sumatera Selatan, 10.94% of the 1.11 million hectares total planted area of South Sumatra, 2.00% of national smallholder managed area and 0.80% of total national planted area) (DJP, 2023).

Sample Collection

Leaf samples from 5,062 oil palms were randomly sampled from 81 smallholdings in 13 of 15 sub-regencies of Musi Banyuasin. Leaf samples from 4,417 oil palms were randomly sampled from 74 smallholdings collected from 5 of 12 sub-regencies of Pelalawan regency. The selection of the combined 155 smallholders from both regencies, established between 2001 to 2020, was assisted by the corresponding regency estate crops department. On average, 61 palms were sampled at each smallholder site by using a barcoded leaf collection device (Orion Biosains "leaf punch"), representing ~22% (61/272) of the palms present on a typical 2 ha smallholding. Quick response (QR) barcodes were tracked in a laboratory information management system through all steps of the automated DNA testing process to provide reliable mapping of test results back to tissue source palms.

DNA Extraction and Analysis

DNA was extracted using standard DNA isolation protocols. Optimised high-throughput two-colour fluorescent PCR-based assays that resolve homozygous wild-type, homozygous variant or heterozygous genotypes were performed

for several *SHELL* fruit-form determining single nucleotide variants known to be present in commercial planting materials (Ooi *et al.*, 2016; 2023; Singh *et al.*, 2020). All DNA processes were performed in an ISO 17025 accredited laboratory. Successful genotype results (meaning that all tested variants were successfully genotyped in a given sample) were generated in 98.5% of the samples (9341/9479). Palms were categorised as *dura*, *pisifera* or *tenera* based on the determined genotype at each variant position, as described (Ooi *et al.*, 2016). The categorisation of phenotypes into seed producer *tenera* or *dura* versus illegitimate *tenera*, *dura* or *pisifera* is described below.

Statistical Methods

Statistical significance of the bimodal distribution of the percentage of planting sites *vs.* non-*tenera* contamination rates per planting site was confirmed by Welch's 2-sample t-test assuming unequal variance that compares the location parameters of two statistical populations to test the (null) hypothesis that the two populations have equal means. All other statistical calculations were performed using standard functions in Microsoft Excel.

RESULTS AND DISCUSSION

SHELL Testing Validation

The technical performance of high-throughput *SHELL* genotyping assays was validated in two independent studies utilising blinded control leaf samples of adult palms with known fruit form phenotypes. First, 72 replicated measurements were determined from 13 palms for a total of 936 tests (Ooi *et al.*, 2023). The leaf sample genotype call rate (*i.e.*, genotype called for all tested *SHELL* variants) was 97.6% (914/936), and the reproducibility of called genotypes was 100.0% (914/914). Second, 378 DNA samples [207 field palms from Pelalawan, 59 field and 44 nursery palms from Musi Banyuasin, and 68 adult blinded control palms from Pusat Penelitian Kelapa Sawit (PPKS)] were genotyped four independent times. The genotype call rate was 100.0% (1512/1512), and the reproducibility of genotypes was 100.0% (1512/1512). Combined, the genotype call rate was 99.1% (2426/2448), and reproducibility was 100.0% (2448/2448).

To determine the accuracy of fruit form prediction, 11 *dura*, 11 *pisifera* and 11 *tenera* palms were sampled in triplicate by scientists from PPKS (biological replicates). Leaf collection device barcodes (Materials and Methods) were anonymised and submitted for blind testing at Orion Biosains. Of 94 tested samples (5 punches lacked leaf material),

92 lab predicted phenotypes were concordant with field-recorded phenotypes. One *dura* phenotyped palm tested as a *tenera* and one *tenera* phenotyped palm tested as a *dura*. To explore whether these were due to genotyping error or minor sample recording/phenotyping error in the field, a DNA plate including the two discordant samples was genotyped again, in triplicate. All three additional replicated genotype results for each of the 68 re-tested palms matched the original genotype call. At the time genotyping was performed, it was not possible to re-phenotype the two discordant palms. However, given the 100% reproducibility of multiple re-genotyping events, the two discordances were likely due to misphenotyping of *dura* or *tenera* fruits, which can be difficult to physically distinguish and is estimated to occur in approximately 5% of visual phenotyping calls (Singh *et al.*, 2013). Therefore, the accuracy of genotype-based fruit form prediction after field collection error correction was 100%.

Seed Garden *Dura* and Illegitimate Planting Material Rates

The 13 Musi Banyuasin and five Pelalawan sub-regencies surveyed represent 88.80% and 85.50% of smallholder planted areas, respectively (Figure 1). Overall smallholder non-*tenera* contamination in the two regencies substantially deviated from the Indonesian National Standard (SNI 8211:2015) and Kepmentan No. 26/2021, and they were markedly similar to one another. Planting site-to-site variation was high, ranging from 100% pure *tenera* palms to 100% non-*tenera* contamination in both regencies. In Musi Banyuasin, average non-*tenera* contamination

was 45.66% (41.53% *dura* and 4.13% *pisifera*). High *pisifera* content indicated a legitimacy issue. In Pelalawan, average non-*tenera* contamination was 47.67% (46.72% *dura* and 0.95% *pisifera*). Only 13 sites in Musi Banyuasin (16.00%) and 11 sites in Pelalawan (14.90%) were compliant with the Indonesian SNI 8211:2015 standard of $\leq 2\%$ non-*tenera* contamination (Figure 2). Average rates of *dura* contamination were comparable to those measured for large investment smallholder farmers in Rokan Hulu regency, Riau province (Jelsma *et al.*, 2017). A supply chain purity survey in Malaysia, which evaluated *tenera* purity in 10,244 samples, found 10.90% non-*tenera* in independent smallholders and nurseries. The same team reported similar results (12.80% contamination) when the study was expanded to cover all industry sectors (>1.1 million samples) (Ooi *et al.*, 2023; Singh *et al.*, 2022).

A histogram of planting sites plotted by overall non-*tenera* contamination displayed a highly resolved bimodal distribution. A total of 28.4% of sites fell into the 'left peak' of lower overall non-*tenera* contamination (5.2% average non-*tenera*). The remaining 71.6% fell into the 'right peak' of very high non-*tenera* contamination (63.5% average non-*tenera*) (Figure 3). Welch's 2-sample t-test assuming unequal variance confirmed the statistical significance of the bimodal distribution above vs. below 30% non-*tenera* contamination ($t = 1.976$, $p = 9.60 \times 10^{-58}$). Of 248 *pisifera* palms detected, 246 (99.2%) were observed in 'right peak' planting sites, suggesting planting sites in the 'left peak' were supplied primarily with legitimate planting material, while planting sites in the right peak were supplied entirely with illegitimate planting material (Figure 3).

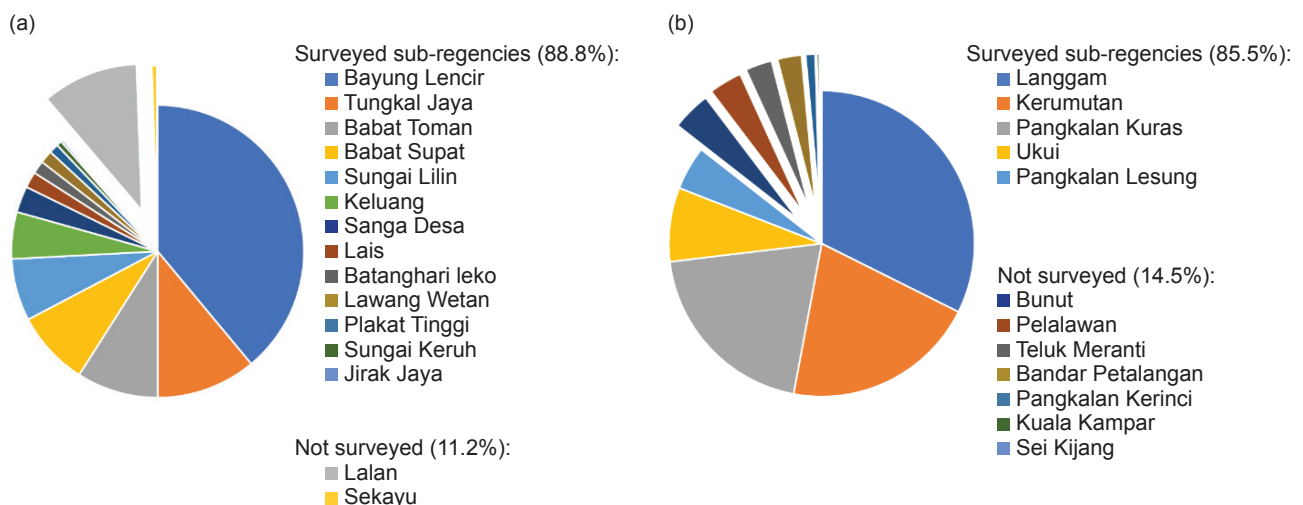


Figure 1. Percentages of Musi Banyuasin and Pelalawan regencies surveyed for the SHELL DNA Census. (a) Field planted palms from 13 sub-regencies (88.8% of smallholder planted area) was surveyed in Musi Banyuasin. (b) Palms from 5 sub-regencies (85.5%) were surveyed in Pelalawan. Sub-regencies are sized by their percentage of total oil palm smallholder planted area in each regency. Sub-regencies not surveyed are shown as offset sections.

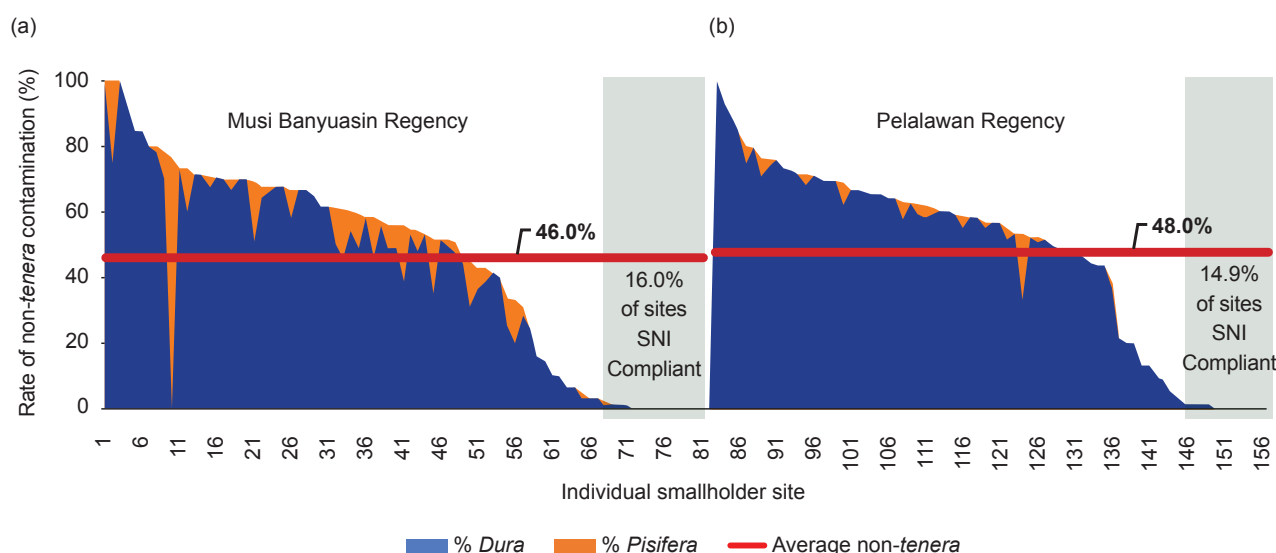


Figure 2. Non-tenera percentages of (a) Musi Banyuasin and (b) Pelalawan. Sub-regencies are sized by their percentage of total oil palm smallholder planted area in each regency.

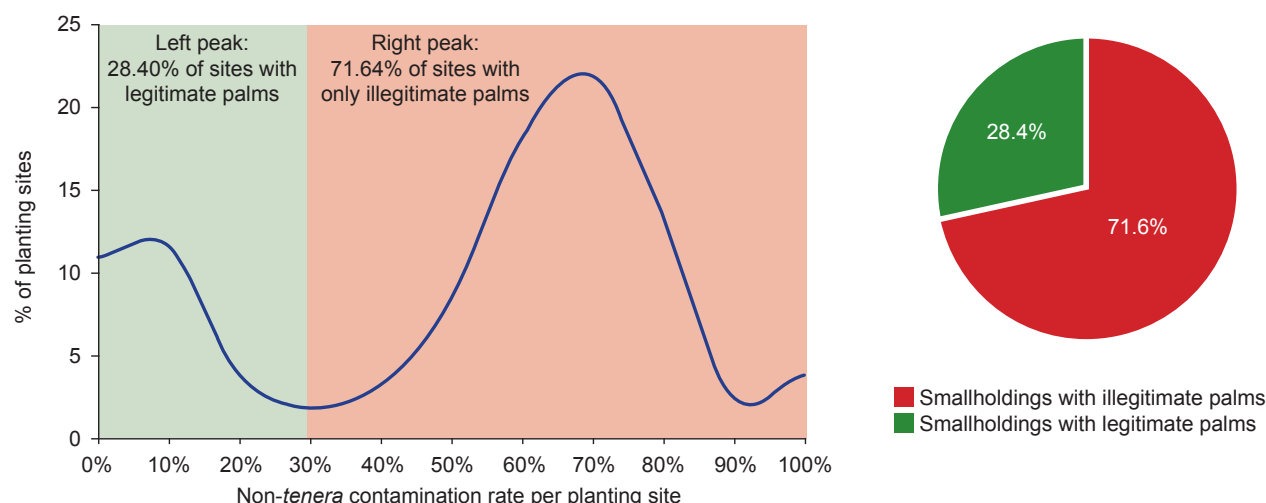


Figure 3. Bimodal distribution of planting sites indicates legitimate and illegitimate planting material. A histogram of smallholder planting sites binned by overall non-tenera contamination displays a bi-modal distribution. Sites supplied primarily with legitimate material (left peak) are distinct from sites which were likely supplied with illegitimate material (right peak). The pie graph depicts a fraction of smallholder sites supplied with legitimate (green) or illegitimate material (red).

Rates of Legitimate *Tenera*, Legitimate *Dura*, Illegitimate *Tenera*, Illegitimate *Dura* and Illegitimate *Pisifera*

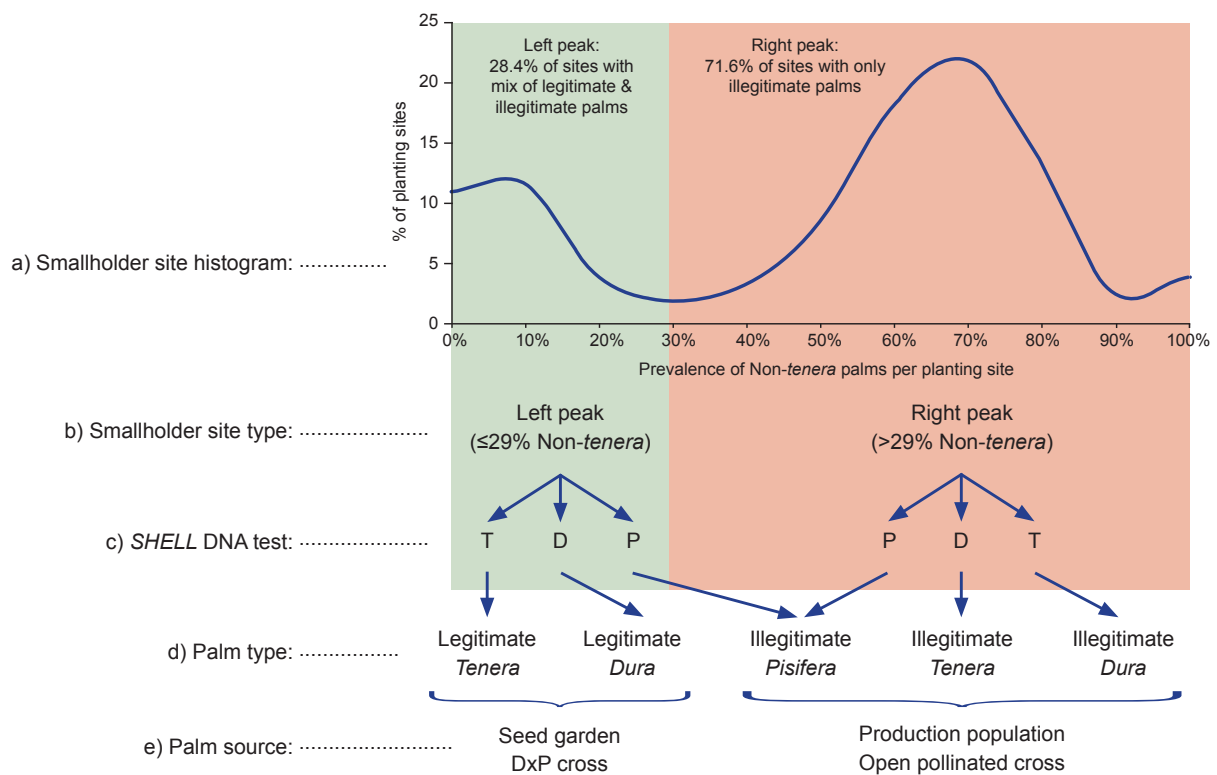
Five oil palm productivity types are present in production fields, two of which originate from legitimate materials (*i.e.*, progeny tested DxP crosses at seed companies), and three of which originate from illegitimate materials (*i.e.*, undesirable open pollination of production palms) (Cochard *et al.*, 2001). Legitimate materials include two palm productivity types: Intended 'seed garden *tenera*' resulting from DxP crosses, and unintended 'seed garden *dura*' resulting from the selfing of mother *dura* palms, or intercrosses between highly

related *dura* palms in the maternal seed garden. Therefore, *pisifera* palms are almost never present in seed company provided material (Hartley, 1988; Rajanaidu *et al.*, 2000). Illegitimate material typically has three productivity types (*dura*, *tenera* and *pisifera*) which originate from open pollination of production populations. Of the five productivity types, only 'seed garden *tenera*' is desirable, while the other four types are undesirable as they have lower FFB production as the result of inbreeding depression, as well as lower mesocarp production from the suboptimal inheritance of *SHELL* alleles (Figure 4) (Cochard *et al.*, 2005; Corley, 2005; Hardon, 1970; Luyindula *et al.*, 2005; Singh *et al.*, 2022).

To determine the prevalence of each of the five productivity types in the survey, we assumed palms present in planting sites with >29% non-*tenera* contamination (i.e., in the 'right peak') were supplied from sources providing illegitimate materials. Therefore, *tenera*, *dura* and *pisifera* palms observed in these sites are each considered illegitimate. Similarly, we assumed palms present in planting sites with ≤29% non-*tenera* contamination (i.e., in the 'left peak') were supplied from sources providing legitimate materials, except the two detected *pisifera* palms (Figure 4).

Rates of all five productivity types were determined for each smallholder site, and the averages of these rates were determined for smallholdings in each sub-regency in Musi Banyuasin and Pelalawan. Sub-regency averages were then weighted by the total oil palm smallholder planted area in the sub-regency. On a weighted average basis, 73.80% (3.30% illegitimate *pisifera*, 43.50% illegitimate *dura*, 26.30% illegitimate *tenera*, 0.68% legitimate *dura*) of palms in Musi Banyuasin smallholdings were of a low-productivity type (Figure 5). Unweighted averages were 4.10% illegitimate *pisifera* (207 palms), 40.30% illegitimate *dura* (2,018 palms), 26.00% illegitimate *tenera* (1,300 palms) and 1.20% legitimate *dura* (62 palms).

Similarly, 72.60% (1.10% illegitimate *pisifera*, 44.00% illegitimate *dura*, 25.70% illegitimate *tenera*, 1.80% legitimate *dura*) palms in Pelalawan smallholdings were of a low-productivity type (Figure 5). Unweighted averages were 0.90% illegitimate *pisifera* (41 palms), 45.00% illegitimate *dura* (1951 palms), 26.50% illegitimate *tenera* (1148 palms) and 1.70% legitimate *dura* (73 palms). Legitimate seed garden *tenera* in smallholdings in Musi Banyuasin and Pelalawan were 26.20% and 27.40%, respectively, well below the required 98.00% *tenera* purity of Indonesian SNI standard and Kepmentan No. 26/2021. In open pollinations, resulting in illegitimate progeny, maternal palms may be either *dura* or *tenera* (but not *pisifera*) while paternal palms may be *dura*, *tenera* or *pisifera*. Therefore, even in a population with all fruit form types equally represented (which is not expected in open pollinated fields due to the low occurrence of *pisifera*), one would expect a higher proportion of illegitimate *dura* and a lower proportion of *pisifera* (when possible), relative to 1:2:1 (D:T:P) *tenera* x *tenera*, 1:1 (D:T) *dura* x *tenera* or 1:1 (T:P) *tenera* x *pisifera* ratio. Furthermore, as *dura* and *pisifera* representation in the population increases and decreases, respectively, there will be a natural shift in the population in favour of illegitimate *dura*



Note: T - *tenera*; D - *dura*; P - *pisifera*.

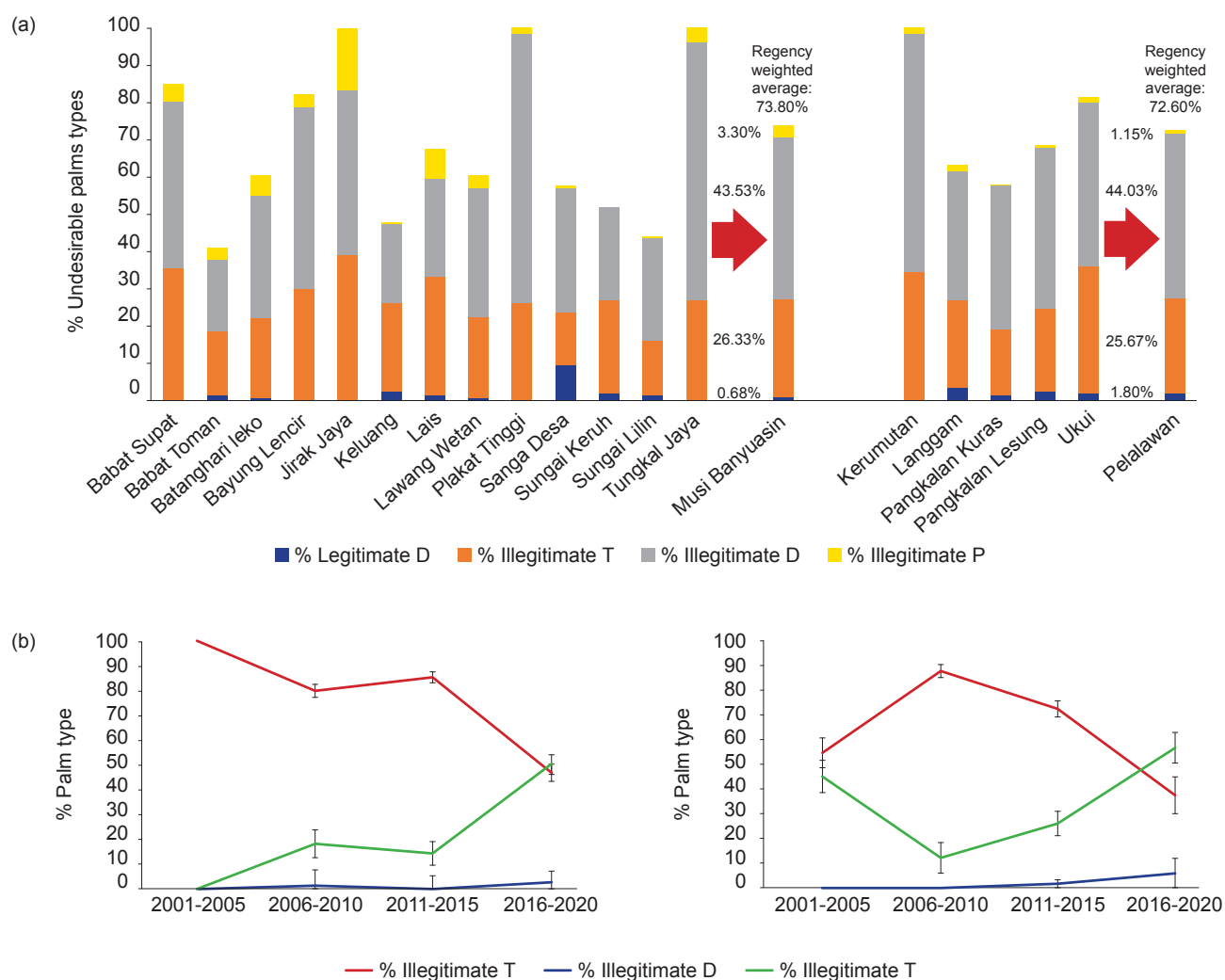
Figure 4. Determining rates of five oil palm productivity types. (a) Histogram of smallholder sites sorted by non-*tenera* contamination. (b) Smallholder site types: Sites with ≤29% and sites with >29% non-*tenera* contamination. (c) and (d) SHELL test results from each site type can be mapped to the 5 palm productivity types based on the assumption that left and right peak sites are supplied from sources providing legitimate or illegitimate material, respectively. (e) Legitimate vs. illegitimate palm sources.

relative to illegitimate *tenera*. This behaviour is reflected in the relative proportions of illegitimate *dura* and illegitimate *tenera* observed (Figure 5a). Compared to sites planted in 2006 to 2015, legitimate *tenera* rates have increased at sites planted in 2016–2020 in both regencies, likely due to improvements in the quality of planting materials in more recent years (Figure 5b). This does not correlate with an associated decline in legitimate *dura* rates because even in controlled DxP crosses, legitimate *dura* will still be obtained at low levels due to self-pollination of *dura* maternal palms.

Relative FFB, CPO and PKO Production Gains with SHELL Testing

FFB, CPO and PKO gains in Musi Banyuasin and Pelalawan regencies. A recent publication concluded that in comparison to seed garden *tenera*,

three of the undesired palm types (seed garden *dura*, illegitimate *dura* and illegitimate *tenera* palms) suffering from various degrees of in-breeding depression will produce lower FFB, CPO and PKO yields (Singh *et al.*, 2022). The study revealed that FFB yield potential for *dura* resulting from a DxP cross designed for seed production is 62.5%, lower than that of illegitimate *dura* and illegitimate *tenera* which are each 70.0%. PKO yield potential for all three undesired types matches the FFB yield potential. The CPO oil yield potential of the same *dura* is 43.8% while that of the illegitimate *dura* and illegitimate *tenera* is 49.0% and 70.0%, respectively. Such vast differences in the yield of undesired types, in combination with surveyed palm type frequency rates among smallholders in both regencies, could indicate the relative productivity gain if the four low-productivity palm types could be identified and removed from the supply chain before field planting.



Note: D - *dura*; T - *tenera*; P - *pisifera*.

Figure 5. Rates of four contaminant low-productivity oil palm types in Musi Banyuasin and Pelalawan. (a) Rates are plotted for sub-regencies in Musi Banyuasin (left panel) and Pelalawan (right panel). Overall weighted average rates are plotted in the right most bar. (b) Percent illegitimate palms, legitimate *dura* palms and legitimate *tenera* palms for all smallholders in Musi Banyuasin (left) and Pelalawan (right) over 5-year intervals spanning 2001 to 2020. Error bars represent 95% confidence intervals.

Based on the actual census rate for each of the palm productivity types (Figure 5), productivity gains were determined by comparing the actual reported productivity of Musi Banyuasin and Pelalawan to the expected productivity of these regions if *SHELL* testing were deployed. Calculations by which FFB, CPO, PKO and relative yield potential, as well as expected gains, are provided in the caption to Table 1. We estimate that *SHELL* testing would increase smallholder FFB production by 31% and 28% in Musi Banyuasin and Pelalawan, respectively, and this would directly translate to a 31% and 28% increase in smallholder income. Similarly, CPO production arising from smallholder derived FFB would increase by 49% and 46% while PKO productivity would increase by 31% and 28% in the two regencies, respectively.

FFB, CPO and PKO gains in Indonesia. To determine relative production gains in FFB, CPO and PKO made possible by incorporating *SHELL* testing into the seed and seedling supply chain for the smallholder replanting program across all of Indonesia, we assumed that contamination

throughout the country is similar to that directly measured in the Musi Banyuasin and Pelalawan surveys (Table 1). We estimate that *SHELL* testing applied nationally would lower contamination by 37-fold (from 73.2% to less than 2.0%) and increase the production of FFB, CPO and PKO in the smallholder sector by 30.0%, 48.0% and 30.0%, respectively (Figure 6).

Value of CPO and PKO Production Gains with *SHELL* Testing

To determine the economic gains of incorporating *SHELL* testing into the smallholder seedling supply chains in Musi Banyuasin and Pelalawan, we first determined the actual quantity of CPO and PKO produced by smallholders in these regencies for a seven-year period from 2017-2023. We then determined the average price (local delivery) for CPO and PKO paid annually in this time period and computed the dollar value of smallholder production. Finally, we determined the value of the additional production that would have resulted if *SHELL* testing had been deployed in the past.

TABLE 1. NATIONAL POTENTIAL RELATIVE YIELD GAINS IN SMALLHOLDINGS ARE MADE POSSIBLE WITH *SHELL* TESTING

		a	b	c	d	e	f	g	h
Palm productivity type		Census rate (a)	FFB yield (b)	SHELL factor (c)	CPO yield (=b*c)	PKO yield (=b)	FFB yield potential (=a*b)	CPO yield potential (=a*d)	PKO yield potential (=a*e)
Yield potential (SHELL Testing, SNI Compliant)	Legitimate <i>Tenera</i>	98.00%	100.00%	100.00%	100.00%	100.00%	98.00%	98.00%	98.00%
	Legitimate <i>Dura</i>	2.00%	62.50%	70.00%	43.80%	62.50%	1.25%	0.88%	1.25%
	Illegitimate <i>Tenera</i>	0.00%	70.00%	100.00%	70.00%	70.00%	0.00%	0.00%	0.00%
	Illegitimate <i>Dura</i>	0.00%	70.00%	70.00%	49.00%	70.00%	0.00%	0.00%	0.00%
	Illegitimate <i>Pisifera</i>	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	Relative yield potential - with SHELL testing:						99.25%	98.88%	99.25%
National Impact - Gain from SHELL Testing	Legitimate <i>Tenera</i>	26.76%	100.00%	100.00%	100.00%	100.00%	26.76%	26.76%	26.76%
	Legitimate <i>Dura</i>	1.240%	62.50%	70.00%	43.80%	62.50%	0.77%	0.54%	0.77%
	Illegitimate <i>Tenera</i>	26.00%	70.00%	100.00%	70.00%	70.00%	18.20%	18.20%	18.20%
	Illegitimate <i>Dura</i>	43.78%	70.00%	70.00%	49.00%	70.00%	30.65%	21.45%	30.65%
	Illegitimate <i>Pisifera</i>	2.23%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
	Relative yield potential - Avg 2 Surveys:						76.37%	66.95%	76.37%
	Relative yield potential - with SHELL testing:						99.25%	98.88%	99.25%
% gain in smallholdings:							30.00%	47.70%	30.00%

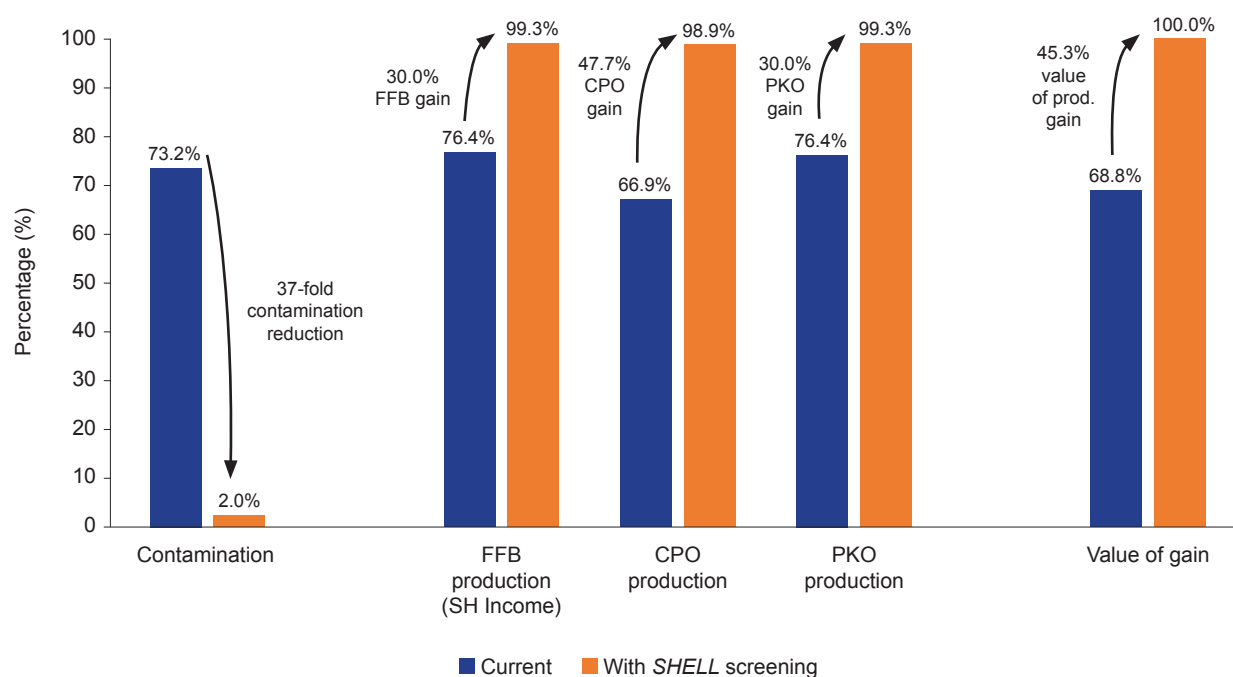
Note: '*SHELL* Testing, SNI compliant' shows expected potential yields when *SHELL* testing is implemented to allow SNI-compliant planting materials. 'National Impact - Gain from *SHELL* Testing' shows the expected net gain in small holdings upon implementation of *SHELL* testing when the baseline is the average result of two regency census rates. FFB Yield potential (f) is the product of the Census Rate (a) times the FFB Yield for each of the palm types (b). CPO Yield Potential (g) is the product of the Census Rate (a) times the CPO Yield for each of the palm types (d). PKO Yield Potential (h) is the product of the Census Rate (a) times the PKO Yield for each of the palm types (e). The relative yield potential of FFB, CPO and PKO is the sum of the yield potentials in columns f, g and h, respectively. Expected gain in yield is the ratio of the Relative Yield Potential in the current survey divided by the Relative Yield Potential if *SHELL* testing had been in place - where all smallholder sites are made SNI compliant (<2% contamination) through the use of testing.

Historic CPO and PKO production. We constructed an estimate of smallholder CPO and PKO production for a seven-year period between 2017 and 2023 in Musi Banyuasin and Pelalawan regencies. Actual smallholder CPO production values for three years from 2017 to 2019 were supplied by the Estate Crop Department of Musi Banyuasin government. To approximate production in 2020 through 2023, we used the average production for 2017-2019. Similarly, actual smallholder CPO production values for 2019 and 2020 were supplied by the Estate Crop Department of Pelalawan Regency Government. To estimate production in 2017, 2018 and 2021 through 2023, the average of the CPO production for 2019 and 2020 years was used. To estimate PKO production for all seven years in both regencies, the ratio of the national average of PKO/CPO was used for each year where national PKO production was between 11.1% and 11.5% of national CPO production, (<https://www.indexmundi.com>) (Table 2).

Historic CPO and PKO price. Historic CPO and PKO prices were determined as the average annual CPO and PKO price (Rotterdam) minus the international delivery cost to estimate local CPO and PKO delivery prices for 2017-2023. The CPO and PKO prices in 2023 were estimated as the average of the monthly settlement prices for January through September 2023 (the most recent

values available at the time of analysis), minus international costs of delivery (www.indexmundi.com) (Table 2).

Estimated value of production gain of CPO and PKO in the two regencies. We estimated that with a screening program that removes contaminant palms from the seedling supply chain based on SHELL testing, CPO production in the smallholder sector would increase on a relative basis by 49% and 46% in Musi Banyuasin and Pelalawan, respectively. Similarly, we estimated that testing planting material would increase PKO production by 31% and 28% on a relative basis in Musi Banyuasin and Pelalawan, respectively. Therefore, to estimate the quantity of additional oil that would have been produced if a DNA based testing program had been in place, the regency CPO and PKO production in each of the seven years from 2017-2023 was multiplied by the estimated relative gains from testing (Table 2). The value of the gains in production was determined as the estimated gain in production (in tonne) times the historic average price for each of the years (USD/t oil). In Musi Banyuasin, the annual value of additional CPO and PKO production from testing would range from USD31.09 million to USD68.47 million (USD45.50 million 7 year average), while in Pelalawan, the annual value of additional CPO and PKO production would range from USD118.08 million to USD244.22 million (USD162.01 million



Note: SH - smallholder.

Figure 6. Summary of smallholder SHELL test survey. SHELL testing would lower contamination from 73.2% to less than 2.0% (37-fold). By lowering contamination, FFB production and smallholder income would increase by 30.0%. With more FFB and higher mesocarp per bunch, CPO production would increase by 47.7%, and PKO production would increase by 30.0%. The value of gain would increase by 45.3% (fifth series).

7-year average), depending on production levels in the years and CPO and PKO price fluctuation (Figure 7a-b).

Estimated value of production gain of CPO and PKO nationally, with *SHELL* testing. Assuming that non-*tenera* and illegitimate contamination found in Musi Bayuasin and Pelalawan regencies was representative of smallholdings across Indonesia, we used the averages of the census rates of the five palm productivity types from the two regencies as estimates for non-*tenera* and illegitimate contamination nationally. To estimate the quantity of additional oil that would have been produced if a *SHELL* testing program had been in place in the smallholder sector across Indonesia, CPO and PKO production for the smallholder sector in each of the seven years from 2017-2023 (provided by the DJP, Kementerian Pertanian), was multiplied by the estimated relative gains made possible through testing (Table 2). The value of the gains in production was determined as the estimated gain in production (in t) times the historic average price for each of the years (USD/t oil).

In the Indonesian smallholder sector, the value of additional CPO and PKO production from testing would range from USD4.16 billion to USD9.80 BN (7-year average USD6.41 billion) annually, depending on production levels and CPO and PKO price fluctuation over the period (Table 2, Figure 7c).

With an estimated smallholder mature planted area of 5.14 million hectares (7-year average; provided by the DJP, Kementerian Pertanian), the annual value of the gains of smallholder production would have been USD1,246/ha/yr (Table 2).

Economic Impact of CPO and PKO Production Gains with *SHELL* Testing

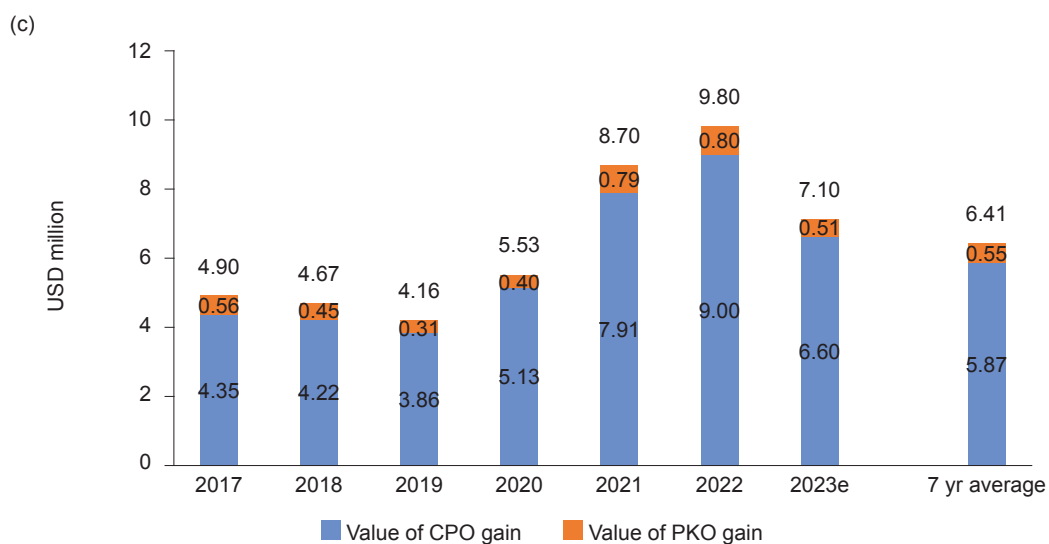
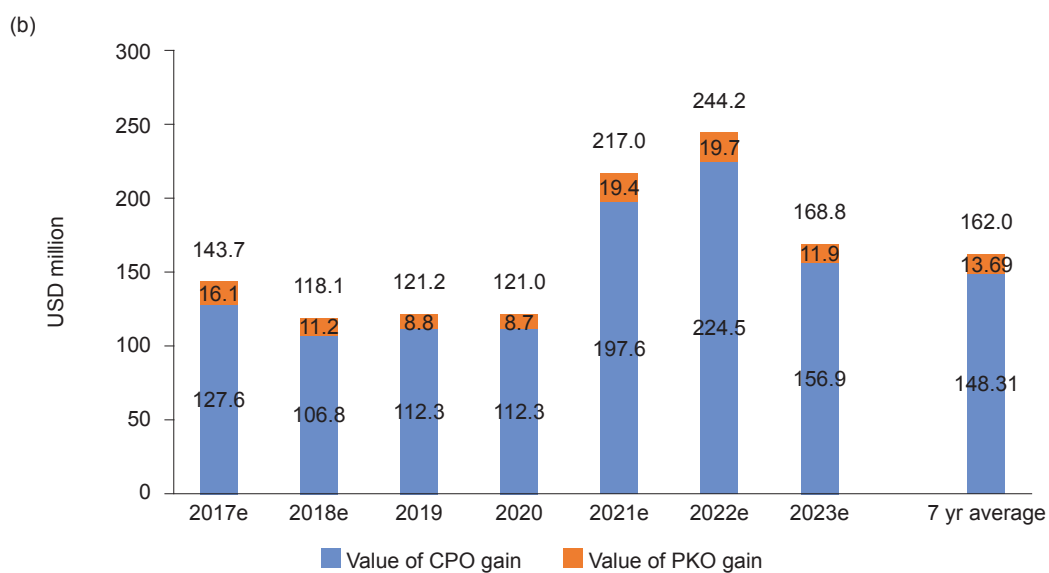
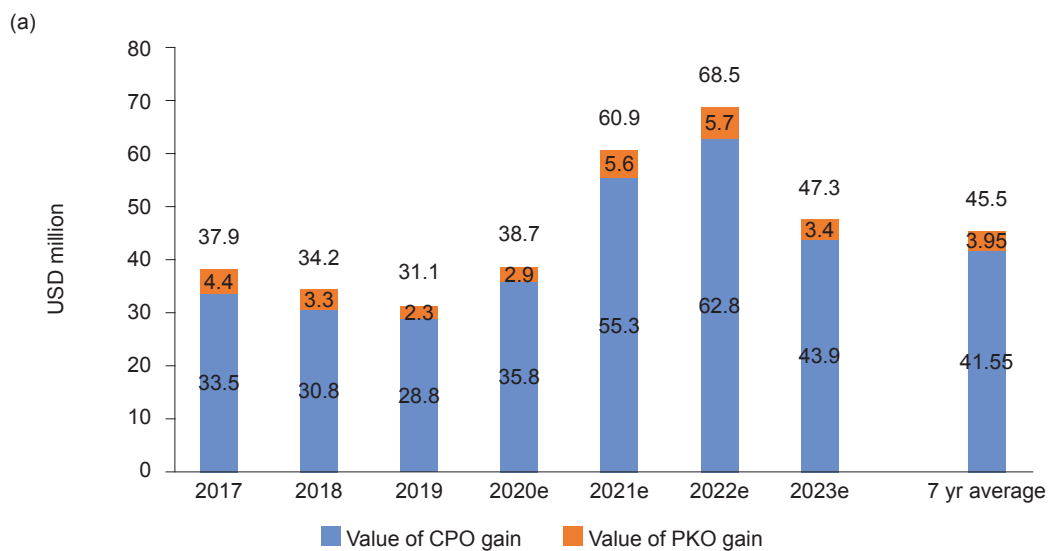
To determine the economic impact on Indonesian Gross Nation Income (GNI) of a national *SHELL* testing initiative in the smallholder replanting program, estimated gains in the production of CPO and PKO (7-year average) (Table 2, Figure 8) were multiplied by an economic multiplier of 1.711x (Palm Oil Agribusiness Strategic Policy Institute [PASPI], 2017). To compute increases in national tax receipts, a corporate tax rate of 25% was assumed. To estimate the gains per hectare per year increases in production, GNI and tax receipts were divided by 5.14 million hectares, the average estimated mature planted area for smallholders in Indonesia over the seven years. Based on the smallholder contamination observed in Musi Banyuasin and Pelalawan, *SHELL* testing would increase annual oil production by USD6.41 billion/yr (USD1,246/ha/yr). Gross National Income would increase by USD10.96 billion/yr (USD2,131/ha/yr) and tax receipts would grow by USD2.74 billion/yr (USD533/ha/yr) (Figure 8).

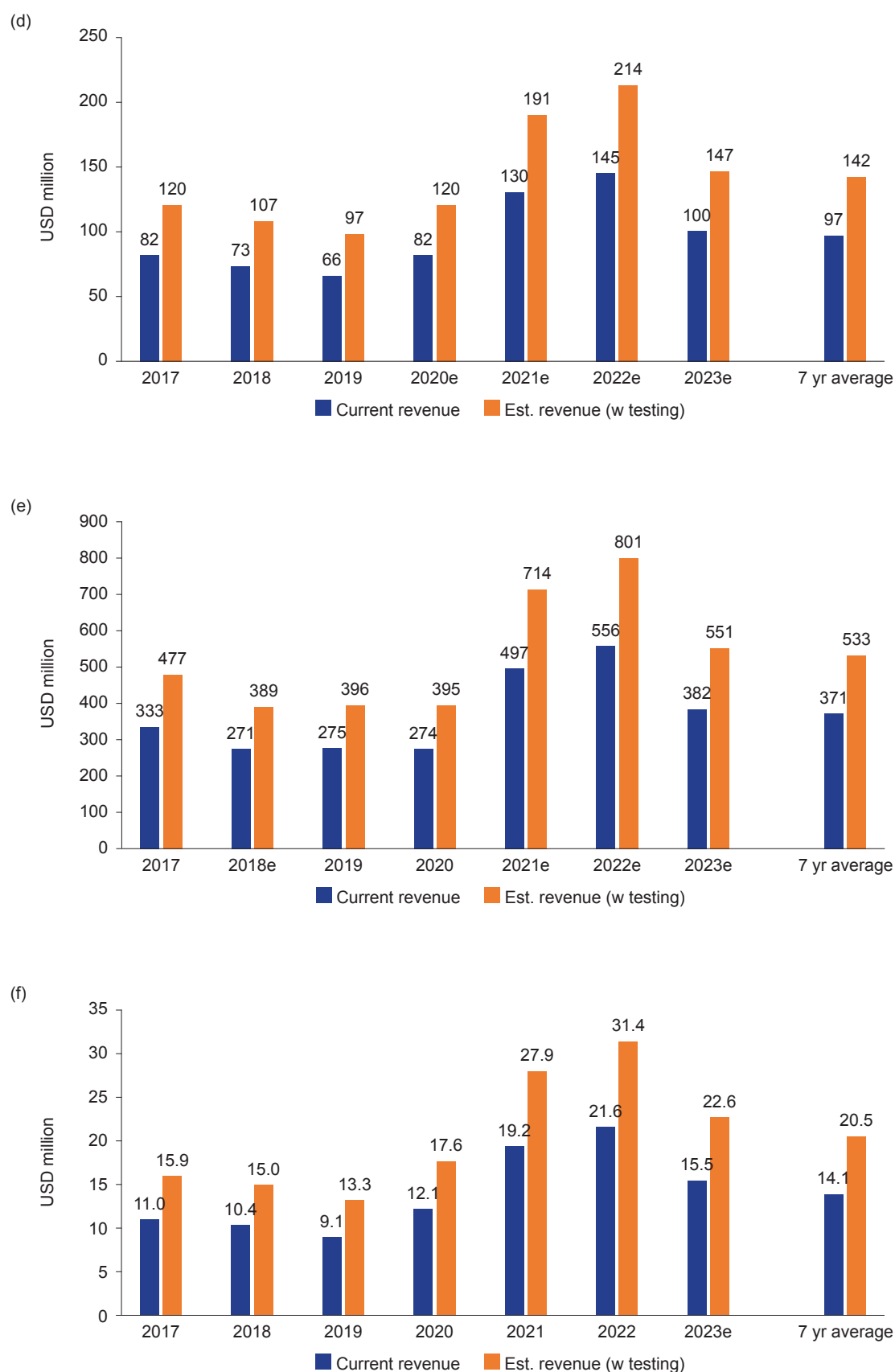
TABLE 2. NATIONAL ECONOMIC GAIN FROM *SHELL* TESTING

	2017	2018	2019	2020	2021	2022	2023	7 yr average
Smallholder mature area (ha)	4,240,588	4,751,827	4,836,168	5,052,497	5,719,840	5,698,546	5,707,400 ^e	5,143,838
SH CPO production (t)	13,191,189	15,296,801	14,925,877	15,495,427	15,503,840	15,519,234	16,273,170 ^e	15,172,220
SH PKO production (t)	1,517,822	1,739,781	1,708,925	1,766,122	1,790,324	1,721,960	1,800,436 ^e	1,720,767
CPO price (USD local)	691	579	542	694	1,070	1,216	850 ^e	806
PKO price (USD local)	1,228	867	602	763	1,473	1,557	944 ^e	1,062
CPO gain w testing (t)	6,290,573	7,294,691	7,117,806	7,389,411	7,393,423	7,400,764	7,760,299	7,235,281
PKO gain w testing (t)	454,613	521,093	511,851	528,983	536,232	515,755	539,260	515,398
Value of CPO gain (USD billion)	4.35	4.22	3.86	5.13	7.91	9.00	6.60	5.87
Value of PKO gain (USD billion)	0.56	0.45	0.31	0.40	0.79	0.80	0.51	0.55
Total revenue gain (USD billion)	4.90	4.67	4.16	5.53	8.70	9.80	7.10	6.41
Gain/ha (SH mature area)	1,156.59	983.34	860.89	1,094.60	1,521.54	1,720.12	1,244.85	1,246.46
Revenue (baseline) (USD billion)	10.98	10.36	9.11	12.10	19.23	21.55	15.53	14.05
% Revenue gain	44.70	45.10	45.70	45.70	45.30	45.50	45.70	45.60
Current revenue (USD billion)	10.98	10.36	9.11	12.10	19.23	21.55	15.53	14.05
Est. revenue (w testing) (USD billion)	15.88	15.03	13.28	17.63	27.93	31.35	22.64	20.47

Note: Theoretical economic gains from national *SHELL* testing in the smallholder sector are determined. CPO and PKO price for local delivery is based on historic Rotterdam price minus the cost of transportation to Europe. The value of CPO and PKO gains for each year is the estimated gain from *SHELL* testing (determined in Table 1) times the actual production for each of the seven years. The value of the gain in production is the estimated gain (t) times the price (USD/t oil).

e - estimated values for the full year of 2023 are based on January-September 2023 data; SH - smallholder.





Note: e - estimated value as described in result and discussion.

Figure 7. Estimated annual gain in production value from smallholder sector SHELL testing. Estimated value of additional production of CPO (light-blue bar) and PKO (orange bar) is plotted for 2017-2023 and the 7-year average for (a) Musi Banyuasin, (b) Pelalawan and (c) Nationally. The current combined revenue from CPO and PKO (blue bar) and what revenue would have been if SHELL testing had been in place in the past (orange bar) is plotted for the (d, e) two regencies and (f) nationally.

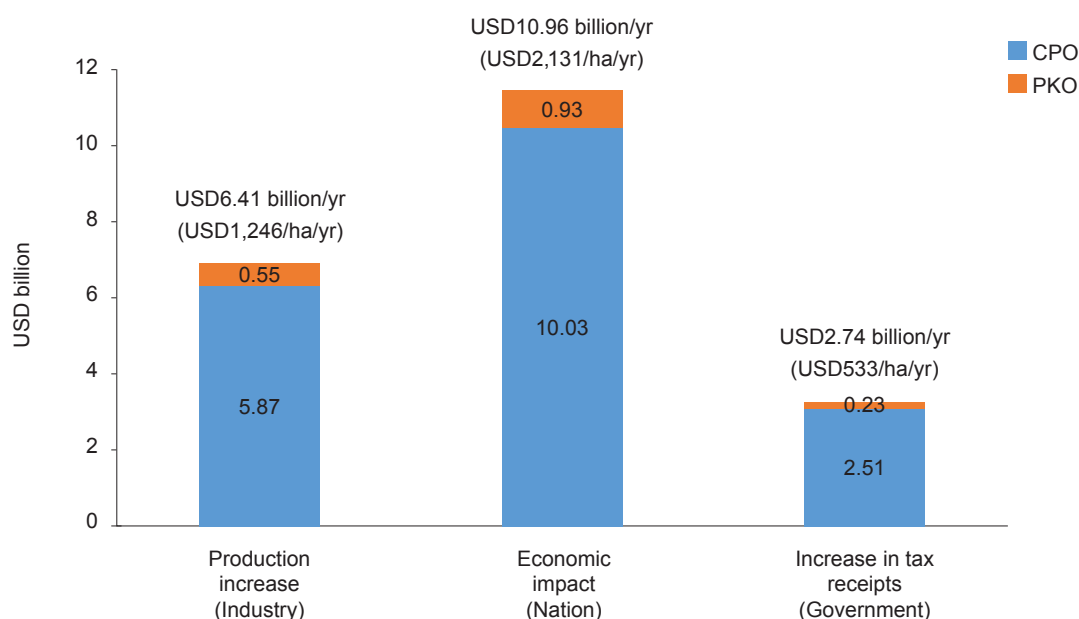


Figure 8. The annual economic impact of SHELL testing in the Indonesian smallholder replanting program. Based on average expected gains over seven years from 2017 to 2023, the estimated value of additional production of CPO (blue) and PKO (orange) (left), gains in GNI (center) and gains in tax receipts (right) are shown in billions USD. Gains in production, GNI and tax receipts are annualised. Estimated gains/ha/yr are reported above each series.

CONCLUSION

This report represents the first *SHELL* DNA based seed and seedling purity study reported for the Indonesian palm oil industry. Results from 81 smallholdings in 13 subregencies (Kecamatan) representing 88.5% of smallholding plantation area in Musi Banyuasin and 74 smallholdings in five subregencies representing 85.5% of smallholding plantation area in Pelalawan reveal high levels of non-*tenera* and illegitimate contamination: 73.8% and 72.6% in Musi Banyuasin and Pelalawan, respectively. The study highlights a fundamental problem in the secure supply of high-purity DxP planting material to independent smallholders, and likely, the results are representative of smallholders nationally.

Efforts by BRIN and PSN to establish an IVO supply chain integrating Indonesian smallholders with second generation biofuel conversion technologies rely not only on the construction of biofuel plants near areas of smallholder cultivation but also on the establishment of a smallholder replanting supply chain that is not constrained by high rates of low yielding oil palm planting material. Implementation of *SHELL* testing in the smallholder supply chain promises to provide a means to achieve significantly increased yields while also providing substantial economic gains to the palm oil industry, the government and the country of Indonesia as a whole. These gains further translate to improved sustainability in

the Indonesian oil palm industry, as yield goals could be achieved from a smaller total utilised land mass.

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The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Muhammad A. Budiman, Abdul R. Purba, Nathan D. Lakey and Jared M. Ordway are employees and/or directors of Orion Biosains, Sdn. Bhd. and/or PT-Orion Biosains, affiliate companies of Orion Genomics, LLC that offer genetic testing to the oil palm industry. Muhammad A. Budiman, Abdul R. Purba, Nathan D. Lakey and Jared M. Ordway are employees and stockholders of Orion Genomics, LLC.

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OBJECT-BASED IMAGE ANALYSIS (OBIA) ON HYPERSPECTRAL IMAGERY FROM DRONE FOR *Ganoderma* BASAL STEM ROT DISEASE DETECTION IN OIL PALM

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ABSTRACT

Ganoderma basal stem rot (BSR) disease infection in oil palm causes significant yield loss to the industry. An efficient disease control application is practical during early and moderate infection of Ganoderma in oil palm. Early detection of disease caused by Ganoderma has been conducted in the lab and on the ground manually. This approach requires inspection and sampling of each oil palm in the field, which is laborious, time-consuming, and costly. Airborne detection using a drone provides a good alternative for fast Ganoderma BSR disease detection in oil palms. This study uses hyperspectral images obtained from a camera mounted on a drone and Object-based Image Analysis (OBIA) to detect Ganoderma infection in oil palms and classify the Ganoderma BSR Disease Severity Index (GDSI). In OBIA, segmentation parameters, such as the Edge were set to 30 while Merge was set to 70 to demarcate respective oil palm without overlaps. Two classifiers namely 1) Support Vector Machine (SVM) and 2) K-Nearest Neighbour (KNN) were used to classify the segmented samples for each GDSI. The results demonstrate that the SVM classifier provides a good classification of the raster image with an overall accuracy of 92.5%. The study shows that hyperspectral images captured by drones provide a viable technique for detecting Ganoderma infection and classifying its severity.

Keywords: drone, *Ganoderma*, hyperspectral, image segmentation.

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INTRODUCTION

The basal stem rot (BSR) disease caused by *Ganoderma boninense* has caused significant economic loss to oil palm plantations (Basiron, 2007; Roslan & Idris, 2012) ranging from 31% to 67% resulting in the decreased yield of fresh fruit bunches (FFB) from 26% to 45% which is a huge loss to oil palm planters

(Singh, 1991). The indicator of the disease is the presence of basidiomata of the pathogen in the stem base or frond bases or the root (Idris *et al.*, 2004). The *Ganoderma* invades the oil palm through the root and spreads towards the bole and the stem base. Moderate and severe infection of *Ganoderma* BSR disease in the bole causes visual symptoms in the oil palm canopy (Flood *et al.*, 2022). The understanding of symptoms and physiological changes of oil palms after *Ganoderma* infection is important to develop a practical approach to detecting *Ganoderma* BSR infection as early as possible.

Several ground and lab-based technologies have been developed for detecting *Ganoderma* in oil palms, namely through the use of the *Ganoderma* Selective Medium (GSM) (Idris *et al.*, 2006); Polyclonal Antibodies Enzyme-Linked Immunosorbent Assay (PABs-ELISA) (Idris & Rafidah, 2008) and GanoSken Tomography (Idris *et al.*, 2010; Mazliham *et al.*, 2006). These ground and lab-based detection

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methods require inspecting and taking samples from individual oil palms in the field. They are costly, time-consuming, and laborious. Thus, there is a need for a more practical technology that can detect *Ganoderma* in oil palms rapidly and efficiently, suitable for a large area of oil palm plantation. Aerial-based detection of *Ganoderma* infection can assist plantation managers in monitoring the health status of many oil palms in the field without the need to inspect individual oil palms on the ground. This approach provides an effective way to control disease and conduct a viable rehabilitation programme.

Previously, the Malaysian Palm Oil Board (MPOB) in collaboration with the University of Malaya (UM) and Universiti Putra Malaysia (UPM) had conducted preliminary diagnostic studies on the application of ground-based remote sensing technologies or hyperspectral field spectroscopy for the detection of *Ganoderma* in oil palms (Izzuddin, 2010; Nisfariza, 2012; Shafri *et al.*, 2011). Following the success of the ground-based investigations, the efforts were expanded to airborne platforms using manned fixed-wing aircraft mounted with hyperspectral cameras which generated promising results. By analysing the data, *Ganoderma* infection was detected and classified into three classes of disease severity which are healthy, early infected and severely infected (Nisfariza, 2012). The work was then extended further using a lightweight drone mounted with a multispectral camera (Izzuddin *et al.*, 2018; 2020). However, there are challenges in the development of early detection techniques using hyperspectral images for the *Ganoderma* disease infection in oil palm due to the complexity of spatial and spectral properties of the hyperspectral image cube. The challenges are in developing a new procedure for colour-balancing of image mosaic, image transformation to enhance spectral properties, denoising to remove noise due to illumination and climate effects, and also selection of significant wavelengths that give higher spectral-separability between features (Izzuddin *et al.*, 2018; Liang, 2017; Nisfariza, 2012).

Thus, this study attempts to examine the capabilities of hyperspectral remote sensing images acquired from drones to detect *Ganoderma* infection in oil palms. Previous studies conducted using moderate spatial and spectral resolution multispectral and hyperspectral remote sensing showed that the healthy and early *Ganoderma* BSR infected oil palms were hard to discriminate due to similar canopy morphology and spectral signatures for both categories. The introduction of new and advanced upgrades of the Automated Orders and Resource System (AORS) that can be mounted on drones, which is more precise, smaller in size, durable, and provide higher spatial and spectral resolution images had given a new opportunity for

further research for the detection of *Ganoderma* in oil palm. The high spatial and spectral resolution of the new generation multispectral and hyperspectral technologies also need new and advanced data classifier that can handle higher spectral dimensions of AORS imagery data.

Advanced imagery such as OBIA that integrate image segmentation analysis with an advanced classifier such as a Support Vector Machine (SVM) can increase classification accuracy for AORS images. Several studies on the application of these advanced classifiers for optical remote sensing images have shown remarkable accuracy of results. Therefore, this study was conducted to develop enhanced image analysis techniques based on these advanced data classifiers to discriminate between all levels of *Ganoderma* infection severity in oil palms.

MATERIALS AND METHODS

Figure 1 shows the flow of work involved in this study. The data are acquired from drone and ground census and both data were pre-processed to prepare the datasets for further processing using OBIA. Ground data was used for accuracy assessment of the classification results.

Area of Study

The study area was located in Lekir, Perak, Malaysia (4°12'3.43"N, 100°47'7.87"E). The area covers an estimated 30 ha and is planted with nine-year old commercial *Dura* × *Pisifera* (DxP) planting materials. The annual rainfall is 2,100 mm/yr. The area consisted of coastal soil which is the Sogomana-Setiawan series. The area only reported the presence of *Ganoderma*, and no other diseases, pests, or nutrient deficiencies have been reported.

Equipment and Devices

In this study, a hyperspectral drone was used for airborne image acquisition in September 2019. The hyperspectral drone system consisted of a RESONON Pika L hyperspectral camera (RESONON Inc., Montana, USA) mounted on a DJI M600 Pro hexacopter drone (Figure 2). The camera captured hyperspectral images consisting of image scanlines of 300 bands from 350-1,018 nm. The drone was flown at 80 m altitude to obtain a 10 cm spatial resolution hyperspectral image for each scanline. A calibration tarp was also laid on the open space near the study area during drone hyperspectral image acquisition. The gray tarp was used for image calibration during hyperspectral image radiometric correction. The image acquisition was conducted from 10:00 to 11:30 am on a sunny day, with a low amount of cloud cover (< 30%).

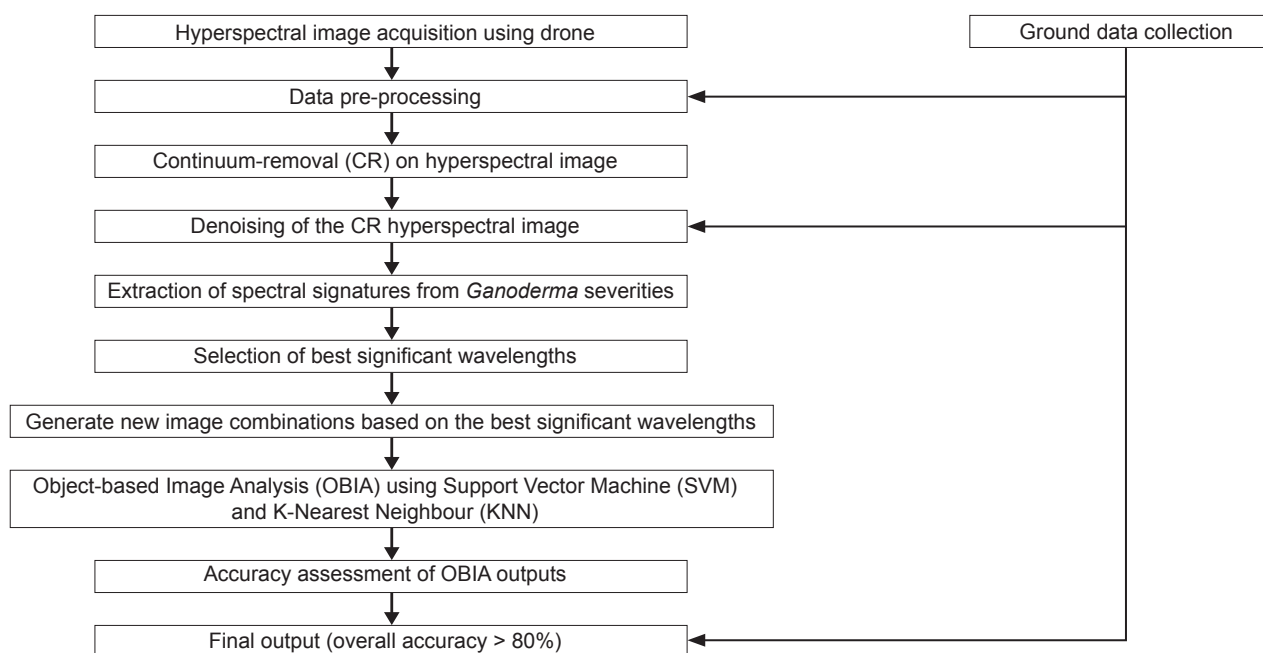


Figure 1. Flowchart of methodology.

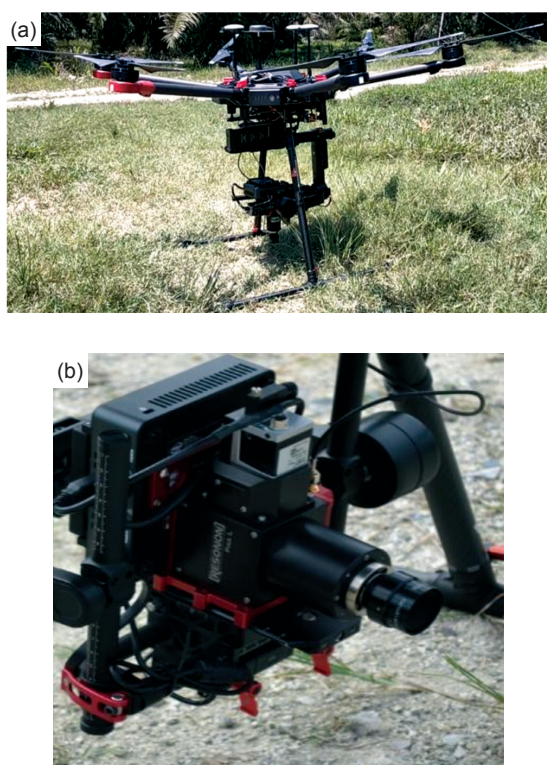


Figure 2. (a) The DJI M600 Pro hexacopter drone with hyperspectral camera and (b) RESONON Pika L camera.

The RESONON Pika L camera is linked to an Eclipse Inertial Measurement Unit (IMU) system that assisted in tagging each scanline recorded during flight to a defined coordinate system. The coordinate system used in this study is World Geodetic System 84 (WGS 84), Universal Transverse (UTM) Zone 47 North (N). The flight plan and image

acquisition parameter settings were defined and set using RESONON Ground Station software while the drone navigation setting parameters were set using DJI GSPRO software.

Ground Data Collection

The ground data collection was conducted during drone data acquisition. The ground data consisted of a ground census of the severity of the infection which is based on the *Ganoderma* Infection Severity Index (GDSI) mentioned in *Standard Operating Procedures (SOP) Guidelines for Managing Ganoderma Disease in Oil Palm* (Idris *et al.*, 2016) (Table 1). The ground census procedure is conducted by manual inspection of the base of the oil palm stem for the presence of *Ganoderma* basidiocarp, small white button, white mycelium, or rotting. Tissue samples are taken from the base stem using drilling techniques and then tested using the *Ganoderma* Selective Medium (GSM). Then, the severity of the infection was determined by inspection of the oil palm frond morphology.

Ground measurement of spectral signatures from healthy oil palm foliar was also conducted during the ground census. The spectral signatures were measured in the field using a portable spectroradiometer GER 1500 that recorded the spectral signatures from 350-1,050 nm. The spectral signatures were measured using a measurement probe with a leaf clipper attached to the spectroradiometer using a fibre optic cable. The probe was equipped with a halogen-lamp as a source of illumination. The leaf clipper has white calibration reference attached with the probe for calibration reference before each measurement was taken.

TABLE 1. *Ganoderma* BASAL STEM ROT DISEASE SEVERITY INDEX (GDSI)

Severity	0	1	2	3
Description				
Category	Healthy	Early/Mild	Moderate	Severe
				
0	No existence of <i>Ganoderma</i> fruiting bodies, no foliar symptoms, and no evidence of rotting at the base of the stem, negative results for GSM and PCR-DNA test.			
1	Existence of <i>Ganoderma</i> fruiting bodies or rotting or both at the base stem of oil palm and no foliar symptoms.			
2	Existence of white mycelia fruiting bodies of <i>Ganoderma</i> at the base of the stem, the existence of foliar symptoms such as yellowing and lowering of older fronds (<30%). Presence of rotting at the base of the stem (<30%), positive results for <i>Ganoderma</i> using GSM or PCR-DNA test.			
3	Existence of white mycelia fruiting bodies of <i>Ganoderma</i> at the base of the stem. Severe foliar symptoms (>30%) and rotting at the base of the stem (>30%), positive results for <i>Ganoderma</i> using GSM or PCR-DNA test.			

Data of Pre-processing and Denoising

The raw hyperspectral images were captured in the form of scanlines saved in .bip format. Each of the scanlines consisted of 300 bands. The scanlines were radiometrically and geometrically corrected using Spectronon Pro software to generate an orthorectified image (RESONON Inc., Montana, USA). Post correction, the best orthorectified scanlines with good visual were used as the reference image for mosaicking, generate orthomosaiced image and also colour balance correction processes using the mosaicking tool provided by ENVI 5.0 software. Meanwhile, ground census data were digitised into vector files containing the infection severity attributes of each oil palm using ArcGIS Pro software. The digitised data was saved in shapefile (.shp) format file.

Following that, the continuum-removal (CR) process was applied to the mosaiced hyperspectral image by dividing it into actual spectrum for each pixel in the image:

$$S_{cr} = (S/C) \quad (1)$$

where, S_{cr} is continuum-removed spectra, S is original spectrum and C is continuum curve.

The resulting image spectra equals to 1.0 is when the continuum and the spectra matches, and less than 1.0 when absorption features occur. The CR was used to normalise reflectance spectral to compare individual absorption features from a common baseline. Then, the CR hyperspectral image in spectral reflectance (SR) was converted into the first derivative of spectral reflectance (FDSR) image using the algorithm below.

The FDSR was generated using:

$$FD = \frac{y_2 - y_1}{x_2 - x_1} \quad FD = \frac{y_2 - y_1}{x_2 - x_1} \quad (2)$$

where FD is the denoised first derivative that was computed with inputs y_2 and y_1 namely, the reflectance spectra at the second and first wavelengths. The values x_2 and x_1 represent the second and first wavelengths.

In this study, two types of hyperspectral imageries were prepared as input for denoising and analysis which are: 1) Denoised and continuum removed spectral reflectance image (D-CR SR) and 2) denoised and continuum-removed first derivative of spectral reflectance image (D-CR FDSR) was used in the selection for best significant wavelengths and then the OBIA was used for the image classification into the disease severities.

Denoising started with the manual removal of noisy bands. The process needs manual visual assessment on each of the image displays from each wavelength one-by-one. The ENVI 5.0 software was used to display the images in each discrete wavelength. Then, the image from wavelengths which showed a blurred appearance containing lots of speckles and noise that made it difficult to visually interpret oil palm canopies were removed from the hyperspectral image. The remaining bands that show a clear image of the oil palm canopies were retained for further denoising process using the Savitzky-Golay (S-G) denoising technique (Ruffin *et al.*, 2008). The best denoising settings were conducted using comparison with ground-based spectral signatures obtained from

TABLE 2. SAVITZKY-GOLAY (S-G) DENOISING PARAMETER SETTINGS

Set	Denoising parameters	1	2	3	4	5
A	Filter width	1	5	10	15	20
	Order of derivative	1	1	1	1	1
	Degree of polynomial	1	1	1	1	1
B	Filter width	1	5	10	15	20
	Order of derivative	1	1	1	1	1
	Degree of polynomial	2	2	2	2	2
C	Filter width	1	5	10	15	20
	Order of derivative	1	1	1	1	1
	Degree of polynomial	3	3	3	3	3

field spectroscopy (Izzudin *et al.*, 2013). The images were denoised using three different S-G denoising parameter settings which are the filter width and degree of polynomial, namely Set A, Set B and Set C (Table 2). The image denoising using S-G was conducted using ENVI 5.0 software.

Table 2 shows the 15 S-G denoising parameter settings that were used to denoise the CR hyperspectral image. The denoising parameters are Filter Width (FW), Order of Derivative (OD) and Degree of Polynomial (DoP). The best denoised image was chosen based on Root Mean Square Error (RMSE) and visual comparative analysis of denoised spectral signatures with the spectral signatures obtained from the ground-based field spectroscopy. The field spectroscopy used a field spectroradiometer to record spectral reflectance from the foliar of oil palm frond directly. In this study, a GER 1500 spectroradiometer is used to measure the spectral reflectance of a healthy oil palm foliar as the benchmark to select the best image denoising parameter.

Selection of Best Significant Wavelengths

The D-CR SR and D-CR FDSR hyperspectral images were overlaid with the ground census of GDSI vector file in ENVI 5.0 software to tag the oil palm canopies in the images with their respective GDSI. Then, the spectral signatures of the oil palm canopy were extracted from the fronds within 3 m radius from the middle of the canopy using ENVI 5.0 software. About 50 spectral signatures were extracted from each frond randomly selected on the oil palm canopy. The spectral signatures were extracted from 65/634 oil palms of GDSI 0, 35/51 oil palms of GDSI 1, 35/52 oil palms from GDSI 2 and 5/10 oil palms from GDSI 3. The number of oil palms for GDSI 2 and 3 is low because the incidence of moderate and severe *Ganoderma* disease is low in young to mature oil palms.

The number of healthy and early severity of the disease is adequate (>30 samples) for classification analysis using OBIA. The spectral signatures for

each GDSI were stored in ASCII format (.txt). The spectral signatures from the bands for each D-CR SR and D-CR FDSR were analysed using Minimum Redundancy Maximum Relevance (MRMR) band by band to select the best 3, 5 and 7 significant wavelengths that can differentiate between all GDSI. The MRMR algorithm selects a subset of features having the most correlation with the class output and the least correlation between themselves. The algorithm ranks features according to minimal-redundancy-maximal-relevance criterion which is based on mutual information (Radovic *et al.*, 2017). The MRMR was conducted using MATLAB 2022a software. Then, the hyperspectral image from D-CR SR and D-CR FDSR were subset into 3, 5 and 7 best significant spectral bands image sets. The images were then used as input in OBIA.

Object-based Image Analysis (OBIA)

The OBIA comprises two major processes which are: 1) Segmentation and 2) classification. The first process is to select the best segmentation parameters that can demarcate a single oil palm canopy that does not overlap with adjacent trees in the image. The OBIA was conducted according to Izzuddin *et al.* (2020) and was made possible by the use of the watershed segmentation technique to segment the oil palm canopy to its respective canopy crown. The values are tested using a trial and error technique to select the best value combinations that reduce overlaps between oil palm canopy fronds.

In this analysis, the subset of hyperspectral images from 3, 5 and 7 best significant wavelengths are used as input for OBIA. The OBIA was conducted using ENVI 5.0 software. In this study, the best parameter settings that can segment the fronds of each canopy without overlapping into adjacent oil palm stands and visually provide differences at the edge between the oil palm canopy and the background were selected. The three best segments and merged parameters in this study are named Set 1, Set 2 and Set 3 with Edge chosen as segment settings, while the scale level is set to 20, 30 and 40

with merge settings at Full Lambda Schedule with respective merge level of 80, 70 and 60 and texture kernel size are set to 3. The scale and merge level are used to delineate features for detecting edges of features where objects of interest have sharp edges. The scale level delineates the boundaries of features as much as possible without over-segmenting the features. Increasing the scale value can generate fewer segments while decreasing the scale level value generates more segments. The merge level is used primarily to merge or combine segments with similar spectral information.

The workflow for this process involves multiple steps from preprocessing to segmentation, sample selection, training, classifying and accuracy assessment. Each step needs iteration with in-depth knowledge of the input imagery, classification schema, classification methods, expected results, and acceptable accuracy. The second process is a classification of the segmented image using: 1) OBIA with SVM and 2) OBIA with KNN.

The SVM classifier can process a segmented raster input or a standard image. It is less susceptible to noise, correlated bands and an unbalanced number or size of training sites within each class. The SVM usually use one of the four basic kernels which are linear, polynomial, sigmoid and radial basis functions (Gholami & Fakhari, 2017). The SVM has been used successfully in many data classification analyses with high accuracy (Azmi *et al.*, 2020). The SVM is a non-parametric classifier that requires a small sample size for training in the classification processes. This method is suitable for high-dimensional data with a limited training set (Cortes & Vapnik, 2015; Lan *et al.*, 2020). There are several parameter settings that need to be conducted to obtain the best classification model. The SVM hyperparameters input are: 1) Kernel scale; 2) kernel function; 3) box constraint level; 4) multiclass method and 5) standardised data. The SVM optimiser options are: 1) Optimiser; 2) acquisition function; 3) iterations and 4) training time limit.

Other than that, the KNN classifier computes the Euclidean distance from each segment in the segmentation image to every training region that is defined. The distance is measured in n-dimensional space, where n is the number of attributes for that training region. The classification process uses the area of attribute values and spectral mean attribute values and calculates the distance from the segment of training regions. In KNN, one parameter that needs to be set is the neighbour values that are comprised of odd values that depend on the number of total numbers of training regions for all classes. Other than that, the threshold value is set to 95% to indicate the level of confidence that the closest segments of any given segment represents the same class.

Accuracy Assessment

The accuracy of OBIA classification outputs of D-CR SR and D-CR FDSR were assessed using the percentage of correct classification over ground truth data. The method was also used by Izzuddin *et al.* (2020) to assess the accuracy of OBIA output for multispectral image analysis.

RESULTS AND DISCUSSION

Hyperspectral Orthomosaiced Image

The geometrically and radiometrically corrected hyperspectral image scanlines are orthomosaiced and colour-balanced using ENVI 5.0 software (Figure 3). The colour balance correction was conducted by selecting one reference image scanline that has uniform brightness and tone. The selection of the reference image scanline was conducted manually using visual eye inspection. Then, the statistics of max and min pixel values of the reference scanline are measured and the colour balance process was conducted using complete overlap scanline statistics for the mosaic image.

This section also consists of the results of the image denoising, selection of the best significant wavelengths, segmentation and classification of new images generated from the significant wavelengths and the accuracy of the classification into different degrees of *Ganoderma*-infection severity.

Hyperspectral Denoising

The results of spectral denoising of the hyperspectral image were selected based on comparison with the ground-based field spectroradiometer measurements of oil palm foliar on fronds. The comparison of ground-based spectroradiometer and the denoised spectral signatures is shown in Figure 4. The results showed that the S-G with Filter Width 5, Level of Derivative 1 and Degree of Polynomial 2 is the best denoised parameter setting to denoise the image.

Figure 4 shows the comparison of denoised spectral signatures from the hyperspectral image and clean spectral signatures measured using field spectroscopy in the field from healthy oil palm. The denoising outputs show that Set B2 give the best denoising results compared to Set C3 output visually not similar to the field spectroscopy spectral signature. The results showed that the denoised spectral signatures of hyperspectral using Set B2 have similar spectral signatures with the reference spectral signatures in terms of pattern and magnitude meanwhile denoising using Set C3 resulted in over denoising effect where the slope of the derivative seems to be a very smooth.

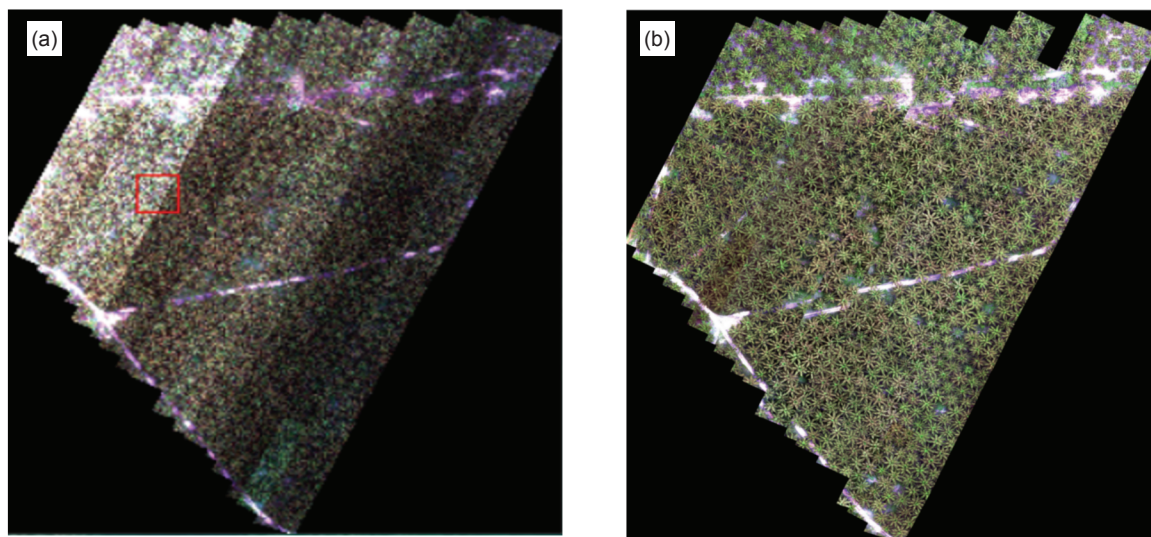


Figure 3. The geometrically and radiometrically corrected airborne hyperspectral image mosaic of the study area: (a) True colour (band combination: 35, 79 and 179 (450.10, 540.41 and 664.12 nm) before colour balance process and (b) true colour after colour balance process.

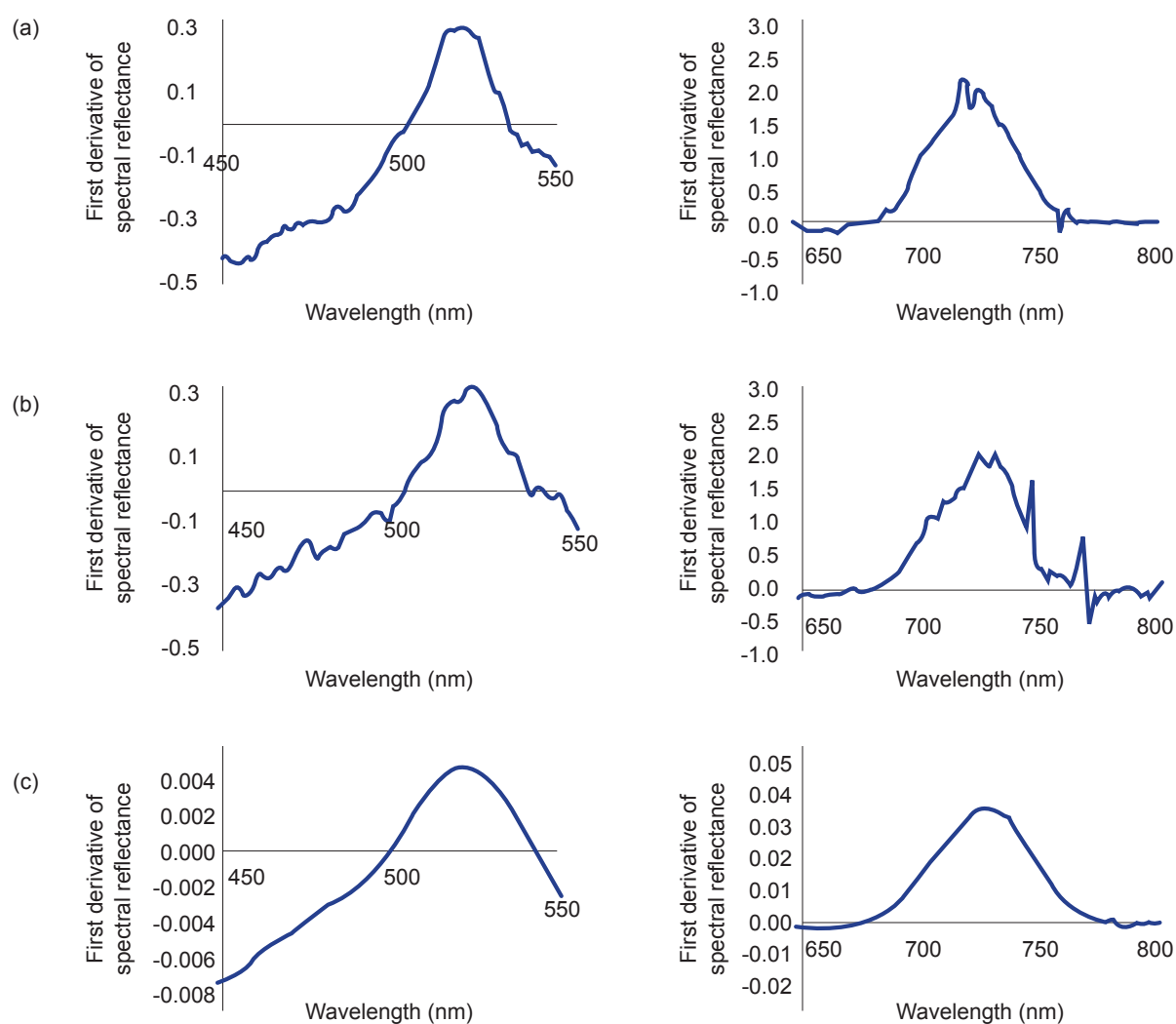


Figure 4. Spectral comparison between (a) clean first derivative spectral signature from field spectroscopy measurement while (b) the denoised first derivative of spectral signature from hyperspectral image using Set B2 and (c) using Set C3 from 450 to 550 nm and 650 to 800 nm.

The best denoising parameter settings were analysed using Root Mean Square Error (RMSE). Fifty-two wavelengths were chosen as sample points from the CR FDSR image for RMSE calculation. The 52 wavelengths comprised 10 wavelengths (401.70 to 417.11 nm), 10 wavelengths (531.48 to 547.75 nm), 10 wavelengths (654.26 to 668.66 nm), 10 wavelengths (732.22 to 746.41 nm), 10 wavelengths (850.63 to 864.46 nm) and 2 wavelengths (901.18 and 902.71 nm). The FDSR values from these wavelengths were used to calculate the RMSE. A larger RMSE value indicates worse denoising output. Smaller RMSE indicates better denoising output that is similar and fits with the reference spectral signatures from ground measurement. The results showed that the RMSE for Set B2 was 0.009934903 while the RMSE for Set C3 was 0.010271097 (Table 3).

For further processing, the denoised hyperspectral image was analysed to select the best 3, 5 and 7 significant wavelengths. In this study, MRMR technique was used on the D-CR-SR and D-CR-FDSR. Table 4 shows the best 3, 5 and 7 significant wavelengths from D-CR-SR and D-CR-FDSR. Then, three new spectral subsets of hyperspectral image were generated using ENVI 5.0 software based on the 3, 5 and 7 best significant wavelengths. The best significant wavelengths were arranged according to

their discriminatory capability index obtained from the MRMR analysis. The OBIA was conducted using ENVI 5.0 software. The Example-based Feature Extraction (EFE) tool was used to process and analyse the 3, 5 and 7 spectral subset hyperspectral image in ENVI 5.0.

In this study, three settings of image segmentation were used, and the best setting is Set 2 which used the value 30 for the segment and 70 for merging with Kernel value of 3. The segmentation process had segmented the fronds of the canopy with good separation between adjacent oil palms and the background Figure 5.

Then, the segments from the oil palm canopy from each GDSI were selected as training samples for each *Ganoderma* disease severity. The classifiers used in OBIA were SVM and KNN. The classification results of OBIA using SVM and KNN classifier are shown in Figure 6. The classification results classified oil palm canopies into four GDSI which are green (0), blue (1), yellow (2) and red (3). The classification outputs are in raster format. The OBIA was conducted on each of the generated images in Table 5 which are: a) D-CR-SR 3; b) D-CR-SR 5; c) D-CR-SR 7; d) D-CR-FDSR 3; e) D-CR-FDSR 5 and f) D-CR-FDSR 7. The classification accuracy of OBIA for each image is shown in Table 5.

TABLE 3. THE ROOT MEAN SQUARE ERROR (RMSE) VALUES COMPARISON BETWEEN SPECTRORADIOMETER AND DENOISED HYPERSPECTRAL IMAGE

Section	1	2	3	4	5	6
A	-	0.026111233	0.009738559	0.008359466	0.006673	0.00563
B	0.032150344	0.009934903	0.006768957	0.006176466	0.005866852	-
C	-	0.013242752	0.010271097	0.00846282	0.00741182	

TABLE 4. THE BEST SIGNIFICANT WAVELENGTHS IDENTIFIED FROM DENOISED HYPERSPECTRAL IMAGE USING MRMR

Number of best significant wavelengths	D-CR-SR (nm)	Index	D-CR-FDSR (nm)	Index
3	749.69	0.3766	550.00	0.3943
	602.94	0.2759	650.00	0.3794
	922.76	0.0523	790.83	0.3172
5	749.69	0.3766	550.00	0.3943
	602.94	0.2759	650.00	0.3794
	922.76	0.0523	790.00	0.3172
	411.61	0.0374	662.00	0.2204
	490.94	0.0368	889.40	0.2125
7	749.69	0.3766	550.00	0.3943
	602.94	0.2759	650.00	0.3794
	922.76	0.0523	790.00	0.3172
	411.61	0.0374	662.00	0.2204
	490.94	0.0368	889.40	0.2125
	896.11	0.0366	601.00	0.1121
	803.88	0.0362	908.00	0.1098

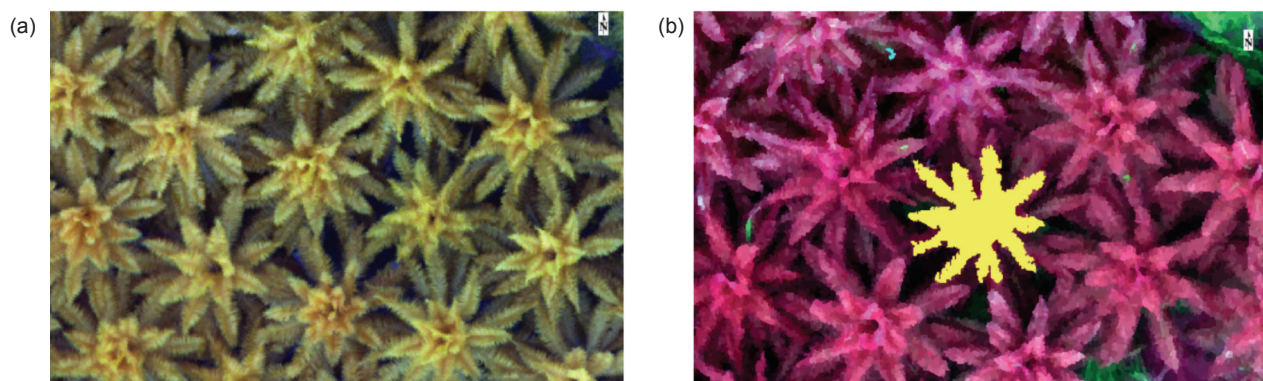


Figure 5. Outputs of the segmentation process: (a) Original image, and (b) segmented image of the oil palm canopy from the hyperspectral image.

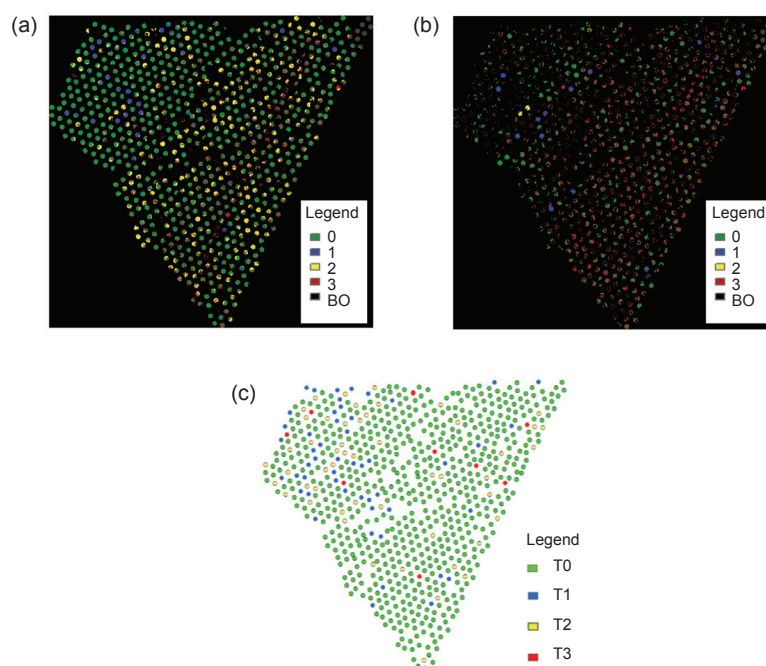


Figure 6. The OBIA classification output image using (a) SVM and (b) KNN classifiers for D-CR-FDSR3 image. Green (healthy), blue (early/mild), yellow (moderate) and red (severe) degree of Ganoderma infection in oil palm, and (c) ground census map.

TABLE 5. THE OUTPUT IMAGES OF CLASSIFICATION USING OBIA WITH SVM FOR D-CR-SR AND D-CR-FDSR FOR THE 3, 5 AND 7 BEST SIGNIFICANT WAVELENGTHS

Image	Accuracy (%)		Overall accuracy (%)	Image	Accuracy (%)		Overall accuracy
D-CR-SR 3	T0	90	60.00	D-CR-FDSR 3	T0	95	92.50
	T1	20			T1	85	
	T2	30			T2	90	
	T3	100			T3	100	
D-CR-SR 5	T0	98	83.25	D-CR-FDSR 5	T0	60	71.25
	T1	60			T1	50	
	T2	75			T2	75	
	T3	100			T3	100	
D-CR-SR 7	T0	30	40.00	D-CR-FDSR 7	T0	70	70.00
	T1	0			T1	50	
	T2	30			T2	60	
	T3	100			T3	100	

From the classification results in Table 5, the best classifier is SVM as compared to KNN. The best dataset for the SVM Classifier is the D-CR-FDSR 3 and D-CR-SR 5 with overall accuracy of 92.50% and 83.25%, but D-CR-SR 5B shows low accuracy for T1 classification. Meanwhile, D-CR-FDSR 3 provides good accuracy for T1 classification (85.00%) and very good classification accuracy for T0, T2 and T3. The results have shown that the integration of image segmentation with classifiers can enhance the capability of hyperspectral images for the detection of early infection of *Ganoderma* in oil palm.

Our study has successfully developed a new method for hyperspectral image processing, which requires a 2-step image pre-processing that involves continuum-removal and is followed by denoising using field spectral signatures as the benchmark, then OBIA to integrate the morphology of oil palm canopy with the significant spectral responses from the best wavelengths. Our approach manages to enhance the classification accuracy of each GDSI. Our study also suggested that the SVM classifier performed far better than KNN in the OBIA based on the comparison of the classification raster image with the ground census map in Figure 6. Our study also identified the seven most significant wavelengths from the MRMR technique which are 550.00, 650.00, 790.00, 662.00, 745.38, 704.59 and 501.21 nm. We then compared the position of our significant wavelengths with the other five studies as shown in Figure 7.

Figure 7 shows significant wavelength positions for the detection of *Ganoderma* BSR disease in oil palm from five previous studies and compared them with our findings. Figure 7 shows that the significant wavelengths are located between 500 to below 1,000 nm. Most of the wavelengths are located at an overlap between 500-550 and 650-800 nm. This region has been reported to have a significant correlation with chlorophyll content changes, stomatal conductance and

other photosynthesis-related parameters. There is a limited number of studies that have been conducted using hyperspectral images from drones for *Ganoderma* BSR disease detection in oil palm (Kurihara *et al.*, 2022). Our results agreed with the findings by Kurihara *et al.* (2022) that identified wavelengths of 560, 690, 710, 730 and 750 nm are suitable to be used in spectral indices generated from hyperspectral images from drones for *Ganoderma* BSR disease detection in oil palm. These regions must be explored in depth to identify what physiological changes in the oil palm foliar caused the subtle changes in the spectral responses when infected with *Ganoderma*. The significant wavelengths identified from our studies have a very good correlation with the significant wavelength regions suggested by Azmi *et al.* (2020) and Nisfariza (2012) except for the 800 nm and above spectral region.

These results concurred with the study conducted by Azmi *et al.* (2020) where they used a FirefLEYE S185 (Cubert GmbH, Ulm, Germany) snapshot camera with wavelengths ranging from 450 to 950 nm (125 bands) covering the visible (blue, green, and red) and near-infrared (NIR) regions with a sampling interval of 4.0 nm was used. They used 28 seedlings of DxP where 15 seedlings were inoculated with the *Ganoderma* pathogen. The image acquisition was conducted five months after transplanting. The camera was mounted horizontally on a custom tripod and was positioned 2.6 m from the ground consisting of eight-month oil palm seedlings from healthy and *Ganoderma*-infected oil palms. They suggested that significant wavelengths from the NIR band have the capability to 100% discriminate between healthy and *Ganoderma*-infected oil palms.

On the contrary, other studies that used ground-based hyperspectral sensors suggested that only NIR played the most significant role in discriminating between healthy and other *Ganoderma* infection severity (Izzuddin *et al.*, 2010;

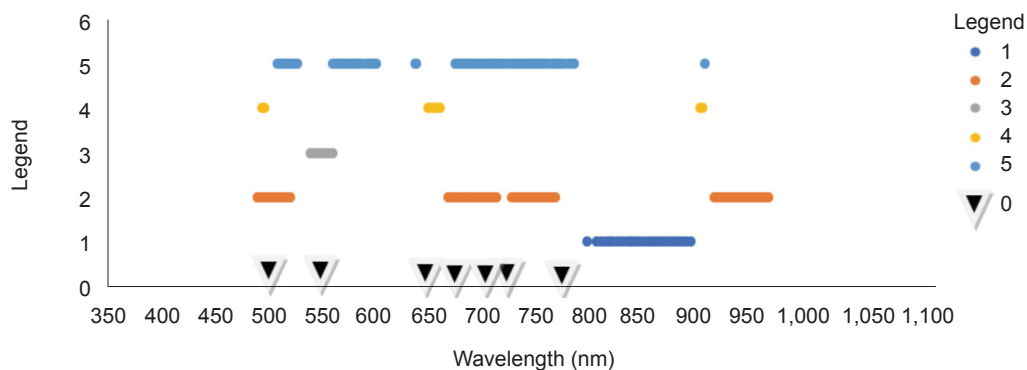


Figure 7. Position of significant wavelengths for detection of *Ganoderma* disease in oil palm from five existing studies: 0) Significant wavelengths from CR-SG-FDSR-3B; 1) Azmi *et al.* (2020); 2) Lelong *et al.* (2010); 3) Ahmadi *et al.* (2017); 4) Izzuddin (2010) and 5) Nisfariza (2012).

2017; 2018; Khairunniza-Bejo *et al.*, 2021; Nisfariza, 2012). The contradiction between the ground-based hyperspectral studies with our drone-based hyperspectral studies was due to the difference in samples. The ground-based hyperspectral studies used seedlings and immature oil palms in a controlled environment such as oil palm nurseries and glass houses meanwhile our study took samples from mature oil palms in the field. The morphology and physiology of immature and young oil palms are different.

These physiological changes in the oil palm foliar between healthy and *Ganoderma*-infected or other are likely stresses in oil palm also reported by several ground and lab-based studies (Haniff *et al.*, 2005; Putranto, 2018; Wei *et al.*, 2021). These physiological changes in the oil palm foliar also cause changes to the spectral properties of the oil palm canopy. Putranto (2018) agreed with Haniff *et al.* (2005) on the suggestion that water stress in oil palm causes a decrease in stomatal conductance.

Wei *et al.* (2021) also mentioned that abiotic stress due to inefficient water intake from pathogen infection affects the physiology of the oil palm. The abiotic stress decreased chlorophyll content, variable fluorescence (Fv), maximum fluorescence (Fm), variable fluorescence and maximum fluorescence ratio (Fv/Fm) of oil palm leaves, whereas initial fluorescence (F0) and non-photochemical quenching coefficient (NPQ) increased, indicating that the plants were affected by water stress.

These studies supported the fact that disruption to the root of the plant, including oil palm can affect the physiology traits in the foliar. These changes can be asymptomatic and can be measured using spectral signatures from hyperspectral remote sensing imagery. Finally, our findings showed that hyperspectral cameras are useful for conducting a thorough diagnosis via spectral responses from plants for disease detection. The results from the diagnosis provide a set of several narrow wavelengths that are specific for GDSI classification. Our results could open opportunities to develop a new camera that is specific for *Ganoderma* detection in oil palm and can reduce the cost of drone operation in the field. Our findings also confirmed that hyperspectral images provide higher accuracy as compared to multispectral images for the detection of *Ganoderma* infection in oil palms (Izzuddin *et al.*, 2020).

CONCLUSION

In this study, the hyperspectral image from the drone was accessed using CR and denoised using the S-G technique before conducting OBIA methods that integrate segmentation and merging with SVM and KNN classifiers to categorise oil

palm into four GDSI namely T0, T1, T2 and T3. The results showed different performances of each band and different band combinations in the classification of the disease severity categories. Nevertheless, the combinations of three, five and seven bands' images of the FDSR had shown good results (70.0%-92.5%). This study showed that the three-band combination of D-CR-FDSR image analysed using OBIA analysis can detect early, moderate, and severe infection of *Ganoderma* disease in oil palm. For future work, extensive experiments of OBIA analysis with deep learning should be conducted to enhance the detection of early infection of *Ganoderma* in oil palm using multispectral band combinations. Specific cameras for drones could also be developed for *Ganoderma* detection in oil palm using the new set of significant wavelengths, thus reducing the cost of operation in the field.

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BATCH SYSTEM CLARIFICATION OF TWO VARIETIES OF PALM OIL: KINETICS AND THERMODYNAMICS

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ABSTRACT

The kinetics and thermodynamics of palm oil clarification, a major and critical unit operation along the palm oil chain especially at local levels, were studied in this work. The effects of temperature, oil/water ratio and stirring speed on oil yield were investigated. No significant difference in oil yield with respect to temperature was observed for the *dura* variety while for the *tenera* variety oil yield decreased with an increase in temperature. Increase in oil water ratio significantly ($p < 0.05$) increased oil yield for both varieties. Optimum clarification conditions for both varieties were established as temperature of 80°C, oil/water ratio of 1:2, 30 min settling time and a stirring speed of 95 rpm. These conditions gave oil clarification yields of 73.33% and 90.39% for *dura* and *tenera*, respectively. The kinetics of palm oil clarification was modelled using a modified Newton and Lewis equation and the Peleg's model. The thermodynamic study gave enthalpy change (ΔH) values of -1809.87 and -17182.54 kJ/mol for *dura* and *tenera* variety respectively showing that the clarification process is exothermic. The corresponding values for entropy change (ΔS) were negative while free energy changes (ΔG) were positive indicating the process is random, non-spontaneous and irreversible.

Keywords: clarification, kinetics, oil yield, palm oil, thermodynamics.

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INTRODUCTION

The oil palm (*Elaeis guineensis*) is one of the most important species in the genus *Elaeis* which belongs to the family Palmae grown in the tropics. It originated from the Guinea Coast of West Africa (Henson, 2012). World's demand for cooking oils is expected to go well above 240 million tonnes by 2050 for both industrial and domestic consumption and palm oil would contribute to more than 50% of global world vegetable production (Xin *et al.*, 2022).

Three main varieties are generally recognised, *i.e.*, *dura* (thick shelled), *tenera* (medium or thin shelled) and *pisifera* (shell-less). It is the cross between *dura* and *pisifera* that results in *tenera* variety that makes up the basis of modern plantations (Henson, 2012; Teoh, 2002).

One interesting feature of oil palm cultivation is the production of two different types of edible oils; palm and palm kernel oils with distinct physicochemical properties from the pulp and the kernel, respectively. Palm oil is extracted from fresh fruits which on reception are washed, sterilised, and cooked to soften the pulp and facilitate detachment from the fresh fruit bunches and subsequent oil flow during extraction. The oil is then extracted by press followed by clarification and subsequent drying and bottling. Palm kernel oil is extracted from the kernel after cracking of the nuts, a byproduct from the palm oil process. The nuts are crushed, cooked and extracted by press and/or solvent extraction. Palm kernel oil is then clarified in a filter press or by sedimentation, refined and packaged. In addition, the empty fruit bunches and husks left behind

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after oil processing can be used as combustion fuels and in pyrolysis to produce bio-oils and char for various applications (Claoston *et al.*, 2014; Omar *et al.*, 2011; Sukiran *et al.*, 2011; Sulaiman & Abdullah, 2011). These make oil palm cultivation very appealing because all parts of the plant have income-generating potential. The oils extracted from the oil palm are used in pharmaceuticals and cosmetics and recently for biodiesel production (Ayanlowo *et al.*, 2022; Suryani *et al.*, 2020).

Clarification is the last but most important unit operation of the palm oil process. Clarification is aimed at separating the sludge consisting of water, fibrous material, debris and other unwanted solids that are left in the oil after extraction (Nurulhuda, 2009). Several parameters such as the volume of oil to water ratio, operating temperature, stirring speed and retention time affect oil clarification yields. Hence, mastery of how these parameters affect oil clarification yields is important in the design of clarifiers for palm oil, to reduce oil losses, energy use and the overall cost of the clarification process.

Kinetic studies are useful to identify factors (temperature, reactants, catalysts) that influence a reaction significantly and how they can be controlled to maximise the amount of product produced and to determine the mechanism of a reaction. Thermodynamics determines if the reaction is endothermic or exothermic as well as the entropy changes that are involved. Thermodynamics thus explains the driving force for a reaction. There is an inherent relation between the kinetics and thermodynamics of a reaction (Van Boekel & Tijsken, 2001).

Modelling batch kinetic data is important for clarification process scale-up and design of clarifiers as it provides information that could be used to compare different modelling approaches under different operating conditions for the process.

Although a substantial amount of palm oil is produced industrially in most palm oil-producing countries, there is a non-negligible contribution that comes from smallholder farmers. For example, in Cameroon, it is estimated that smallholder farmers contribute about 30% of the total palm oil production (Frank *et al.*, 2011). Smallholder farmers, therefore, derive substantial incomes from oil palm production to partially or completely fulfil their household needs which is essential for poverty reduction in their communities (Koczberski 2007; Qaim *et al.*, 2020). Industrial palm oil processing is elaborate, mastered and already vulgarised for more than a century but is very expensive and beyond the reach of smallholder farmers. Because industrial processing techniques are not directly transposable to smallholder levels, there is a need to adapt them to those of smallholder farmers to reduce the drudgery that is involved in processing at this level. Processing at this level produces doubtful or variable

quality oil because of the difficulties encountered at the level of clarification. For the smallholder farmers to meet with expected production targets that will contribute significantly to global palm oil production, processing techniques at their level need to be optimised.

Crude palm oil (CPO) contains water, cell debris, fibrous material, non-oily solid and oil as it is released from the press. This mixture undergoes clarification to obtain pure palm oil with little or no moisture. In small-scale palm oil processing units, clarification is done manually after the release of press liquor from the press using locally fabricated equipment. Two clarification methods are popular at this level. In the first case, during pressing, a small amount of water is added to the mashed mixture and the press liquid is left to settle in drums beneath the press at the temperature at which the oil was pressed. In the second case, the oil is boiled in drums using uncontrolled amounts of water and allowed to settle after boiling and then the top oil layer is scooped out (Nchanji *et al.*, 2013). Clarification of palm oil by local farmers is therefore a cause for concern as the process conditions are not mastered and most of the oil is left in the sludge leading to visible but unquantified oil losses. These farmers require well-designed and clearly defined processing conditions to enable them to produce high yield clarified oils of recommended quality to attract high prices for their produce. Palm oil clarification methods reported in the literature include the use of ultrasound assisted clarification (Juliano *et al.*, 2013a; 2013b) and the simultaneous clarification and dehydration of CPO using specially designed membranes (Wenten *et al.*, 2019). These methods are expensive and not easily adaptable to local processors working on small household units. Unfortunately, therefore, there is a dearth of information in the literature on studies that can facilitate the modification of existing local clarification methods or clarifiers and/or the development of new ones entirely. A major step towards achieving this is to carry out kinetic and thermodynamic studies on the clarification process of the different varieties in order to put at the disposal of the stakeholders, reliable information that can be exploited to design and construct clarifiers locally for small-scale processing of palm oil. This study examines the processing parameters that must be mastered in order to reduce the losses encountered by smallholder farmers during CPO clarification. The work lays down a reliable basis for defining parameters that will subsequently be used in the design of small-scale clarifiers for use by smallholder farmers to enhance oil yields and quality. The study examines the kinetics and thermodynamics of palm oil clarification, a critical unit operation in the palm oil process, as a major step to optimising oil clarification at smallholder processing levels.

MATERIAL AND METHODS

Sample Collection

Samples of the two varieties (*dura* and *tenera*) of CPO were bought from local women processors in Mbengwi (6.0126°N, 10.0192°E) Cameroon and transported in polyethylene containers to the Food Science Laboratory of the Biochemistry Department in the University of Dschang and stored in a refrigerator until use. The impurity of the oil was 0.12%. A block diagram for the work carried out is presented in Figure 1.

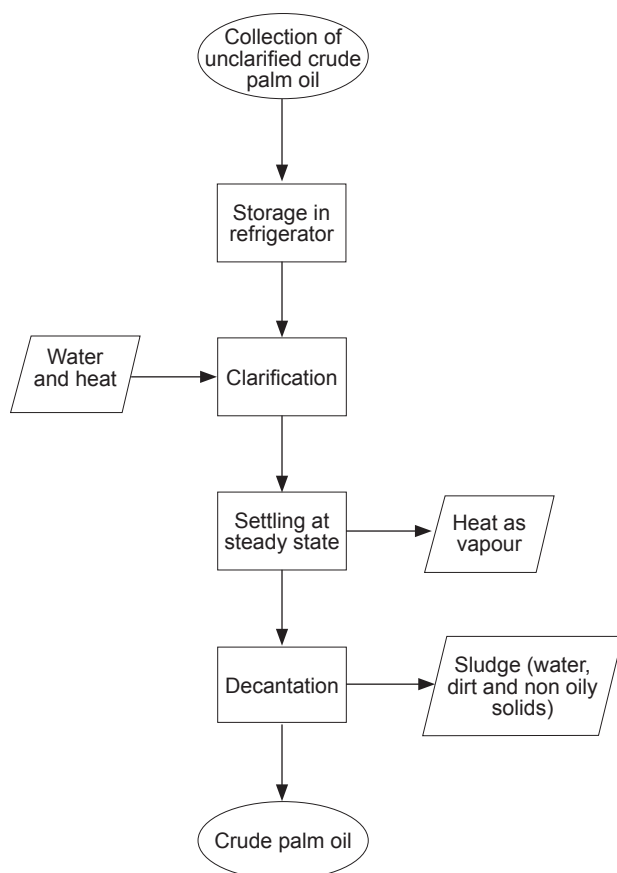


Figure 1. Block diagram for the clarification process.

The Experimental Set Up and Design

Laboratory beakers, 500 mL, were used as reactors for this study. Unclarified crude palm oil was accurately measured and transferred into the beaker. The beaker was covered with a polyethylene material and a hole with a diameter equal to that of the stirrer rod was made through the plastic such that when the stirrer was put in, the system functioned in a closed system. The system was then placed in a hot water bath and the temperature was adjusted to target values. The experimental setup is presented in Figure 2.

The effect of three parameters namely temperatures (80°C, 90°C, 100°C), agitation or stirring speeds (60, 95, 115 rpm), and oil/water ratio (1:1, 1:2, 1:3) were investigated at different settling times using a 3 x 3 x 3 experimental design. Oil yield from the clarification process was measured under different experimental conditions. The effect of mole ratios and agitation speed were first evaluated. To evaluate the effect of temperature, the mole ratio with the best yield was selected from the experiments at 80°C and carried out using a rotation speed of 60 rpm. While the effect of rotation speed was investigated at a constant mole ratio and temperature of 80°C.

The Clarification Process

A 500 mL laboratory beaker was used as a batch clarifier. The required quantity of water and CPO following the mole ratio was poured into the clarifier. The required stirrer speed and temperature were set. When the prescribed temperature was reached, the clarification process was allowed to go on for 20 min. The mixture was then allowed to sediment naturally for 0, 5, 10, 20, 30 and 40 min. After clarification, the mixture was separated by decantation and the weight of the oil and that of the sediment were determined using an electronic

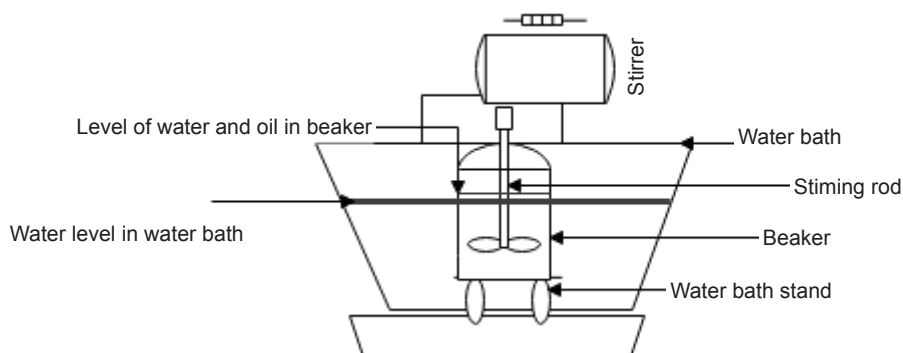


Figure 2. Sketch for clarifier setup.

balance. The percentage oil yield (Y) and quantity (Q) of sediment were calculated using Equations (1) and (2), respectively.

$$Y = \frac{W_0}{W} \times 100 \quad (1)$$

$$Q = \frac{W_1}{W} \times 100 \quad (2)$$

where, W , W_0 and W_1 are the weights of samples of press liquor or raw CPO, clarified oil (g), and sediment respectively. All experiments were carried out in triplicates.

Kinetics of Oil Clarification

The kinetics of oil clarification were modelled by two equations: A proposed modified Newton and Lewis equation and the Peleg model. For the proposed modified Newton model, it was assumed that once the reaction temperature is attained, palm oil clarification with respect to time occurred at a steady state and that there are no chemical reactions occurring at a steady state. For Newton's law, it was assumed that the rate of oil clarification is governed by the mass transfer of palm oil from sludge (non-oily solids and dirt particles) to the surface of the clarification medium (water). Therefore, following Newton's law, the rate of oil transfer to the surface of the clarification medium for the unsteady state can be written as (Liauw *et al.*, 2008; Westerman *et al.*, 1973):

$$Y = \exp(-kt) \quad (3)$$

A new term $[Y/t]$ which replaces Y in Equation (3) representing the quantity of oil clarified per unit time was assumed to increase exponentially with sedimentation time and therefore followed an empirical relation given by a modified Newton and Lewis equation of the form [Equation (4)]. K_{nl} replaces $-k$ in Equation (3) to depict the increase in the oil clarification rate with time.

$$\frac{Y}{t} = e^{tK_{nl}} \quad (4)$$

Linearisation of Equation (4) gives Equation (5):

$$\ln\left(\frac{Y}{t}\right) = K_{nl}t \quad (5)$$

Y is the oil clarification yield (%), t is sedimentation or clarification time (min), K is the constant from the Newton equation and K_{nl} is clarification constant from the modified Newton and Lewis equation; the gradient of the graph of $\ln(Y/t)$ against t .

The linearised form of the Peleg model [Equation (6)] (Peleg, 1988) was also tested for the

modelling of the clarification kinetics which can be rearranged to give Equation (7).

$$Y - Y_0 = \frac{t}{K_1 + K_p t} \quad (6)$$

$$\frac{t}{Y + Y_0} = K_p t + K_1 \quad (7)$$

Y_0 and Y are the initial and oil clarification yields at time t respectively. At $t = 0$, $Y_0 = 0$, and Equation (7) reduces to Equation (8).

$$\frac{t}{Y} = K_p t + K_1 \quad (8)$$

The clarification constant, K_p , from the Peleg equation is obtained from the gradient of the graph of t/Y against t . K_1 is the intercept.

Thermodynamics of Oil Clarification

Thermodynamic parameters were determined from Equation (9) and (11) (Liauw *et al.*, 2008).

$$\ln K = \frac{-\Delta G}{RT} = \frac{-\Delta H}{RT} + \frac{-\Delta S}{R} \quad (9)$$

where, K , ΔG , ΔH , ΔS and T are clarification constant, Gibbs energy (KJ/mol), enthalpy change (KJ/mol), entropy change (KJ/mol.C) and temperature (K) respectively. R is the universal gas constant (8.314 KJ/Kmol).

K values determined from the modified Newton model and the Peleg equation did not give good fits for the thermodynamic Equation (9). A new value of K (K_{YQ}) was then defined by Equation (10) (Nwabanne, 2012).

$$K_{YQ} = \frac{Y}{Q} \quad (10)$$

Y is the percentage oil yield at temperature T , and Q is the percentage of sludge or sediment. The values of K_{YQ} for each treatment was then determined and used to plot (K_{YQ}) vs $1/T$ from which ΔH and ΔS were obtained from the slope and intercept respectively. The Gibbs energy (ΔG) was estimated from Equation (11) (Nwabanne, 2012; Santos *et al.*, 2015).

$$\Delta G = -\Delta H - T\Delta S \quad (11)$$

Statistical Analysis

The One-way ANOVA was used on JMP Pro 16 to detect differences between means and the DUNCAN multiple range test was used to separate the means.

RESULTS AND DISCUSSION

Effect of Process Parameters on the Clarification Yields

Effect of temperature on the yield of clarified palm oil. Figure 1 present the temperature effect on the clarification yield of the *dura* variety of palm oil. For all three agitation speeds studied, no significant difference ($p < 0.05$) was observed in the clarification yields because of the change in temperature. Oil yields for the *dura* variety ranged from 0.00% to 68.13%, 0.00% to 71.89% and 0.00% to 72.62% at 80°C, 90°C and 100°C respectively which were not significantly different ($p < 0.05$). Unlike the *dura* variety, temperature had a profound effect on the clarification yields of the *tenera* variety (Figure 2). For the *tenera* variety, the oil yield decreased as the temperature increased from 80°C to 100°C when the clarification was carried out at an agitation speed of 60 rpm. Similar observations were made at higher stirring speeds of 90 rpm and 115 rpm where oil clarification yields were consistently lower at 90°C compared to 80°C. The ranges of oil yields were from 0.00% to 92.81%, 0.00% to 89.32% and 0.00% to 78.44% at 80°C, 90°C and 100°C, respectively at constant agitation speed of 60 rpm; the highest oil yield was obtained at 80°C, 60 rpm. The reduction in clarification yield with an increase in

temperature is likely because the solubility of the oil in water increases as temperature increases. At high temperatures, more of the oil then dissolves in water which is removed as part of the sludge thereby leading to the observed decrease in oil clarification yields. These results suggest that for both varieties, clarification should be carried out at 80°C using a stirring speed of 60 rpm.

Effect of mole ratio on the yield of clarified palm oil.

The effect of mole ratio on the clarification yields for the *dura* and *tenera* varieties at 80°C are presented in Figure 3 and 4. The oil yield for the *dura* variety decreased as the mole ratio increased from 1:1 to 1:3. The highest oil yield was obtained at 1:1 mole ratio. For the *dura* variety and at a lower stirring speed of 60 rpm, oil clarification yield decreased with an increase in CPO to water ratio from 1:1 to 1:3, which was contrary to our expectation. At higher rotation speeds of 95 and 115 rpm there was no significant difference in the oil clarification yields of the *dura* variety with respect to mole ratio (Figure 3). Therefore, for the *dura* variety, the optimum oil to water ratio which gives high oil yield after clarification is 1:1. For the *tenera* variety, significant differences ($p < 0.05$) were observed between the three different mole ratios investigated in this study. Oil clarification yield increased with an increase in CPO/water ratio as expected when

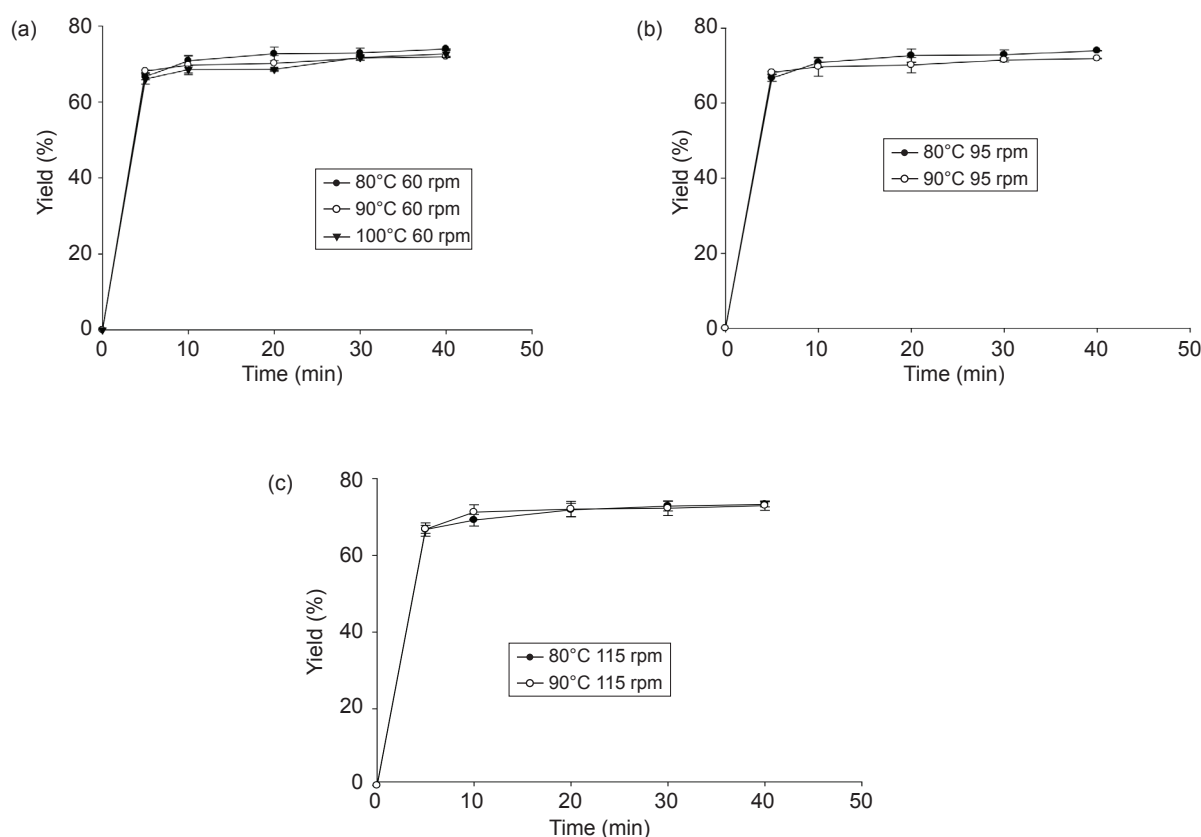


Figure 1. Effect of temperature on the clarification of palm oil *dura* variety at (a) 60 rpm, (b) 95 rpm and (c) 115 rpm.

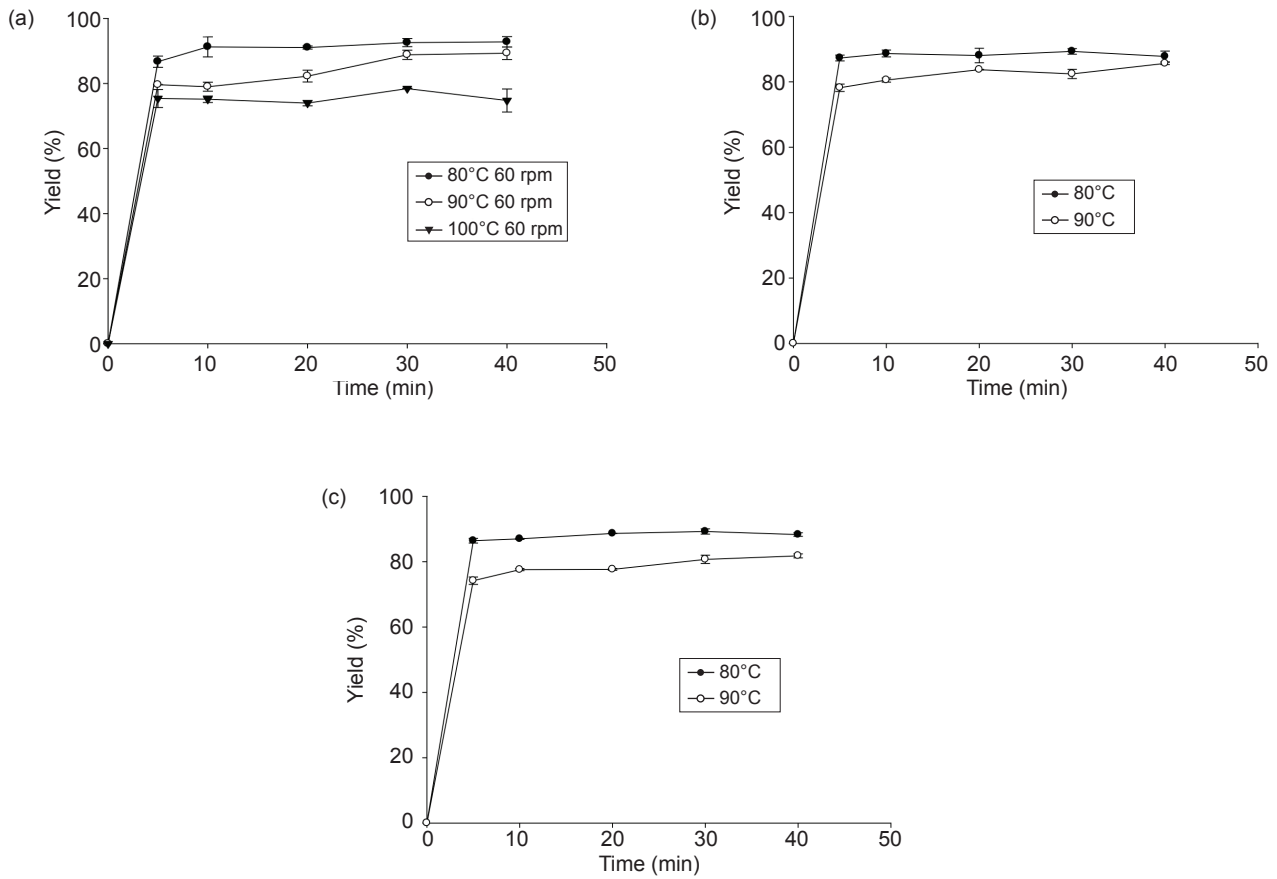


Figure 2. Effect of temperature on the clarification of palm oil tenera variety at (a) 60 rpm, (b) 95 rpm and (c) 115 rpm.

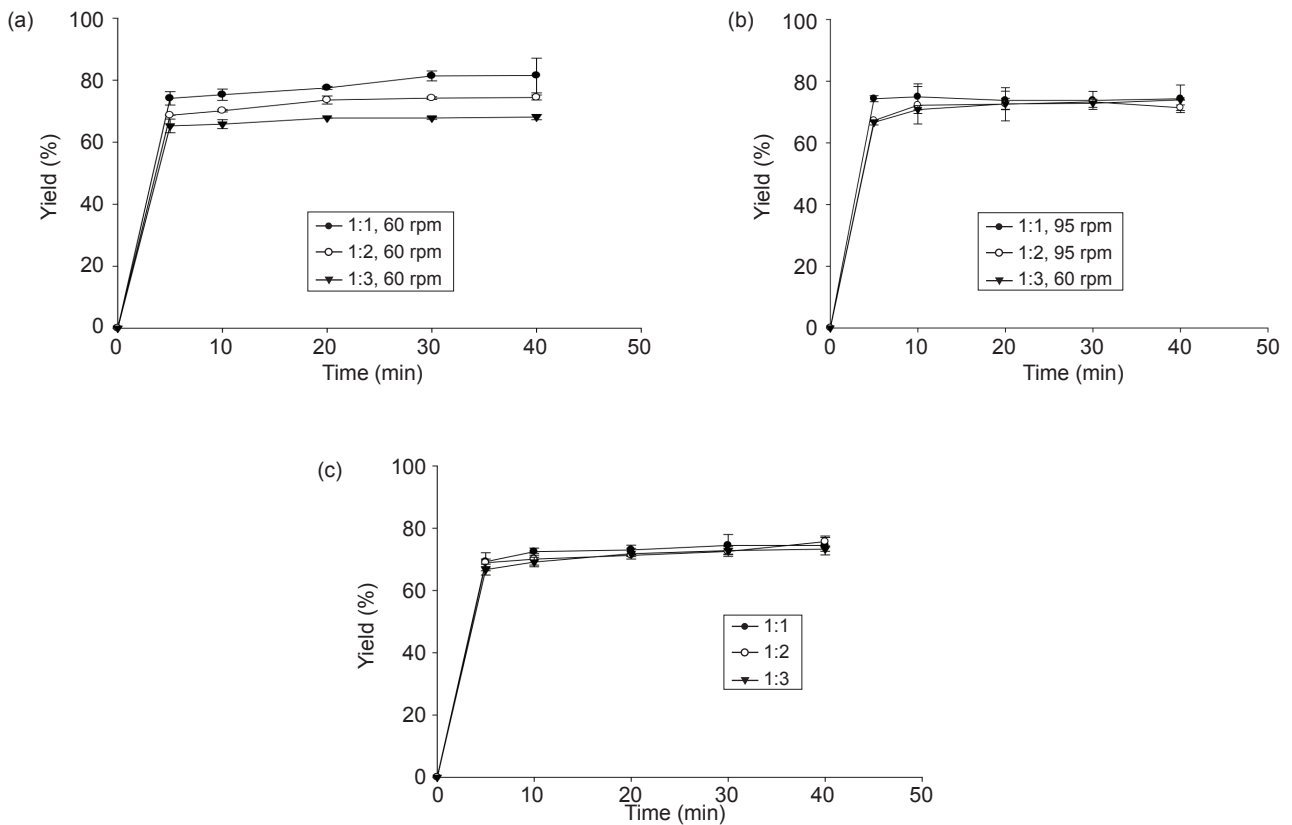


Figure 3. Effect of mole ratio on palm oil clarification dura variety, at (a) 60 rpm and 80°C, (b) 95 rpm and 80°C and (c) 115 rpm and 80°C.

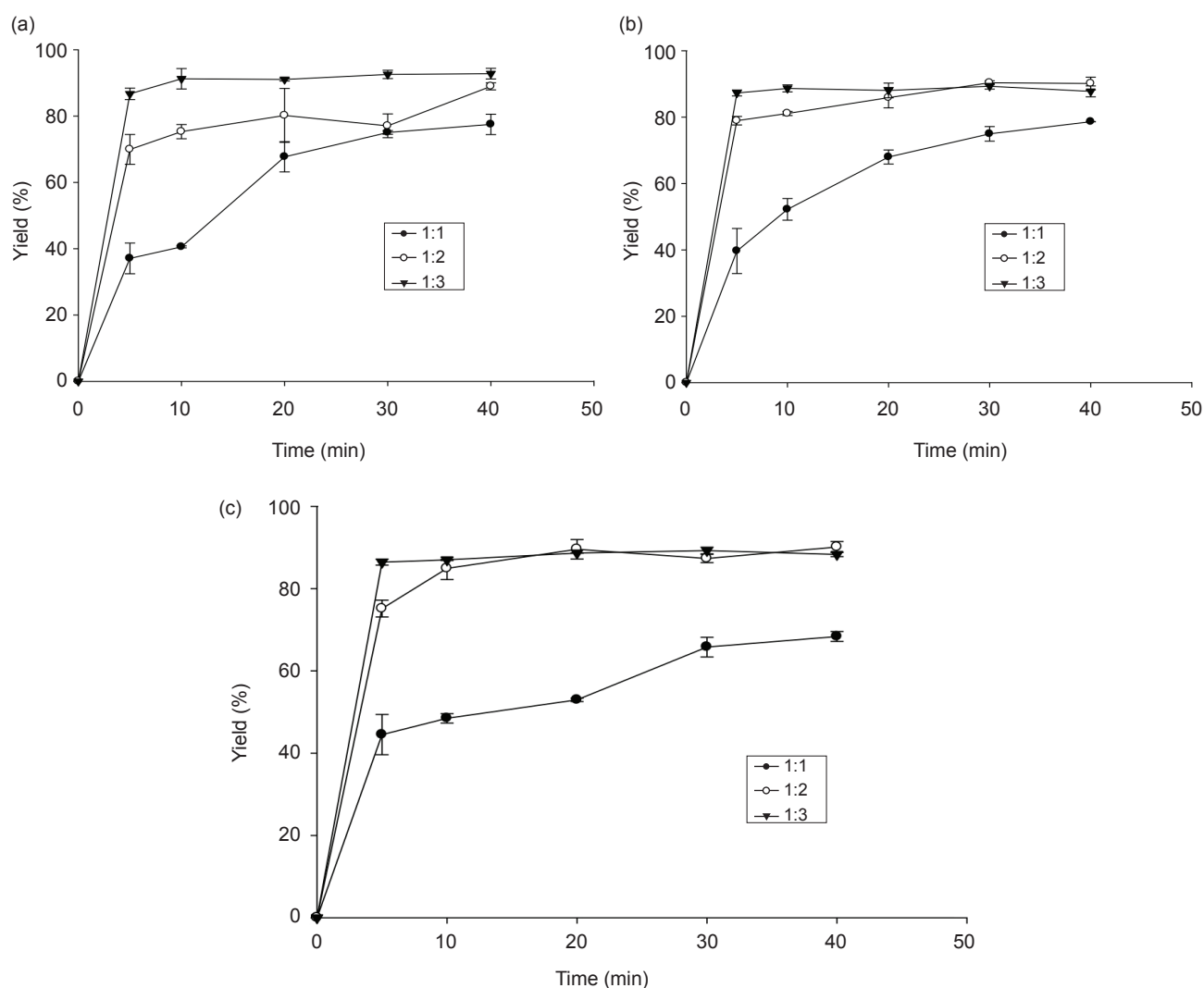


Figure 4. Effect of mole ratio on palm oil clarification tenera variety, at (a) 60 rpm and 80°C, (b) 95 rpm and 80°C and (c) 115 rpm and 80°C.

the temperature was kept constant. At constant temperature, more impurities are expected to dissolve in an increased quantity of water to form a sludge thereby facilitating the separation of sludge from the oil and hence the high oil clarification yields. At 95 and 115 rpm there was no significant difference ($p < 0.05$) between mole ratio 1:2 and 1:3. Therefore optimum mole ratio for the clarification of oil from the *tenera* variety was retained as 1:2.

Effect of stirring speed on the yield of clarified palm oil. The effects of stirring speed on the clarification of palm oil from the *dura* and *tenera* varieties are shown on Figure 5 and 6, respectively. Oil clarification yield decreases as speed increases from 60 to 115 rpm at mole ratio 1:1 and increases as stirring speed increases from 60 to 115 rpm at mole ratio 1:2 and 1:3 respectively for both varieties. One of the advantages of agitation is to cause the dirt particles to form aggregates which become heavier

and then sediment to ease separation. The slight reduction in oil yield with increase in stirring speed was attributable to the fact that at low mole ratio (1:1), high agitation did not allow sufficient contact time for the particles to aggregate and sediment hence the observed decrease in the clarified oil yield. Adopted stirring speed for both varieties was 95 rpm.

Effect of variety on the yield of clarified palm oil. The effect of variety on the clarification of palm oil is shown in Figure 7 and 8. It can be observed that except for the treatments at mole ratio 1:1, temperature 80°C each at 60, 95 and 115 rpm where clarification yields for *dura* were significantly higher ($p < 0.05$) compared to *tenera* (Figure 7), all other treatments gave oil yields for *tenera* which were significantly higher than that of *dura*. The observed significant differences obtained for the two varieties can be linked to the oil composition, method of oil extraction, quantity of debris or dirt contained in the CPO.

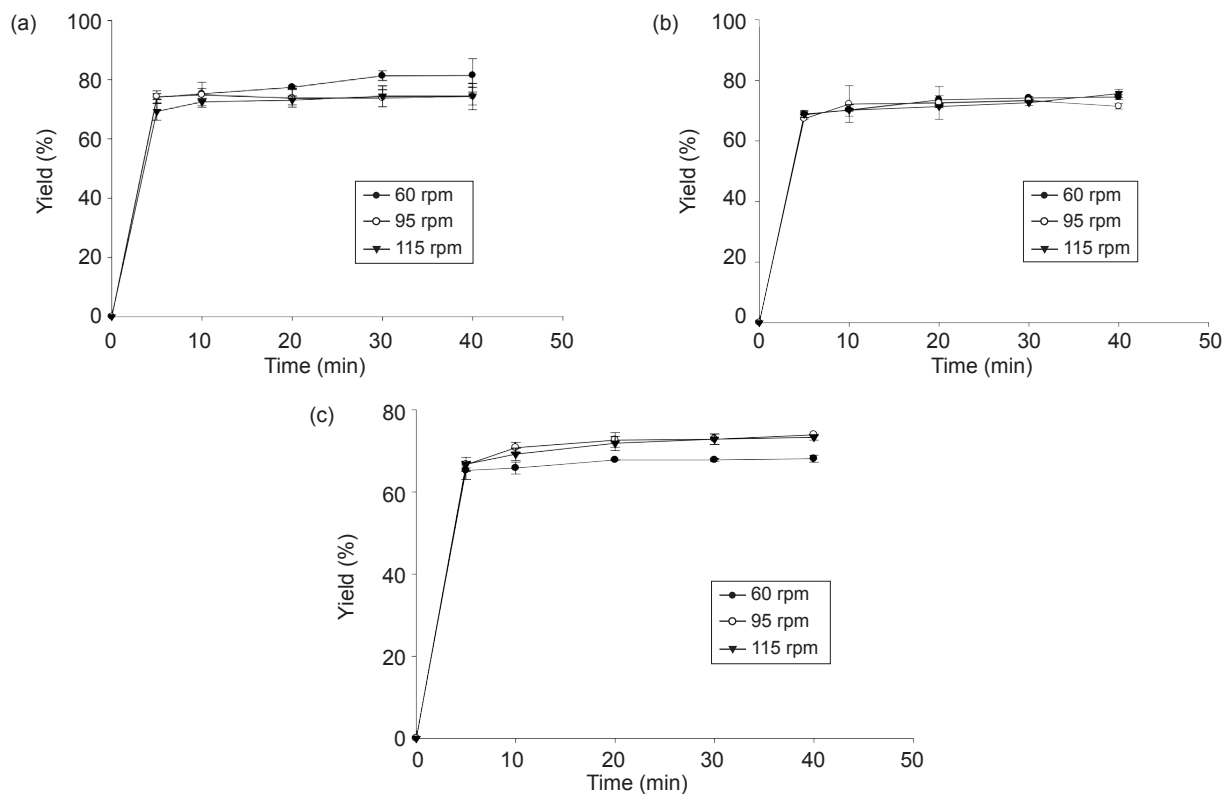


Figure 5. Effect of stirring speed on palm oil yield at temperature 80°C and mole ratio (a) 1:1, (b) 1:2 and (c) 1:3 for dura variety.

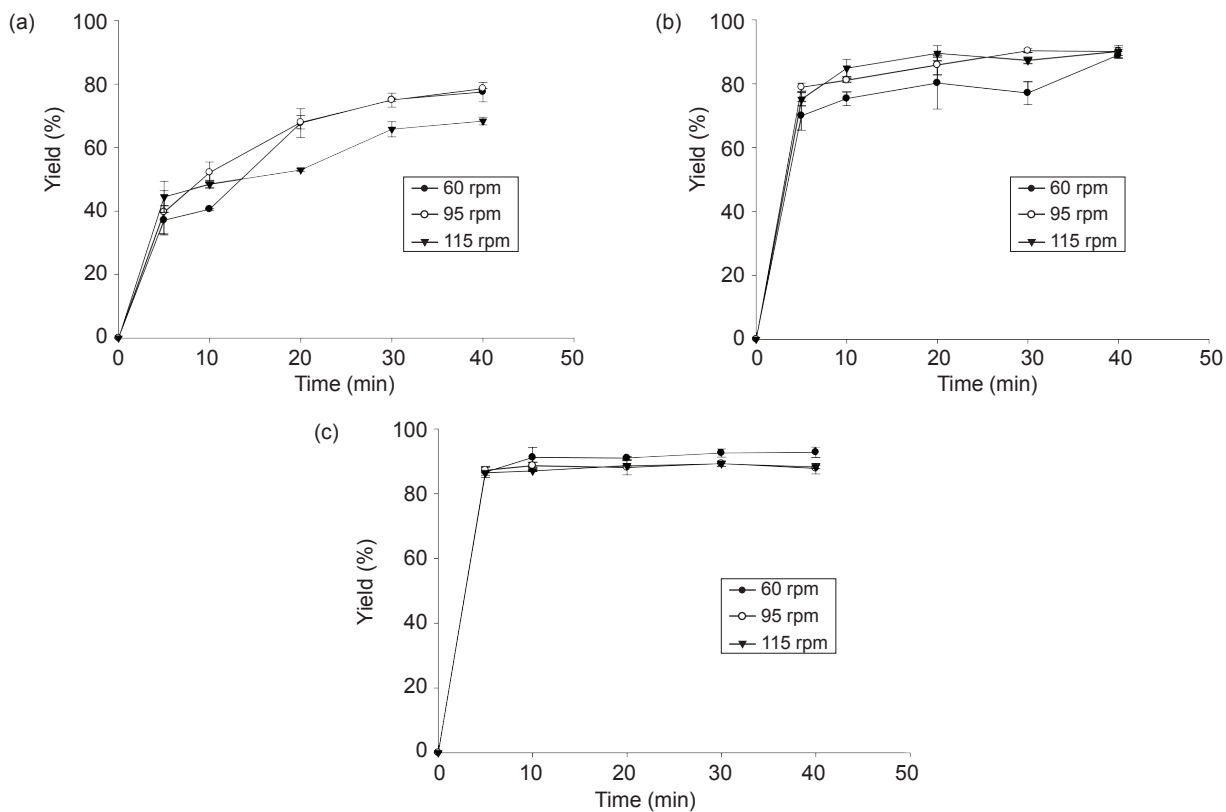


Figure 6. Effect of stirring speed on palm oil yield at temperature 80°C and mole ratio (a) 1:1, (b) 1:2 and (c) 1:3 for tenera variety.

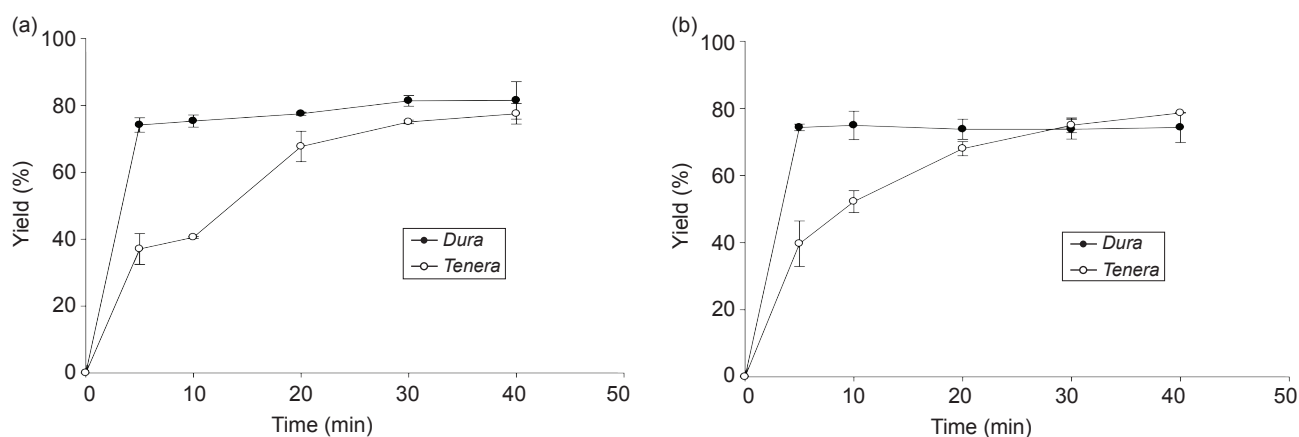


Figure 7. Effect of variety on oil yield at 80°C, 1:1 mole ratio, (a) 60 rpm and (b) 95 rpm.

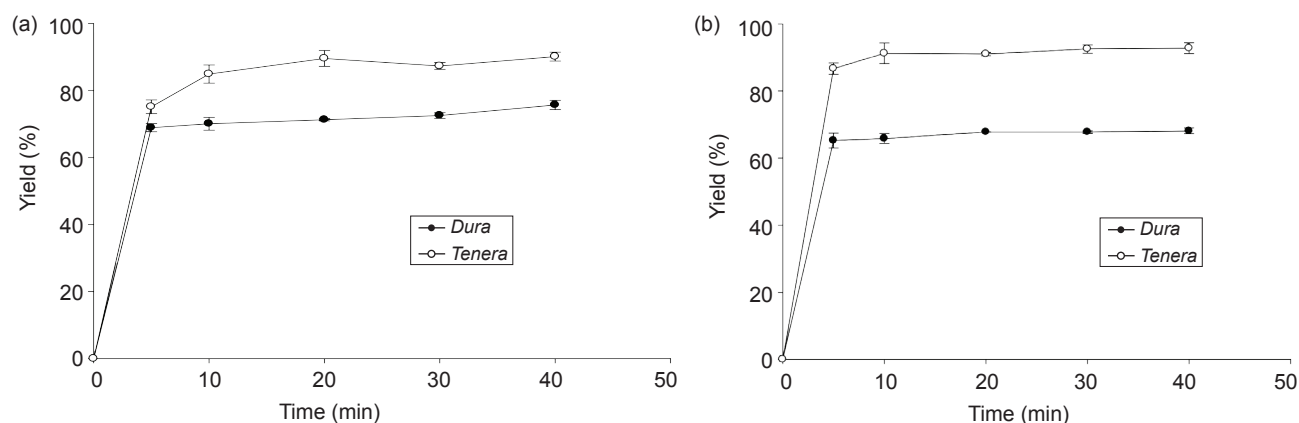


Figure 8. Effect of variety on oil yield at (a) 80°C, 1:2 mole ratio, 95 rpm, 115 rpm and (b) 80°C, 1:3 mole ratio, 60 rpm.

Palm oil separation generally adopts a vertical or horizontal settling tank. Depending on the preferred method, parameters (variables) to be considered include fluid flow rate, feed composition, settling volume, retention time, and temperature (Abikoye, 2014). Nurulhuda (2009) reported optimum oil clarification efficiency of palm oil at 95°C. However, crude palm oil obtained from the two varieties of oil palm treated was not taken into consideration. In this study, it was found that temperature significantly affected the clarification yields of CPO from two different varieties of oil palm. As temperature increases, oil yield in the *tenera* variety decreases while in the *dura* variety, the situation was the reverse even though no significant difference was observed at 90°C and 100°C. Nurulhuda (2009) and Abikoye (2014) found out that the feed flow rate and the settling volume are the major controlling factors in the separation of oil from sludge in a continuous system. A batch system was used in this work. John *et al.* (2019) studied the conventional method of clarifying CPO where the temperature in the clarification tank was set at 70°C. Most industrial palm oil processing units deal with the *tenera* variety of CPO commonly called the commercial variety. Kandiah and Batumalai (2013) used evaporation method to replace the

centrifugation stage in the conventional clarification of palm oil. In this method, water addition to facilitate oil-sludge separation was avoided. Sulung and Tan (1996; 1999) used the membrane filter press to clarify palm oil. In this method, the diluted crude slurry produced by mechanical pressing of the cooked fruitlets is settled in a vertical or horizontal clarifier.

These studies were aimed at standardising the traditional clarification of palm oil which is very costly and unaffordable for local farmers. It was found out that at a temperature of 80°C, mole ratio of 1:2 and stirring speed of 95 rpm, maximum oil yield will be obtained for the two varieties.

Kinetic Parameters

Model constants for the modified Newton/Lewis and Peleg models are presented on Table 1 and 2, respectively. All R^2 values for both models were close to 1; therefore the two equations can be used to describe the clarification process of palm oil for both varieties. These results are in accordance with that of Bup *et al.* (2012), who used Peleg's model to evaluate the water adsorption kinetics of cooked and uncooked shea nut kernels (*Vitellaria paradoxa* Gaertn).

TABLE 1. VALUES OF K_{NL} AND R^2 OBTAINED FROM THE PLOT OF THE MODIFIED NEWTON AND LEWIS EQUATION

Temperature (°C)	Mole ratio (g)	Speed (rpm)	<i>Dura</i>		<i>Tenera</i>	
			K_{nl}	R^2	K_{nl}	R^2
80	1:1	60	4.218	0.999	2.908	0.967
80	1:1	95	4.317	1.000	3.161	0.996
80	1:1	115	4.193	0.999	3.415	0.99
80	1:2	60	4.161	0.999	4.100	0.9969
80	1:2	95	4.184	0.999	4.246	0.999
80	1:2	115	4.162	0.999	4.224	0.998
80	1:3	60	4.141	1.000	4.427	0.999
80	1:3	95	4.138	0.998	4.468	0.999
80	1:3	115	4.129	1.000	4.437	0.999
90	1:3	60	4.101	0.999	4.265	0.998
90	1:3	95	4.183	1.000	4.299	0.999
90	1:3	115	4.156	0.999	4.239	0.999
100	1:3	60	4.120	0.999	4.311	0.999

TABLE 2. VALUES OF R^2 AND K_p OBTAINED FROM THE PELEG EQUATION FOR *Dura* AND *Tenera*

Temperature (°C)	Mole ratio (g)	Speed (rpm)	<i>Dura</i>		<i>Tenera</i>	
			K_p	R^2	K_p	R^2
80	1:1	60	0.011	0.999	0.108	0.972
80	1:1	95	0.001	0.999	0.078	0.999
80	1:1	115	0.006	0.999	0.071	0.981
80	1:2	60	0.008	0.999	0.023	0.986
80	1:2	95	0.001	0.999	0.013	0.999
80	1:2	115	0.001	0.99	0.010	0.999
80	1:3	60	0.004	1.000	0.004	0.999
80	1:3	95	0.008	0.9999	0.000	0.999
80	1:3	115	0.009	1.000	0.001	0.999
90	1:3	60	0.010	0.999	0.015	0.998
90	1:3	95	0.006	0.999	0.008	0.999
90	1:3	115	0.006	1.000	0.010	0.999
100	1:3	60	0.012	0.999	0.001	0.998

Thermodynamics Parameters

Entropy (ΔS) values were -9.333KJ/mol and -59.166KJ/mol for *dura* and *tenera* varieties respectively while the corresponding enthalpy change (ΔH) values were -1809.87 KJ/mol and -17182.54 KJ/mol. The negative values of enthalpy change indicate that the process is exothermic; energy is lost to the surrounding during the process, thus oil would clarify best at lower temperatures than at higher temperatures. This corroborates with the highest oil yields which were obtained at 80°C, compared to 90°C and 100°C. The Gibbs free energy at different temperatures is calculated using Equation (11). Gibbs free energy (ΔG) were respectively 1484.67, 1578.00, 1671.3 KJ/mol and

3702.95, 4294.61, 4886.26 KJ/mol for *dura* and *tenera* at 80°C, 90°C and 100°C. ΔG is positive indicating that this process is exothermic, irreversible and not spontaneous.

CONCLUSION

This study has shown that the traditional clarification process of palm oil can be controlled. Temperature, oil/water ratio and stirring speed significantly affect oil yield. The effect of these parameters varies with CPO from the different oil palm varieties. Best conditions to obtain high yield of palm oil (92%) were found to be temperature of 80°C, 1:2 mole ratio, settling time of 30 min

and 95 rpm stirring speed for both varieties. Palm oil clarification followed the modified Newton's and Lewis Equation and the Peleg's Model. It was also found that ΔH and ΔS are negative for both varieties, and ΔG is positive indicating that this process is exothermic, irreversible and not spontaneous.

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MECHANISATION STATUS OF OIL PALM NURSERY AND FIELD CULTIVATION IN WEST MALAYSIA

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ABSTRACT

Implementation of mechanisation has been the prime need for the Malaysian palm oil industry in order to reduce its foreign labour dependency. Mechanisation Index (MI) was used in this study to quantify the current mechanisation status in both oil palm seedling production and oil palm field cultivation. It was computed according to the expression of the ratio of machine energy to the total human and machine energy utilised in conducting the involved operations. The obtained average overall MI based on the 10 common clusters of operations in the oil palm FFB production was 0.43. The average overall MI for nursery operation was slightly lower than that of field cultivation operation (i.e., MI values of 0.36 vs. 0.41). The three most critical operations for mechanisation in the oil palm field cultivation operation were the FFB harvesting, which included pruning operation (0.08), rat control operation (0.06) and loose fruit infield collection operation (0.09). The MI values approaching zero reflect the extent of human labour dependency on the method used in the field operations, signifying their seriousness. In general, the obtained MI values provide useful information for developing a workable mechanisation strategy that aligns with the specific circumstances of crop production.

Keywords: crop production, mechanisation indicator, oil palm.

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INTRODUCTION

The oil palm plantation industry is one of the economic sectors in Malaysia that is still highly dependent on labour. International Organization for Migration (2023) reported that about 80% of the current plantation workforce are foreign labourers.

The working condition in the plantations has been associated by the young locals with the 3Ds stigma - dangerous, dirty and difficult, the conditions which discourage them from working in the plantations (Parveez, 2022). To attract more locals working in the oil palm plantation industry, this sector must be modernised and mechanised. Introducing mechanisation is not meant to displace the presence of the labour force with machines in field operations but rather to reduce the total dependency on the labour force in conducting field operations. Additionally, its implementation in plantations is in line with the government's policy of encouraging the locals to be involved in this field, with the aim to gradually reduce the country's dependency on foreign labours. The ultimate objective is to equip the labourers with proper hand tools or machines in some selected field operations that require significant human effort and energy. Whilst, the field operations that could easily be conducted and consume less human energy are done using proper hand tools.

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Therefore, using mobile machines, instead of hand tools for less demanding field operations, would only increase the cost of field operations. For any application of mechanisation to be effective, as mentioned by Johari *et al.* (2020), it is essential to utilise machines that can increase land and labour productivity, meet the field operation timelines and enhance the rate of work output.

Earlier attempts in the 1980s used land to labour ratio as the means to measure the productivity of plantation workers in terms of machine usage. The overall land to labour ratio of 6:1 to 7:1 in the 1980s increased to 10:1 to 12:1 in 2013. Today, in some oil palm plantations with appropriate adaptation of mechanisation, labour productivity has reached 20:1 (Khalid *et al.*, 2013).

Nawi *et al.* (2012) directly measured the workers' energy expenditures while doing various field operations in an attempt to rank the critical field operations of the crop production system. The human energy expenditure was measured based on the intensity and duration of the workers' activity, age, gender, and body weight (Westerterp, 2001). Pebrian *et al.* (2014) proposed a method of prioritising mechanisation based on workers' workload and productivity in conducting oil palm field operations. However, in this study, an attempt to systematically evaluate human energy expenditure and field operational capacity was done for complete field operations involved in producing oil palm seedlings at an oil palm nursery and oil palm fresh fruit bunches (FFB). Hence, a methodology was used to measure the nursery and field plantation mechanisation level, with the main objectives to identify, prioritise and eventually to design and develop appropriate machines for the most critical tasks within the field operations. Hence, in general, this study can significantly contribute to the development of a viable mechanisation strategy under the specified crop production scenario.

MATERIALS AND METHODS

A field study was conducted to explore, assess and determine the existing practices in the oil palm seedling production centre at Pusat Penyelidikan Pertanian Tun Razak (PPPTR), Jerantut, Pahang, Malaysia, under FELDA Agricultural Services Sdn. Bhd. (FASSB). The oil palm field cultivation was conducted at Ladang Felda Kechau 3, 8 and 10, Kuala Lipis, Pahang, Malaysia, under FELDA Global Ventures Plantations (M) Sdn. Bhd. The palm seedling production at PPPTR employed two-stage nursery techniques, which comprised pre-nursery and main nursery. In the pre-nursery stage, two methods of palm seedling production were used, *i.e.*, closed (indoor field) and open field techniques. Open field technique involved the planting of

germinated seeds in an open area on the ground, while the closed (indoor) technique involved the planting of germinated seeds in planting trays filled with peat moss. The seeds were soaked in a liquid medium to expedite the germination process and enhance the percentage of successfully sprouted seeds prior to planting. Soaking aims to sort out viable from non-viable seeds. If the seeds sink, they are still viable. On the contrary, if they float, it is advisable to discard them as they are less likely to germinate. Meanwhile, peat moss together with NPK fertiliser was crushed and smashed to get the fine particles for better growth of the seeds. The trays planted with the germinated seeds were then placed on a bench above the soil covered with a shelter made of ultraviolet (UV) resistant plastic. A total of 50 field operations were involved; 35 at the oil palm nursery and 15 at the oil palm plantation. Detailed descriptions of the work done in the nursery and field operations are given in Table 1 and 2, respectively.

A total of 100 subjects consisting of 49 for the oil palm nursery and 51 for the oil palm field were randomly selected as participants by using a probability sampling approach. The subjects were field workers. They were healthy males and females who were familiar with the oil palm cultivation activities. The age, weight, and height of the subjects were in the range of 18-54 years old, 37-99 kg and 143-182 cm, respectively. The selected subjects represented a typical sample of the workforce in the oil palm plantations. Three or four workers were involved in each operation and the whole data collection was repeated for at least three full working days with a duration of eight working hr day⁻¹. Time motion and energy expenditure measurements were taken on the assigned workers while conducting the nursery or field operations.

Digital stop watches were used to record the timing of all the tasks carried out by the subjects during the operations. Body Media Armbands were attached to the right arm of each subject to measure the energy expenditure while conducting the stated operations. Detailed technical specifications of the tools, equipment, or machinery used by the subjects in conducting the operations were recorded, as well as technical specifications, crop inputs and other consumable materials used in the operation. After completing the operations, work outputs from the conducted operations were counted and the data recorded by the Body Media Armbands were downloaded into a computer for analysis. The workers' field operational capacities were computed by dividing the quantity of work outputs by the corresponding time required for completing the operations. A value of 110 palms ha⁻¹ to represent a typical planting density for a plantation with a hilly terrain similar to that of the study area was used throughout the calculations of operation capacity

and energy expenditure. Machinery energy refers to tool, equipment or machinery/tractor using energy (J palm⁻¹) at each stage of oil palm nursery or field operations estimated by using Equation (1) (Board on Science and Technology for International Development, 1981), as follows:

$$ME = \frac{CEE \times WT}{TP} \times 10^6 \quad (1)$$

where *ME* is machinery energy (J palm⁻¹), *CEE* is the coefficient of energy equivalent of machinery, equipment, or tool (MJ hr⁻¹), *WT* is working time (hr), and *TP* is total palms, dimensionless. The energy equivalent coefficient serves as an indicator of an object or system's mass

when quantifying its energy content in Joules (J) or Mega Joules (MJ). Working time is the period of time that a machinery uses in a day.

Machinery energy could also be calculated from its known weight in terms of J palm⁻¹ which was calculated using Equation (2) (Board on Science and Technology for International Development, 1981):

$$ME = \frac{CEE \times W}{TP} \times 10^6 \quad (2)$$

where *ME* is machinery energy (J palm⁻¹), *CEE* is the coefficient of energy equivalent of machinery, equipment or tool (MJ kg⁻¹), *W* is weight (kg) and *TP* is total palms, dimensionless.

TABLE 1. FREQUENCY, EQUIPMENT AND MATERIAL USAGE AND WORKERS' INVOLVEMENT IN THE OIL PALM NURSERY OPERATIONS

Operation	Equipment and material	Number of workers
Pre-nursery		
Filling peat moss in trays	Peat moss (Klasmann-Deilmann TS 3) Open technique: Nursery bed, tray (24 seeds per tray), canvas, nursery bed cover (Silvershine) Closed technique: Tray (50 seeds per tray), scissors, table, wheelbarrow	Open technique: 5 Closed technique: 4
Planting oil palm germinated seeds in trays	Tray filled with Klasmann, oil palm germinated seeds, Scissors, small plastic chair (open technique)	1
Fertilising oil palm seedlings in trays	Open technique: Agroblen@SK Cote Precise Fertiliser, pail, hole punch, spoon, small plastic chair Closed technique: Fertiliser system, Felda Liquid Fertiliser 8, water	1
Pest and disease control of oil palm seedlings in trays	Open technique: Conventional Knapsack Sprayer (CKS), container, Zagro, Dithane, Argi-dex, Bayfolan, water Closed technique: Engine and pump, container, Zagro, Dithane, Argi-dex, Bayfolan, water	Open technique: 1 Closed technique: 2
Watering oil palm seedlings in trays	Open technique: Engine pump, pipe line (mist irrigation system), gasoline fuel, diesel fuel, water Closed technique: Irrigation system, water	1
Main-nursery		
Filling soil in large polybags	Pile/stalk, funnel, big scoop plastic polybags (15" x 18"). Cable wire 12G with 20 points by distance of 0.9 m, top soil	3
Transplanting oil palm seedlings from the trays to large polybags	60 kW 4WD Kubota tractor with 2 t trailer, diesel fuel, small scoop, wooden rod, Agroblen@SK Cote Precise Fertiliser, Rock Phosphate Fertiliser, oil palm seedlings from trays in basket, 15" x 18" polybags filled with soil, pail, spoon	3
Mulching fibres around the oil palm seedlings in large polybags	Oil palm seedlings in large polybags, basket, the mulching fibres from oil palm EFB fibres	1
Fertilising oil palm seedlings in large polybags	Pail, Scoop, Agrenas fertiliser, FPM compound fertiliser	1
Clearing grass in large polybags	Polybag, scissor	1
Watering oil palm seedlings in large polybags	3 kW Engine-pump set, pipe line (drip irrigation system), gasoline fuel, diesel fuel, water	1
Weeding oil palm seedlings in large polybags	Conventional Knapsack Sprayer (CKS), sodium chlorate (99%), Agri-dex, Dual G 960, Basta 15, water, container	1
Pest and disease control of oil palm seedlings in large polybags	Conventional Knapsack Sprayer (CKS), Zagro, Dithane, Bayfolan, Dithane, water, container	1

TABLE 2. FREQUENCY, EQUIPMENT AND MATERIAL USAGE AND WORKERS' INVOLVEMENT IN THE OIL PALM FIELD OPERATIONS

Operation	Equipment and material	Number of workers
Clearing old palm trees – cutting and chipping	110 kW Kobelco Yutani excavator, diesel fuel, chipping bucket for chipping, 3 kW generator and sharpening machine for chipping bucket, gasoline fuel	1
Clearing weeds	Mist blower, measuring container, water, Garlon 250, gasoline fuel	1
Lining for planting	Wooden pegs survey tape	1
Holing for planting	37.28 kW 4WD tractor EUROSTAR 4540 with fully mounted post hole digger with 35.6 cm diameter auger bit, diesel fuel	1
Planting ground cover crops	CIRP fertiliser (30 g per each drill at oil palm inter-row), hoe, legume cover crops (LCC)	3
Seedling delivery from nursery to field	3 t bin lorry, diesel fuel, oil palm seedlings, rope	2
Planting oil palm seedlings	CIRP fertiliser (500 g per palm), hoe, wheelbarrow, oil palm seedling	1
Circle weeding	Immature palm: Conventional Knapsack Sprayer (CKS), herbicides Mature palm: Controlled Droplet Applicator (CDA) with battery 6V, herbicides	1
Fertilising	Immature palm: Fertiliser pail, NPK fertiliser Mature palm: Fertiliser pail, SOA fertiliser	1
Rat control	Fertiliser pail, butik S Rat Bait	1
Loose fruit infield collection	50 kg capacity sacks, rake	1
FFB harvesting including pruning	Harvesting sickle, loading spike, parang	2
FFB infield collection transportation	60 kW 4WD Kubota tractor and 2-t trailer, loading spike, collection bin, diesel fuel	2
FFB mainline transportation	8 t bin lorry with 96 kW hook-lift system, canvas or net, diesel fuel	1

Energy consumption by fuel used in terms of J palm⁻¹ could be calculated using Equation (3) (Board on Science and Technology for International Development, 1981), as follows:

$$IE = \frac{CEF \times Q}{TP} \times 10^6 \quad (3)$$

where *IE* is input energy (J palm⁻¹), *CEF* is the coefficient of energy equivalent of fuel (43.3 MJ L⁻¹ for diesel and 39.7 MJ L⁻¹ for gasoline), *Q* is quantity (L), and *TP* is total palms, dimensionless. Energy consumption by water for irrigation used in terms of J palm⁻¹ could be calculated using Equation (4) (Board on Science and Technology for International Development, 1981):

$$IE = \frac{CEI \times Q}{a \times TP} \times 10^6 \quad (4)$$

where *IE* is input energy (J palm⁻¹), *CEI* is the coefficient of energy equivalent of water for irrigation (0.63 MJ m⁻³), *Q* is quantity (L) and *TP* is total palms, dimensionless and *a* is constant (1,000 L m⁻³).

The value of fuel energy consumption in terms of J palm⁻¹ for the tractor used in the infield FFB

collection transportation operation was computed using Equation (5) (Board on Science and Technology for International Development, 1981):

$$IE = \frac{CEH \times A}{TP} \times 10^6 \quad (5)$$

where *IE* is input energy (J palm⁻¹), *CEH* is the coefficient of energy equivalent of harvesting tractor (1,323.15625 MJ ha⁻¹), *A* is area (ha) and *TP* is total palms, dimensionless.

The coefficient of energy is equivalent for different operational inputs for the oil palm nursery and field cultivation operations was used in the calculation (Canakci & Akinci, 2006; Chaichana *et al.*, 2008; De *et al.*, 2001; Fluck, 1992; Ozkan *et al.*, 2004; Pimentel, 1992; Strapatsa *et al.*, 2006; Singh, 2002).

MI is expressed as the ratio of machine energy to the total human and machine energy utilised in conducting the field operations. Machine energy includes machinery and tractor energy, fuel energy consumption and energy consumption by water for irrigation. The MI value could be calculated using Equation (6) (Isaak *et al.*, 2020):

$$MI = \frac{MCE}{MCE + HE} \quad (6)$$

where *MI* is mechanisation index, dimensionless, *MCE* is machine energy (MJ ha⁻¹) and *HE* is human energy expenditure (MJ ha⁻¹).

Duncan's Multiple Range Test (DMRT) was used to compare significant differences in the subjects' mean energy expenditure, mean quantity of work output, mean machine energy, mean human energy, and mechanisation index (MI) in the palm nursery and field operations.

RESULTS AND DISCUSSION

Field Capacity and Energy Expenditure in the Oil Palm Nursery Operation

Table 3 presents the measured field capacities and the human energy expenditures of individual operations in oil palm nursery operations. Any operation with the highest field operational capacity could be considered an expeditious operation where the involved work is normally referred to be very quick, efficient and productive to accomplish. During the field pre-nursery stage under the open field nursery system, the pest and disease control of oil palm seedlings in trays was the most expeditious level of operation, with the highest field operational capacity of 120,000 seedlings hr⁻¹. Similarly, during the indoor pre-nursery stage under the closed field nursery system, the pest

and disease control of oil palm seedlings in trays was the most expeditious level of operation, with the highest field capacity of 6,000 seedlings hr⁻¹. During the field main-nursery stage for both the open and closed field nursery systems, the pest and disease control of oil palm seedlings in large polybags was the most expeditious level of operation, with the highest field capacity of 6,667 seedlings hr⁻¹, while filling soil in large polybags was the least expeditious level of operation with the lowest field capacity of 108 seedlings hr⁻¹.

Any field operation with the highest energy could be considered as an exhausting operation where the work involved are commonly referred to as backbreaking, taxing and tough to accomplish. During the field pre-nursery stage under the open field nursery system, planting oil palm seeds in trays was the most exhausting level of operation with the highest energy expenditure of 976.28 J seed⁻¹. On the other hand, the pest and disease control of oil palm seedling in the tray was the least exhausting operation with the lowest field energy expenditure of 10.60 J seedling⁻¹. During the indoor pre-nursery stage under the closed field nursery system, filling peat moss in trays was the most exhausting level of operation with the highest energy expenditure of 935.96 J seedling⁻¹, while the pest and disease control of oil palm seedling in tray was stated as the least exhausting operation with the lowest energy expenditure of 18.42 J seedling⁻¹.

TABLE 3. FIELD CAPACITY AND HUMAN ENERGY EXPENDITURE OF OIL PALM NURSERY OPERATION

Oil palm nursery operation	Field capacity*		Human energy expenditure*
	95% confidence intervals seed ⁻¹	Seeds hr ⁻¹ or seedlings hr ⁻¹	95% confidence interval, J palm ⁻¹
Field pre-nursery stage			
Filling peat moss in trays	1.70 ± 0.12	2,118 ^d	598.49 ± 155.12 ^e
Planting oil palm germinated seeds in trays	3.87 ± 0.27	930 ^b	976.28 ± 102.04 ^c
Fertilising oil palm seedlings in trays	3.00 ± 0.21	1,200 ^c	592.16 ± 69.98 ^e
Pest and disease control of oil palm seedlings in trays	0.03 ± 0.002	120,000 ⁱ	10.60 ± 2.40 ^j
Watering oil palm seedlings in trays	0.07 ± 0.003	51,429 ^h	14.92 ± 0.15 ⁱ
Indoor pre-nursery stage			
Filling peat moss in trays	2.66 ± 0.26	1,353 ^c	935.96 ± 18.70 ^c
Planting oil palm germinated seeds in trays	3.75 ± 0.15	960 ^b	735.46 ± 136.98 ^d
Fertilising oil palm seedlings in trays	0.37 ± 0.009	9,730 ^f	85.25 ± 3.83 ^g
Pest and disease control of oil palm seedlings in trays	0.06 ± 0.005	60,000 ^h	18.42 ± 1.64 ⁱ
Watering oil palm seedlings in trays	0.11 ± 0.0003	32,727 ^d	25.98 ± 0.71 ^h
Field main-nursery stage			
Filling soil in large polybags	33.30 ± 1.53	108 ^a	11,439.72 ± 2,674.05 ^a
Transplanting oil palm seedlings from the trays to large polybags	23.58 ± 0.89	153 ^a	7,555.30 ± 1,011.15 ^b
Mulching fibres around the oil palm seedlings in large polybags	20.22 ± 1.48	178 ^a	6,060.43 ± 717.14 ^c
Fertilising oil palm seedlings in large polybags	2.91 ± 0.003	1,237 ^c	638.70 ± 25.71 ^d
Clearing grass in large polybags	1.03 ± 0.096	3,495 ^d	243.58 ± 24.76 ^f
Watering oil palm seedlings in large polybags	0.79 ± 0.008	4,557 ^e	221.36 ± 9.65 ^f
Weeding oil palm seedlings in large polybags	1.96 ± 0.28	1,837 ^c	555.65 ± 97.12 ^e
Pest and disease control of oil palm seedlings in large polybags	0.54 ± 0.036	6,667 ^e	165.50 ± 12.17 ^f

Note: *Superscripts with different letters are considered significantly different at 0.001 level.

During the field main-nursery stage under both the open and closed field nursery systems, filling soil in large polybags was the most exhausting level of operation with the highest energy expenditure of 11,439.72 J seedling⁻¹, while the pest and disease control of oil palm seedlings in large polybags was the least exhausting level of operation, with the lowest energy expenditure of 165.50 Joule seedling⁻¹. Meanwhile, the human energy expenditure of the workers in conducting the field operation was extremely affected by the nature of work of individual operation, whereby a low field capacity operation was normally and generally associated with high energy expenditure.

Figure 1 is the column chart graphical representation of the critical level of the individual operation involved in the open field nursery and closed field nursery systems. The critical level values were computed based on the ratio of the ranking levels of the field operational capacity and energy expenditure obtained for each field operation. The field operation having a critical level of 1.0 was categorised as the most critical field operation to be mechanised, while the field operation showing a critical level of 0.00 was categorised as the least critical field operation to be mechanised. As

observed, the three most critical operations in both the open and closed field nursery systems, based on the highest hierarchy order, were filling soil in large polybags operation, transplanting oil palm seedlings from tray to large polybags operation, and mulching fibres around oil palm seedlings in large polybags. However, the three least critical operations based on the lowest hierarchy order in both the open and closed nursery systems were watering oil palm seedlings in trays, fertilising oil palm seedlings in trays, and planting oil palm germinated seeds in trays.

Field Capacity and Energy Expenditure in the Oil Palm Field Cultivation Operation

At the immature crop stage, rat control operation in the plantation field was categorised as the most expeditious operation, with the highest field capacity of 472 palms hr⁻¹ (Table 4). The rat control operation requires workers to walk carrying a pail containing the rat baits along the harvesting path in the plantation field while distributing the rat baits at the targeted spots. Such a task was easy to conduct in the field even though it required frequent refilling of the rat baits in the pail at predetermined filling

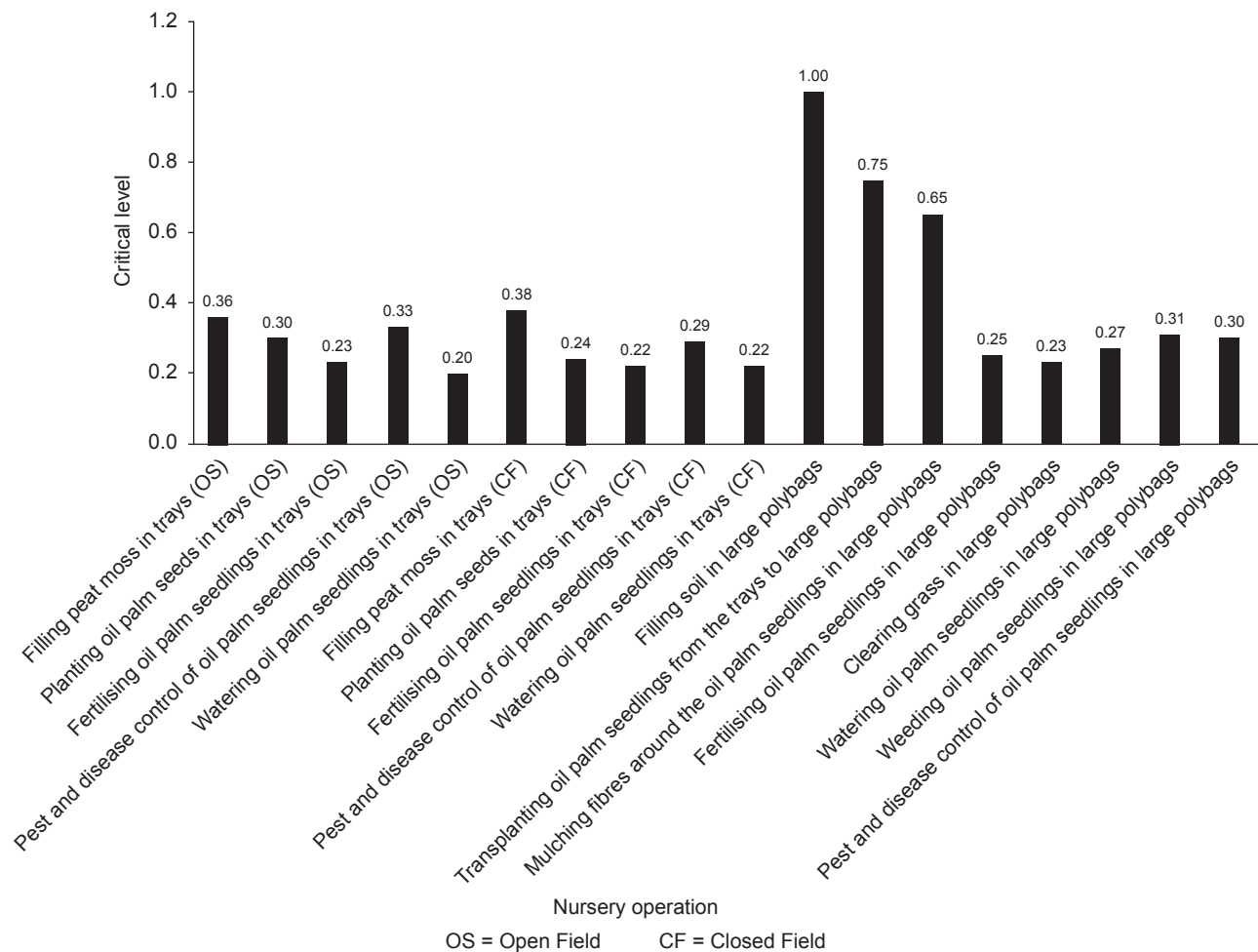


Figure 1. Critical levels of oil palm nursery operations.

points in the plantation field. On the other hand, planting oil palm seedlings in the plantation was the least expeditious operation as the workers had to manually complete the task of removing plastic polybags from the seedling soil body mass, lifting to place the seedlings into available prepared holes on the ground, filling space around the planted seedlings with available soil mass around the planting holes, and finally compacting the filled soil around the planted seedlings. On top of these, the workers had to walk carrying a hoe from one planting hole to the next adjacent planting hole along the planting row in the plantation field to complete the operation for the day. The next least expeditious level operation was the clearing of old palm trees operation, with the rated field operational capacity of 18 palms hr⁻¹. Even though the task was machine-assisted, it was time-consuming and demanding due to the cutting and chipping of old palm trees.

At the mature crop stage, rat control operation in the plantation field was once again the most expeditious level of operations. However, the loose fruit infield collection operation was categorised as the least expeditious level of operations with the lowest field capacity of 8 palms hr⁻¹. The loose fruit infield collection operation requires the workers to carry a sack and a rake while walking along the harvesting path and stopping at every location where a significant amount of loose fruits are found on the ground. The task was to manually rake and collect all the scattered loose fruits on the

ground and load them into the sack. The ultimate task was to gather the most amount of loose fruits and minimise the number of uncollected loose fruits left on the ground. The next least expeditious operation that was close to the loose fruit in the field collection operation was the FFB harvesting. The FFB harvesting including pruning had a field capacity of 16 palms hr⁻¹. The operation requires the workers to walk along the harvesting path while carrying a harvesting pole. Among others, the task was to identify any ripe FFB on the palm, cut the frond underneath it and the identified FFB, and relocate the cut fronds to the nearby frond stack.

At the immature crop stage, planting oil palm seedlings in the plantation field was categorised as the most exhausting level of operations with the highest human energy expenditure of 80,285.32 J hr⁻¹. The next exhausting level operation was delivering the seedlings from the nursery to the field with the energy expenditure of 72,693.54 J hr⁻¹. To complete both operations requires a number of tasks to be done manually with a substantial effort by the workers. The tasks of placing the seedlings in the planting hole, covering them with the surrounding soil and then compacting the filled soil around the planted seedlings in the planting oil palm seedlings operation were done manually with the use of hands. In the seedling delivery operation, much of the manual effort involved lifting individual

TABLE 4. FIELD CAPACITY AND ENERGY EXPENDITURE OF THE OIL PALM FIELD CULTIVATION OPERATION

Oil palm field operation	Field capacity*		Human energy expenditure*
	95% confidence intervals palm ⁻¹	Palms hr ⁻¹	95% confidence interval (J palm ⁻¹)
Immature crop stage			
Clearing old palm trees – cutting and chipping	194.61 ± 11.78	18 ^a	39,457.56 ± 299.90 ^c
Clearing weeds	57.49 ± 0.32	63 ^d	12,131.49 ± 437.10 ^f
Lining for planting	53.28 ± 2.20	68 ^d	16,276.13 ± 141.52 ^e
Holing for planting	79.26 ± 0.39	45 ^c	24,308.07 ± 169.68 ^d
Planting ground cover crops	55.65 ± 0.64	65 ^d	17,491.88 ± 335.44 ^e
Seedling delivery from nursery to field	129.97 ± 4.65	28 ^b	72,693.54 ± 1,971.68 ^b
Planting oil palm seedlings	265.66 ± 1.18	14 ^a	80,285.32 ± 2,773.37 ^a
Circle weeding by using CKS	44.58 ± 0.03	81 ^e	11,865.82 ± 683.17 ^f
Fertilising	38.62 ± 3.54	93 ^f	10,451.81 ± 1,204.80 ^g
Rat control	7.63 ± 0.85	472 ^g	1,926.09 ± 212.62 ^h
Mature crop stage			
Circle weeding by using CDA	39.49 ± 0.98	91 ^c	10,632.16 ± 468.69 ^d
Fertilising	33.63 ± 1.68	107 ^d	8,416.04 ± 890.69 ^e
Rat control	7.63 ± 0.85	472 ^g	1,926.09 ± 212.62 ^g
Loose fruit infield collection	464.02 ± 59.92	8 ^a	107,656.25 ± 16,502.23 ^a
FFB harvesting including pruning	221.53 ± 19.16	16 ^b	70,865.81 ± 20,025.98 ^b
FFB infield collection transportation	22.86 ± 0.79	157 ^e	16,859.50 ± 1,350.76 ^c
FFB mainline transportation	16.58 ± 1.33	217 ^f	7,233.77 ± 1,149.83 ^f

Note: *Superscripts with different letters are considered significantly different at 0.001 level.

seedlings onto the trailer of the tractor at the nursery site, unloading at the field site, and carrying them to the nearest available planting holes in the field. On the other hand, the rat control operation was the least exhausting level of operation with the energy expenditure of $1,926.09 \text{ J hr}^{-1}$, followed by the fertilising operation with the energy expenditure of $10,451.81 \text{ J hr}^{-1}$. The fertiliser operation requires the workers to walk along the harvesting path in the field carrying a pail containing granular type of fertilisers. In this task, the workers would grab a cup-full of fertilisers from the pail and toss them into a smaller cup to the targeted cut palm front heap row next to the palm row. Just like the rat control operation, this task was fairly easy and consumed less amount of time in the field even though frequent refilling of the granular fertilisers was required at the earlier predetermined filling point in the field.

At the mature crop stage, loose fruit infield collection operation had the most exhausting level of operation with the highest energy expenditure of $107,656.25 \text{ J hr}^{-1}$. The rat control operation was once again the least exhausting level of operation with energy expenditure of $1,926.09 \text{ J hr}^{-1}$, followed by the FFB mainline transportation with the energy expenditure of $7,233.77 \text{ J hr}^{-1}$. Not much of the manual handling of the FFB was done in the FFB mainline transportation because 8 t bin main transporter lorry was equipped with a mechanical hydraulically-assisted hook-lift system for pulling the fully loaded bin of the harvested FFB onto the chassis.

Figure 2 shows the column chart graphical representation of critical levels of the individual operations involved in the oil palm field cultivation operations. The top three most critical level of oil palm field cultivation operations in the highest hierarchy order were loose fruit infield collection operation (0.77), planting oil palm seedlings and seedling delivery from nursery to field operations (0.58 and 0.57), and oil palm FFB harvesting and frond pruning operation (level of 0.53). However, the three least critical oil palm field cultivation operations in the lowest hierarchy order were rat control operation (0.13), clearing weeds (0.17) and fertilising operation (0.18).

MI of the Oil Palm Nursery and Field Cultivation

Table 5 and 6 present the computed MI for the open field system and closed field system in the oil palm nursery operations. The overall MI for the oil palm nursery operations was 0.31 for the open nursery system and 0.30 for the closed nursery system.

With the open nursery system, the average MI of the open field nursery operation and closed field nursery was very close to each other (*i.e.*, 0.31 *versus* 0.36). The main critical operations were filling peat moss in trays operation and planting oil palm seedlings in tray operation at the field pre-nursery stage (0.00) and filling soil in large polybags operation at the field main nursery stage (0.06). Meanwhile, watering oil palm seedlings in tray operation at field pre-nursery stage and watering oil palm seedlings

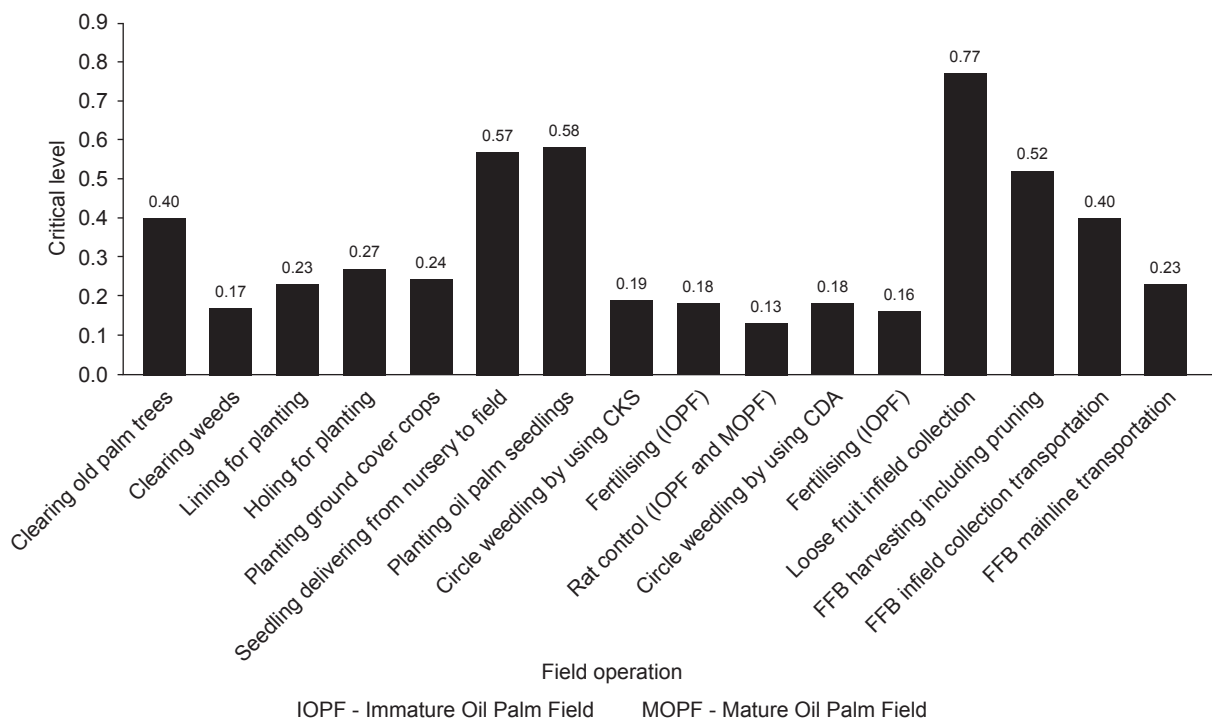


Figure 2. Critical levels of oil palm field cultivation operations.

in large polybags operation at the field main nursery showed the highest MI of 0.76 and 1.00, respectively.

With the closed nursery system, the MI of indoor pre-nursery was slightly higher than the field main nursery stages (*i.e.*, 0.44 *versus* 0.30). Similarly, for the open nursery system, the main critical operations were filling peat moss in trays operation and planting oil palm germinated seeds in tray operation at the field pre-nursery stage (0.00) and filling soil in large polybags operation at the field main nursery stage (0.06).

Once again, watering oil palm seedlings in tray operation at the field pre-nursery stage and watering oil palm seedlings in large polybags operation at the field main nursery showed the highest mechanisation levels with MI of 0.75 and 1.00, respectively. There were still a number of operations in both the open and closed nursery systems with the MI values of below 0.50, indicating significant human labour involvement instead of proper machines in performing the operations.

Table 7 presents the average MI value for the immature stage of oil palm cultivation, which was slightly higher than the average MI for the mature stage of oil palm cultivation (*i.e.*, 0.44 *versus* 0.37). The overall average MI for the field cultivation operation was slightly higher than the

overall average MI for both the open and closed field nursery operations (*i.e.*, 0.41 *versus* 0.31 and 0.36). This reflects a slightly higher level of machinery used at the field level compared to the nursery level in the current oil palm cultivation in Malaysia.

At the immature crop stage, the most critical operations were planting oil palm seedlings and planting ground cover crops operations (0.00) and rat control and fertilising operations (0.08). The four field operations that include clearing old palm trees – Cutting and chipping, clearing weeds and holing for planting could be classified to be fully mechanised.

At the matured crop stage, the most critical field operations to be mechanised was FFB harvesting that included pruning operation with the MI of 0.00. This was followed by two critical field operations rat control and loose fruit infield collection operations with MI of 0.08 and 0.09, respectively. Generally, the mechanisation levels with the most involved field operations were relatively very low (0.00 to 0.28), with the exception of FFB infield collection-transportation operation and FFB mainline transportation operation (1.00 and 0.97). Such low values generally indicated that the techniques currently employed in the operations required more human labour involvement in conducting and completing the field operations.

TABLE 5. MECHANISATION INDEX FOR THE OIL PALM OPEN FIELD NURSERY SYSTEM

Oil palm nursery operation	Labour energy (J palm ⁻¹)	Machinery energy (J palm ⁻¹)	Mechanisation index	Level
Field pre-nursery stage				
Filling peat moss in trays	598.49	-	0.00	4 ^d
Planting oil palm germinated seeds in trays	976.28	-	0.00	4 ^d
Fertilising oil palm seedlings in trays	592.16	798.24	0.57	2 ^b
Pest and disease control of oil palm seedlings in trays	10.60	3.10	0.23	7 ^c
Watering oil palm seedlings in trays	14.92	48.06	0.76	1 ^a
Average			0.31	
Field main-nursery stage				
Filling soil in large polybags	11,439.72	793.48	0.06	5 ^f
Transplanting oil palm seedlings from the trays to large polybags	7,555.30	13,551.20	0.64	2 ^b
Mulching fibres around the oil palm seedlings in large polybags	6,060.43	477.32	0.07	5 ^f
Fertilising oil palm seedlings in large polybags	638.70	68.74	0.10	4 ^d
Clearing grass in large polybags	243.58	5.87	0.02	6 ^e
Watering oil palm seedlings in large polybags	221.36	155,647.41	1.00	1 ^a
Weeding oil palm seedlings in large polybags	555.65	206.47	0.27	3 ^c
Pest and disease control of oil palm seedlings in large polybags	165.50	57.29	0.26	3 ^c
Average			0.30	
Overall			0.31	

Note: ¹Decreasing order and superscripts with different letters are considered significantly different at 0.001 level.

TABLE 6. MECHANISATION INDEX FOR OIL PALM CLOSED FIELD NURSERY SYSTEM

Oil palm nursery operation	Labour energy (J palm ⁻¹)	Machinery energy (J palm ⁻¹)	Mechanisation index	Level
Indoor pre-nursery stage				
Filling peat moss in trays	935.96	-	0.00	3 ^c
Planting oil palm germinated seeds in trays	735.46	-	0.00	3 ^c
Fertilising oil palm seedlings in trays	85.25	254.32	0.75	1 ^a
Pest and disease control of oil palm seedlings in trays	18.42	41.60	0.69	2 ^b
Watering oil palm seedlings in trays	25.98	76.92	0.75	1 ^a
Average			0.44	
Field main-nursery stage				
Filling soil in large polybags	11,439.72	793.48	0.06	7 ^g
Transplanting oil palm seedlings from the trays to large polybags	7,555.30	13,551.20	0.64	2 ^b
Mulching fibres around the oil palm seedlings in large polybags	6,060.43	477.32	0.07	5 ^f
Fertilising oil palm seedlings in large polybags	638.70	68.74	0.10	4 ^e
Clearing grass in large polybags	243.58	5.87	0.02	6 ^g
Watering oil palm seedlings in large polybags	221.36	155,647.41	1.00	1 ^a
Weeding oil palm seedlings in large polybags	555.65	206.47	0.27	3 ^c
Pest and disease control of oil palm seedlings in large polybags	165.50	57.29	0.26	3 ^c
Average			0.30	
Overall			0.36	

Note: ¹Decreasing order and superscripts with different letters are considered significantly different at 0.001 level.

TABLE 7. MECHANISATION INDEX FOR OIL PALM FIELD CULTIVATION OPERATION

Oil palm field operation	Labour energy (J palm ⁻¹)	Machinery energy (J palm ⁻¹)	Mechanisation index	Level ¹
Immature crop stage				
Clearing old palm trees – cutting and chipping	39,457.56	15,239,407,027.30	1.00	1 ^a
Clearing weeds	12,131.49	1,389,886.71	0.99	1 ^a
Lining for planting	16,276.13	-	0.00	
Holing for planting	24,308.07	365,332,464.42	1.00	1 ^a
Planting ground cover crops	17,491.88	6.18	0.00	5 ^e
Seedling delivery from nursery to field	72,693.54	1,074,586.37	0.94	2 ^b
Planting oil palm seedlings	80,285.32	29.54	0.00	5 ^e
Circle weeding by using CKS	11,865.82	4,447.08	0.27	3 ^c
Fertilising	10,451.81	911.88	0.08	4 ^d
Rat control	1,926.09	175.77	0.08	4 ^d
Average			0.44	
Mature crop stage				
Circle weeding by using CDA	10,632.16	4,167.71	0.28	3 ^c
Fertilising	8,416.04	1,588.24	0.15	4 ^d
Rat control	1,926.09	175.77	0.08	5 ^f
Loose fruit infield collection	107,656.25	10,931.67	0.09	5 ^f
FFB harvesting including pruning	70,865.81	16.10	0.00	6 ^g
FFB infield collection transportation	16,859.50	9,918,308.35	1.00	1 ^a
FFB mainline transportation	7,233.77	225,229.48	0.97	2 ^b
Average			0.37	
Overall average			0.41	

Note: ¹Decreasing order and superscripts with different letters are considered significantly different at 0.001 level.

TABLE 8. AVERAGE MECHANISATION INDEXES AND FIELD CAPACITIES FOR 10 COMMON CLUSTER OPERATIONS IN OIL PALM CULTIVATION

Main operation	Average field capacity	Mechanisation index	Mechanisation status ranking
Seedling preparation	39.07 seedlings hr ⁻¹	0.34	4
Land preparation	0.10 ha hr ⁻¹	1.00	1
Seedling planting	0.68 ha hr ⁻¹	0.39	3
Circle spraying	0.55 ha hr ⁻¹	0.28	5
Fertilising	0.72 ha hr ⁻¹	0.12	6
Rat control	2.05 ha hr ⁻¹	0.08	8
Loose fruit infield collection	0.14 ha hr ⁻¹	0.09	7
FFB harvesting including pruning	0.87 ha hr ⁻¹	0.00	9
FFB infield collection-transportation	1.49 ha hr ⁻¹	1.00	1
FFB main line transportation	1.50 ha hr ⁻¹	0.97	2
Average		0.43	

Table 8 summarises the average MI and field capacities for the 10 common clusters of operations in oil palm cultivation. A common average MI of 0.43 was obtained in the cultivation of oil palm, which was slightly higher than the average MI of 0.31 for coconut and much lower than the average MI of 0.65 for all oilseed crops (Elsoragaby *et al.*, 2019). The three most critical operations for mechanisation in the oil palm field cultivation operation were the FFB harvesting including pruning operation (0.00), rat control operation (0.06), and loose fruit infield collection operation (0.09). This important information could be used by the government in setting up the R&D direction priorities in developing mechanisation and automation technologies for the oil palm cultivation plantation industries in the country. Additionally, it can aid the top management in the oil palm plantation industries to plan proper mechanisation programme for the oil palm plantations. The basis of their planning was to place the highest priority in the order of operations, *i.e.*, from the lowest rank in mechanisation status table. Secondly, for any new mechanisation and automation technology to be developed or used, the technology should give an average field capacity that is much higher than the given average field capacity for the said field operation.

CONCLUSION

This study has successfully demonstrated the use of MI in quantifying the overall mechanisation level of the oil palm FFB production, the mechanisation level of individual nursery, and the field operations in the oil palm FFB production. It was found that the MI values of the operations ranging from 0.00 to 1.00, whereby the operations with MI values

close to zero require prompt mechanisation. An average MI of 0.43 was obtained for the 10 common clusters of operations in oil palm cultivation. The information obtained is useful in the preparation of a viable mechanisation programme under the specified crop production scenario. Besides that, the column chart has been shown to be useful in identifying the most critical operations or tasks within any operation that need to be improved to fulfil the overall crop production target. Future works are recommended to quantify the overall mechanisation level of the oil palm FFB production in the plantations in East Malaysia.

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AN INVESTIGATION OF 30% PALM BIODIESEL BLEND FUEL (B30) UNDER INDONESIAN OPERATING CONDITIONS

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ABSTRACT

A study was conducted under Indonesian operating conditions to verify that utilising 30% palm methyl ester (B30) did not negatively impact vehicle performance, ensuring the smooth nationwide implementation of B30 in Indonesia. A comprehensive study involved a 50,000 km road test and periodic laboratory assessments to evaluate the long-term effect on vehicle performances, including engine power, fuel economy, and exhaust emissions. These investigations were conducted in compliance with UNECE R85, R101 and R83 regulations. As a comparison, 20% palm biodiesel blend fuel (B20) were also evaluated alongside B30. The results showed that both vehicles fuelled with B20 and B30 had no problem until a distance travelled of 50,000 km. The power B30 varied in average by 4.8 kW when compared to B20, while fuel economy was 0.2 km/L. B30 exhibited lower emissions of CO, HC, and PM in comparison to B20, with average reduction of 0.10, 0.01 and 0.02 g/km, respectively. However, there was an average increase of 0.11 g/km in NO_x emissions when compared to B20. Furthermore, this study revealed that the fuel filter replacement interval for B30 and B20 remained within the range recommended by the manufacturer, with no issues observed regarding cold start-ability.

Keywords: engine performance, filter blocking, monoglycerides, palm biodiesel blend fuel, road test.

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INTRODUCTION

Indonesia requires to evaluate an alternative fuel due to its high reliance on fossil fuels. Biofuel

from palm oil is a promising alternative fuel due to the abundant palm oil supply in Indonesia. Gheidan *et al.* (2022) reported that biofuel is one of the potential fuels to be used as a substitute for fossil fuels. Furthermore, biofuel is also known as carbon neutral fuel, despite the fact that it has the potential to emit carbon dioxide (CO₂) during the production process (Kuncoro & Purwanto, 2020). Reksowardojo *et al.* (2020) reported a study on the implementation of 20% palm biodiesel blend with diesel fuel (B20) in light-duty vehicles. Their results revealed that vehicles running on B20 exhibited comparable durability to those using pure diesel fuel (B0). Moreover, as the mileage increased, these B20-fuelled vehicles demonstrated enhanced fuel economy, despite a slight 3% reduction in maximum power. Implementing biodiesel at a higher ratio, such as palm methyl ester with a blend ratio of 30% (B30) shows promise as a method to further decrease CO₂ and reduce dependence on fossil diesel fuel.

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Various research has been conducted for the implementation of B30 to prevent problems in real-world applications. Paryanto *et al.* (2019a; 2019b) have reported that to increase the palm biodiesel ratio, the monoglyceride (MG) value must be a maximum of 0.6% to prevent a decrease in fuel filter mileage. Cardeno *et al.* (2020) made a similar report, recommending limiting MG content to a maximum value of 0.4% for applications in advanced engine technologies. Other studies have also been carried out to support the implementation of B30 which is related to the compatibility of materials in the fuel systems, influence in exhaust after treatment, degradation of lubricant, and the formation of deposits due to metal contaminant parameters in biodiesel (Hartono & Cahyono, 2020; Hidayat & Sugiarto, 2020; Iswantoro *et al.*, 2021; Liaquat *et al.*, 2013; Maharani *et al.*, 2022; Petiteaux & Monsallier, 2009; Sadashiva *et al.*, 2022). Reksowardojo *et al.* (2023) studied the effect of B30 in the performance and emissions of the advanced vehicle on Indonesia market. Utilisation of palm B30 could result in a slight increase in fuel economy and a decrease in power, while exhaust emissions could comply with EURO4 regulation limit. Other studies have also been carried out by Wu *et al.* (2020) who have provided a comprehensive review regarding the use of biodiesel in engines by reviewing various parameters including performance, emissions, noise, vibration, and material compatibility. Another study conducted by several researchers on the application of biodiesel in various applications showed that the use of biodiesel in general could reduce emissions by optimising the engine parameters. Moreover, an optimisation of engine parameters could improve performance close to that of diesel fuel (Altarazi *et al.*, 2022; Doppalapudi *et al.*, 2023; Fernandes *et al.*, 2022; Ge *et al.*, 2020; Huang *et al.*, 2022; Pawar *et al.*, 2022; Singh *et al.*, 2021; Soudagar *et al.*, 2021).

Most studies reported that biodiesel quality and vehicle technology are two important factors that directly influence performance, emissions and engine components lifetimes. In principle, alternative fuel should be compatible with engine technology and *vice versa* that comply with a standard (Ahn *et al.*, 2016). Furthermore, it is essential to consider the optimisation of various engine performance parameters, emissions and prices when establishing the optimal biodiesel blending ratio (Yilmaz *et al.*, 2022). For these purposes, Indonesia government has made some improvements on biodiesel properties, including oxidation stability, MG content, water content and total acid number for shifting from B20 to B30. As described in the previous paragraph, while there have been numerous laboratory-scale studies on B30, there is a noticeable lack of research that explores the nationwide implementation of B30. The objective of this study is to address the gaps in B30 research by examining enhanced

biodiesel properties and assessing its performance for nationwide implementation under Indonesian operating conditions. Moreover, this study was conducted to ensure that the utilisation of B30 had no adverse effects on the performance parameters and engine components of diesel vehicles currently available in the Indonesia market. This article presents the results of a 50,000 km vehicle road test conducted in Indonesia, evaluating its effects on power, fuel economy, emission, maintenance and low-temperature operation. This similar method was also employed when the Indonesian government planned to implement B20 to replace B10 in 2016 (Reksowardojo *et al.*, 2020).

MATERIALS AND METHODS

Test Vehicle Specifications

In this article, the discussion will include samples of light-duty vehicles from two different brands. These two brands were based on the representation of the vehicle population and on the recommendation of the association of Indonesia automotive industries for evaluating B30 on common rail vehicle technology. Each pair consist of two vehicles fuelled by B20 and B30. In this case, B20 fuel was used as the reference fuel because it had been implemented in Indonesia since 2016 and was available as market fuel. The specifications of the test vehicle used for the study in this article are shown in *Table 1*.

Test Fuels

In this road test, diesel fuel (B0) has a maximum sulphur content of 2,000 ppm and a cetane number (CN) of 48. Palm methyl ester (B100) for blending fuel of B30 has an MG content of 0.55% and a maximum water content of 500 ppm, while B20 had an MG of 0.80% and a water content of 500 ppm. B20 was readily available in the market, produced by Indonesia State Owned Oil and Gas Corporation, PT. Pertamina. Meanwhile, B30 was a custom-blended fuel, with PT. Pertamina provided the diesel fuel (B0), while the pure palm methyl ester (B100) was supplied by the Indonesian Biofuel Producer Association (APROBI). The fuel properties of the B20 and B30 for the road test are shown in *Table 2*. B30 has the advantages of higher CN, lower sulphur, and particulate contaminants and better lubricity compared with B20. However, the pour point and cold filter plugging point (CFPP) of B30 was higher than B20. Therefore, it is more likely to form precipitate at low temperature operation.

The test procedure for road and laboratory tests are shown in *Figure 1*. During the road test, all vehicles were examined for its performance,

TABLE 1. TESTED VEHICLE SPECIFICATIONS

Specification	Vehicle 1 (V1)	Vehicle 2 (V2)
Model	Mitsubishi-Pajero	Nisan-Terra
Engine type	DOHC	DOHC, Turbocharger with VGS
No. of cylinder	4	4
Displacement	2,500 cc	2,488 cc
Max. power	181 PS/3,500 rpm	190 PS/3,600 rpm
Max. torque	430 Nm/2,500 rpm	450 Nm / 2,000 rpm
Transmission	A/T	A/T
Additional information	Turbocharged Intercooler Common rail	Turbocharged Common rail

TABLE 2. TESTED FUEL PROPERTIES

Parameter	Unit	B20	B30	Method
Cetane number	-	51.9	53.2	ASTM D613
Cetane index	-	48.3	50.2	ASTM D4737
Sulphur	%	0.143	0.136	ASTM D4292
Distillation T90°C	°C	347	335	ASTM D86
Flash point	°C	68	77	ASTM D93
Pour point	°C	9	12	ASTM D5949
Carbon residue	%	Nil	Nil	ASTM D4530
Copper strip corrosion	merit	1A	1A	ASTM D130
Ash content	%	0	0	ASTM D482
Sediment content	%	Nil	Nil	ASTM D473
Strong acid number (SAN)	mg KOH/g	0	0	ASTM D664
FAME content	%v	20.6	30.5	ASTM D7371
Lubricity (HFRR wear scar dia.)	micron	198	174	ASTM D6079
Appearance	-	bright and clear	bright and clear	-
ASTM color	No. ASTM	2.5	2.8	ASTM D1500
Oxidation stability (Rancimat)	Hours	>48	>48	EN 15751
CFPP	°C	12	16	ASTM D6371
Particulate contaminant	Code rating	4.21	3.32	ASTM D5425

fuel economy, emissions, and filter blocking test as shown in *Figure 1*.

Route of road test was determined so that it can represent the conditions of daily operation with road type and condition in main island of Indonesia. The general route for road test includes 10.6% flat terrain, 49.9% climbing and downhill roads and 39.5% highway. The total distance for a one-day route was an average of 570 km.

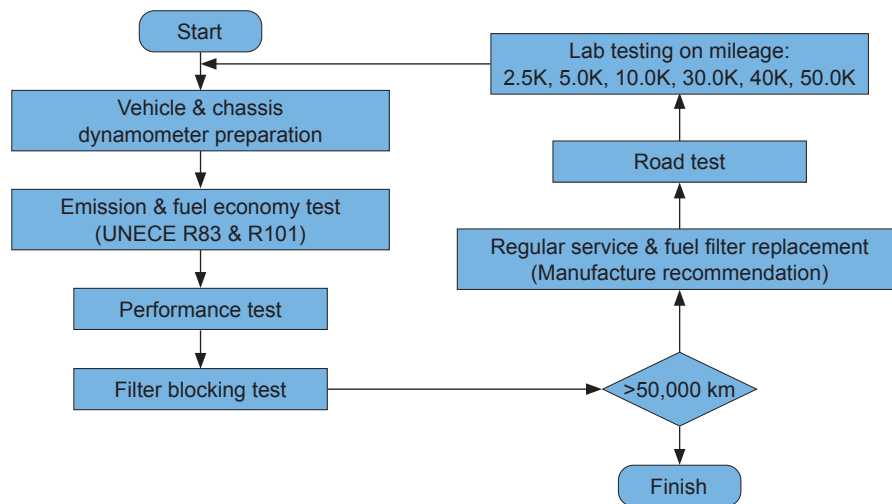
The power of the vehicle was tested on a chassis dynamometer with the adopted UN-ECE R85, which measures the maximum power of the vehicle on wheels. The power absorption and speed of the chassis dynamometer are 150 kW and 200 km/hr, respectively. Fuel economy was calculated using the carbon balance method from measured exhaust gases as shown in Equation 1. Hydrocarbon (HC), carbon monoxide (CO), CO₂ and nitrogen oxides (NOx) were measured

using an emission analyser with specific sensors. Hydrocarbon was detected with heated flame ionisation detector (HFID), while CO and CO₂ were assessed through non-dispersive infrared (NDIR) sensors. Additionally, NOx was analysed with chemiluminescence detector (CLD). A carbon balance calculation is conducted using a Equation (1) in R101 as follows:

$$FE = \frac{100 \times D}{[0.115 \times (0.866 \times \text{mHC}) + (0.429 \times \text{mCO}) + (0.273 \times \text{mCO}_2)]} \quad (1)$$

where, FE is the fuel consumption in km/L, D is the density of the fuel, and mHC, mCO, and mCO₂ are measured emission quantities of hydrocarbon, carbon monoxide, and carbon dioxide in g/km, respectively.

(a) Procedure of road test



(b) Scheme of laboratory test

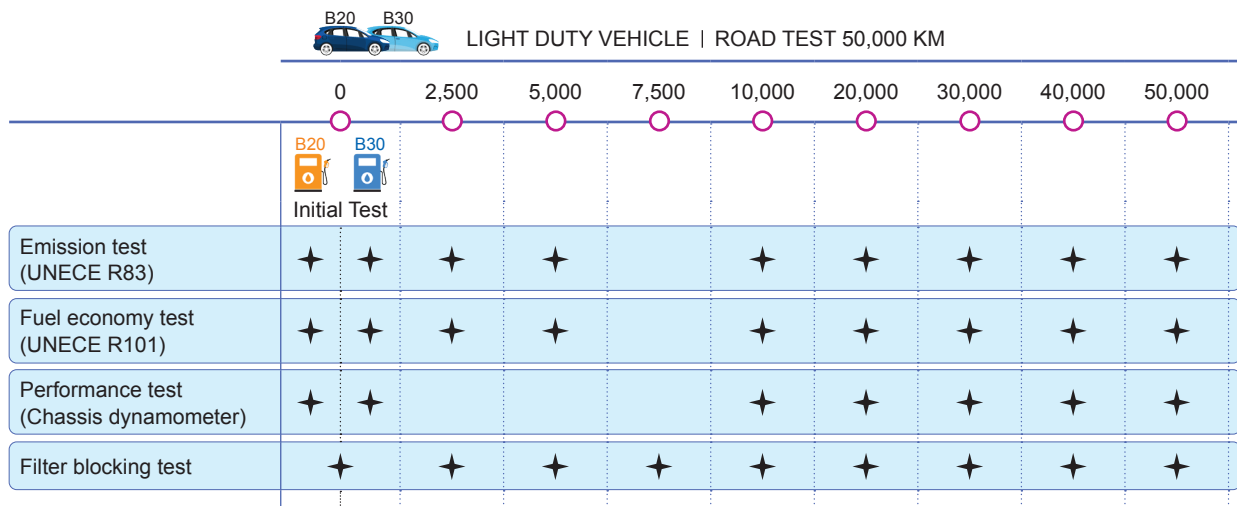


Figure 1. Procedure of road and laboratory test.

In this study, measurement of exhaust gas emission referred to the standard of United Nation Economic Commission for Europe (UNECE) R83-04 and was conducted at the laboratory of the National Research and Innovation Agency of Indonesia (BRIN). In addition, an evaluation of fuel filter mileage was also conducted to find out how much influence B30 has on the occurrence of blockages in the fuel filter in the vehicle. This test was done by checking the pressure difference that occurred in the fuel line before and after the fuel filter.

RESULTS AND DISCUSSION

An examination of uncertainty has been conducted to assess the precision of measurements. This analysis was based on the widely recognised reference standard, Joint Committee for Guide Metrology

(JCGM) 100:2008, which is commonly used for evaluating uncertainty in testing and calibration laboratories. The measurement of uncertainty was determined by considering a sum of influential parameters outlined in the testing standard. Among these parameters, the measurement data and equipment calibration play a substantial role in combined uncertainty, surpassing the impact of other factors. In quantifying the uncertainty, a confidence level of 95% is applied along with a coverage factor of two.

Cross-test Before Road Test

A cross-test was conducted for all vehicles prior to the road test (0 km). This aims to determine the effect of the use of fuel B20 and B30 on the initial condition of the vehicle. The vehicle had been set up in accordance with the recommendations of

the manufacturers for fuel properties in Indonesia, namely B20. The cross-test at 0 km was conducted by means of a vehicle that uses B20 fuel on the road to perform a performance test using B30 fuel and *vice versa*, B30-fueled vehicles during road tests were tested using B20 fuel.

The results of the cross-test of measured power for all test vehicles are shown in Figure 2. V is the type of vehicle and the following number indicates the brand or type of the test vehicle. B20 fuel is indicated by the number 20 and B30 by 30. For example, the V1-20 is a type/brand number one vehicle with B20 fuel. In this study, measurement uncertainties for power and fuel economy (FE) have been determined, with values of 3.3 kW and 0.24 km/L, respectively. These values are represented as error bars in the Figure 2.

Figure 2 shows that utilisation of B30 fuel increased the power of V1-20 and V2-20 compared to B20, while V1-30 and V2-30 showed the opposite trend. Theoretically, the use of biodiesel will reduce the calorific value, resulting in a decrease in power. In the UNECE R85 performance test regulation, it is stated that the maximum power tolerance produced at rated power is 2%. Therefore, it can be assumed that the utilisation of B20 and B30 does not lead to a significant difference in the maximum power of the vehicle, which can be considered due to their different vehicle settings. Figure 2 also showed that V1-20 and V1-30 had similar power characteristics at the initial condition of 0 km, while V2-30 had a slightly higher power maximum compared with V2-20.

Road Test Results 50,000 km

The effect of using B30 on vehicles during the 50,000 km road test was evaluated through various parameters such as power, fuel consumption, exhaust emissions, component degradation, cold start ability, fuel storage stability, and others. However, this article only discussed an effect of B30 on power, fuel consumption, emissions, fuel filter mileage, and cold start ability.

An Evaluation of Power During Road Tests

Power evaluation by using R85 test method was conducted at 10,000 km interval, with the exception in the initial range of 0 to 10,000 km, during which evaluations were also conducted at 2,500 and 5,000 km. All vehicles were tested after they had passed periodic maintenance services. Here, the service intervals for V1 and V2 were at every 10,000 km mileage. Figure 3 shows the results of power measurements for two vehicles using B20 fuel and two vehicles using B30 fuel at km 0 to 50,000 km. V1 and V2 vehicles show a trend of increasing power for each distance travelled for both B20 and B30 fuel. For the V1-20, the maximum power increase could reach a maximum of 4.0% at km to 30,000 km while the V1-30 could reach a maximum increase of 2.0% at 20,000 km and 40,000 km. For V2, the increase is higher than V1, where the V2-20 reaches a maximum increase of 5.1% at 30,000 km and V2-30 with a maximum increase of 9.7% at 40,000 km. Figure 3 shows that the use of B20 and B30 fuel does not have

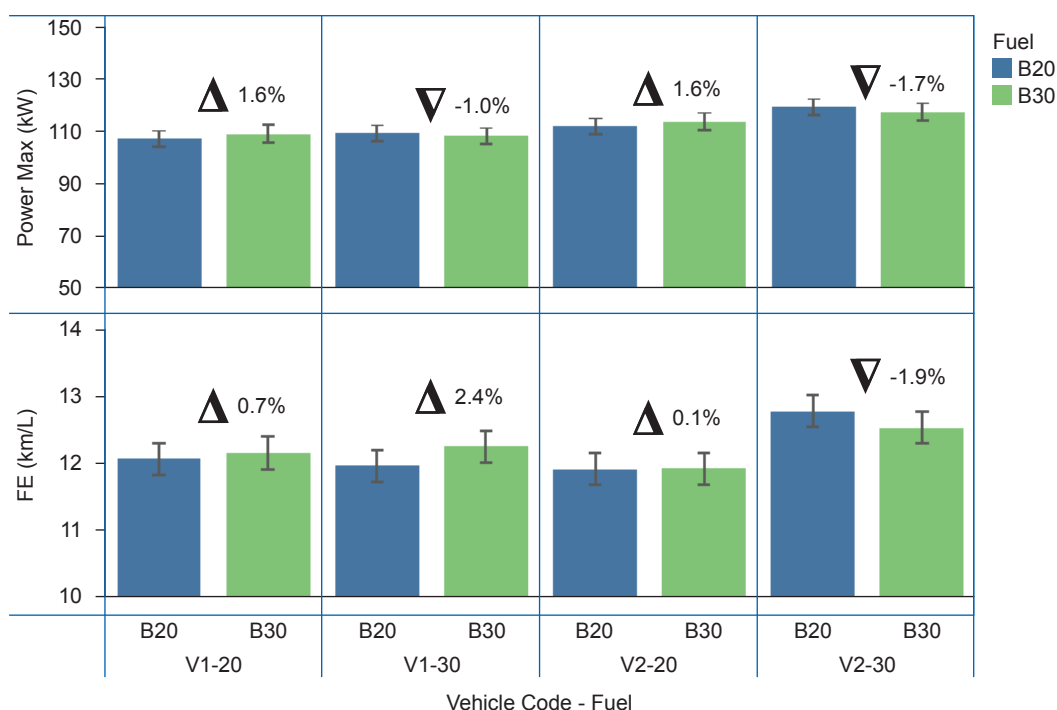


Figure 2. Cross test result before road test.

an impact on reducing power up to a mileage of 50,000 km with a maintenance pattern according to current standards for both V1 and V2 vehicles.

An evaluation for 50,000 km also revealed that V1 fueled with B20 has comparable averaged power with V1-30, while V2-30 has higher averaged power than V2-20. According to various studies, a higher biodiesel ratio will result in lower power output due to its lower heating value (Ogunkunle & Ahmed, 2020; Reksowardojo *et al.*, 2023). Reksowardojo *et al.* (2023) also reported that the reduction of lower heating value of biodiesel which resulted on lower power output could be suppressed by the higher thermal efficiency of biodiesel. While the percentage of reduction in power output differed according to the test method, operating conditions, and engine technology. However, the higher power result of V2-30 compared to V1-30 could be attributed to V2-30 having higher power output characteristics as elucidated in the subsection of cross test prior to the road test, rather than differences in fuel characteristics as shown in Table 2.

An Evaluation on Fuel Economy (FE) During Road Tests

Utilisation of high ratio biodiesel fuel would lead to a reduction in lower heating value and an increase in density and viscosity. Those characteristics could result on lower fuel economy of biodiesel (Reksowardojo *et al.*, 2023; Soudagar *et al.*, 2021; Wu *et al.*, 2020).

Figure 4 shows the results of the FE test for vehicles with B20 and B30 fuel by using UNECE R101 standard. The FE of V1 and V2 type vehicles

has resulted in an improvement trend of FE for all mileage compared to the initial conditions for both B20 and B30. Here, the initial durability of the FE for V2-30 has decreased at 5,000 km and 10,000 km distances. The results of the B30 test show the same trend as B20 for the V1 and V2 types. The V1-30 and V2-30 vehicle produced the optimum FE of 7.1% and 2.9%, respectively. The improvement in the FE value indicates that vehicle condition had more effect than calorific value of the fuel during the 50,000 km durability test. The test results also showed that V2 vehicles with B30 had slightly better FE values than B20. This characteristic was also reported by William *et al.*, (2021) who found that vehicle with B30 was not significantly different from those with B5. Further, a similar study conducted on a single cylinder diesel engine with common rail systems fueled with waste cooking oil methyl ester showed that deterioration of fuel economy could also be minimised with an optimisation on engine parameters, including pilot injection and nozzle opening pressure. The result was considered due to an improvement in the combustion process (Fernandes *et al.*, 2022). Therefore, the improved FE observed for V2-30 in comparison to V2-20 could be considered due to optimum engine setting for V2-30, which enhances the higher cetane number, thereby promoting more efficient combustion.

Emission Test Results

Exhaust emissions were evaluated using the UNECE R83-04 (EURO2 standard), which was implemented in Indonesia. Emission testing under UNECE R83-04 is subjected to several factors that

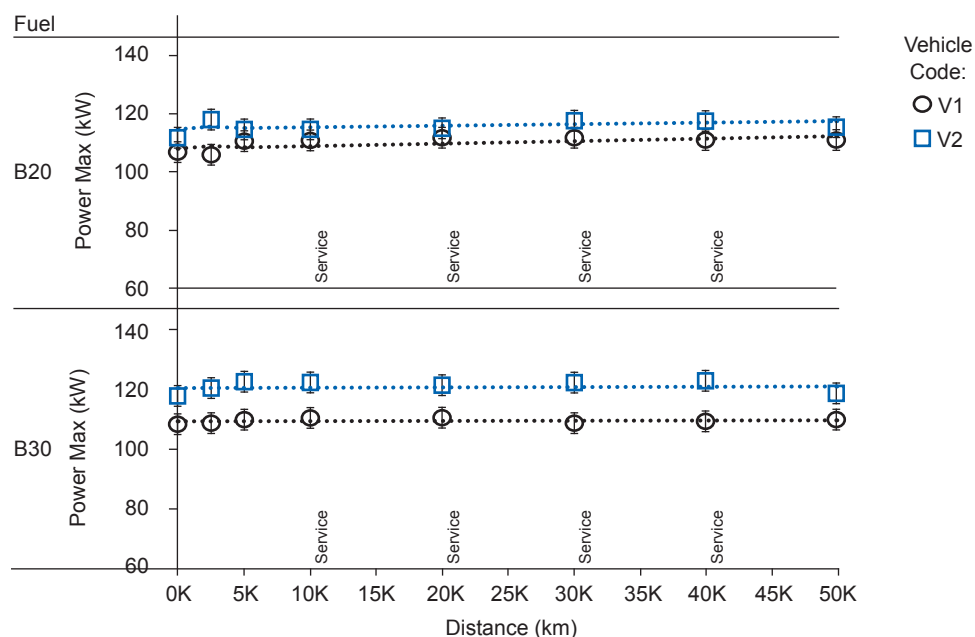


Figure 3. Power during road test.

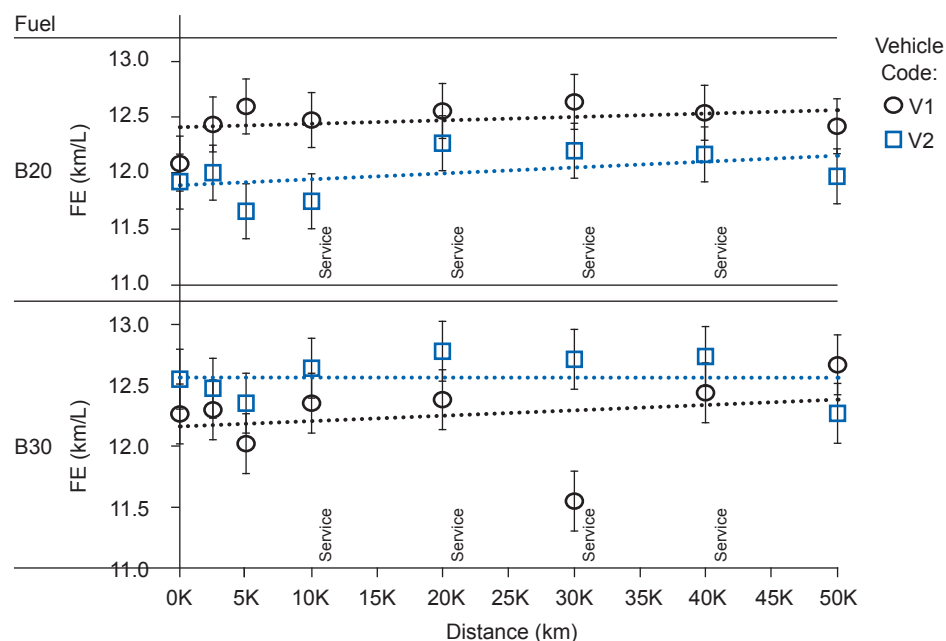


Figure 4. Fuel economy (FE) during road test 50,000 (50K) km.

play a significant role in uncertainty analysis. These factors encompass exhaust diluted volume, emission concentration, weighted particulate mass, distance and environmental conditions. In particular, the uncertainty measurements for CO, HC, NO_x, and PM are 0.063, 0.006, 0.039 and 0.007 g/km, respectively. Figure 5 shows the results of exhaust gas emissions of all vehicles during road test 50,000 km. The V1-20 vehicle shows a decreasing trend of CO, while the V2-20 shows an increasing trend that could reach twice the initial condition. The same trend is shown for B30, where V1-30 vehicles showed a decrease in CO while V2-30 produced a significant increase that can reach up to 50%. The use of biodiesel with a higher cetane number and oxygen content can theoretically reduce CO emissions (Ge *et al.*, 2020; Kodate *et al.*, 2022; Williams *et al.*, 2021). The trend of increasing CO emissions for V2 with both B20 and B30 for all road test mileages could be considered to correlate with the power results in Figure 3.

The V2 power results, indicating a power increase for both B20 and B30, which can lead to more amount of fuel supplied to the combustion chamber, thereby affecting an increase in CO emissions. Furthermore, the test results also show that V1 and V2 vehicles fueled with B30 exhibit lower CO levels compared to B20 across all mileage intervals during the 50,000 km road test. This finding aligns with the study conducted by Rajaeifar *et al.* (2021) and Soudagar *et al.* (2019) who also observed an increase in CO emission during low engine speed operations.

HC emissions for V1-20 show a decreasing trend with increasing road test mileage, while for V2-20, the results are reversed, where HC emissions increase with distance travelled. The B30 vehicle tends to produce similar HC emissions for the whole travelled distances, while for the V2-30 the emissions increase along with the distance travelled. HC emissions exhibit a similar pattern to CO emissions, with V1 and V2 vehicles consistently showing slightly lower HC values when using B30 in contrast to B20 across all mileage during the conducted road test. This decrease in HC emission with B30 can be attributed to the higher cetane number and oxygen content, as shown in Table 2, resulting in improved combustion.

Biodiesel with higher cetane number and increased oxygen content tends to raise NO_x emissions, and a higher ratio of biodiesel in the blend fuel further contributes to increased NO_x emission. This issue presents a challenge when it comes to utilising biodiesel blends since emission reduction requires to encompass not only CO and HC but also for NO_x (Yilmaz *et al.*, 2022). Figure 6 shows that NO_x emissions of V1-20 vehicles tend to increase along with distance travelled, with a maximum increase of 7.4% at a distance of 20,000 km. V2-20 produced different results, in which emissions decreased with mileage and could reach over 20%. B30-fueled vehicles have the same trend of NO_x emissions as B20 where the V1-30 tends to increase while the V2-30 has a decreasing NO_x emission along with the distance travelled. An increase in NO_x emissions

with biodiesel is usually a result of an increase in maximum combustion temperature due to the oxygen content of the biodiesel (Abu-Hamdeh & Alnefaie, 2015; Hadhoum *et al.*, 2021). NO_x emissions for vehicles with B20 and B30 fuel are comparable for V1 vehicle types, but for V2 vehicle types, NO_x emissions with B30 are higher than B20 for each mileage due to its higher cetane number as shown in Table 2. This comparable NO_x result for V1 can be attributed to engine settings including exhaust gas recirculation (EGR) rate and pilot injection (Altarazi *et al.*, 2022; Fernandes *et al.*, 2022).

Particulate matter (PM) measurement results for B20 fuelled vehicles increase significantly, potentially increasing by 60% over a 20,000 km travelled distance, as shown in Figure 6. Here, the V1-20 vehicle produced a maximum PM increase

of 60% at a mileage of 20,000 km, while the V2-20 produced a maximum PM emission of 44%. B30-fueled vehicles resulted in a different trend for V1 and V2. The V1-30 vehicle resulted in increased emissions for each road test distances, while the V2-30 vehicle resulted in a reduction in PM emissions of up to 30%. Most of studies concluded that utilisation of biodiesel could reduce PM emission by shortening the ignition delays and promoting the PM oxidation due to its oxygenate and high cetane number (Chiavola & Recco, 2018; Ge *et al.*, 2020). An increase in PM with biodiesel fuel was also reported by Hadhoum *et al.* (2021). A high PM value was considered due to the larger droplet size of fuel spray due to injector degradation as well as higher viscosity and high density of biodiesel (Altarazi *et al.*, 2022; Ge *et al.*, 2020).

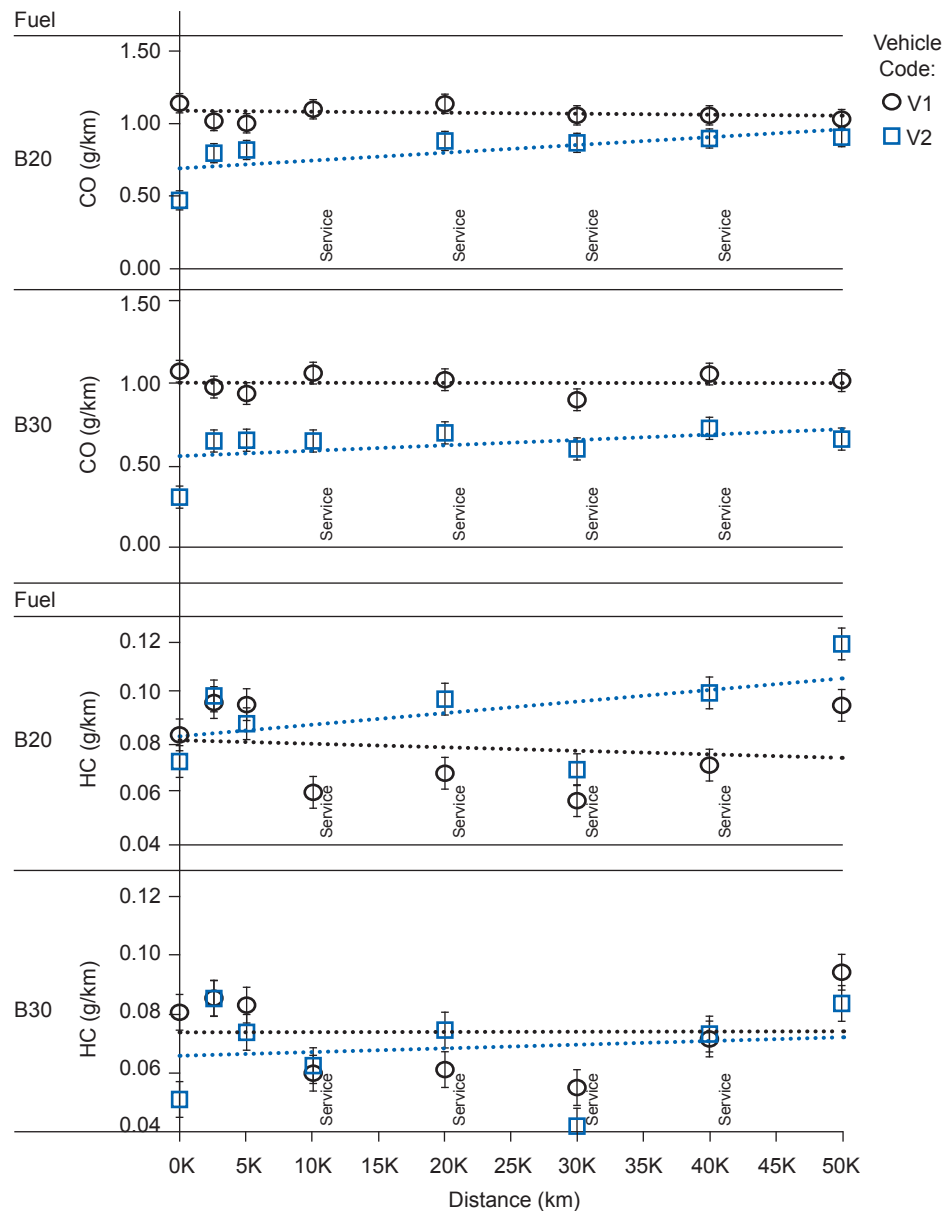


Figure 5. An emission of CO and HC during road test.

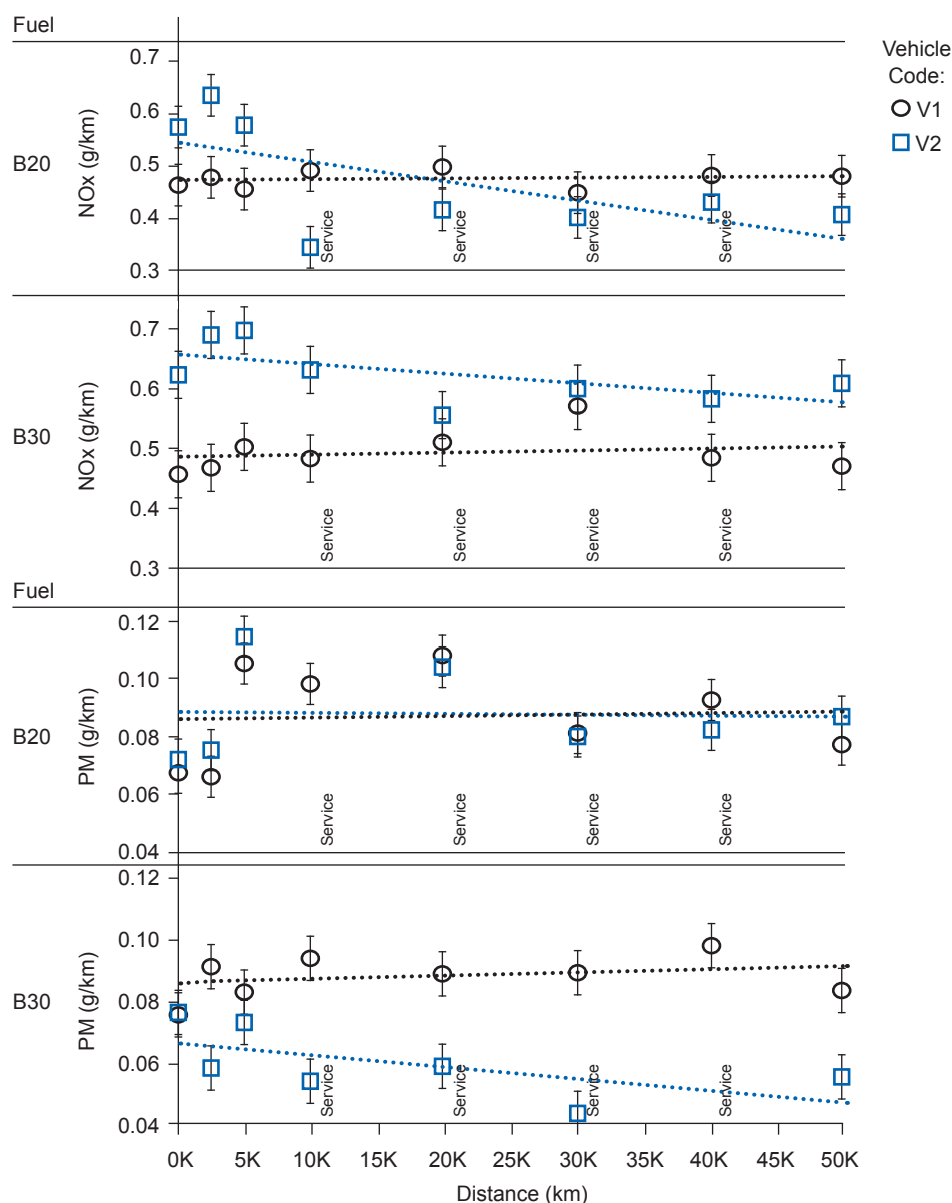


Figure 6. An emission of NOx and PM during road test.

Fuel Filter Mileage and Cold Start Ability

One of the issues at the beginning of B20 implementation in Indonesia in 2016 was shortened fuel filter mileage. In this road test, an evaluation of fuel filter changing was investigated by measuring the pressure difference at the inlet and outlet of the fuel filter. Here, replacement of the fuel filter on V1 and V2 vehicles, according to the recommendation from the vehicle manufactures, was at every 20,000 km. Figure 7 shows the value of the pressure difference at the fuel inlet and outlet of the fuel filter for V1 and V2 vehicles. The fuel filter was changed three times for both V1 and V2 during the 50,000 km, as shown in Figure 7. The value of delta pressure increases slowly at the beginning and increased

sharply at 10,000 km, indicating that the filter started to clog due to various impurities presented in the fuel filter for V1 and V2 vehicles. This phenomenon also occurred after the second and third filter replacements, where the differential pressure increased algorithmically after 10,000 km. Figure 7 also shows that the trend of increasing differential pressure (dP) at each filter change is identical for both of B20 and B30. The sudden increase in the value of the differential pressure indicates that it is time for filter replacement to be carried out for both V1 and V2 vehicles (Petiteaux & Monsallier, 2009). The maximum differential pressure for V1 during the first filter change could exceed 40 kPa for both of B20 and B30, which is notably higher than V2, where the maximum remains around 20 kPa. However, the

variance in differential pressure between B20 and B30 was not particularly significant for travelled distances of below 10,000 km for both V1 and V2, especially when considering an uncertainty measurement of 0.419 kPa.

Fuel filter replacements of V1 were shorter in the first replacement period but return to periodic filter replacements in the next km. While fuel filter replacement for V2 vehicles was still in accordance with manufacturer recommendations. The shorter fuel filter lifetime of biodiesel was also revealed by Graver *et al.* (2016), Komariah *et al.* (2018) and Paryanto *et al.* (2022). Meanwhile, an improvement of biodiesel quality as well as biodiesel handling and storage could enhance fuel filter mileage (Ma'ruf & Haryono, 2020). The mileage of the fuel filter is not only determined by the amounts of impurities and contaminants contained in B20 or B30 but is also influenced by the specifications of the fuel filter. The porosity of the filter paper determines the life of the filter. The smaller the pores of the filter paper, the faster the filter blocking occurs. Field and laboratory tests concluded that the biodiesel fuel filter will have filter blocking for a porosity of 5 μ , but not for porosity of 8 μ (Petiteaux & Monsallier, 2009).

In addition, this road test also investigated the effect of low temperature ambient in Indonesia on vehicle fuelled with B30. Cold-start ability was

studied at Wonosobo in Central Java, Indonesia, which represents the operability of a vehicle fuelled with B30 in a low-temperature environment. Twelve vehicles were prepared for various soaking periods of 3, 7, 14 and 21 days (Figure 8). Here, three vehicles were prepared for each soaking period which fuelled with pure diesel fuel (B0), B30 with MG of 0.40% and B30 with MG of 0.55%. Its goal is to investigate the effect of MG content on cold start ability at low ambient temperature area in Indonesia. MG could influence on precipitation of B30 so that it is possible to clog the fuel filter (Camerlynck *et al.*, 2012; Paryanto *et al.*, 2019). As a result, the vehicle will either fail to start or start after longer times.

The cold start test was conducted at varying ambient temperature of 12°C to 18°C with an average of 16°C. In this study, the measurement uncertainty for cold start test was determined and found to be 0.12 s. The cold start time for all tested fuels were comparable across all soaking periods as shown in Figure 8. It can be considered that the cold start with B30 is still similar with ordinary diesel fuel in Indonesia, as the average testing temperature was still above the pour point temperature for the whole tested fuels. Furthermore, utilisation of B30 at temperatures lower than its pour point is still possible with the addition of a surfactant to improve its operability in cold temperatures (Azaria *et al.*, 2020).

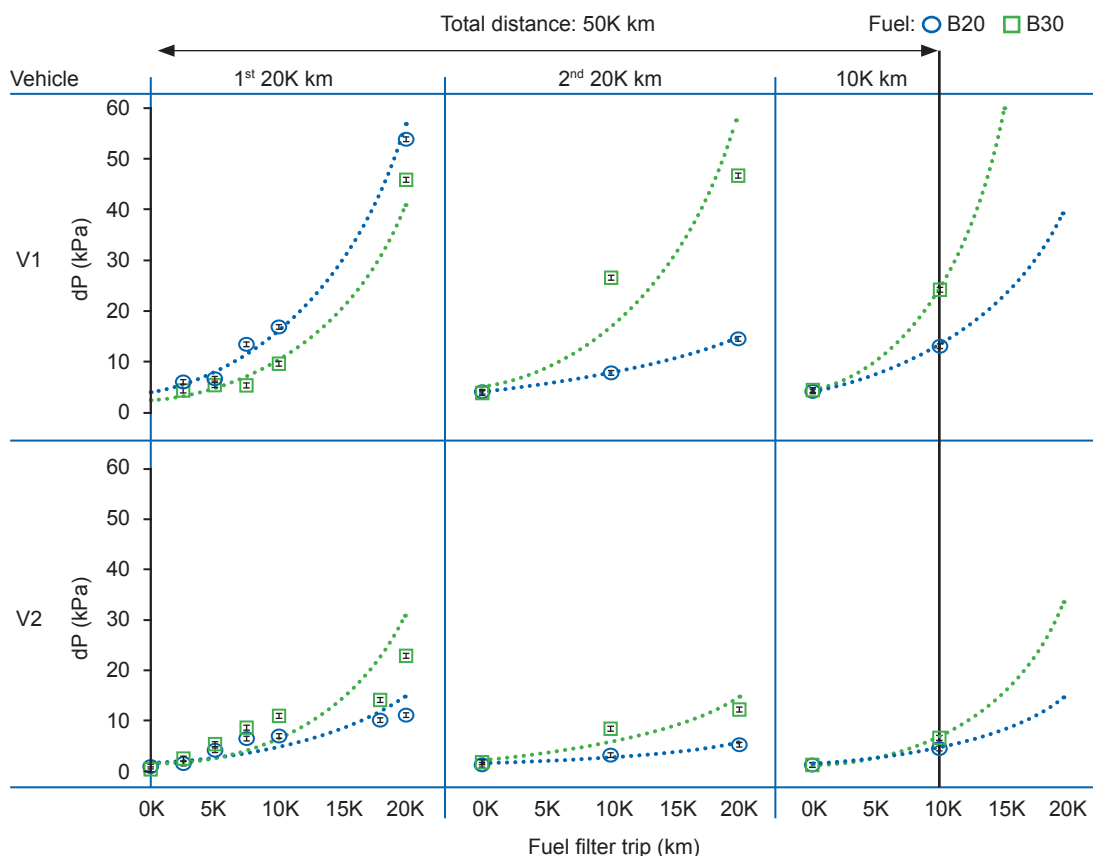


Figure 7. Measurement results of dP fuel filter during road test of V1 and V2 vehicles.

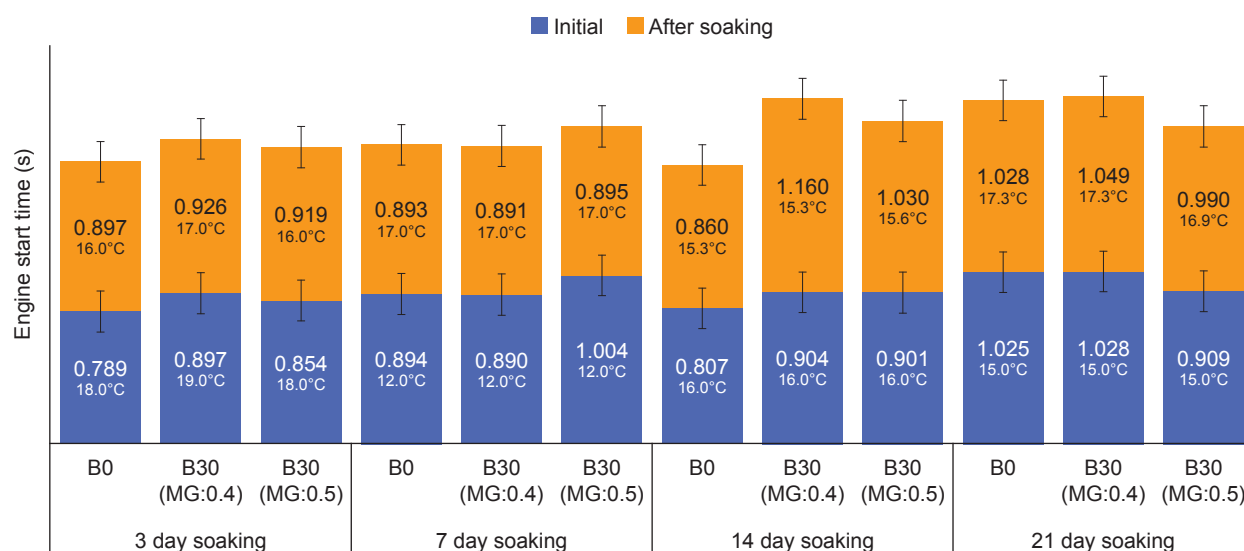


Figure 8. Cold start ability at various soaking period at low ambient temperature.

CONCLUSION

A comprehensive assessment showed that vehicles running on B20 and B30 could successfully cover 50,000 km in Indonesian operating conditions without encountering any operational problems. The road test further revealed that there was no need to change the fuel filters at the recommended 20,000 km interval, as both B20 and B30 maintained a differential pressure below 55 kPa. Moreover, even in Indonesia low-temperature conditions, B20 and B30 performed admirably, with engines starting times in less than five seconds without any noticeable issues.

Moreover, several conclusions have been derived from the laboratory testing of vehicles running on B20 and B30 fuel. The power and fuel economy of the B20 and B30 vehicles exhibited an average variation of 4.8 kW and 0.2 km/L, respectively. Notably, increasing the biodiesel ratio to 30% did not always lead to decreased power and FE, with results dependent on vehicle settings. Furthermore, B30 resulted in lower CO, HC and PM, but exhibited a higher NO_x value when compared to B20. This NO_x increase can be attributed to the higher combustion temperature in B30, indicating more efficient combustion. Additionally, the differential pressure was consistently higher for B30, implying that the existing B20 fuel filter may have a shorter lifetime when used with B30. However, fuel filter lifetime was still comparable with fuel filter changing as manufacturer recommendations. Finally, B30 utilisation did not have a significant impact on the cold start ability of the vehicle.

This study demonstrates that the improved biodiesel properties for B30 implementation could maintain vehicle performance similar to that of

B20 without requiring any changes to the periodic maintenance of the vehicles. Nevertheless, further research is crucial to verify that B30 meets the requirements for sustainable renewable fuel, which necessitates various assessment methods, including life cycle analysis. These studies are important to ensure that supply, demand, fuel prices, and manufacturing technology align adequately for the nationwide implementation of B30.

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GROWTH CHARACTERISTICS AND SUSTAINABILITY INDEX AT DIFFERENT DEVELOPMENT STAGES OF IMMATURE OIL PALM UNDER MONO- AND INTERCROPPING SYSTEMS

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ABSTRACT

Oil palm (Elaeis guineensis) is one of Indonesia's important oil-based plantation crops. The problem with oil palm planting is the long duration from initial planting to harvesting of the fresh fruit bunches. A possible solution to improve farmers' income is to plant oil palm in an intercropping system. This study compared the monocropping and intercropping systems in the immature oil palm stage. This study aimed to evaluate the growth performance of oil palm in an intercropping system with maize using the Sustainability Index (SI) model. The experiment was conducted at the Ciparanje Experimental Field, Universitas Padjadjaran, Indonesia in February-May 2019, 2020 and 2021. In terms of treatment, a monocropping system and an intercropping system of oil palm with maize were replicated four times. The observation variables were plant height, frond number, stem diameter, leaf chlorophyll index, and stomatal conductance. The SI analysis was calculated to observe plant growth consistency. The evaluation results showed no significant effect on the growth performance of immature oil palms through monocropping and intercropping over three years. The SI value showed the variation level in the sustainability of immature oil palm. The majority of SI showed a moderate category, which led to the conclusion that immature oil palm in various cropping systems was stable for the first three years. The intercropping system with oil palm and maize showed similar result to the monocropping system in the three years of the immature oil palm phase.

Keywords: intercropping, maize, oil palm, sustainability.

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INTRODUCTION

Oil palm (*Elaeis guineensis*) is a primary oil-producing crop in Indonesia, and nearly every part of the tree offers substantial advantages (Ibitoye *et al.*, 2011). Therefore, this plant holds a high economic advantage, leading to yearly expansion in cultivated areas (Setyamidjaja, 2006). In 2016, the oil

palm plantation in Indonesia covered 11,300,307 ha, consisting of 22.74% immature, 75.20% mature and 2.06% unproductive trees (Direktorat Jenderal Perkebunan, 2014).

The establishment of small-scale oil palm smallholdings has seen rapid growth, particularly in tropical climates such as Indonesia. These developments are prevalent in communities surrounding oil palm agro-industrial companies. Despite these improvements, several key production factors continue to pose constraints, including challenges related to land availability and the required investment for expanding oil palm cultivation among smallholder communities. The high costs associated with establishing new

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plantations, specifically expenses linked to field preparation and seedlings, as well as production expenses during the initial growth stages of immature trees, present significant challenges (Vermeulen & Goad, 2006). The time required for oil palm to start fruiting also presents a significant challenge for smallholders. Large companies investing substantial resources and labour also face similar issues before generating income from their plantations. Consequently, both smallholders and large plantation groups are turning to intercropping oil palm with faster-harvesting crops (Zen *et al.*, 2006). The generous spacing of oil palm trees, approximately 9 x 9 m, leaves gaps that can be used for growing other crops, such as annual cereals and legumes. Several studies have reported that intercropping could effectively control weed density within the plantations (Liebman & Davis, 2000; Liebman & Dyck, 1993; Tonye *et al.*, 2005). This cropping system is often implemented to take advantage of the 60%-75% of open space between the rows of oil palm trees, specifically during the initial 24 months (Nuraisah *et al.*, 2019; Suherman *et al.*, 2022). In the oil palm monoculture system, open spaces are reserved for planting cover crops such as legumes. Intercropping can enhance profits by incorporating edible plants compared to the monoculture system. This cropping system provides a multitude of benefits in both economic and ecological domains (Nchanji *et al.*, 2016). Maize, a significant cereal crop, is commonly intercropped with oil palm due to its ecological and economic advantages. Furthermore, this particular crop is an important staple food with high economic value in Indonesia (Ruswandi *et al.*, 2023). This shows that intercropping between oil palm and maize is recognised as a profitable undertaking.

Oil palm cultivation offers opportunities for intercropping with other crops beyond maize. Numerous studies have reported successful intercropping with soybeans, pueraria, cassava, banana, plantain, cocoyam, groundnut, ginger and turmeric (Dissanayake & Palihakkara, 2019; Dissanayake *et al.*, 2022; Nchanji *et al.*, 2016; Okyere *et al.*, 2014; Putra *et al.*, 2012). This indicates the versatility of this cropping system, particularly with profitable crop options.

Despite the potential benefits, intercropping remains untapped and not commonly practised by farmers and companies. The economic viability of intercropping on oil palm yield has often been questioned, even though several experiments have shown consistent positive results. However, environmental conditions can easily affect the profitability of intercrop when it is not effectively managed. Agricultural systems, including intercropping, are susceptible to environmental stress, and this complexity can worsen the situation (Okyere *et al.*, 2014). Additionally, the uncertainty of

oil palm yield can deter farmers from adopting this cropping system (Pridham & Entz, 2008). Brainard and Bellinder (2004) explained that mismanagement of crop selection or plant life cycle variations can lead to competition for water and nutrient sources in the soil, affecting yield unfavourably. Furthermore, the planting density can also create conditions favourable to pests, diseases, and weeds, which can pose risks to plantations and neighbouring crops. This shows that careful selection of suitable intercrops is essential in the intercropping system.

Nuertey *et al.* (2009) reported that smallholders in Ghana successfully practiced intercropping by planting food and cash crops within oil palm smallholdings, particularly during the early growth stages, approximately the first three years after oil palm planting. Similar practices are observed in many oil palm producing countries, including Indonesia. Integrating oil palm cultivation with intercrops, comprising both food and commercial crops, serves the economic interests of the local community.

Oil palm demands substantial nutrients to sustain vigorous growth and high yields. According to Supriatna *et al.* (2022), managing these nutrients is essential to achieve optimal yields and this is necessary for establishing a sustainable and profitable planting pattern. It is important to combine scientific and practical efforts to evaluate oil palm productivity under various cropping systems, including intercropping. Therefore, this study aimed: (i) To evaluate the growth performance of oil palm in an intercropping system with maize, and (ii) to identify the most effective intercropping pattern for oil palm and maize using the sustainability index (SI) model.

MATERIALS AND METHODS

Plant Material and Field Experiment

This study was conducted at the Ciparanje Field Trial, Agricultural Faculty, Universitas Padjadjaran, Jatinangor, Indonesia situated at an altitude of ± 780 m above sea level. The soil in the experimental field fell under the Inceptisol classification. The climate was categorised as type C based on the classification of Schmidt-Ferguson. The experiment took place from February to May 2019, 2020 and 2021. The materials used included hybrid maize variety Pertiwi 3 and immature oil palm varieties at SEUM stages I (aged 12-16 months), II (aged 24-27 months) and III (aged 36-40 months). Additional materials used included NPK fertiliser and chicken manure as the base fertiliser. The NPK fertiliser dose for oil palm was 200 g plant⁻¹, and the chicken manure dose was 10 kg plant⁻¹; the NPK fertiliser dose for maize was 200 kg ha⁻¹, and the chicken manure dose was 500 g plant⁻¹.

The field experiment consisted of two treatments, repeated four times each year. These treatments included monocultured oil palms and intercropping between oil palm and maize. The experimental plot size was 1 ha; the planting distance between oil palms was 8 x 8 m. Maize was planted within oil palm plantations, with a spacing of 1.5 m. Oil palm growth performance was assessed based on plant height, frond number, and stem diameter. Meanwhile, leaf chlorophyll index and stomatal conductance (gs) were measured using a chlorophyll meter (Opti Sci., US) and a porometer (Decagon Device Inc. US) respectively.

Data Analysis

The average value for each treatment was analysed using a T-test to evaluate variances between monocropping and intercropping of oil palm. The p -values ≤ 0.05 were considered significant for all growth parameters. SI was calculated using the following Equation (1):

$$SI = (Y - \sigma_n) / Y_M \times 100 \quad (1)$$

where Y represented the mean parameter of oil palm, σ_n was the standard deviation, and Y_M was the best performance of immature tree at any stage. SI values were arbitrarily classified into five groups, including deficient (up to 20%), low (21% to 40%), moderate (41% to 60%), high (61% to 80%) and very high (above 80%) (Atta *et al.*, 2009). SI calculations were performed using Microsoft Excel 2013.

RESULTS AND DISCUSSION

Growth of Immature Oil Palm in Different Cropping Systems

Growth of immature oil palm under monocropping and intercropping with maize was assessed across three stages, as presented in Table 1, 2 and 3. No significant differences were observed in any of the variables between the two-cropping systems across all stages (Figure 1). Variables such as plant height, frond number and stem diameter continued to have consistent development in each phase. The result was supported by the statement of Maxiselly *et al.* (2022a) that growth parameters, such as leaves, stems and branches, increased at each stage, indicating total plant development. Meanwhile, stomatal conductance and chlorophyll index showed fluctuating values per week after transplanting (WAT). The chlorophyll index indicated a decrease from stages I to stage III in both monocropping and intercropping treatments. Several factors, with soil nutrition being a prominent one, significantly influence oil palm growth.

According to Almatholib *et al.* (2017), micronutrients such as Cu and Ca affect the leaf number, leaf length, and canopy size of the plant. These results indicated that intercropping oil palm with maize did not affect oil palm development. This observation was further supported by the results of Nuerthey *et al.* (2009) and Nchanji *et al.* (2016), who reported that intercropping oil palm with annual plants had no adverse effects during the growth phase of the tree. Intercropping immature oil palm with annual plants such as maize not only had a positive impact on oil palm growth but also increased farmer income through the products derived from the intercrop (Dissanayake & Palihakkara, 2019). In summary, maize is one of the crops that does not have a negative impact on oil palm growth. So, it has the potential to become a useful intercrop for increasing farmers' economic benefits.

Sustainability Index (SI) on Oil Palm Cropping System

Table 4 and 5 present SI for oil palm cropping system. Table 4 outlines SI estimation for a monocropping oil palm system across three stages spanning from 2019 to 2021. The observed growth characteristics resulted in varying SI values, categorised as low, moderate, high and very high (Filio *et al.*, 2023; Wicaksana *et al.*, 2022). Certain parameters had low sustainability, such as chlorophyll content at 8 WAT and stem diameter at 4 and 6 WAT. The majority of growth parameters expressed a moderate SI with values ranging between 41.45 (for plant height at 10 WAT) and 58.54 (for frond number at 10 WAT).

The frond number at 8 WAT, as well as stomatal conductance at 4 and 8 WAT, had a high SI, as opposed to the very high SI observed at 6 WAT. The presence of low SI values indicated that the tested traits had inconsistent values over the three-year planting period. Based on previous studies, a low SI value suggested that the characteristics being tested were inherently unstable (Atta *et al.*, 2009; Filio *et al.*, 2023; Utami *et al.*, 2023). A high SI value indicated that the traits under examination remained consistent despite environmental changes. Both chlorophyll content (8 WAT) and stem diameter (4 and 6 WAT) had varying values across the three-year testing period, showing optimal performance in specific years only. To achieve the best chlorophyll content and stem diameter, selection should have been carried out during particular seasons that represented these traits optimally. In the monocropping system, traits falling within the moderate to high SI category, with values above the average represented the best and most stable conditions for three years. These traits incorporated plant height (at 4, 6 and 8 WAT), frond number (at 8 and 10 WAT), chlorophyll content (at 10 WAT),

TABLE 1. CROPPING SYSTEM EFFECT ON GROWTH PARAMETER OF IMMATURE OIL PALM STAGE I

Variable	Treatments	Means (WAT)			
		4 WAT	6 WAT	8 WAT	10 WAT
Plant height (cm)	Oil palm monocropping	92.45	106.49	112.69	122.43
	Oil palm + maize	107.09	114.00	121.56	126.36
Frond number	Oil palm monocropping	8.75	10.00	11.88	12.67
	Oil palm + maize	9.62	11.88	13.25	13.88
Stem diameter (cm)	Oil palm monocropping	16.74	20.15	22.75	23.54
	Oil palm + maize	20.38	22.71	29.25	29.85
Stomatal conductance (mmol m ⁻² s ⁻¹)	Oil palm monocropping	140.97	139.10	209.46	229.46
	Oil palm + maize	132.89	168.78	241.81	251.18
Chlorophyll index (CCI)	Oil palm monocropping	57.50	59.77	62.88	65.25
	Oil palm + maize	56.60	60.67	61.09	64.53

Note: The T-test shows no significant difference in all parameters between monocropping and intercropping on immature oil palm stages I ($p \leq 0.05$).

TABLE 2. CROPPING SYSTEM EFFECT ON GROWTH PARAMETER OF IMMATURE OIL PALM STAGE II

Parameter	Treatments	Means (WAT)			
		4 WAT	6 WAT	8 WAT	10 WAT
Plant height (cm)	Oil palm monocropping	221.73	223.45	225.10	227.30
	Oil palm + maize	212.00	216.25	219.00	221.88
Frond number	Oil palm monocropping	12.25	13.00	13.50	13.50
	Oil palm + maize	14.75	14.50	15.00	15.50
Stem diameter (cm)	Oil palm monocropping	58.00	59.50	61.25	62.00
	Oil palm + maize	56.25	57.50	58.50	60.50
Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)	Oil palm monocropping	132.75	128.83	127.30	115.50
	Oil palm + maize	130.35	97.00	115.75	145.50
Chlorophyll index (CCI)	Oil palm monocropping	45.00	40.25	48.75	47.00
	Oil palm + maize	45.00	40.00	48.00	44.25

Note: The T-test shows no significant difference in all parameters between monocropping and intercropping on immature oil palm stages II ($p \leq 0.05$).

TABLE 3. CROPPING SYSTEM EFFECT ON GROWTH PARAMETER OF IMMATURE OIL PALM STAGE III

Parameter	Treatments	Means (WAT)			
		4 WAT	6 WAT	8 WAT	10 WAT
Plant height (cm)	Oil palm monocropping	247.50	258.75	272.38	284.38
	Oil palm + maize	244.44	255.76	272.10	281.00
Frond number	Oil palm monocropping	114.00	119.18	123.15	126.26
	Oil palm + maize	113.50	118.62	122.84	125.72
Stem diameter (cm)	Oil palm monocropping	19.63	21.00	22.13	23.06
	Oil palm + maize	19.38	21.31	22.88	25.25
Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)	Oil palm monocropping	177.60	155.38	164.01	211.54
	Oil palm + maize	178.38	157.20	168.73	216.20
Chlorophyll index (CCI)	Oil palm monocropping	20.58	19.38	18.55	22.03
	Oil palm + maize	20.13	19.48	18.08	21.95

Note: The T-test shows no significant difference in all parameters between monocropping and intercropping on immature oil palm stages III ($p \leq 0.05$).

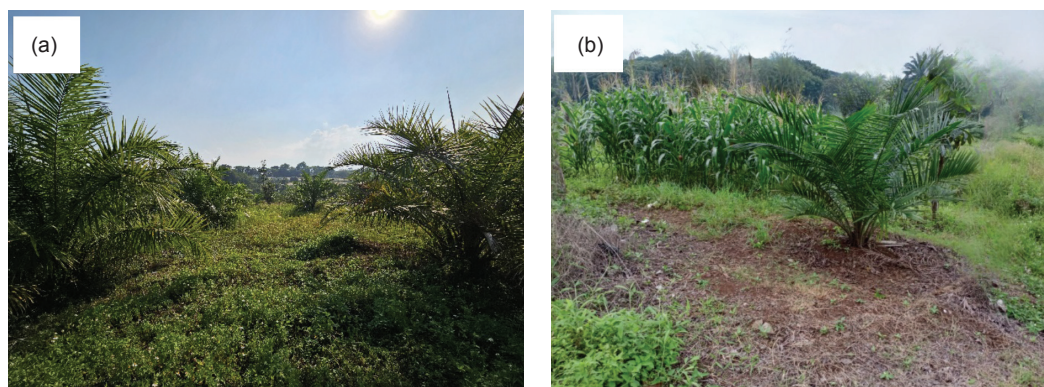


Figure 1. (a) Oil palm monocropping and (b) oil palm intercropping with maize.

TABLE 4. ESTIMATION FOR SI ON MONOCROPPING IMMATURE OIL PALM DURING THREE STAGES (2019-2021)

Variable		Y	σ	YM	SI	Category
Plant height (cm)	4 WAT	187.22	247.50	67.83	48.24	moderate
	6 WAT	196.23	258.75	65.07	50.69	moderate
	8 WAT	203.39	272.38	66.98	50.08	moderate
	10 WAT	44.76	65.25	17.72	41.45	moderate
Frond number	4 WAT	13.54	19.63	4.53	45.89	moderate
	6 WAT	14.67	21.00	4.64	47.73	moderate
	8 WAT	17.82	22.13	4.32	61.00	high
	10 WAT	18.28	23.06	4.78	58.54	moderate
Chlorophyll content (CCI)	4 WAT	41.03	57.50	15.33	44.69	moderate
	6 WAT	29.82	40.25	10.44	48.15	moderate
	8 WAT	33.65	48.75	15.10	38.05	low
	10 WAT	211.37	284.38	67.07	50.74	moderate
Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2} \text{s}^{-1}$)	4 WAT	150.44	177.60	19.50	73.73	high
	6 WAT	141.10	155.38	10.93	83.78	very high
	8 WAT	145.66	164.01	18.35	77.62	high
	10 WAT	163.52	211.54	48.02	54.60	moderate
Stem diameter (cm)	4 WAT	62.91	114.00	39.86	20.22	low
	6 WAT	66.28	119.18	40.71	21.45	low
	8 WAT	92.20	123.15	30.95	49.74	moderate
	10 WAT	94.13	126.26	32.13	49.11	moderate

Note: Y - the mean parameter of an oil palm; σ - standard deviation; YM - the best performance of the immature oil palm at any stage.

stomatal conductance (at 4 and 10 WAT), and stem diameter (at 8 and 10 WAT). The observation was supported by the results of Wicaksana *et al.* (2022), where moderate criteria with yield values above the average yield showed stable performance across diverse environmental conditions.

The intercropping system of oil palm with maize had distinct SI values, as presented in Table 5. Parameters such as chlorophyll contents at 8 WAT and stem diameter at 4 and 6 WAT showed low SI values. These traits consistently had low SI values in both monocropping and intercropping systems,

implying that they could only be selected under certain conditions to attain the desired values. On the other hand, the high SI category included frond numbers at 6 and 8 WAT, along with all variables related to stomatal conductance.

Most variables showed moderate values for SI. Traits falling within the high SI category showed consistency in the face of environmental changes. Conversely, traits with low SI values indicated susceptibility to environmental changes (Maulana *et al.*, 2023). Other studies have also reported that the high SI category had stability throughout three

TABLE 5. ESTIMATION FOR SI ON INTERCROPPING IMMATURE OIL PALM AND MAIZE DURING THREE STAGES (2019-2021)

Variable		Y	σn	YM	SI	Category
Plant height (cm)	4 WAT	187.84	244.44	58.62	52.87	moderate
	6 WAT	195.34	255.76	59.73	53.02	moderate
	8 WAT	204.22	272.10	62.34	52.14	moderate
	10 WAT	209.75	281.00	63.71	51.97	moderate
Frond number	4 WAT	14.58	19.38	3.99	54.68	moderate
	6 WAT	15.90	21.31	3.97	55.95	moderate
	8 WAT	18.94	22.88	3.94	65.56	high
	10 WAT	20.38	25.25	4.88	61.39	high
Chlorophyll content (CCI)	4 WAT	40.58	56.60	15.21	44.81	moderate
	6 WAT	29.74	40.00	10.26	48.70	moderate
	8 WAT	33.04	48.00	14.96	37.67	low
	10 WAT	43.58	64.53	17.39	40.58	moderate
Stomatal conductance (mmol H ₂ O m ⁻² s ⁻¹)	4 WAT	147.21	178.38	22.07	70.15	high
	6 WAT	140.99	168.78	31.47	64.89	high
	8 WAT	142.24	168.73	26.49	68.60	high
	10 WAT	180.85	216.20	35.35	67.30	high
Stem diameter (cm)	4 WAT	63.38	113.50	38.35	22.05	low
	6 WAT	66.28	118.62	39.64	22.45	low
	8 WAT	90.67	122.84	32.17	47.62	moderate
	10 WAT	93.11	125.72	32.61	48.12	moderate

Note: Y - the mean parameter of an oil palm; σn - standard deviation; YM - the best performance of the immature oil palm at any stage.

growing seasons, as observed in butterfly pea (Filio *et al.*, 2023). Traits categorised as having moderate SI values and high average values indicate stability across various locations and seasons (Tuteja, 2006; Verma *et al.*, 2013; Wicaksana *et al.*, 2022). In this study, frond number (at 8 and 10 WAT) and stomatal conductance (at 10 WAT) had optimal performance over three years of intercropping with maize. Frond number was an important trait associated with fruit sets, where a greater number of fronds correlated with a higher number of fruit sets (Balakrishna *et al.*, 2018). Stomatal conductance plays a significant role in metabolic processes such as respiration and photosynthesis, which indirectly enhance crop yield, particularly oil palm (Sales *et al.*, 2023; Suresh & Nagamani, 2006). Therefore, these two traits had substantial consistency in response to environmental changes, spanning both intercropping systems and various growing seasons.

Sustainability in the agricultural industry is important, particularly in the development of sectors such as oil palm plantations. Murphy *et al.* (2021) emphasised the importance of maintaining continuous oil palm production for the economies of key countries, including Indonesia and Malaysia, which are major oil palm exporters. As supported by Tang and Al Qahtani (2020), the sustainability

of oil palm plantations depends on a multitude of factors, comprising policy-making, a conducive environment, and biodiversity. The monoculture system commonly used for oil palm cultivation has contributed significantly to declining biodiversity. In a broader context, sustainable agriculture becomes one of the important factors in the advancement of crops (Maxiselly *et al.*, 2022b). This shows that the use of the intercropping system holds promise for fostering sustainable agricultural practices and increasing biodiversity.

The results from this study indicated that intercropping with immature oil palm did not adversely affect future growth. In contrast, this cropping system could yield economic benefits for farmers even before oil palm starts fruiting. The right selection of crops for intercropping can reduce labour costs for maintenance during the growing period (Nchanji *et al.*, 2016). Intercropping in oil palm during immaturity appeared to offer substantial enhancements in both economic and environmental aspects. The use of this cropping system also indicated the multifunctionality of the land in use (Khasanah *et al.*, 2020). Therefore, the vacant land within the oil palm plantations could be effectively used, mitigating weed growth and offering numerous advantages.

CONCLUSION

The result showed no significant effect between monocropping and intercropping on immature oil palms over three years. The SI value showed the variation level in the sustainability of immature oil palm. The majority of SI showed a moderate category that led to the conclusion that immature oil palms in both cropping systems were stable. The intercropping system between oil palm and maize showed similar result with monocropping for three years during the immature oil palm phase. The oil palm cultivating system (mono and intercrop) showed yearly consistency based on the Sustainability Index on growth variables. Overall, oil palm can be cultivated by mono or intercropping systems, good intercropping will potentially elevate the farmers' income.

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FORMULATION OF FORTIFIED MEDIA CONSISTING OF OIL PALM EMPTY FRUIT BUNCH BIOCOMPOST AND OIL PALM KERNEL SHELL BIOCHAR FOR THE ENHANCEMENT OF BIOACTIVE COMPOUNDS IN *Centella asiatica* (L.) URBAN

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ABSTRACT

Centella asiatica, commonly known as Pegaga, is renowned in Malaysia for its abundant bioactive compound content, making it a highly valued herb. These bioactive compounds, including phenolics, antioxidants, and triterpenes, possess pharmacological activities that are beneficial for health. To optimise the properties of these bioactive compounds, Pegaga Kampung, a superior variety of *C. asiatica* in Malaysia, was cultivated in 14 different formulations of fortified media. The fortified media were formulated by incorporating various ratios of oil palm empty fruit bunch (OPEFB) biocompost, oil palm kernel shell (OPKS) biochar, organic fertiliser, inorganic fertiliser and soils. Our findings showed that fortified media with a high biocompost ratio of 25:75 (soil:biocompost) and enriched with inorganic fertiliser had a 33% enhanced total phenolic content (TPC). The combination of soil, biocompost and biochar at a ratio of 50:25:25, enriched with organic fertiliser, was found to increase the TPC by 30% and the antioxidant properties by 16% compared to cultivation in soil alone (control). This suggested that the use of fortified media containing OPEFB biocompost and OPKS biochar, along with organic or inorganic fertilisers, significantly improves the bioactive compound characteristics of *C. asiatica*.

Keywords: bioactive compound, biochar Pegaga, biocompost, *Centella asiatica*.

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INTRODUCTION

Biocompost and biochar have been recognised for their beneficial effects on crop production, as they have been shown to improve the physical, chemical and biological properties of soils (Alfano *et al.*, 2009; Bastida *et al.*, 2010). For example, biocompost is commonly used as a fortification medium with soil. It works as the replenishment of nutrients in the soil and a nutrient source for plants. The amendment of soil with biocompost increases the water retention capacity (Zhou *et al.*, 2020). The application of biocompost in agriculture is gaining attention since chemical fertilisers and pesticides can significantly contribute to soil infertility

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(Jahangir *et al.*, 2021), environmental pollution (Singh & Prabha, 2017), and health hazards (Kalyabina *et al.*, 2021). Therefore, biocompost offers a sustainable and environmentally-friendly solution in the agricultural sector by reducing the reliance on chemical fertilisers and pesticides. It also provides an alternative approach to waste management, as it can be produced from waste materials, making it a greener option for improving soil health and crop productivity (Ozores-Hampton *et al.*, 1994). Hence, there is increasing demand worldwide for the application of biocompost from crop residues to croplands as a key countermeasure for enhancing soil carbon stock and reducing greenhouse gases emission from the agricultural sector (Jahangir *et al.*, 2021).

The ability of biochar to enhance the structure, porosity, particle size distribution and texture of soil is well known (Ding *et al.*, 2016). It has also been demonstrated to improve the chemistry of soil, including the pH, carbon content and cation exchange capacity (CEC) (Laghari *et al.*, 2016). The high surface area and porous nature of biochar contribute to its ability to retain water and nutrients (Li *et al.*, 2021; Wang *et al.*, 2019). Biochar has been recognised as a beneficial soil amendment that can enhance overall soil health and productivity. Research has shown that the application of biochar can increase the immobilisation of inorganic nitrogen and reduce ammonia volatilisation, indicating its potential for improving nitrogen management and minimising nitrogen losses in agricultural systems (Jindo *et al.*, 2020; Zhang & Guan, 2022). Although the impact of biochar on soil has been well documented, the knowledge of the soil environment due to the biochar implications is still in progress, especially on soil microbial communities and biogeochemical cycles. Research also showed that biochar helps in bioremediation (Patel *et al.*, 2022; Sharma *et al.*, 2020), carbon sequestration (Mona *et al.*, 2021) and nutrient adsorption (Gong *et al.*, 2019). Therefore, due to the beneficial effect of biocompost and biochar, this present study applied both biocompost and biochar produced from oil palm empty fruit bunch (OPEFB) and oil palm kernel shell (OPKS), respectively, to the soil media for the planting of *C. asiatica* and thus, find the optimum formulation that can enhance the production of the bioactive compound.

Due to the extensive utilisation of wild resources and limited industrial-scale cultivation, *C. asiatica* has been categorised as an endangered plant species. The overexploitation of wild populations and inadequate cultivation practices have raised concerns about the sustainability and conservation of this herb (Heidargholinezhad *et al.*, 2023). In addition, there is no inconclusive determination of the best variety of *C. asiatica* in Malaysia (Bakar

et al., 2022; Hazirah *et al.*, 2017). Exploiting the best variety of *C. asiatica* for its pharmacological active ingredients is also challenging. This is because different environmental conditions (abiotic or biotic stress) of *C. asiatica* influence the concentration of those ingredients in the plant (Hazirah *et al.*, 2017; Seong *et al.*, 2023). Finding the ideal planting conditions, which include the soil formulation, is crucial in the efforts to preserve the *C. asiatica* species as well as to make it possible to grow this herb commercially. Hence, it is necessary to devise systematic cultivation of *C. asiatica* to ensure the sustainability and promising quality of the production of its biological compounds. This is because the cultivation conditions will influence the concentration of the bioactive compounds of *C. asiatica*. For example, the geographical area, environmental growth conditions and genetic material have been reported to significantly affect the bioactive compounds production in these herbs (Kunjumon *et al.*, 2022).

Balancing plant growth rate and bioactive compound yield is a laborious process. This is because, plant growth depends mostly on the primary metabolic pathways, utilising polysaccharides, fats, proteins and sugars that make up the plant's bulk. However, the bioactive compounds that contribute to medicinal benefits are derived from the secondary metabolic pathway. Since they are not essential for the survival of the plant, these compounds are produced at a much lower concentration compared to primary metabolites. To increase these metabolites, plant cell culture was explored as one of the techniques developed to increase metabolite concentration produced by plants. On a large scale, plant cell culture was able to produce an exiguous amount of compounds that can meet commercial demands. Some other approaches are the formulation of planting medium, such as by using biocompost and biochar (Ashokkumar *et al.*, 2022; De Moraes *et al.*, 2022). Therefore, this study is aimed to enhance the bioactive compounds in *C. asiatica* by applying the OPEFB biocompost in combination with OPKS biochar in the fortified media formulation to grow *C. asiatica*.

MATERIALS AND METHODS

Production of Biocompost

OPEFB biocompost was produced based on Baharuddin *et al.* (2009). This biocompost was produced using the pressed and shredded OPEFB collected from Hulu Langat Palm Oil Mill, Dengkil, Selangor, Malaysia and palm oil mill effluent (POME) sludge collected from Felda Seriting Palm Oil Mill, Negeri Sembilan, Malaysia. The OPEFB were mixed

with POME sludge at the ratio of 1:1 (w/v) using a compost turner (Backhus, Germany) to form a compost windrow. The composting process was carried out for 20 days and the maturing for 10 days. Matured compost was stored in bags or drums prior to the formulation of fortified media for the planting of *C. asiatica*. The compost nutrient was analysed for quality control monitoring using inductively coupled plasma (ICP) analysis (Hammid *et al.*, 2022).

Production of Biochar

OPKS biochar was produced at Malaysian Palm Oil Board (MPOB) based on the method developed by Zainal *et al.* (2017). The microwave-assisted pre-carbonisation system with a maximum temperature of 300°C was used to produce OPKS biochar. The three magnetrons were set to shut down once the temperature of the system reached 250°C. The resulting heat from the combustion of OPKS was used to sustain the carbonisation process. The particle size of the biochar ranged from 6 to 15 mm. The biochar produced was sundried before being fortified in the media formulation.

Cultivation of *C. asiatica*

The study was conducted under greenhouse conditions. The mother plants of *C. asiatica* (Pegaga Kampung) were obtained from a local nursery in Sungai Buloh, Selangor, Malaysia. Small planting pots with drainage holes were used to grow the plant samples. Each pot was planted with five seedlings. The soil mixture was composed of topsoil, peat and sand at a ratio of 2:2:3 (v/v). All types of soil were supplied by Taman Pertanian Universiti, UPM and prepared accordingly. The formulation of fortified media was prepared as shown in Table 1. The water was provided twice daily to maintain the field's capacity.

TABLE 1. FORMULATION OF FORTIFIED MEDIA

Treatments	Compositions
T1	Soil 100% (Control)
T2	Soil enriched with IF
T3	Soil enriched with OF
T4	Soil: Biocompost [50:50]
T5	Soil: Biocompost [50:50] with IF
T6	Soil: Biocompost [75:25] with IF
T7	Soil: Biocompost [25:75] with IF
T8	Soil: Biocompost [50:50] with OF
T9	Soil: Biocompost [75:25] with OF
T10	Soil: Biocompost [25:75] with OF
T11	Soil: Biochar [50:50]
T12	Soil: Biocompost: Biochar [50:25:25]
T13	Soil: Biocompost: Biochar [50:25:25] with IF
T14	Soil: Biocompost: Biochar [50:25:25] with OF

Note: OF - organic fertiliser; IF - inorganic fertiliser.

Harvesting of Plants

The matured *C. asiatica* was harvested after 90 days of planting. Four plant samples from each of the treatments were selected randomly. The samples were used to determine the plant morphology, growth measurements, bioactive compounds, total phenolic content (TPC) and antioxidant activity. The leaves, petioles and roots were also oven-dried at 60°C for 48 hr and the dry weight was determined (Zin & Osman 2002).

Plant Morphology and Growth Measurements

All the plant samples were separated accordingly based on the plant parts, *i.e.*, leaves, petioles and roots for the measurement of quantitative traits. The measurement was based on the method explained by Pandey and Singh (2011). The fresh samples were characterised according to their number of leaves, leaf width and petiole length as an indicator of the plant growth measurement.

Plant Sample Preparation and Extraction

To prepare the samples for analysis, they were first washed with tap water to remove dirt and contaminants. The plant samples were then dried in a convection oven at 45°C for 48 hr until they reached a constant weight (Zin & Osman 2002). The dried samples were ground using a blender and sealed in polyethylene bags to prevent moisture exposure. The bioactive compounds, antioxidants, and TPC were extracted from 10 g of each powdered plant sample using 100 mL of ethanol as a solvent in an incubator shaker at $25 \pm 2^\circ\text{C}$ for 24 hr. The filtrates obtained through filtration with Whatman No. 1 filter paper were concentrated using a rotary evaporator at a temperature of 40°C for approximately 30 min. The dried extracts were weighed, dissolved in ethanol to a concentration of 1 mg/mL and stored at 4°C until use.

Analytical Procedures

Bioactive compounds. The presence of asiaticoside, madecacosside and asiatic acid in the extracted sample was analysed using high-performance liquid chromatography (HPLC) with a DAD detector. A Phenomenex Gemini 5 m NX-C18 LC column 250.0×4.6 mm was used as the stationary phase with a mobile phase of water-acetonitrile (70:30). The separation was performed with a flow rate of 1 mL/min and 0.01 mL volume of samples injected into the column at 25°C. Standards were prepared by dissolving 1.0 mg samples of asiaticoside (Sigma-Aldrich, Germany), madecacosside (Sigma-Aldrich, Germany) and

asiatic acid (Sigma-Aldrich, Germany) in 1.0 mL of ethanol and analysed at 220 nm wavelength. This method was adopted by Siddiqui *et al.* (2011).

Total phenolic content. The determination of TPC of *C. asiatica* plant extracts was carried out based on Singleton *et al.* (1999). The Folin-Ciocalteu reagent (0.5 mL) was added to 0.5 mL of plant extract. The distilled water was used to adjust the volume of mixture to 8.5 mL of working volume. The mixtures were subjected to incubation for 10 min at room temperature and subsequently followed by the addition of 1.5 mL of 20% sodium carbonate (Na_2CO_3). The tubes were then incubated at 755 nm using the UV-spectrophotometer. The blank was prepared using distilled water. Analyses were performed in triplicates. The gallic acid standard curve was plotted against concentrations 50, 100, 150, 250 and 500 mg/L and calculated for the gallic acid equivalent (GAE)/100 g of the extracted sample.

Antioxidant activity. The antioxidant activity of *C. asiatica* plant extracts was determined based on their ability to scavenge free radicals as measured by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay (Akowuah *et al.*, 2004). The assay was conducted by adding 2 mL of a 0.1 mM DPPH methanolic solution to test tubes containing 200 μL of the plant extracts. The well-mixed solution was incubated in the dark for 1 hr. A control sample was prepared by replacing the plant extracts with 200 μL of methanol. After 1 hr, the absorbance was measured at 517 nm using a UV-spectrophotometer. All analyses were performed in triplicate. The percentage of DPPH scavenging activity was calculated using the following Equation (1):

$$\text{DPPH scavenging activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} \times 100 \quad (1)$$

Statistical analysis. All analyses were performed in triplicates and the data reported in this study are mean with standard deviation. The linear correlation coefficient analysis was calculated using MS Office Excel 2010. The significant difference of $p < 0.05$ between each treatment was analysed using Tukey's test performed in SAS software.

RESULTS AND DISCUSSION

Effect of Different Formulations on the Growth of *C. asiatica*

Plant growth can be boosted by enhancing the physicochemical properties and nutrient content of the soil, which will be affecting the nutritional effects

and secondary metabolite compounds of the plants. It has been recognised that the biocompost and biochar from plant organic matter act as a substitute for agrochemicals (Bernal-Vicente *et al.*, 2008). This study investigated the plant growth performance using 14 formulated fortified media. Results on the growth measurements of *C. asiatica* on different treatments are provided in Table 2. It can be observed that the addition of biocompost in the fortified media increased the width of the leaf as compared to soil only or soil with biochar only. The number of leaves showed that the highest treatment bearing a value of 32.75 ± 12.28 was seen in T2 followed by T14 with a value of 32.00 ± 5.41 whereas the lowest number of leaves were seen in T9 and T1 (control) with a value of 17.75 ± 1.50 and 18.75 ± 8.65 number of leaves, respectively. T2, which is soil with inorganic fertiliser is the planting medium applied by most nurseries in Malaysia. Therefore, T2 has been observed to exhibit better growth compared to other treatments. Among the treatments, T8 showed the highest petiole length, while T11 showed the lowest. It is possible that climatic differences or variations in plant response to the formulated fortified media may lead to conflicting outcomes. However, it is important to note that the statistical analysis of treatment effects, conducted with Tukey's test (HSD), showed that the number of leaves and petiole length were not statistically significant. This study also showed the effect of the formulated fortified medium on the leaves width. It can be observed that the T1-T2 generates the smallest leaves width between 3.88-3.58 cm. There is no significant difference for treatment T3-T14, except T7 which has the highest average leaves width of 4.48 ± 0.36 cm. The small variation of the leaf width among the treatment might be due to different amounts of nutrients being adsorbed by the plants, attributed to the similar factors discussed for number of leaves.

Effect of Different Formulations on the Total Phenolic Content of *C. asiatica*

Diverse environmental stresses influence a plant's TPC production, concentration and accumulation levels (Samaniego *et al.*, 2020). The results of TPC in *C. asiatica* on different treatments are shown in Table 3. It should be highlighted that when compared to T1, the treatments designated T2, T7, T8 and T14 exhibited an improvement in TPC in leaves (control). According to Duncan's Multiple Range tests, the TPC value in treatments T2, T7, T8 and T14 differs significantly from T1.

Several factors contribute to the enhancement or decrement of phenolic content. The concentration of different secondary plant products is heavily dependent on growing conditions and affects the metabolic pathways responsible for the accumulation of associated natural products

TABLE 2. GROWTH MEASUREMENTS OF *Centella asiatica* ON DIFFERENT TREATMENTS

Treatments	Compositions	No. of leaves	Leaf width (cm)	Petiole length (cm)
T1	Soil 100% (control)	18.75 ± 8.65 ^a	3.38 ± 0.23 ^b	5.58 ± 1.74 ^a
T2	Soil enriched with IF	32.75 ± 12.28 ^a	3.58 ± 0.34 ^b	8.95 ± 2.14 ^a
T3	Soil enriched with OF	21.50 ± 8.88 ^a	3.82 ± 0.23 ^{ab}	6.08 ± 1.81 ^a
T4	Soil: Biocompost [50:50]	21.30 ± 11.20 ^a	3.55 ± 0.34 ^b	6.83 ± 2.94 ^a
T5	Soil: Biocompost [50:50] with IF	25.50 ± 7.04 ^a	3.83 ± 0.30 ^{ab}	7.20 ± 1.85 ^a
T6	Soil: Biocompost [75:25] with IF	20.30 ± 5.90 ^a	3.55 ± 0.20 ^{ab}	6.78 ± 0.80 ^a
T7	Soil: Biocompost [25:75] with IF	23.80 ± 9.21 ^a	4.48 ± 0.36 ^a	7.23 ± 2.94 ^a
T8	Soil: Biocompost [50:50] with OF	25.50 ± 13.96 ^a	3.95 ± 0.42 ^{ab}	10.23 ± 2.97 ^a
T9	Soil: Biocompost [75:25] with OF	17.75 ± 1.50 ^a	4.00 ± 0.35 ^{ab}	8.18 ± 1.55 ^a
T10	Soil: Biocompost [25:75] with OF	26.30 ± 15.41 ^a	4.18 ± 0.45 ^{ab}	7.32 ± 2.16 ^a
T11	Soil: Biochar [50:50]	20.00 ± 12.02 ^a	3.70 ± 0.29 ^{ab}	5.23 ± 2.99 ^a
T12	Soil: Biocompost: Biochar [50:25:25]	24.00 ± 5.88 ^a	3.55 ± 0.47 ^{ab}	5.50 ± 1.04 ^a
T13	Soil: Biocompost: Biochar [50:25:25] with IF	28.50 ± 7.76 ^a	3.88 ± 0.25 ^{ab}	6.75 ± 1.72 ^a
T14	Soil: Biocompost: Biochar [50:25:25] with OF	32.00 ± 5.41 ^a	4.00 ± 0.78 ^{ab}	7.30 ± 2.22 ^a

Note: *Mean of growth measurements, values in row followed by the same letter indicates no significant difference of growth measurements at $p < 0.05$ level by Tukey's tests. OF - organic fertiliser; IF - inorganic fertiliser.

(Akula & Ravishankar, 2011). In this study, different components and amounts of fortified media were used. The nutrients present in the fortified media could be one of the factors influencing the phenolic content of the samples. Further investigation is needed to elucidate the specific role of fortified media in the observed increase in phenolic concentration. When *C. asiatica* is being planted using the nursery's treatment (T2), it showed that this herb had a great TPC with a value of 12.44 g/mg. However, when the soil is fortified with biocompost at a ratio of 25:75 and then enriched with inorganic fertiliser biweekly, it shows a better result with a value of 13.06 g/mg. Since both treatments were enriched with inorganic fertiliser biweekly, the enhancement of phenolic levels could probably be due to the plant being supplied with adequate nutrients as the characteristics of the inorganic fertiliser itself. Inorganic fertiliser is known as a readily formed nutrient where it releases its nutrients rapidly once applied (Belay *et al.*, 2002).

On the other hand, the treatments which are enriched with organic fertilisers also showed a positive effect in the enhancement of TPC in *C. asiatica*. However, it requires other materials such as biocompost and biochar to be fortified with soil to achieve this significant difference. Treatment labelled T8, which is fortified with soil and biocompost with a ratio of 50:50 showed a better amount of TPC when compared with T1. This observation was also supported by a study conducted by Siddiqui *et al.* (2011) where the composition of 50% of compost tea and 50% of NPK had the highest value for plant growth and yield.

T8 had a value of 12.13 mg/g of TPC. However, when biochar was added to the composition of fortified media with a ratio of (50:25:25) and supplemented with organic fertiliser, the TPC was recorded at 12.73 mg/g, which is higher than T2 and T8. This result is in agreement with Schulz *et al.* (2013), whereby, the beneficial effects of biochar and compost on plant growth might be because of the rising levels of total organic carbon and total nitrogen, both of which are essential for plant growth. Although the value of phenolic content in T14 is much lower compared to T7, it should be emphasised that these treatments were in the same group (a) when statistically analysed, with no significant difference between treatments labelled T2, T7, T8 and T14.

The usage of inorganic and organic fertilisers has been debated for many centuries in the agricultural world. Both have their benefits and circumstances. It has been documented that organic fertiliser had lower performance compared to inorganic fertiliser (Pan *et al.*, 2020). Meanwhile, organic fertilisers are a sustainable choice for nourishing plants, as they release their nutrients slowly, in response to the plant's specific needs and environmental conditions. This gradual nutrient release promotes a healthier growing environment by avoiding soil exhaustion, as noted by Oad *et al.* (2004). However, it is important to note that organic fertilisers may require more time and investment due to their lower growth performance, which can be a concern for farmers. Nevertheless, the long-term benefits of using organic fertilisers outweigh the initial challenges, as they contribute to sustainable agriculture practices and promote

TABLE 3. TOTAL PHENOLIC CONTENT OF *Centella asiatica* IN DIFFERENT TREATMENTS

Treatments	Compositions	Leaves (mg/g)	Petiole (mg/g)	Roots (mg/g)
T1	Soil 100% (control)	9.83 ± 0.20 ^{cd}	1.89 ± 0.07 ^{cd}	1.55 ± 0.03 ^a
T2	Soil enriched with IF	12.44 ± 0.47 ^a	1.89 ± 0.02 ^{cd}	1.39 ± 0.09 ^{ab}
T3	Soil enriched with OF	9.23 ± 0.25 ^{de}	1.07 ± 0.05 ^{ab}	0.99 ± 0.23 ^c
T4	Soil: Biocompost [50:50]	5.36 ± 0.11 ^h	1.57 ± 0.08 ^h	1.18 ± 0.08 ^{bc}
T5	Soil: Biocompost [50:50] with IF	4.50 ± 0.48 ^h	2.89 ± 0.21 ^{ef}	1.53 ± 0.19 ^a
T6	Soil: Biocompost [75:25] with IF	5.15 ± 0.30 ^h	1.76 ± 0.07 ^a	0.99 ± 0.03 ^c
T7	Soil: Biocompost [25:75] with IF	13.06 ± 0.15 ^a	2.12 ± 0.05 ^{de}	1.16 ± 0.02 ^{bc}
T8	Soil: Biocompost [50:50] with OF	12.13 ± 0.55 ^a	1.37 ± 0.08 ^{bc}	1.50 ± 0.10 ^a
T9	Soil: Biocompost [75:25] with OF	11.02 ± 0.42 ^b	1.71 ± 0.09 ^{de}	1.40 ± 0.07 ^{ab}
T10	Soil: Biocompost [25:75] with OF	8.02 ± 0.33 ^f	2.30 ± 0.16 ^b	1.06 ± 0.07 ^c
T11	Soil: Biochar [50:50]	6.71 ± 0.31 ^g	1.24 ± 0.10 ^{hg}	1.00 ± 0.02 ^c
T12	Soil: Biocompost: Biochar [50:25:25]	10.37 ± 0.26 ^{bc}	1.36 ± 0.07 ^{fg}	1.20 ± 0.02 ^{bc}
T13	Soil: Biocompost: Biochar [50:25:25] with IF	8.59 ± 0.44 ^{bc}	2.18 ± 0.17 ^{fg}	1.00 ± 0.12 ^{bc}
T14	Soil: Biocompost: Biochar [50:25:25] with OF	12.73 ± 1.07 ^a	1.63 ± 0.06 ^e	1.20 ± 0.05 ^{bc}

Note: *Mean of growth measurements, values in row followed by the same letter indicates no significant difference of growth measurements at $p < 0.05$ level by Tukey's tests. OF - organic fertiliser; IF - inorganic fertiliser.

soil health. Biochar, on the other hand, can play a vital role in enhancing the total plant count (TPC) due to its remarkable absorption and adsorption capabilities (Rawat *et al.*, 2019). This study found that the high concentration of phenolic compounds in T14 might be due to a greater uptake of nutrients from the fertiliser. Therefore, it can be concluded that the combination of this fortified media (T14) is beneficial since it aids in the release of nutrients when the plant most needs them, resulting in probable less leaching and nutrient waste. The potential of biochar to act as a soil conditioner and improve the chemical composition of soil could make it healthier and better suited for growing plants, herbs, or crops. Biochar is also known to absorb heavy metals from soils (Mustafa *et al.*, 2018). Different types and amounts of fertiliser had different effects on the TPC of *C. asiatica* which was also supported by other studies (Nguyen *et al.*, 2010).

Effect of Different Formulations on the Antioxidant Activity of *C. asiatica*

Enhancement of antioxidant activity was also determined based on several treatments formulated in this study. *C. asiatica* is one of the herbs that are well-known to have high antioxidant activity. Previous researchers have documented the great benefits of this herb, especially in its antioxidant activity since this herb can scavenge radical components thus providing consumers with a nourished and youthful appearance. Biologically, consuming foods containing high antioxidant activity could also help maintain health as the

radicals, if not being scavenged could cause detrimental effects to the cells such as cancers. In this study, the results obtained from different formulations of fortified media showed different values in antioxidant activity as shown in Table 4.

When compared to the control (T1), the treatments T8 and T14 are among those that are able to increase the antioxidant activity in *C. asiatica*. The highest antioxidant activity was obtained from T14 with a value of 56.13% in its leaf extracts. This treatment was also in line to show an enhancement in the TPC of *C. asiatica*. This could probably be due to the phenolic compounds being a combination of various compounds (phenolic acid and alcohols, flavonoids, stilbenes, tocotrienols and tocopherols), which are a good source of antioxidants (Zheng *et al.*, 2011). Several studies reported that high TPC also resulted in high antioxidant activity (Cai *et al.*, 2004; Shan *et al.*, 2005; Wong *et al.*, 2006). The combination of soil, biocompost at 50:50 and soil, biocompost and biochar at (50:25:25) with the enrichment of organic compounds might be suitable for the enhancement of both antioxidant activity and TPC.

However, in comparison to other treatments, apart from T14 and T8, the control group (T1) exhibited a higher value (48%). This observation could potentially be attributed to the plant experiencing stress, which could have resulted in a contrasting effect. It is well noted that T1 is not being supplied with other nutrients besides the soil only, however, this treatment still had a high antioxidant activity value. Plant exposure to stress conditions, for example, stress from drought, salt or heavy metal could induce reactive oxygen species

TABLE 4. ANTIOXIDANT ACTIVITY OF *Centella asiatica* IN DIFFERENT TREATMENTS

Treatments	Compositions	Leaves (%)	Petiole (%)	Roots (%)
T1	Soil 100% (control)	48.39 ± 2.33 ^{ab}	5.13 ± 1.88 ^{bcd}	2.56 ± 1.10 ^{bc}
T2	Soil enriched with IF	38.30 ± 1.06 ^{cd}	6.85 ± 0.41 ^{abcd}	5.78 ± 0.58 ^{abc}
T3	Soil enriched with OF	31.47 ± 4.39 ^d	3.85 ± 3.25 ^{cd}	2.43 ± 1.12 ^c
T4	Soil: Biocompost [50:50]	8.80 ± 7.26 ^e	2.78 ± 4.29 ^d	5.80 ± 2.38 ^{abc}
T5	Soil: Biocompost [50:50] with IF	3.80 ± 3.11 ^e	2.75 ± 1.58 ^d	6.37 ± 1.19 ^{abc}
T6	Soil: Biocompost [75:25] with IF	4.17 ± 3.02 ^e	5.46 ± 1.02 ^{bcd}	7.76 ± 0.71 ^{abc}
T7	Soil: Biocompost [25:75] with IF	41.35 ± 0.01 ^{bc}	8.06 ± 0.04 ^{abcd}	5.54 ± 0.01 ^{abc}
T8	Soil: Biocompost [50:50] with OF	49.33 ± 0.03 ^{ab}	6.53 ± 0.01 ^{abcd}	6.18 ± 0.00 ^{abc}
T9	Soil: Biocompost [75:25] with OF	41.89 ± 0.02 ^{bc}	7.25 ± 0.01 ^{abcd}	8.62 ± 0.01 ^a
T10	Soil: Biocompost [25:75] with OF	43.15 ± 0.01 ^{bc}	9.48 ± 0.06 ^{ab}	6.77 ± 0.01 ^{abc}
T11	Soil: Biochar [50:50]	30.25 ± 0.01 ^d	7.92 ± 0.01 ^{abcd}	8.94 ± 0.04 ^a
T12	Soil: Biocompost: Biochar [50:25:25]	47.91 ± 0.01 ^b	8.83 ± 0.01 ^{abc}	8.70 ± 0.02 ^a
T13	Soil: Biocompost: Biochar [50:25:25] with IF	46.31 ± 0.01 ^{bc}	11.16 ± 0.00 ^a	7.82 ± 0.02 ^{ab}
T14	Soil: Biocompost: Biochar [50:25:25] with OF	56.13 ± 0.04 ^a	11.62 ± 0.01 ^a	10.76 ± 0.03 ^a

Note: *Mean of growth measurements, values in a row followed by the same letter indicates no significant difference of growth measurements at $p < 0.05$ level by Tukey's tests. OF - organic fertiliser; IF - inorganic fertiliser.

(ROS) to mass-produce (Moradi & Ismail, 2007; Murata *et al.*, 2007; Takahashi & Murata, 2008). The mass-produce of this ROS could disrupt the stability of the cell membrane and thus accelerate protein deformation and nucleic acid damage resulting from the inhibition of plant growth (Choudhury *et al.*, 2013; 2016). This phenomenon can be reflected as shown in Table 2, where almost all T1's growth measurements are seen to be lower compared to other treatments. Plants connected by stolons or rhizomes are more prone to encountering heterogeneous availability of resources. This variability in resource availability can pose challenges for these plants in capturing essential resources, which may affect their growth and development (Klimesova & Bello, 2011; Ye *et al.*, 2013; You *et al.*, 2016). Therefore, treatments that are enriched with organic fertilisers showed enhancement in the value of the antioxidant activity. The usage of organic fertiliser is advisable as it provides a suitable and healthier growing environment, thus, giving benefits not only to the plant but also to the soil properties, including functionality, structure, porosity as well as water holding capacity (Assefa & Tadesse, 2019).

Effect of Different Formulations on the Triterpene Composition of *C. asiatica*

Madecacosside, asiaticoside and asiatic acid are secondary metabolites that fall under the category of triterpene compounds. These three triterpenoids serve as the main biomarkers of *C. asiatica*, contributing to the distinctive properties and uniqueness of this herb. Results on the

occurrence of triterpenes compounds of *C. asiatica* on different treatments are tabulated in Table 5. It can be observed that madecacosside was only present in T1-T5 and T14, where the highest value was observed in T2. On the other hand, it was observed that the presence of asiaticoside compound, which is known for its potential effects on collagen synthesis, anti-wrinkle properties, brain tonic properties, and wound healing effects, was not detected in any of the treatments. The preliminary sample showed the highest presence of asiaticoside compound with a value of 1.30 mg/g, while the market sample had a lower value of 0.24 mg/g. On the other hand, asiatic acid was found to be highest in T10 as compared to other treatments.

Asiatic acid was enhanced in T10, with a value of 32.55 mg/g. This increase in asiatic acid production can be attributed to the synergistic effect of the combination of soil, biocompost and biochar enriched with either inorganic or organic fertilisers (Siddiqui *et al.*, 2011). However, this compound was observed only in treatments T6 to T13. It should be noted that asiaticoside is a derivative of asiatic acid, and asiatic acid is a derivative of asiaticoside due to the synthesis and hydrolysis processes involved in their formation. Therefore, although asiaticoside was not detected in all treatments, it is possible that asiatic acid, which is a precursor of asiaticoside, could be present. This suggests that the functions and benefits of asiaticoside in *C. asiatica* may still be available in the form of asiatic acid. Both asiatic acid and asiaticoside are known to have anti-aging, wound-healing and collagen synthesis properties.

TABLE 5. TRITERPENE COMPOUNDS OF *Centella asiatica* IN DIFFERENT TREATMENTS

Treatments	Compositions	Madecacosside (mg/g)	Asiatic acid (mg/g)
T1	Soil 100% (control)	0.8 ± 12.3 ^a	ND
T2	Soil enriched with IF	0.9 ± 8.9 ^a	ND
T3	Soil enriched with OF	0.9 ± 11.2 ^a	ND
T4	Soil: Biocompost [50:50]	0.8 ± 7.0 ^a	ND
T5	Soil: Biocompost [50:50] with IF	0.8 ± 5.9 ^a	ND
T6	Soil: Biocompost [75:25] with IF	ND	31.1 ± 2.9 ^a
T7	Soil: Biocompost [25:75] with IF	ND	29.0 ± 3.0 ^a
T8	Soil: Biocompost [50:50] with OF	ND	28.4 ± 1.6 ^a
T9	Soil: Biocompost [75:25] with OF	ND	28.0 ± 2.2 ^a
T10	Soil: Biocompost [25:75] with OF	ND	32.6 ± 3.0 ^a
T11	Soil: Biochar [50:50]	ND	26.4 ± 1.0 ^a
T12	Soil: Biocompost: Biochar [50:25:25]	ND	26.9 ± 1.7 ^a
T13	Soil: Biocompost: Biochar [50:25:25] with IF	ND	27.8 ± 1.7 ^a
T14	Soil: Biocompost: Biochar [50:25:25] with OF	0.8 ± 5.4 ^a	ND

Note: *Mean of growth measurements, values in row followed by the same letter indicates no significant difference of growth measurements at $p < 0.05$ level by Tukey's tests. ND - Not detected; OF - organic fertiliser; IF - inorganic fertiliser.

However, it was reported that asiatic acid is less bioavailable due to the rapid metabolism measured (Meeran *et al.*, 2018; Rush *et al.*, 1993). This is because, derivatives of asiatic acid by the modifications at C-11 and C-28 positions are more potent and had higher bioavailability to exhibit its beneficial effect (Meeran *et al.*, 2018).

The variation in the values of triterpene compounds could be attributed to factors such as harvesting time and geographical location, which may vary across different months or seasons. These factors can influence the growth and development of the plant, as well as the accumulation of secondary metabolites, including triterpenes. Environmental conditions, such as temperature, humidity, sunlight and soil composition, can affect the synthesis and accumulation of triterpenes in plants (Meeran *et al.*, 2018). Studies conducted by Alqahtani *et al.* (2015) have suggested that *C. asiatica*, which originates in Australia, exhibits higher amounts of triterpenoids during the summer season, indicating an optimum time for harvesting. Similarly, research by Puttarak and Panichayupakaranant (2012) has documented that different provenance in Thailand and cultivation during different months also greatly influence the yield of bioactive compounds in *C. asiatica*. This highlights the potential impact of geographical location and harvesting time on the triterpenoid content of *C. asiatica*. For example, *C. asiatica* harvested in Thailand during March provided higher triterpenoids with a value of 37.2 mg/g dry powder whereas *C. asiatica* from Songkhla had the highest amount of triterpenes compounds (37.4 mg/g dry powder) when it was harvested in December. However, *C. asiatica*

collected from Nakornsrihammarat and Ratchaburi (different provinces in Thailand) produces the lowest content of triterpene compounds across all harvesting periods.

CONCLUSION

A suitable and appropriate formulation of fortified media as a planting medium of *C. asiatica* determined is treatment T14. This treatment is a mixture of soil, biocompost and biochar with a ratio of 50:25:25 enriched with organic fertiliser. This treatment resulted in an enhancement in the TPC and antioxidant activity of *C. asiatica*. The formulated fortified media could be a suitable alternative to organic farming in the agriculture world as most of these materials are a mixture of recycled waste products of oil palm biomass, which is utilised as a value-added product. The best treatment for the specific chemical properties depends greatly on the needs of the plants and preferences in terms of cost and environmental impact. If one plans on producing a plant using organic materials without having to think of the cost, it is advisable to use the organic method such as treatment T14, which specifically focuses on using organic materials such as biocompost, biochar and organic fertilisers. However, if one is concerned about the cost and time and neglects the environmental pollution, it can be advisable to use treatment T2 and treatment T6, where treatment focuses on using inorganic fertiliser which may cause detrimental to the environment after a long period of usage. Treatment T6 on the other hand

gives an option of organic and inorganic materials, which comprises biocompost enriched with inorganic fertiliser.

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SPECIES IDENTIFICATION OF THE FATTY ACID COMPOSITION OF THE MARGARINES AND SPREADS

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ABSTRACT

Fat-and-oil products (margarine and spreads) are included in the daily diet and meet the requirements for “wholesome foods”. It is a substitute for butter used in cooking. The purpose of a series of studies regarding the fat phase of margarine and spreads was to determine the amounts of fatty acids and their isomers with the highest possible degree of detail in fat-and-oil products, as well as to improve the methodology using gas chromatographic methods in combination with mass spectrometry. Seventeen samples of fat-and-oil products were used for analysis and comparison in this study. All measurements were performed using a triple quadrupole GC-MS/MS Thermo Scientific™ TSQ 8000™ system equipped with GC Thermo Scientific™ TRACE™ 1310 with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The article shows the possibility of using information about fatty acid composition to establish the species of fat-and-oil products. The study presents the fatty acid composition of margarine and spreads. It is shown that the high-resolution mass spectrometry method for inferring the fatty acid composition is very useful for identifying things that come from plants and animals.

Keywords: dietary fats, fatty acid composition, products of natural origin, species identification.

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INTRODUCTION

Vegetable oils or fats are product of natural origin, which are obtained during the processing of vegetable raw materials. Margarine was developed as an alternative to butter, the fat content in them is 80%. Margarine usually contains suitable ratios of solid vegetable fats from coconut, palm kernels, transesterified vegetable oils and/or hydrogenated vegetable oils (Jala & Kumar, 2018). Basically, in the process of industrial catalytic hydrogenation, some natural fatty acids are destroyed and new artificial *trans* isomers are formed, which behave similarly to saturated fats. These isomers lack

the necessary metabolic activity of the starting compounds and they inhibit the enzymatic desaturation of essential fatty acids (Kandhro *et al.*, 2008).

Industrial margarines are designed for diverse types of baking. Both butter and spreads are emulsions wherein a matrix of solid fat crystals binds liquid oil and small drops of water containing aromatic compounds. For the manufacture of spreads, a hard, refractory fat is needed (Triantafillou *et al.*, 2003). By choosing its distinct types, as well as processing conditions, various spreads are obtained – softer and harder at room temperature and in a cooled form, as well as described by a higher or lower total fatty acid content and saturated fatty acid (SFA) content. Fats with a high SFA content have a higher non-oxidising ability (Garsetti *et al.*, 2016; Perna & Hewlings, 2023; Takeuchi *et al.*, 2023).

One of the strategies to encourage the replacement of *trans* fatty acids (TFA) with oils with a higher content of polyunsaturated fatty acids

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(PUFA) to establish preferential prices. One of the factors contributing to the frequent use of palm oil as a substitute for TFA is its affordability (Kodali *et al.*, 2001).

Industrially produced TFAs remain a global killer and considerably contribute to the development of non-communicable diseases (NCDs). The consumption of TFA in the amount of 2% of the daily calorie intake increases the risk of death from cardiovascular diseases by 20%-32%. High consumption of TFA is one of the main risk factors for the development of coronary heart disease (Roe *et al.*, 2013) and causes obesity, type II diabetes (Willett, 2006), oncological diseases, ovulatory infertility, diseases of the nervous, immune systems and gastrointestinal tract (Islam *et al.*, 2019; Owais *et al.*, 2023; Salemi *et al.*, 2023; Xu *et al.*, 2022). Based on the accumulated data on the adverse impact of TFA on human health, the Food and Agriculture Organization (FAO)/ World Health Organization (WHO) recommended reducing their consumption to 1% of the daily caloric content of the daily diet, which, with the recommended FAO/WHO daily caloric content of the daily diet (2,000 kcal) is less than 2.2 g/day (The NCD Alliance, 2022; WHO, 2018).

It is estimated that eliminating TFA from food can annually prevent 500,000 deaths from coronary heart disease (CHD) (Restrepo & Rieger, 2016). The highest mortality rates from cardiovascular diseases (CVD) in the region of the Eurasian Economic Union (EAEU) are observed in Russia and Belarus for men and in Kyrgyzstan for women (Wang *et al.*, 2016). In Kazakhstan, the average TFA content in publicly available street products was up to 144.3% of the recommended maximum daily intake (Nichols *et al.*, 2014).

By using world practices and following the recommendations of the World Health Organization, the European Economic Commission (EEC) introduced restrictions on harmful TFAs in products of vegetable oil and animal fat processing: Margarine, milk fat substitutes, special-purpose fats, vegetable-cream and vegetable-fat spreads, and rendered vegetable-cream and vegetable-fat mixtures. For this, the EEC suggests reducing, or better, eliminating industrially hydrogenated fats from the diet, which will help preserve the health of consumers.

However, the regulations on fat-and-oil products on the content of TFAs do not apply to other types of food products. They are subject to the norms of other technical regulations of the Union. For example, the technical regulation "on the safety of milk and dairy products" (Padrão *et al.*, 2019) in creamy vegetable spreads and rendered creamy vegetable mixtures limits the number of *trans* isomers of oleic acid in fat isolated from the product—their mass fraction should not

exceed 8%. The technical regulations "on food safety" (Customs Union Commission, 2013) in food products for young children normalise the content of TFA in breast milk substitutes—no more than 4% of the total content of such acids. Therewith, the technical regulations of the Union "on food safety" do not regulate the content of TFAs as a percentage of the total fat content, for example, in cakes, cookies, sweets, and other confectionery products. The high information content and specificity of the fatty acid composition allow using it to identify fat-and-oil products—margarine and spreads.

The purpose of a series of studies on the fat phase of margarine and spreads was to determine the amounts of fatty acids and their isomers with the highest possible degree of detail in fat-and-oil products, as well as to improve the methodology using gas chromatographic methods in combination with mass spectrometry.

MATERIALS AND METHODS

A total of 17 samples of fat-and-oil products were analysed and compared in this study. The samples included margarine, and vegetable-fat spreads since these products mainly contain SFAs and TFAs. Samples were purchased in several Almaty's local supermarkets, including national and imported brands. Standards of *trans*- and *cis*-fatty acid methyl esters of the 37 component FAME Supelco mixture (Supelco, USA) (purity; $\geq 99\%$ (GC); Sigma-Aldrich, Germany) were purchased from LaborPharm (Almaty, Kazakhstan). All chemicals (methanol, toluene, glacial acetic acid, hydrochloric acid, potassium hydroxide and sodium hydroxide, n-hexane) were pure for analytical reagents and were purchased from LaborPharm (Almaty, Kazakhstan), which had a higher purity (System, Malaysia, for GC $\geq 99\%$).

Approximately 1 g of samples of fat-and-oil products were melted in an oven (Binder BD115 thermostat, Germany) at 40°C–50°C to obtain the fat phase. The upper-fat phase was removed after centrifugation at 500 g (centrifugal acceleration) for 4 min (Sigma 2-16P, Germany) (Customs Union Commission, 2011), then dried by adding anhydrous sodium sulphate to remove moisture from the margarine. The fat obtained from the samples of fat-and-oil products was transferred to 5 mL glass vials.

Two mL of hexane were added to 20 μ L of fat obtained from samples of fat-and-oil products. Subsequently, 100 mL of sodium methylate solution (2.7 g of sodium metallic Na in 25 mL of methanol CH₃OH) was added, and the mixture was shaken for 30 s (Ika, Vortex Genius 3, Germany). It was incubated at room temperature for 10 min to separate the solution of the transparent layer

containing FAME from the opaque water layer. Then the solution was centrifuged at 3,000 rpm, for 5 min (Çetin *et al.*, 2003).

All measurements were carried out using the Thermo Scientific™ TSQ 8000™ triple quadrupole GC-MS/MS system equipped with the Thermo Scientific™ TRACE™ 1310 GC with SSL Instant Connect™ SSL module and Thermo Scientific™ TriPlus™ RSH autosampler. The method details are presented in *Table 1*.

TABLE 1. TRACE 1310 GC AND TSQ 8000 MS/MS METHOD PARAMETER

TRACE 1310 GC	
Injection mode	Splitless
Splitless time	1.0 min
GC column	Restek™ RTX™-5Sil MS, 60 m × 0.25 mm × 0.25 µm
Carrier gas	He (99.999%)
Flow	1.5 mL/min, constant flow
Temperature programme	50°C, 5 min 5°C/min to 220°C, 20 min
Transfer line temperature	230°C
Total analysis time	14.6 min
TriPlus RSH Autosampler	
Injection volume	1 µL
TSQ 8000 MS/MS	
Ionisation mode	EL, 70 eV
Ion source temperature	285°C
Scan mode	SRM using timed SRM
SRM transition setup	Automatically build-up by AutoSRM software

RESULTS AND DISCUSSION

The results of the fatty acid composition of the analysed samples of fat-and-oil products – margarine (M-A, M-B, M-C, M-D, M-E, M-F, M-G), are presented in *Table 2* and 3, while spreads (S-A, S-B, S-C, S-D, S-E, S-F, S-G, S-H, S-I, S-J) are presented in *Table 4* and 5.

Differentiation of vegetable fats of various origins is primarily associated with a certain set of fatty acids that make up their composition and determine the species of vegetable raw materials. Fatty acids are a biological marker that allows determining with a high degree of probability whether a fat belongs to a certain type due to their specificity. They may contain one or more double bonds in their structure, and therefore, are classified into saturated and unsaturated.

The biologically complete fat base of margarine has a balanced fat composition. It is dominated by unsaturated fatty acids, the content of linoleic acid should be about 40.00% and the ratio of polyunsaturated to saturated acids should approach 2:1.

The presence of linoleic acid C18:2 in the studied samples M-B (9.58%) and M-C (4.35%) showed a very low content of fatty acid. In the remaining samples, the content of linoleic acid C18:2 is in the range of 16.24% to 27.54%.

Table 2 demonstrates that the dominant fatty acids among the saturated group in the analysed margarine samples: Capronic C6:0, caprylic C8:0 and capric C10:0 are almost all below the detection limit of 0.10% or completely absent. Lauric fatty acid C12:0 content is in the amount of up to 0.90%. The content of myristic acid C14:0 is in the range of up to 2.00%.

TABLE 2. FATTY ACID COMPOSITION OF MARGARINE SAMPLES (SATURATED FATTY ACIDS, AVERAGE PERCENTAGE)

Component name	Rt	M-A	M-B	M-C	M-D	M-E	M-F	M-G
C4:0	6.22	N/A	N/A	0.00	0.01	N/A	0.02	0.00
C6:0	11.18	N/A	N/A	0.00	0.01	0.00	0.02	0.00
C8:0	16.46	0.01	0.03	0.01	0.04	0.06	0.05	0.03
C10:0	21.07	0.01	0.04	0.02	0.11	0.08	0.08	0.08
C11:0	23.10	N/A	0.00	0.00	0.00	N/A	N/A	N/A
C12:0	25.06	0.18	0.07	0.04	0.41	0.92	0.55	0.45
C13:0	26.86	0.01	0.00	N/A	0.01	0.00	0.00	0.01
C14:0	28.61	1.21	0.42	0.24	1.62	1.36	1.48	1.45
C15:0	30.21	0.06	0.07	0.03	0.13	0.06	0.09	0.09
C16:0	31.82	28.49	15.94	7.90	25.52	30.20	31.05	30.30
C17:0	33.24	0.14	0.14	0.08	0.18	0.12	0.15	0.14
C18:0	34.72	5.41	12.19	6.40	4.92	6.15	5.72	6.70
C20:0	36.63	0.06	0.66	0.56	0.04	0.03	0.03	0.03

TABLE 2. FATTY ACID COMPOSITION OF MARGARINE SAMPLES (SATURATED FATTY ACIDS, AVERAGE PERCENTAGE)
(continued)

Component name	Rt	M-A	M-B	M-C	M-D	M-E	M-F	M-G
C21:0	38.54	0.10	0.01	0.68	0.07	0.01	0.01	0.00
C22:0	39.19	0.07	0.03	0.39	0.03	0.00	0.00	0.00
C23:0	40.27	0.88	0.13	4.74	0.77	0.09	0.01	0.03
C22:2	41.03	1.89	0.35	5.94	1.64	0.36	0.08	0.06
C24:0	41.22	0.73	0.08	4.40	0.62	0.05	0.03	0.05
C22:6	44.49	0.18	0.00	2.15	0.18	0.02	0.01	0.01

Palmitic acid (C16:0) is predominant in all samples, with its highest presence in M-F (31.05%) and the lowest in M-C (7.90%). Stearic acid (C18:0) shows variability across samples, ranging from 4.92% in M-D to 12.19% in M-B. Shorter-chain fatty acids, such as C4:0 and C6:0, are either in trace amounts or not detected in the samples. Fatty acids like C23:0, C22:2 and C24:0 show considerably high percentages in the M-C sample, indicating a distinct fat source or processing method for this sample. Although the focus is on saturated fatty acids, it is noteworthy that certain unsaturated fatty acids like C22:2 and C22:6 are listed, indicating the presence of polyunsaturated fats in the samples.

The content of palmitic acid C16:0 does not exceed 35.00%. Fatty acid C17:0 is present in an amount not exceeding 0.20%. The content of stearic fatty acid C18:0 in margarine samples does not exceed the amount of up to 7.00% (Nurgalieva, 2019). The exception is a sample of margarine of the M-B brand, where a sufficiently high content of stearic acid C18:0 12.19% was found, which suggests the presence of animal impurities in this margarine, or rather pork fat (Pedersen & Kirkhus, 2008). The introduction of animal fats complicates the identification of the product and requires additional identification criteria and methods for their determination. The content of arachinic acid C20:0 is in the range of up to 0.60% and begenic acid C22:0 to 0.20%.

Table 3 indicates the content of monounsaturated (MUFA) and PUFA in margarine samples. In the samples of margarine M-B, the content of fatty acid C18:1 turned out to be in the amount of 12.19%, and the content of C18:2 – 10.87% of *trans* isomers, indicating the presence of hydrogenated fats in the composition of the product. In the sample of margarine M-C, the content of fatty acid C18:2 (7.27%) also considerably exceeds the limit, C18:1 within the normal range up to 0.30%. In other samples, *trans* isomers are present in extremely small amounts (up to 0.40%). In the M-A sample, there are no *trans* fats at all.

Sample M-C shows a different composition profile, with higher values of certain fatty acids

compared to other samples. High in MUFA: Margarines across the board seem to have a high percentage of MUFA, especially C18:1 n9c (oleic acid). The inclusion of omega-6 and omega-3 fatty acids indicates these margarines could contribute to essential fatty acid intake. Some samples contain TFAs. Consumption of these is generally advised against due to potential health concerns. The varied composition across samples suggests the use of different oils or processing techniques in the production of these margarines. Thus, according to Table 2, the spectrum of fatty acids and the ratio of their content indicate the presence of oils with a mass fraction of palmitic acid of more than 17% (Jala & Kumar, 2018). This group includes the liquid fraction of palm oil, referred to in commercial and industrial practice as palm olein, and the solid fraction, known as palm stearin. A fraction that is a substitute for cocoa butter can also be obtained from palm oil by double fractionation. Due to the high content of solid triglycerides, palm oil and its fractions are widely used in the production of margarines and spreads, manifesting the so-called post-crystallisation property–solidification upon storage (Jala & Kumar, 2018).

Analysis of the fatty acid composition of margarine in Table 2 indicates a complete absence of butter (milk) fat, this is evidenced by the absence of butyric acid C4:0, capronic acid C6:0 and myristoleic acid C14:1 inherent in milk fat. Caprylic C8:0 and capric C10:0 show a fatty acid content of less than 0.1%. In mixtures, vegetable fats are usually in free or hydrogenated form. In hydrogenated forms, the content of TFA that is harmful to health is elevated. When interpreting the data in Table 2, the content of *trans* isomers in the studied margarine products showed a result of less than 2.0%. However, two samples M-B (C18:1 n9t – 12.19%, C18:2 n6t – 10.87%) and M-C (C18:2 n6t – 7.27%) contain TFAs in an amount that is higher than the permissible requirements of technical regulations. The fat component of spreads is dominated by unsaturated fatty acids (both MUFA and PUFA), and the amount of linoleic acid varies from 5.00% to 45.00% depending on the type of spread.

TABLE 3. FATTY ACID COMPOSITION OF MARGARINE SAMPLES (UNSATURATED FATTY ACIDS, AVERAGE PERCENTAGE - FAMES)

Component name	Rt	M-A	M-B	M-C	M-D	M-E	M-F	M-G
C14:1	29.46	0.01	0.02	0.01	0.07	0.01	0.03	0.03
C15:1	31.07	0.01	0.00	0.00	0.02	0.03	0.00	0.01
C16:1	32.41	0.27	0.18	0.11	0.33	0.22	0.24	0.23
C17:1	33.88	0.03	0.05	0.06	0.05	0.03	0.03	0.03
C18:1_n9t	34.99	0.00	12.19	0.27	0.19	0.23	0.07	0.38
C18:1_n9c	35.20	25.35	24.62	14.25	23.65	28.02	27.93	27.98
C18:2_n6t	35.57	0.01	10.87	7.27	0.47	0.06	0.56	0.11
C18:2_n6c	36.06	16.24	9.58	4.35	24.04	24.77	27.54	27.03
C18:3_n6c	37.10	0.14	0.96	0.86	0.30	0.21	0.28	0.27
C20:1_n9c	37.34	1.23	1.33	3.26	1.07	0.79	0.78	0.82
C18:3_n3c	37.80	0.44	0.73	1.22	0.42	0.29	0.30	0.30
C20:3_n3	39.57	2.78	2.29	0.16	0.00	1.20	0.76	0.94
C22:1_n9	39.64	2.78	2.29	9.64	2.90	1.22	0.77	0.95
C20:3_n3	39.77	2.78	2.29	9.64	2.90	1.22	0.77	0.95
C20:4_n6	40.68	1.89	0.35	0.29	1.64	0.36	0.08	0.06
C22:2	41.03	1.89	0.35	5.94	1.64	0.36	0.08	0.06
C20:5_n3	42.53	4.42	1.69	8.75	3.78	1.45	0.42	0.42
C24:1_n9c	43.22	2.06	0.37	5.59	1.78	0.39	0.06	0.04
C22:6	44.49	0.18	0.00	2.15	0.18	0.02	0.01	0.01

TABLE 4. FATTY ACID COMPOSITION OF SPREAD SAMPLES (SATURATED FATTY ACIDS, AVERAGE PERCENTAGE)

No.	Component name	Expected RT	Area %									
			S-A	S-B	S-C	S-D	S-E	S-F	S-G	S-H	S-I	S-J
1	C4:0	6.22	N/A	0.48	0.38	0.00	0.54	0.01	0.08	0.04	0.47	0.47
2	C6:0	11.18	0.00	0.49	0.37	0.00	0.53	0.01	0.09	0.04	0.45	0.50
3	C8:0	16.46	0.03	0.45	0.34	0.02	0.47	0.04	0.1	0.09	0.38	0.43
4	C10:0	21.07	0.04	1.29	0.91	0.03	1.26	0.07	0.26	0.23	0.96	1.07
5	C11:0	23.1	N/A	0.03	0.02	N/A	0.02	N/A	0.01	0	0.02	0.02
6	C12:0	25.06	0.53	1.99	1.22	0.32	1.98	0.53	0.61	0.24	1.47	1.43
7	C13:0	26.86	N/A	0.07	0.04	0.00	0.06	0.00	0.01	0.00	0.04	0.04
8	C14:0	28.61	1.10	6.35	4.17	1.39	6.57	2.14	2.12	1.01	4.58	5.16
10	C15:0	30.21	0.05	0.82	0.50	0.07	0.8	0.10	0.20	0.12	0.61	0.67
12	C16:0	31.82	23.15	31.01	22.35	34.47	31.82	0.78	26.31	16.33	24.2	21.33
14	C17:0	33.24	0.15	0.56	0.33	0.15	0.55	0.16	0.22	0.16	0.40	0.44
16	C18:0	34.72	4.03	10.6	13.44	7.11	8.23	0.66	5.37	23.31	7.88	13.09
21	C20:0	36.63	0.06	0.15	0.11	0.07	0.14	0.32	0.09	0.20	0.11	0.09
25	C21:0	38.54	0.64	0.02	0.02	0.01	0.01	N/A	0.01	0.00	0.00	0.01
26	C20:2	38.65	0.64	0.02	0.00	0.01	0.02	N/A	0.01	0.00	0.00	0.01
27	C22:0	39.19	0.39	0.06	0.09	0.02	0.06	1.45	0.11	0.51	0.15	0.44
31	C23:0	40.27	3.71	0.02	0.01	0.00	0.00	0.13	0.01	0.08	0.01	0.06
33	C22:2	41.03	4.59	0.02	0.03	0.01	0.00	0.15	0.00	0.00	0.01	0.01
34	C24:0	41.22	4.59	0.01	0.03	0.02	0.00	0.15	0.01	0.02	0.03	0.03
37	C22:6	44.49	1.82	N/A	N/A	N/A	N/A	0.04	N/A	N/A	N/A	N/A

The right choice of fat compositions and their optimal combination with milk fat in a suitable ratio of fat component and milk plasma allows this product to be considered as an improvement with a more balanced fatty acid composition.

The distinctive features of the composition of fatty acids of natural milk fat are the presence of butyric C4:0, capronic C6:0, caprylic C8:0, capric C10:0, and myristoleic C14:1 acids, as well as minor components (pentadecanoic C15:1, palmitoleic C16:1, and margaric C17:1 acids). In the spread samples, these acids are present in an amount below the permissible limit, which indicates the absence of a fatty phase of milk fat in the composition of this product (butyric acid C4:0 varies from 0.00% to 0.54%, capronic acid C6:0 (0.00% to 0.53%), caprylic acid C8:0 (0.02% to 0.47%), caprine C10:0 (0.03% to 1.29%), myristolein C14:1 (0.00% to 0.74%).

The presence of pentadecanoic C15:1 (from 0.00% to 0.22%), palmitoleic C16:1 (0.20% to 1.15%) and margaric C17:1 acids (from 0.05% to 0.24%) also show a low content. Thus, almost all samples lack butyric acid and other low molecular weight acids, including myristic acid. These spread samples have no fat derived from milk, only fats of non-dairy origin are present.

The following spread samples contain oils with a mass fraction of palmitic acid C16:0 of more than 17%: S-A (23.15%), S-B (31.01%), S-C (22.35%), S-D (34.47%), S-E (31.82%), S-G (26.31%), S-I (24.2%),

and S-J (21.33%). The samples of the spreads S-F (0.78%) and S-H (16.33%) have a very low content of palmitic acid.

A sufficiently high content of stearic acid C18:0 in the following spread samples suggests the presence of animal fat in the composition of the product: S-B (10.60%), S-C (13.44%), S-D (7.11%), S-E (8.23%), S-H (23.31%), S-I (7.88%), S-J (13.09%). The introduction of animal fats complicates the identification of the product by its fatty acid composition and requires other identification criteria and methods for their determination.

The samples S-A, S-B, S-C, S-D, S-E, S-G, S-H, S-I, and S-J, with high oleic acid content (C18:1) ranging from 19.38% to 35.67%, indicate the presence of palm kernel oil or its fractions, such as palm stearin, contrasting with palm kernel olein of 22.80% and palm kernel oil with 15.10% oleic acid content.

The analysis of the data in Table 4 showed a low content of up to 2.00% of fatty acids C6:0-C12:0 in the samples under study. This indicates that the fatty acid composition of these samples does not contain lauric group oils or low molecular weight fatty acids of more than 2.00%.

The content of erucic acid C22:1 is in the range of 0.02 to 2.27%, which indicates the absence in these samples of oils with a high mass fraction of erucic acid, such as rapeseed, cabbage oil (more than 3.00%), mustard (more than 5.00%). The spread samples have an extremely low content of linolenic

TABLE 5. FATTY ACID COMPOSITION OF SPREAD SAMPLES (UNSATURATED FATTY ACIDS, AVERAGE PERCENTAGE – FAMES)

No.	Component name	Expected RT	Area %									
			S-A	S-B	S-C	S-D	S-E	S-F	S-G	S-H	S-I	S-J
9	C14:1	29.46	N/A	0.71	0.42	0	0.74	0.01	0.13	0.07	0.51	0.59
11	C15:1	31.07	0	0.22	0.09	0.01	0.21	0.07	0.02	0.06	0.13	0.15
13	C16:1	32.41	0.23	1.06	0.7	0.25	1.15	0.47	0.43	0.2	0.77	0.76
15	C17:1	33.88	0.06	0.24	0.12	0.03	0.22	0.05	0.07	0.05	0.21	0.17
17	C18:1_n9t	34.99	0.04	0.66	0.05	0.00	0.06	9.32	0.00	0.00	0.00	0.25
18	C18:1_n9c	35.20	19.43	26.23	28.91	33.35	29.06	9.32	25.28	35.67	22.01	19.38
19	C18:2_n6t	35.57	0.34	0.58	11.71	0.64	0.65	32.86	25.28	9.18	22.01	19.38
20	C18:2_n6c	36.06	12.09	11.94	7.39	20.19	13.33	22.27	11.43	5.55	9.36	7.72
22	C18:3_n6c	37.1	0.27	0.32	0.23	0.27	0.37	0.09	0.30	0.28	0.52	0.67
23	C20:1_n9c	37.34	2.81	0.80	0.95	0.82	0.60	0.60	0.30	0.55	0.74	0.92
24	C18:3_n3c	37.8	0.92	0.24	0.34	0.31	0.22	0.31	0.19	0.42	0.21	0.46
28	C20:3_n3	39.57	0.12	0.1	0.03	0.02	0.10	1.20	0.02	0.51	0.05	0.05
29	C22:1_n9	39.64	0.12	1.17	2.27	0.16	0.08	1.59	0.02	0.28	0.06	0.05
30	C20:3_n3	39.77	6.55	1.17	2.27	0.16	0.1	1.58	0.02	0.28	0.06	0.33
32	C20:4_n6	40.68	0.29	0.02	0.03	0.05	0.02	0.14	0.01	0.03	0.02	0.04
33	C22:2	41.03	4.59	0.02	0.03	0.01	0.00	0.15	0.00	0.00	0.01	0.01
35	C20:5_n3	42.53	6.98	0.09	0.07	0.03	0.02	13.18	0.85	4.38	1.53	3.75
36	C24:1_n9c	43.22	4.21	0.00	0.01	N/A	N/A	0.21	0.01	0.09	0.02	0.04
37	C22:6	44.49	1.82	N/A	N/A	N/A	N/A	0.04	N/A	N/A	N/A	N/A

acid C18:3 (0.09%-0.67%). These spreads do not contain oil, with a high content of linolenic acid (from 2.00% to 20.00%). Oils with the maximum mass fraction of linoleic acid C18:2 (26.00%-81.00%) are also not present in these spreads, the content of linoleic acid varies from 5.55% to 22.27%.

When interpreting the data in Table 4, the content of *trans* isomers in the samples showed the highest possible content of C18:2 n6t – S-F (32.86%), S-G (25.28%), S-I (22.01%) and S-J (19.38%), S-C (11.71%), S-H (9.18%). In the S-F sample (9.32%), the content of C18:1 n9t *trans* isomers also shows a result above the level allowed by the technical regulations. In the remaining samples of the spreads under study, *trans* fats are found in an amount of less than 2.00%.

The positive health effects of all categories of emulsified fat products (from margarine to spreads) made from mixtures of natural vegetable oils should contain less SFA. C18:1 MUFA and C18:2 PUFA are oxidised, respectively, 10 and 100 times faster than C18:0 PUFA (Kodali *et al.*, 2001). The content of SFA in emulsion fat products should be as low as possible, and PUFA - as high as possible and preferably include both omega-6 and omega-3.

Unsaturated fatty acids of natural origin contained in raw and delicatessen oils and fats have only *cis*-double bonds (Figure 5). The main source of TFA is partially hydrogenated oils (PHOs), which are formed as a result of partial hydrogenation. PUFAs are usually converted into MUFA-TFA, and therefore PHOs have increased oxidation resistance. The REPLACE initiative by the World Health Organization (WHO) provides guidelines for regulating food safety issues. It proposes a strategic approach to implementing legislative measures that aim to eliminate industrially produced TFAs from fat products. The ultimate goal is to achieve a worldwide ban on the use of TFAs by 2023 and replace them with oils that have a higher proportion of PUFAs and a lower proportion of SFAs.

Palm oil is rich in saturated fats, specifically palmitic acid, comprising about 44% of its content. Palm oil does not contain *trans* fats, the hydrogenation process used to produce margarine or spreads can lead to the formation of these unhealthy fats. *Trans* fats can increase LDL cholesterol and decrease 'good' cholesterol, known as high-density lipoprotein (HDL), which further contributes to heart disease risk (Urugo *et al.*, 2021).

According to the results of the fatty acid composition of the analysed margarine samples (Figure 1), the total content of saturated acids is as follows:

M-A (37.36%), M-B (29.78%), M-C (25.49%), M-D (34.49%), M-E (39.13%), M-F (39.29%), M-G (39.36%), unsaturated fatty acids (both monounsaturated M-A (31.74%), M-B (28.86%), M-C (32.92%), M-D

(29.87%), M-E (30.71%), M-F (29.84%), M-G (30.09%) and polyunsaturated M-A (30.86%), M-B (18.25%), M-C (34.04%), M-D (34.97%), M-E (29.88%), M-F (30.25%), M-G (30.04%). Fatty acid *trans* isomers account for M-B (23.06%), M-C (7.54%), M-D (0.66%), M-E (0.29%), M-F (0.63%) and M-G (0.49%).

According to the results of the fatty acid composition of the analysed samples of spreads (Figure 2), the total content of saturated acids is S-A (38.47%), S-B (54.4%), S-C (44.33%), S-D (43.68%), S-E (52.5%), S-F (6.55%), S-G (35.61%), S-H (42.34%), S-I (41.76%), S-J (45.28%), unsaturated fatty acids (both monounsaturated S-A (26.86%), S-B (30.43%), S-C (33.47%), S-D (34.62%), S-E (32.06%), S-F (12.32%), S-G (26.26%), S-H (36.97%), S-I (24.45%), S-J (22.06%), and polyunsaturated S-A (34.27%), S-B (13.92%), S-C (10.39%), S-D (21.05%), S-E (14.18%), S-F (38.92%), S-G (12.83%), S-H (11.45%), S-I (11.76%), S-J (13.04%).

Fatty acid *trans* isomers account for S-A (0.38%), S-B (1.24%), S-C (11.76%), S-D (0.64%), S-E (0.71%), S-F (42.18%), S-G (25.28%), S-H (9.18%), S-I (22.01%), S-J (19.63%).

As a result of the study performed, the opportunity to use an improved technique for identifying fat traces and objects of biological origin by fatty acid composition using high-resolution mass spectrometry with the highest possible degree of detail in fat-and-oil products is shown (Figure 2).

Thus, in the composition of the fat phase of spreads and margarines, the best and most beneficial for human health is the use of mixtures of vegetable oils with a balanced composition of PUFA, which are free of *trans* isomers or presence in minimal amounts (Figure 1). To this end, many manufacturers of spreads limit the use of hydrogenated fats, which are the main sources of *trans* isomers. However, the prototypes of most of the spreads showed the highest possible content from 9.18% to 42.18%. In the margarine M-B sample, the content of *trans* isomers is also extremely high at 23.06%.

Gas chromatography-mass spectrometry (GC-MS) is a vital analytical tool for the qualitative and quantitative analysis of complex mixtures. GC-MS is a powerful hyphenated technique that has emerged as an indispensable tool for the analysis of complex mixtures, particularly in identifying and characterising volatile and thermally stable constituents in various samples. GC-MS couples the separating power of gas chromatography with the detection and identification capability of mass spectrometry. In the process, the compounds separated by the GC column enter a mass spectrometer, where they are ionised and fragmented. The resulting ion fragments are then detected and can be used to identify the compound based on its unique mass spectrum. GC-MS is extensively used to detect and quantify pollutants in air, water and soil samples (Nekoei & Mohammadhosseini, 2014).

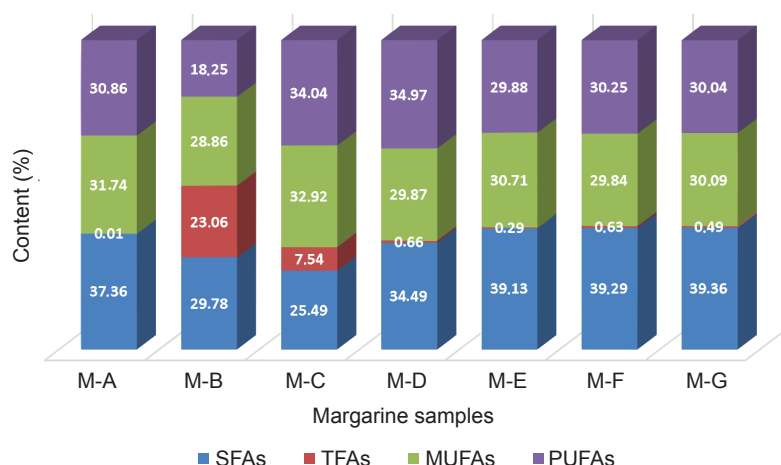


Figure 1. The content of SFAs, TFAs, MUFAs and PUFAs in margarine samples.

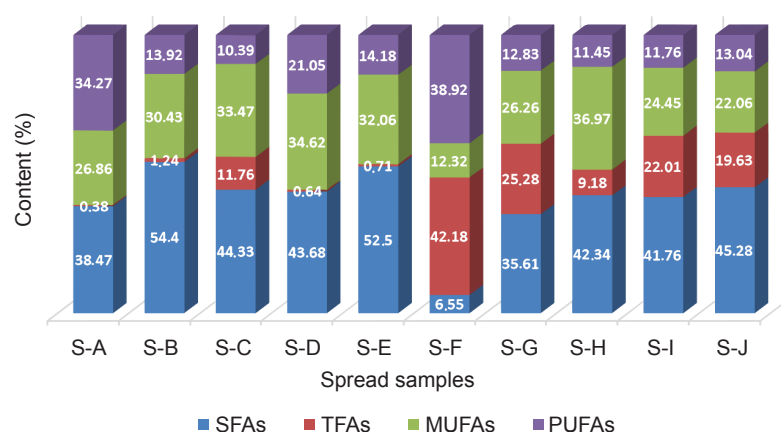


Figure 2. The content of SFAs, TFAs, MUFAs and PUFAs in spread samples.

This technique can identify trace amounts of harmful substances and contaminants, as well as profile the flavour and aroma compounds (Quan *et al.*, 2023). GC-MS can determine substances in blood and urine samples, playing a crucial role in forensic investigations and drug testing (Mohammadhosseini *et al.*, 2016). In drug development and quality control, GC-MS can identify and quantify compounds in formulations. It's a primary tool for identifying constituents of essential oils, plant extracts and other natural products (Mohammadhosseini *et al.*, 2023).

GC-MS stands as a foundational pillar in the realm of analytical methodologies due to its manifold advantages. One of its primary strengths lies in its high sensitivity. This analytical tool is adept at detecting even trace amounts of compounds, making it invaluable in analyses that necessitate the identification of substances present in minute quantities. Coupled with its sensitivity, GC-MS boasts exceptional specificity. This is attributed to the seamless amalgamation of chromatographic separation with mass spectral detection. Such a

combination ensures that compounds undergo not just separation but are also pinpointed with precision based on their unique mass spectra.

The versatility of GC-MS is evident in its capacity to analyse a vast spectrum of volatile and semi-volatile compounds. This trait renders it applicable across a myriad of research arenas, encompassing fields like environmental studies, forensics, food analytics and pharmaceuticals. Beyond its qualitative prowess, GC-MS excels in quantitative analysis, furnishing detailed data about the analytes. This capability is bolstered by the rapidity with which modern GC-MS instruments can yield results, facilitating high-throughput analysis. Another salient feature of GC-MS is its advanced software which possesses deconvolution capabilities. This allows for the disentanglement of overlapping peaks, enhancing the potential to discern compounds even in intricate mixtures. Furthermore, the mass spectra gleaned can be juxtaposed against extensive libraries, simplifying compound identification. From a structural perspective, the fragmentation patterns

discerned in the mass spectra can shed light on the structural nuances of unknown compounds. This is further complemented by the fact that many samples, especially those with volatility, demand minimal preparation pre-analysis. Lastly, in the broader context of societal well-being, GC-MS plays an instrumental role. Its proficiency in detecting pollutants and contaminants underscores its importance in safeguarding environmental and health standards. The multifaceted benefits of GC-MS underscore its pivotal position in analytical chemistry, with its influence permeating diverse scientific fields.

CONCLUSION

The conditions for the preparation of samples of emulsion fat products (margarine, spreads) have been optimised: A solvent was selected for the extraction of the fat phase of the product (hexane); the volume of sodium methylate (2 M) required for the esterification reaction (50 µL) and other non-polar and low-polar organic solvents reaction time for complete saponification of triglycerides (15 min). Using the presented methodology, experimental samples of fat-and-oil products (7 samples) and spreads (10 samples) were analysed.

The obtained fatty acid composition is used to deduce whether the sample belongs to a particular type of object of plant or animal origin. The analytical approaches to the identification of objects by fatty acid composition described in this paper can be used to determine and detect the falsification of fat-and-oil products by an instrumental method using high-resolution mass spectrometry. This method is also applicable for the identification of undeclared animal fats in the composition of fat-and-oil products labelled "halal" but requires further scientific research to develop additional approaches and criteria for their identification.

The data of the literature review and the complex investigations conducted on the problem under study suggest that the production of emulsion fat products (from margarine to spreads) made from mixtures of vegetable and animal fats can be the subject of further scientific research and technological developments aimed at ensuring a healthy diet and food safety issues.

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THE FIRST RECORD OF THE WHITE-THROATED KINGFISHER, *Halcyon smyrnensis*, BREEDING IN BORNEO, INDONESIA

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ABSTRACT

The White-throated kingfisher, *Halcyon smyrnensis*, is widely distributed from the Middle East throughout most of Southeast Asia except for the island of Borneo, where it has been recorded as an occasional visitor but not yet as a resident breeder. During standard biodiversity monitoring activities, adult birds were regularly observed at PT Surya Sawit Sejati (PTSSS) estates. Regular monitoring of adult birds commenced and the location of observations was mapped. Records of birds in pairs suggested that these were indeed pairs and subsequent monitoring confirmed this. Here we present comprehensive evidence of the White-throated kingfisher as a resident breeder in Central Kalimantan, Indonesian Borneo.

Keywords: Borneo, *Halcyon smyrnensis*, resident breeder, White-throated kingfisher.

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INTRODUCTION

The White-throated kingfisher, *Halcyon smyrnensis*, is widely distributed from the Sinai Peninsula in the Middle East, through the Indian subcontinent to the Philippines and South, through West Malaysia, Sumatra to Java, Indonesia (BirdLife International, 2017; Iswandaru *et al.*, 2020; Taufiqurrahman *et al.*, 2019; Woodall & Kirwan, 2020) represented by six different subspecies (Andersen *et al.*, 2017). It is listed as *Least Concern* on the International Union for Conservation of Natures (IUCN) Red list. This is a large kingfisher species, measuring 27-28 cm in length, with a predominantly brown head and chest and blue back and wings (eBird, 2024). Its bill is red and the snow-white patch from the throat through the breast gives rise to its name (eBird, 2024). The adult has a bright blue back, wings, and tail. Its head, shoulders, flanks, and lower belly are chestnut and the throat and breast are white. The large bill and legs are bright red. In most of its distribution range,

it is observed as a resident breeder in a variety of habitats, along waterways and mangroves as well as in human-altered landscapes (Ali *et al.*, 2010; Burton, 1998; Palkar *et al.*, 2009; Reza *et al.*, 2003), where it feeds on a wide variety of prey, including small reptiles, amphibians, crabs and birds (Burton, 1998; Purandare, 2008; Roberts & Priddy, 1965; Tehsin, 1995; Yahya & Yasmin, 1991). The breeding season appears to span over March-June depending on the location in its distribution range (Ali *et al.*, 2010; Prakash *et al.*, 2021; Rehman *et al.*, 2022; Sela-Klein *et al.*, 2023). Nesting holes are excavated in vertical earth or riverbanks and measure approximately 30-152 cm deep (Ali *et al.*, 2010; Palkar *et al.*, 2009). There remain only a few recent records from Western Java (Taufiqurrahman *et al.*, 2019), which may suggest that the species may be dispersing eastwards, where habitat is plentiful, because of its adaptation to human-dominated landscapes. It has been infrequently recorded in Borneo, especially along the western coastal area (eBird, 2023).

Considering the species-wide distribution range, its IUCN status, its adaptability across a wide range of habitats, and its presence already in Borneo, means it is only a matter of time before some individuals establish themselves as residential breeders in Borneo too. Here we report on sightings of White-throated kingfisher breeding in the far south of Central Kalimantan, Indonesia.

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MATERIALS AND METHODS

The observations took place in United Plantations Bhd's (UP) subsidiary plantations, PT Suria Sawit Sejati (PTSSS) in Central Kalimantan, Indonesia (Figure 1), where the White-throated kingfisher was first spotted in the plantations in Lada Estate, in November 2017. The area consists primarily of oil palm monoculture, with patches of degraded mixed tropical rainforest, peat forest and riverine habitat. At the time, it was considered a vagrant to Borneo and we categorised it as a general observation. The first detailed observation took place when an individual *Halcyon smyrnensis* perched on a tree branch on the 19th of October 2019. Subsequently, patrol teams and staff made *ad hoc* observations using binoculars (Nikon Monarch 7 10x48) and a camera (Nikon D800E, Tamron SP 150-600 mm lens) with each entry added to a database. A Digital inspection camera, CEN-TECH®, 2.4-inch LCD was used to check nesting holes for eggs and chicks.

RESULTS AND DISCUSSION

The perch site where the first White-throated kingfisher was observed was used extensively

and made it possible to obtain clear photographic evidence of the bird (Figure 2). From the perching behaviour and the potential time of the year for breeding, we assumed that the perching individual was a male overlooking his territory. After a week of *ad hoc* observations, the bird was observed flying to a small grassy earth bank forming part of a backyard garden of a staff-house, adjacent to a small riparian forest reserve. Here it had excavated a nesting hole (Figure 3) and was later spotted with what we assumed was a female. Subsequent frequent use of the nesting hole suggested that the pair had produced chicks, and this was confirmed using a digital inspection camera (Figure 4). During the following 18 months, White-throated kingfishers were regularly observed, with an average of 14.5 observations/month ($n = 176$, SD 8.3, range 2-31), with December 2019 peaking with 28 observations (Table 1). From November 2017 until March 2021, we observed the species 183 times (Table 1).

We recorded a total of seven nesting holes, all of which were located in earth banks in the oil palm estates. These were either earth banks on roadsides or along ditches and canals in the estates. The diameter of the entrance holes averaged 5.9 cm ($n = 7$, SD ± 0.3 , range 5.5-6.5 cm) with the distance

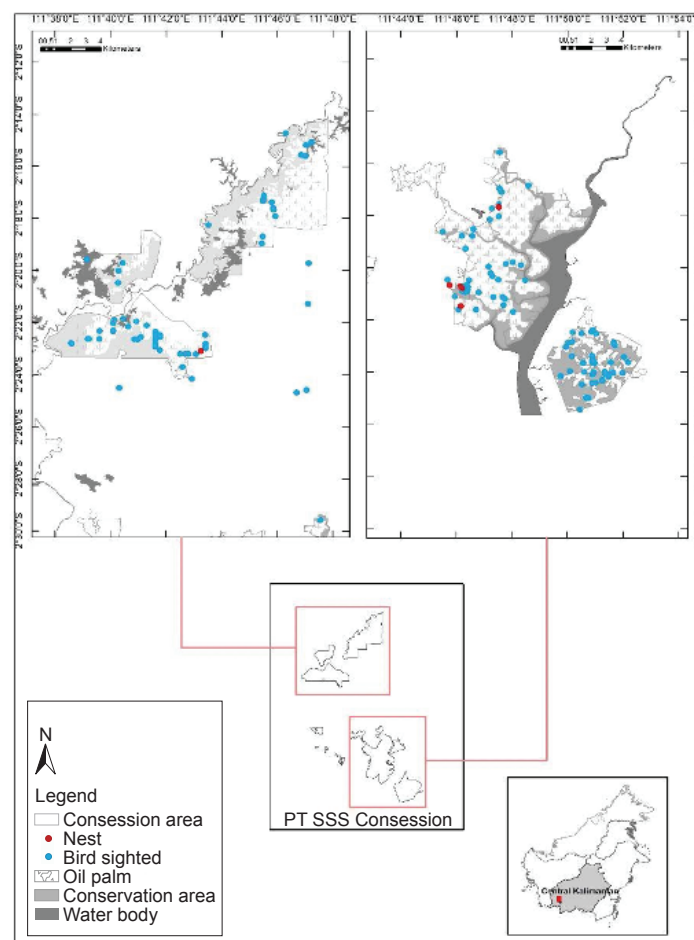


Figure 1. Study location, observation records, and nest locations of the White-throated kingfisher.



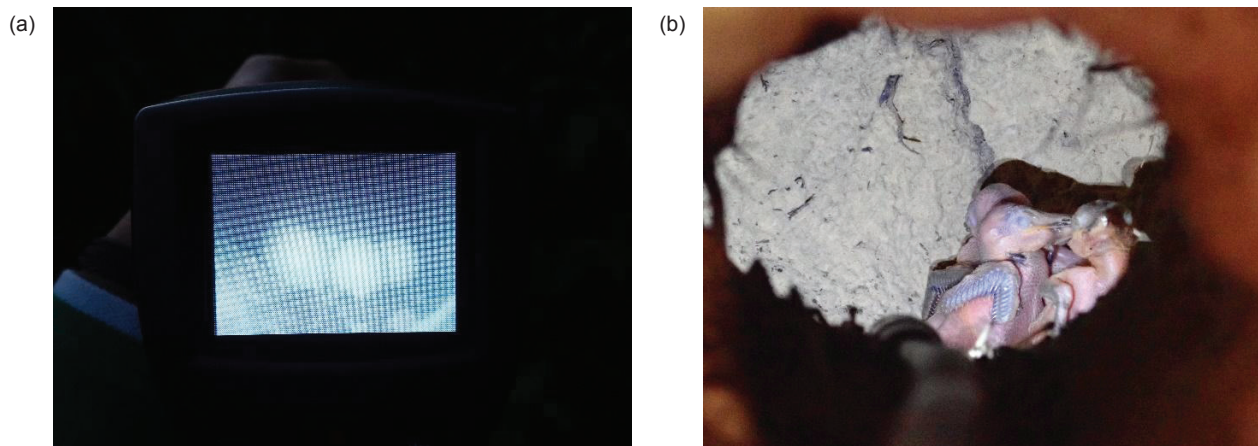
Source: Silmi (2019).

Figure 2. A White-throated kingfisher on its favourite perch site, Lada Estate, Central Kalimantan, Borneo.



Source: Silmi (2019).

Figure 3. Nesting hole at a small earth bank adjacent to staff quarters, Lada Estate, Central Kalimantan, Borneo. The nest was regularly inspected for eggs and chicks.



Source: Silmi (2019).

Figure 4. (a) The kingfisher pair produced five eggs and (b) which all successfully hatched.

from the entrance to the nest chamber at 52 cm (Table 2). From photographic records, we estimated the nest chamber dimensions at 14-20 cm wide and 12-14 cm high. Only three of seven nests had eggs/chicks in them (Table 2). The first record was in a nest at the earth bank in Lada Estate on the 1st of November 2019 (Figure 4). On the 29th of November, 2019, a second nesting hole with four chicks was found, estimated at 4-5 days old. Finally, a third nest was found with three eggs on the 1st of December 2019. This differed from the March-April breeding season recorded in Tamil Nadu, India (Ali *et al.*, 2010), April-May in Israel (Sela-Klein *et al.*, 2023), April-June in Sri Lanka (Prakash *et al.*, 2021) and May-June in Pakistan (Rehman *et al.*, 2022).

Unfortunately, none of the eggs/chicks survived to fledging age, in all the natural nesting holes. The first nest was raided by a small water monitor lizard, *Varanus salvator*, whereas the second and third nests were excavated by humans (Figure 5), who collected

the eggs/chicks, likely for breeding and sale in Indonesia's sprawling bird trade (Harris *et al.*, 2017; Okarda *et al.*, 2022; Sy *et al.*, 2022).

To our knowledge, the observation of breeding White-throated kingfishers is the first of its kind in Indonesian Borneo. The first observed pair successfully produced five chicks (Figure 4) and this, combined with the numerous subsequent observations of other individuals dispersed across a large area, suggests that these are more than merely random incidents. The current population density in the study area, however, remains unknown, but considering the species' habitat and dietary flexibility (Burton, 1998; Purandare, 2008; Reza *et al.*, 2003; Roberts & Priddy, 1965; Tehsin, 1995; Yahya & Yasmin, 1991) our observations suggest that the species has established a local breeding population. It is also very likely that it can disperse further into Borneo and colonise suitable environments that have emerged following extensive loss of natural

TABLE 1. OBSERVATIONS OF WHITE-THROATED KINGFISHER FROM NOVEMBER 2017-MARCH 2021 IN UP/PTSSS' PALM OIL ESTATES AND ADJACENT AREAS, CENTRAL KALIMANTAN, INDONESIAN BORNEO

Month	Number of bird encounters				
	Year				
	2017	2018	2019	2020	2021
January				7	2
February			1	14	6
March			1	7	2
April			1	6	
May				2	
June				17	
July				27	
August			1	12	
September				14	
October			5	10	
November	1		8	6	
December		2	28	3	
Total	1	2	45	125	10

TABLE 2. NESTING HOLE CHARACTERISTICS OF WHITE-THROATED KINGFISHER OBSERVED IN UP/PTSSS' PALM OIL ESTATES AND ADJACENT AREAS, CENTRAL KALIMANTAN, INDONESIAN BORNEO

Nest hole	Entrance diameter (cm)	Hole depth (cm)	No. eggs/chicks
1	6.5	52	5 eggs
2	6.0	55	4 chicks
3	5.5	60	3 eggs
4	6.0	57	no egg and no chick
5	5.8	49	no egg and no chick
6	5.7	50	no egg and no chick
7	6.3	47	no egg and no chick
Ave ± SD	5.97 ± 0.32	52.86 ± 4.32	



Source: Silmi (2019).

Figure 5. (a) A nesting hole before it was raided by humans and (b) after. (c) The hole had clear “cut” marks suggesting it had been excavated by a sharp tool and the size sufficient for an average-sized hand/arm to enter into.

rainforest in the past two decades to agricultural development (Gaveau *et al.*, 2018; Meijaard *et al.*, 2018). Several “generalist” species such as the Leopard cat, *Priornailurus bengalensis* and Sumatran spitting cobra, *Naja sumatrana* thrive in oil palm landscapes (Rochmyaningsih, 2016; Silmi *et al.*, 2013; 2021), due to an abundance of prey and suitable breeding environment (Silmi *et al.*, 2021).

The main challenge for reproducing birds appears to be a large number of snake species in the study area, in particular, Sumatran spitting cobra, Bornean short python, *Python breitensteini* and reticulated python, *Malayopython reticulatus* that are known to predate on a wide variety of prey from eels, mammals and birds, including eggs and young chicks (Asad *et al.*, 2018; Chakravarty & Isaac, 2013; Manchi & Sankaran, 2009). The plantation also has several other species such, as the water monitor lizard and rats, *Rattus* spp., that are known to prey on either chicks or eggs (Duron *et al.*, 2017; Hughes *et al.*, 2019; Karunarathna *et al.*, 2015; Seto & Conant, 1996; Traeholt, 1993). Indeed, the first recorded nest was raided by a water monitor lizard. In addition, the two other nests with eggs/chicks were excavated by humans and are likely either sold to traders and breeders and/or raised until adulthood for sale (Nijman *et al.*, 2022; Rentschlar *et al.*, 2018). Indonesia is home to the world’s largest domestic bird trade and despite strict laws, illegal trade continues to persist across the archipelago. Four of the seven nest holes were empty. While these showed no signs of human excavation, these could have been raided by natural predators too. Although we need more data to predict the reproductive success of the species, three of seven confirmed failures in this study have a very high loss rate. However, during the post-study period, the species continued to be observed regularly, suggesting that it has successfully colonised the study area and possibly, beyond.

CONCLUSION

The ongoing habitat change following agricultural and urban development challenges many species to adapt to a changing environment. These challenges are amplified by the increasing impact of global warming. For some species, such changes can be disastrous with the risk of driving them into extinction. In contrast, species that exhibit high adaptive behavioural capacity, will gradually increase their distribution range and colonise areas where they were once absent, a decade ago. The species have colonised a variety of habitats from the Far West Asia to Southeast Asia and the Andamans. Our study provides solid evidence that the White-throated kingfisher has managed to colonise our study area and, thereby, expand its distribution range. This allopatric speciation suggests that the species will be able to adapt to climatic changes in the near future. Despite the high reproductive failure rate recorded, the abundance of wetlands, mangrove forests, streams, and plantation canals, teeming with small fish, amphibians, small lizards and insects, combined with streambanks and other potential nest hole sites, offer ideal feeding and reproductive conditions for the species. We anticipate that more breeding records of this species will emerge in the future, along the coastal areas of Kalimantan and inland. We recommend that future studies should focus on reproductive ecology and time activity budget.

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