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REVIEW ARTICLE

- Oil Palm Economic Performance in Malaysia and R&D Progress in 2025
- Paradigms and Knowledge Gaps in Oil Palm Stem Rots Caused by *Ganoderma*

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Vol. 38 (2) June 2026

CONTENTS

Cover picture:

1. Economic prosperity driven by Malaysia's oil palm industry.
2. Severe emergence of *Ganoderma* basidiocarps in oil palm infected with basal stem rot.

Journal of Oil Palm Research Vol. 38 (2) June 2026 p. 201–228
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OIL PALM ECONOMIC PERFORMANCE IN MALAYSIA AND R&D PROGRESS IN 2025

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ABSTRACT

The Malaysian oil palm industry recorded a strong performance in 2025, driven by productivity gains, replanting-led structural adjustments, and stabilised labour conditions. Crude palm oil (CPO) production increased by 4.9% to 20.28 million tonnes, supported by higher fresh fruit bunch (FFB) yields and improved labour efficiency, while the total oil palm area expanded marginally to 5.70 million hectares within a strictly capped land-use framework. Despite weaker export demand and elevated stock levels, sustained productivity improvements and ongoing structural reforms enhanced Malaysia's competitive position in the global market. Additionally, the advancements in research and development (R&D) continue to support the industry to remain resilient amid the rising global trade uncertainties and market volatility. This review synthesises recent industry statistics and selected literature on oil palm R&D to evaluate how current innovations address key sectoral constraints. While important progress is evident in breeding, disease management, traceability, waste management, biomass valorisation, and downstream applications, gaps remain in smallholder adoption, technology translation, and critical assessment of long-term competitiveness.

Keywords: competitiveness, economic performance, resilience, value addition and sustainability.

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INTRODUCTION

The year 2025 has been a challenging year for the global vegetable oils industry and the oil palm sector is no exception. Key challenges included uncertainties in weather conditions, fluctuations in commodity prices, as well as the dynamics of supply and demand in vegetable oils. Despite these challenges, the Malaysian oil palm industry has demonstrated resilience and maintained its competitive edge as the world's second-largest producer and exporter of palm oil. The strong fundamentals of the industry continue to drive its progress, underpinned by sustainable development practices. Malaysia remains committed to sustainable palm oil production, balancing environmental protection, biodiversity conservation and socio-economic development.

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Palm oil is the most widely consumed edible oil globally and a key ingredient in a wide range of everyday products, both for food and non-food applications, due to its versatility and functional properties. The growing utilisation of palm oil and its derivatives continues to drive global demand. The extensive efforts and initiatives by Malaysia have positioned the oil palm industry as one of the most advanced sectors in terms of sustainability.

The mandatory implementation of the Malaysian Sustainable Palm Oil (MSPO) certification standard (MSPO, 2022) is one of the approaches by the industry to ensure transparency while promoting sustainable practices across the entire palm oil supply chain. Malaysia is also developing the National Traceability System (SKN) to enable full traceability, providing credible data and science-based evidence to support the sustainable production of Malaysian palm oil and its derivatives in a holistic manner. Continuous engagement and collaboration among stakeholders of the industry, including smallholders, enable the sector to evolve in tandem with global dynamics encompassing policy developments, contributing to both food and energy security.

Additionally, Malaysia launched the Malaysia Aviation Decarbonisation Blueprint on 5 September 2024 (Ministry of Transport, 2024), with the aim of achieving net-zero carbon emissions by 2050 for the aviation sector. This initiative presents potential for growth in the use of palm oil to produce sustainable aviation fuel (SAF) in line with the global transition to a low-carbon economy.

The oil palm industry plays a significant role in alleviating poverty, particularly among oil palm smallholders. It fosters economic growth, creates employment opportunities and facilitates the development of new industries and infrastructure. The importance of the oil palm sector has expanded beyond the two largest producing countries, Indonesia and Malaysia, with its growing presence in regions such as Latin America and Africa. This global expansion underscores the socio-economic contributions of the industry, highlighting its broader impacts on the national economy of these developing countries.

The positive growth of the oil palm industry globally has prompted the intensification of research and development (R&D) across the supply chain. These efforts have continued to progress robustly, with the focus of addressing challenges faced by the industry, including enhancing productivity, reducing greenhouse gas (GHG) emissions, developing downstream value-added palm products as well as tackling global concerns related to sustainability, nutrition and food safety, among others. The R&D is aimed at ensuring the oil palm industry continues to remain sustainable and competitive, while balancing the efforts for conservation of forest and biodiversity as well as social equity with economic growth.

Although annual reports and topic-specific studies are available, there remains limited integrative analysis linking near-term economic performance indicators with the evolving R&D landscape of the Malaysian oil palm sector. This review addresses that gap by critically evaluating how recent scientific and technological advances respond to current production, labour, trade, sustainability, and value-addition challenges. This will enable the oil palm industry to continue to make positive transformation and advancement for the benefit of the global community.

PERFORMANCE OF THE MALAYSIAN OIL PALM INDUSTRY

The Malaysian oil palm industry demonstrated a resilient and structurally transformative performance in 2025, amid intensifying sustainability constraints, heightened global market volatility, and increasing demands for supply chain transparency. Operating within a tightly regulated land-use framework, the sector has shifted from expansionary growth toward a productivity-driven paradigm, whereby output, income generation, and export competitiveness are sustained through efficiency gains rather than area expansion. This transition reflects the cumulative impact of long-term structural reforms, particularly in plantation rejuvenation, labour optimisation, and the adoption of digital traceability systems, which are now yielding measurable economic outcomes.

Unlike earlier cyclical recoveries driven by favourable price dynamics or short-term yield improvements, the 2025 trajectory signals a deeper recalibration of the industry's production base. This recalibration is characterised by accelerated replanting, gradual normalisation of labour availability, and the consolidation of upstream processing and downstream value-added activities within an increasingly stringent global regulatory environment. Despite ongoing external pressures including evolving import policies, competition from alternative vegetable oils, and tightening sustainability-related market access requirements, the sector exhibits enhanced systemic efficiency and adaptive capacity.

Importantly, the industry's performance underscores a structural shift from volume-driven expansion to efficiency-driven value creation. Improvements in fresh fruit bunch (FFB) productivity, stabilisation of crude palm oil (CPO) output, and stronger downstream price realisation indicate a transition toward a more mature growth phase. However, this transformation entails trade-offs, including reduced mature acreage due to accelerated replanting, persistently high labour costs, and uneven spatial distribution of downstream value capture. Collectively, these dynamics define the sector's 2025 performance landscape, highlighting both its developmental progress and the structural constraints shaping its future trajectory.

Oil Palm Planted Area

In 2025, the total oil palm planted area reached 5.70 million hectares, representing a 1.6% adjustment from 5.61 million hectares in 2024 (*Table 1*). This marginal shift underscores a strategic transition towards high-precision data synchronisation in recording active cultivation areas. Concurrent with this spatial refinement, the industry witnessed a robust intensification of replanting activities, spearheaded by estate conglomerates and further catalysed by government-led initiatives, specifically those targeting independent smallholders.

Empirical data from the Malaysian Palm Oil Board (MPOB, 2026) indicates that the national replanting rate ascended from 2.0% in 2024 to 3.4% in 2025. In absolute terms, the replanted area expanded significantly from 0.11 million hectares to 0.19 million hectares, marking a substantial year-on-year increase of 67.1%.

Regional analysis reveals heterogeneous growth patterns: Sarawak recorded the most significant expansion (2.2%), followed by Peninsular Malaysia (1.6%), while Sabah demonstrated a more conservative growth trajectory (0.9%). Conversely, the national mature oil palm area contracted by 0.5%, a direct corollary of the temporary withdrawal of productive stands during the replanting phase. This contraction was most evident in Sabah, whereas Peninsular Malaysia exhibited relative stability, suggesting a more balanced age structure and a well-regulated replanting cycle. Collectively, these trends signify a deliberate, industry-wide pivot toward rejuvenating aging plantations to optimise yield potential and fortify sectoral resilience against future productivity constraints.

In 2025, private and government-owned estates continued to dominate the oil palm sector, accounting for 73.2% (4.17 million hectares) of total oil palm area, reflecting their structural advantage in capital mobilisation and large-scale replanting. Independent smallholders comprised 15.2% (0.87 million hectares), while organised smallholders accounted for 11.6%, indicating a persistently concentrated ownership structure as shown in *Figure 1*.

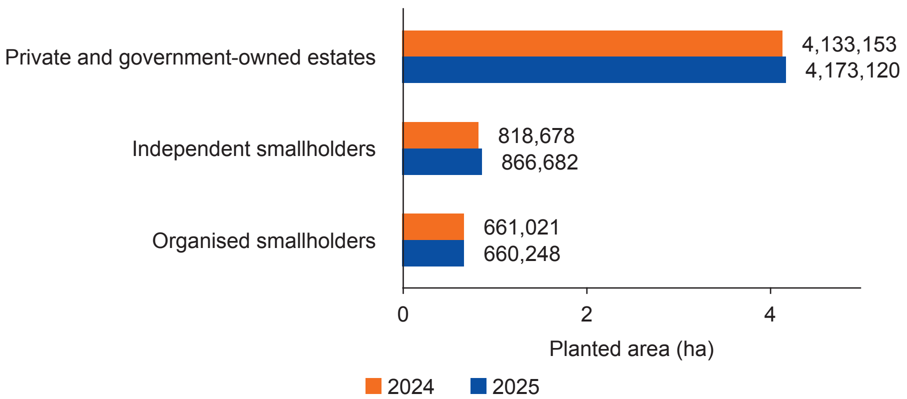
Independent smallholders recorded the fastest expansion, increasing by approximately 48,000 ha, driven by the *Budi Agrokomoditi* initiative and the rollout of the MPOB's Sawit Intelligent Management System (SIMS). SIMS requires dealers and manufacturers to record transactions, including identity card information for purchases from unlicensed independent smallholders, which has facilitated the formal recognition of previously unregistered smallholder activities and contributed to the increase in recorded acreage. In contrast, the organised smallholder segment remained broadly stable, with a marginal contraction (0.12%) likely reflecting administrative consolidation rather than a substantive sectoral exit.

Overall, the sector remains characterised by entrenched estate dominance alongside a gradual, policy-enabled rise in independent smallholder participation. However, without sustained improvements in access to finance, technology, and extension services, this expansion is unlikely to yield proportional productivity gains, limiting its contribution to long-term sectoral resilience.

TABLE 1. MALAYSIAN OIL PALM PLANTED AREA IN 2024 AND 2025

Region	Total area (ha)			Mature area (ha)		
	2025	2024	Difference (%)	2025	2024	Difference (%)
Peninsular Malaysia	2,543,636	2,504,786	1.6	2,275,822	2,268,705	0.3
Sabah	1,496,558	1,483,699	0.9	1,267,999	1,291,591	–1.8
Sarawak	1,659,857	1,624,366	2.2	1,499,656	1,506,228	–0.4
Total	**5,700,050**	**5,612,852**	**1.6**	**5,043,478**	**5,066,524**	**0.5**

Source: MPOB (2026).



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 (2026).

Figure 1. Ownership distribution of oil palm area, 2024 vs. 2025.

Processing Facilities and Downstream Integration

At the upstream level, Malaysia's milling landscape underwent a subtle consolidation, with the total number of active palm oil mills adjusting from 453 in 2024 to 452 in 2025. Paradoxically, total installed capacity ascended from 125.40 to 126.33 million tonnes yr^{-1} (*Table 2*). This divergence signifies a strategic shift from horizontal expansion toward capacity intensification and technological retrofitting of existing infrastructure. Spatially, milling activities remain intrinsically coupled with plantation distribution. Peninsular Malaysia maintains its primacy with 240 mills (64.79 million tonnes yr^{-1}), followed by Sabah (128 mills; 34.93 million tonnes yr^{-1}) and Sarawak (84 mills; 26.60 million tonnes yr^{-1}). The observed stability in mill counts, juxtaposed with incremental capacity gains, suggests a trajectory of productivity optimisation within the established milling framework.

In contrast, the downstream segment exhibits a more concentrated and selectively adjusted structure. While the refinery count increased marginally from 53 to 54, aggregate refining capacity experienced a slight contraction from 26.39 to 26.15 million tonnes yr^{-1}. This phenomenon likely reflects operational recalibration, encompassing capacity rationalisation or tactical underutilisation in response to market fluctuations. Spatially, refining activities demonstrate significant geographical inertia, heavily clustered in Peninsular Malaysia (39 refineries), which commands over 60% of national capacity (16.12 million tonnes yr^{-1}). This highlights the critical role of industrial agglomeration, proximity to major export gateways, and sophisticated logistical ecosystems. Conversely, both Sabah and Sarawak recorded diminished refining capacities, with Sarawak experiencing a decline in refinery numbers indicative of persistent regional disparities in downstream maturation.

The palm kernel crushing sector remained structurally resilient, maintaining 43 plants nationwide. However, total capacity edged upward from 7.36 to 7.39 million tonnes yr^{-1}. Notably, Sarawak registered a meaningful capacity surge (0.76 to 0.79 million tonnes yr^{-1}) despite a static plant count. This underscores a pattern of selective capital deepening, where output growth is driven by asset upgrading rather than new facility establishment. Similarly, the oleochemical industry remains the most spatially polarised segment, with all 20 facilities centralised in Peninsular Malaysia. Although capacity declined slightly (2.68 to 2.63 million tonnes yr^{-1}), its absolute absence in East Malaysia underscores the high entry barriers associated with value-added downstream activities, including specialised infrastructure requirements and the need for economies of scale.

Synthetically, these trends indicate that Malaysia's palm oil industry is undergoing a fundamental structural transition from land-based expansion to efficiency-driven optimisation. Productivity gains are increasingly derived from technological integration and facility consolidation rather than physical footprint growth. The industry exhibits a bifurcated spatial logic: Upstream processing is dispersed according to feedstock proximity, while high-value downstream and oleochemical activities gravitate toward the Peninsular to leverage agglomeration economies. While this configuration enhances global competitiveness, it also reveals a strategic window for policy interventions aimed at regional rebalancing. Encouraging downstream investment in Sabah and Sarawak remains imperative to foster a more inclusive and resilient national value chain.

Crude Palm Oil (CPO) Production Trends

The CPO production rose by 4.9% year-on-year to 20.28 million tonnes in 2025 (*Table 3*), reflecting a cyclical recovery in upstream performance supported by improved FFB yields, stabilised labour availability, and incremental gains in operational efficiency.

A disaggregated analysis of regional production reveals Sarawak as the primary driver of growth in terms of percentage increase. Production in Sarawak surged by 7.8%, rising from 4.17 million tonnes in 2024 to 4.50 million tonnes in 2025. This outsized growth (323,930 t) likely reflects the maturation of newer plantings and the stabilisation of labour availability in the state's expansive plantation landscape.

Conversely, Peninsular Malaysia maintained its status as the largest contributor to national output, accounting for 56.1% of total production in 2025. The region recorded a solid growth rate of 4.5%, with an absolute volume increase of 485,428 t. Meanwhile, Sabah exhibited a more moderate growth profile at 3.2%, reaching 4.41 million tonnes. While Sabah's growth was the most conservative among the three regions, its consistent output remains vital to the national supply chain.

The synchronisation of increased CPO production across all regions, despite diverse geographical and operational challenges, underscores the sectoral resilience of the Malaysian palm oil industry. The notable increase in Sarawak's output suggests a narrowing of the productivity gap between East and Peninsular Malaysia. Overall, the 945,209 t increment at the national level provides a critical buffer for the downstream processing industry, ensuring adequate feedstock for refining and oleochemical activities while strengthening Malaysia's competitive position in the global vegetable oil market (MPOB, 2026).

TABLE 2. NUMBER AND CAPACITIES OF PALM OIL MILLS, REFINERIES, PALM KERNEL CRUSHERS AND OLEOCHEMICAL PLANTS, 2024 AND 2025

Region	Year	Item	Sector			
			Palm oil mill	Palm oil refinery	Palm kernel crusher	Oleochemical plant
Peninsular Malaysia	2025	Cap. number	240	39	27	20
		(Mn t yr^{-1})	64.79	16.12	4.61	2.63
	2024	Cap. number	240	37	27	19
		(Mn t yr^{-1})	64.05	15.42	4.61	2.68
Sabah	2025	Cap. number	128	10	10	-
		(Mn t yr^{-1})	34.93	7.44	2.00	-
	2024	Cap. number	129	10	10	-
		(Mn t yr^{-1})	34.99	7.88	2.00	-
Sarawak	2025	Cap. number	84	5	6	-
		(Mn t yr^{-1})	26.60	2.60	0.79	-
	2024	Cap. number	84	6	6	-
		(Mn t yr^{-1})	26.35	3.10	0.76	-
Total	**2025**	**Cap. number**	**452**	**54**	**43**	**20**
		(Mn t yr^{-1})	**126.33**	**26.15**	**7.39**	**2.63**
	2024	**Cap. number**	**453**	**53**	**43**	**19**
		(Mn t yr^{-1})	**125.40**	**26.39**	**7.36**	**2.68**

Note: Mn t yr^{-1} - million tonnes yr^{-1}, Cap - capacity.
Source: MPOB (2026).

TABLE 3. MALAYSIAN CRUDE PALM OIL (CPO) PRODUCTION, 2024 AND 2025

Region	2025 (t)	2024 (t)	Difference	
			Volume (t)	%
Peninsular Malaysia	11,376,845	10,891,417	485,428	4.5
Sabah	4,410,291	4,274,440	135,851	3.2
Sarawak	4,496,339	4,172,409	323,930	7.8
Total	**20,283,475**	**19,338,266**	**945,209**	**4.9**

Source: MPOB (2026).

Productivity, Extraction Efficiency, and Labour Normalisation

The FFB productivity in Malaysia improved markedly in 2025, increasing by 6.4% to 17.77 t ha^{-1}, supported by favourable weather conditions, improved agronomic practices, and more stable labour availability. Yield gains were observed across all regions, with Sarawak recording the strongest relative improvement, followed by Sabah and Peninsular Malaysia. Despite this recovery, national yields remain below theoretical potential, reflecting persistent structural constraints, including aging planting materials, soil fertility limitations, and uneven adoption of precision agriculture.

Improvements in oil extraction rate (OER) were modest, with the national average rising marginally to 19.74% (*Table 4*). Regional divergence suggests ongoing variability in fruit quality, harvesting timeliness, and mill efficiency, indicating that future output growth will rely more heavily on yield enhancement and harvesting efficiency than extraction gains alone.

Labour normalisation in 2025, driven by targeted foreign worker approvals, improved harvesting execution, and raised labour productivity to 1.49 t man-day^{-1}. While this supported output recovery, continued reliance on foreign labour exposes the sector to policy risks, underscoring the need for mechanisation and automation to sustain competitiveness.

Trade Performance and Market Reconfiguration

A defining feature of Malaysia's palm oil trade performance in 2025 was the decoupling between export volume and export value. Total export volume declined by 7.2% to 24.75 million tonnes, while export value increased by 2.8% to RM112.43 billion (*Table 5*). This divergence reflects improved unit prices and a compositional shift towards higher value-added products.

Exports of palm oil contracted sharply in volume (9.6%) yet experienced only a marginal decline in value (1.8%), suggesting partial price insulation amid weaker global demand. In contrast, palm kernel oil and oleochemicals recorded strong value growth despite lower volumes, pointing to tighter global supply conditions and sustained demand for specialised downstream derivatives. Biodiesel emerged as the most dynamic segment, with substantial increases in both export volume (38.4%) and value (48.1%), highlighting Malaysia's growing exposure to renewable energy markets and policy-driven demand. While biodiesel exports enhance value capture, they also expose the industry to regulatory risk, as demand is closely tied to energy and climate policies in importing countries.

These patterns indicate a structural shift towards downstream value, which has partially mitigated declining bulk exports. However, the uneven performance across product categories underscores persistent vulnerabilities, including reliance on price-sensitive markets and policy-driven demand for biofuels. Sustaining export resilience will require deepening downstream capabilities, diversifying end markets, and managing exposure to regulatory and price volatility in the global energy and chemical sectors.

Figure 2 illustrates the year-on-year changes in Malaysia's palm oil export volumes to the top-10 destinations between 2024 and 2025. Countries are systematically ordered by their 2025 export volumes (thousand tonnes), ranging from the largest market (India) to the smallest (Tanzania), to facilitate consistent cross-country comparison. Over this period, total exports declined from 16.90 million tonnes in 2024 to 15.27 million tonnes in 2025, indicating a notable contraction in external demand. The reduction was particularly pronounced in major importing markets, including India (12.1%), China (35.1%), and the European Union (20.2%). These declines likely reflect a combination of heightened price sensitivity, evolving trade policy environments, and substitution effects within the global vegetable oil complex, where competing oils

TABLE 4. MALAYSIAN FRESH FRUIT BUNCH (FFB) PRODUCTIVITY AND OIL EXTRACTION RATE (OER), 2024 AND 2025

Region	FFB productivity (t ha^{-1})				OER (%)			
	2025	2024	Difference		2025	2024	Difference	
			Volume	%			Volume	%
Peninsular Malaysia	19.49	18.42	1.07	5.8	19.62	19.46	0.16	0.8
Sabah	16.76	15.74	1.02	6.5	20.31	20.53	–0.22	–1.1
Sarawak	15.98	14.89	1.09	7.3	19.51	19.37	0.14	0.7
Total	**17.77**	**16.70**	**1.07**	**6.4**	**19.74**	**19.67**	**0.07**	**0.4**

Source: MPOB (2026).

TABLE 5. MALAYSIAN EXPORTS OF PALM OIL AND PALM-BASED PRODUCTS, 2024 AND 2025

Palm-based product	Volume (t)			Value (RM million)		
	2025	2024	Difference (%)	2025	2024	Difference (%)
Palm oil	15,272,823	16,902,595	–9.6	71,632.49	72,952.58	–1.8
Palm kernel oil	1,082,925	1,151,104	–5.9	9,209.13	6,787.45	35.7
Palm kernel cake	2,431,495	2,396,517	1.5	1,303.49	1,496.60	–12.9
Palm-based oleochemicals	2,684,938	2,993,592	–10.3	19,385.69	17,409.76	11.3
Biodiesel	353,780	255,669	38.4	1,856.18	1,253.27	48.1
Finished products	587,231	585,764	0.3	4,866.53	4,313.02	12.8
Other palm-based products*	2,337,699	2,375,206	–1.6	4,179.21	5,178.60	–19.3
Total	**24,750,892**	**26,660,448**	**–7.2**	**112,432.71**	**109,391.29**	**2.8**

Note: * - sludge oil, mixed acid oil, industrial grade palm oil, residue oil/scavenger oil, lauric fatty acid distillate, used frying oil, lacto plus, spent bleaching earth, fruit waste/oil palm waste, fatty alcohol residues, empty fruit bunch (EFB), mixed vegetable acid oil, high free fatty acid (FFA) acid oil, palm oil mill effluent (POME), sodium fatty acid salt, oil palm fibre/palm fibre, palm kernel shell (PKS), empty fruit bunch pellet.

Source: MPOB (2026).

such as soybean oil (SBO) and sunflower oil (SFO) continue to exert increasing competitive pressure on palm oil demand.

Import contraction in key Asian markets has heightened volatility in Malaysia's palm oil export performance. In India, elevated domestic edible oil inventories and frequent tariff recalibrations have strengthened substitution towards SBO and SFO when relative price advantages emerge, increasing Malaysia's exposure to policy-driven demand fluctuations in its largest export destination (Jadhav & Bhardwaj, 2025; Organisation for Economic Co-operation and Development & Food and Agriculture Organization of the United Nations [OECD-FAO], 2025). China experienced the steepest decline, reflecting strong substitution towards domestically crushed SBO amid unfavourable palm oil price differentials. Such price-sensitive responses are particularly pronounced in markets with substantial domestic oilseed processing capacity, amplifying demand volatility during periods of palm oil premium pricing (OECD-FAO, 2025). Concurrently, Indonesia's biodiesel blending policies, including the proposed B50 mandate, have tightened regional supply, supported prices but also altered Malaysia's competitive positioning. Intermittent policy adjustments in 2025 eased supply pressure, intensifying competition and constraining Malaysia's export opportunities in price-sensitive markets (Reuters, 2025).

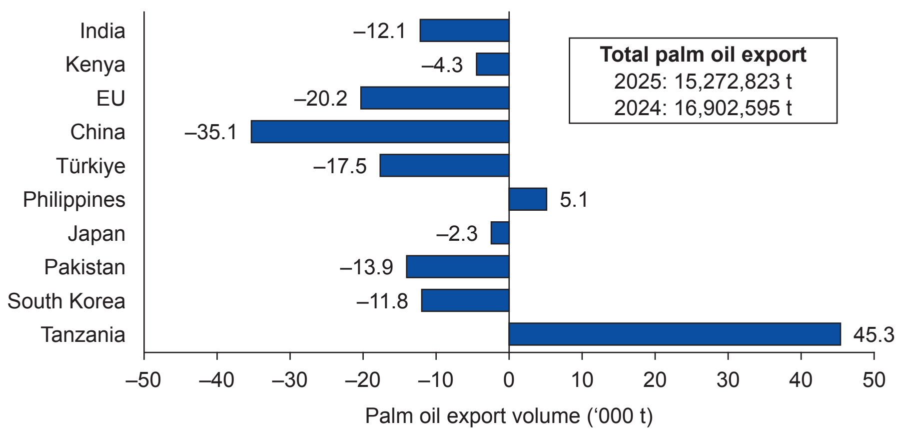
Together, the contraction in Malaysia's palm oil exports in 2025 reflects the interaction of reciprocal trade policies, substitution effects, and regional biofuel strategies rather than demand weakness alone. While emerging markets such as Tanzania and the Philippines recorded growth, these gains were insufficient to offset losses in large, policy-sensitive markets. Strengthening Malaysia's export resilience will therefore depend on managing exposure to tariff volatility, enhancing price competitiveness, and deepening downstream value capture to reduce reliance on bulk commodity trade.

Inventory Dynamics and Price Formation

Figure 3 illustrates the inverse relationship between Malaysia's palm oil stocks and CPO prices in 2025, highlighting the interaction between supply accumulation and price adjustment. Stocks were relatively tight in the first quarter (1.51–1.58 million tonnes), supporting elevated CPO prices of around RM4,700.00 t^{-1}. From April onwards, inventories rose sharply, exceeding 2.00 million tonnes by mid-year and peaking at 3.05 million tonnes in December, reflecting seasonal production recovery and weaker export offtake.

As stocks accumulated, CPO prices declined markedly between April and May, before partially recovering in the third quarter despite continued inventory growth. This divergence suggests that price formation was influenced not only by domestic stock levels but also by expectations of downstream demand, global vegetable oil market conditions, and cost-related price floors. By year-end, prices stabilised despite historically high inventories, indicating a market adjusting to higher supply rather than experiencing a demand-driven collapse.

The 2025 price-stock dynamics underscore the continued role of inventory accumulation in shaping short-term price movements, while the observed price resilience at elevated stock levels points to structural support mechanisms that moderated downside risk. This pattern highlights the importance of managing stock build-ups through export responsiveness and downstream absorption to reduce price volatility in periods of supply expansion.



Source: MPOB (2026).

Figure 2. Year-on-year change in Malaysia's palm oil export volume to major destinations (2024 vs. 2025).

Price movements varied across product segments. The CPO prices increased modestly by 2.7% to RM4,292.50 t^{-1}, while refined products recorded stronger gains. Palm kernel-related products experienced the largest price increases, driven by tight lauric oil supply. At the upstream level, FFB prices rose by 6.3%, improving grower incomes but reinforcing cost pressures downstream.

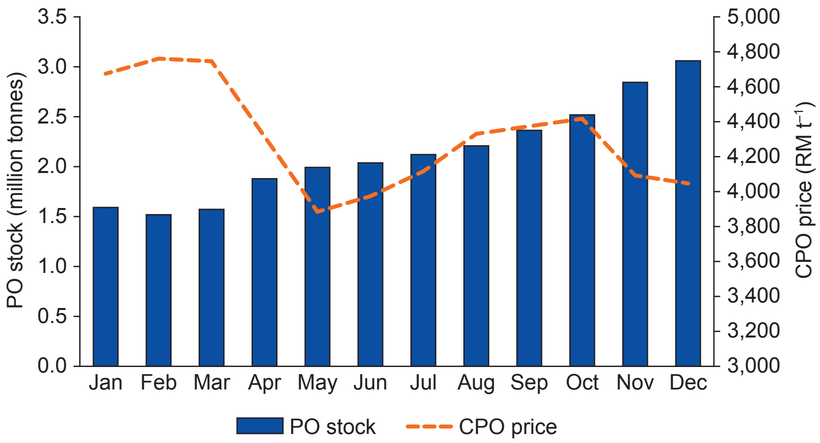
Stronger price gains were observed in refined products, with refined, bleached and deodorised (RBD) palm oil and palm olein increasing by 1.3% and 1.2%, respectively, reflecting sustained downstream demand and refiners' ability to pass through higher costs. In contrast, RBD palm stearin recorded a slight decline (1.6%), pointing to segment-specific demand weakness or relative oversupply.

Palm kernel-related products exhibited the most pronounced price increases, with palm kernel and crude palm kernel oil (CPKO) rising by 29.4% and 33.9%, respectively, driven by tight lauric oil supply and robust demand from the oleochemical and food sectors. At the upstream level, FFB prices increased by 6.3%, improving grower returns but also reinforcing cost pressures across the value chain.

The 2025 price structure highlights increasing differentiation across oil palm product segments, with price strength concentrated in higher value and supply-constrained products (*Table 6*). While this supported producer and processor margins, it also underscores growing exposure to cost inflation and segment-specific demand risks rather than broad-based market expansion.

Figure 4 compares monthly prices of CPO with SBO and SFO in 2024–2025, highlighting divergent price trajectories within the global vegetable oil complex. All three oils recorded higher prices in 2025, particularly in the first quarter, reflecting tight global supplies and strong demand. However, price increases for CPO were generally smaller than those for SBO and SFO, indicating a relative price discount for palm oil.

During the first half of 2025, CPO prices rose by 10%–28% year-on-year but remained consistently below competing oils, reinforcing



Note: PO - palm oil; CPO - crude palm oil.
Source: MPOB (2026).

Figure 3. Palm oil stocks and crude palm oil (CPO) prices, 2025.

TABLE 6. MALAYSIAN PRICES OF OIL PALM PRODUCTS

Item	2025 (RM t^{-1})	2024 (RM t^{-1})	Difference	
			RM t^{-1}	%
CPO	4292.50	4179.50	113.00	2.7
RBD palm oil	4456.50	4400.00	56.50	1.3
RBD palm olein	4471.50	4417.00	54.50	1.2
RBD palm stearin	4353.50	4425.50	–72.00	–1.6
Palm kernel	3424.50	2645.50	779.00	29.4
CPKO	7329.50	5475.50	1854.00	33.9
FFB	930.00	875.00	55.00	6.3

Note: CPO - crude palm oil; RBD - refined, bleached and deodorised; CPKO - crude palm kernel oil; FFB - fresh fruit bunches.
Source: MPOB (2026).

palm oil's cost competitiveness in price-sensitive markets. Price convergence across oils in mid-2025 suggests active substitution effects, with buyers adjusting procurement in response to relative price movements. In contrast, towards the year-end, CPO prices weakened on a year-on-year basis, coinciding with rising inventories, while SBO and SFO prices remained supported by tighter supply conditions. This divergence underscores palm oil's greater exposure to stock accumulation and demand volatility.

On an annual average basis, CPO prices increased by 12.7%, compared with stronger gains for SBO (17.6%) and SFO (19.6%). While this relative underperformance enhanced palm oil's affordability and role in global food security, it also reflects structural vulnerabilities linked to inventory build-up and competitive pressures within the edible oil complex.

In 2025, the Malaysian oil palm industry demonstrated improved system-level performance, underpinned by higher yields, stabilised labour availability, and resilient price conditions. However, competitiveness remained largely price-based, underscoring the importance of sustained productivity enhancement, improved stock cycle management, and deeper downstream integration.

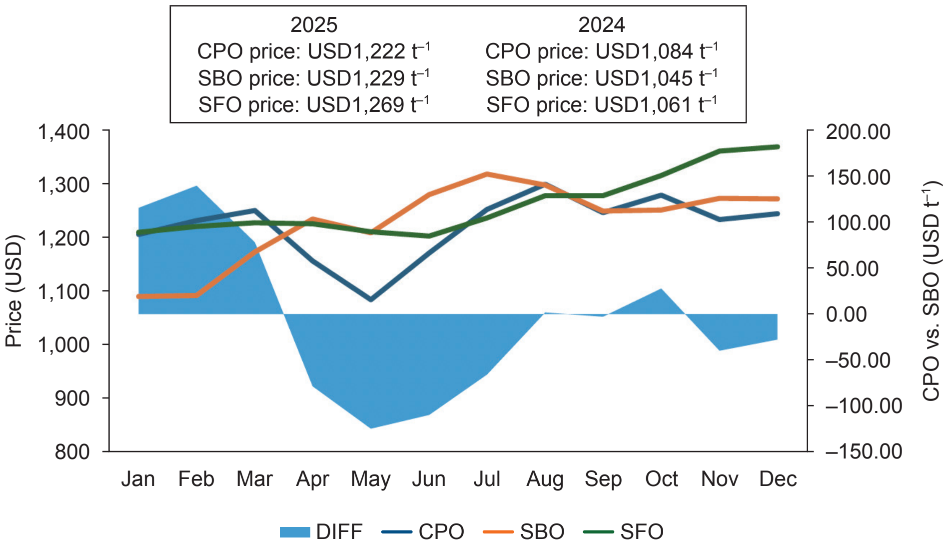
The implementation of digital monitoring systems under the stewardship of MPOB and the acceleration of replanting have established a stronger structural foundation for future growth. As cyclical recovery effects dissipate, the industry's next growth phase will depend on its ability to generate technology-driven productivity gains, enhance climate resilience, and move decisively up the value chain.

The 2025 data suggest moderate recovery in productivity and output, supported by improved labour availability and ongoing replanting, although evidence for deeper structural transformation remains partial and uneven across regions and value-chain segments. However, some sectoral constraints have shaped and prioritised R&D efforts toward targeted, solution-driven innovations. In order to ensure the long-term resilience and competitiveness of the sector, it is crucial to accelerate the development of high-yielding breeding and elite planting materials to address replanting and declining mature areas, enhance mechanisation and automation to reduce labour dependency, strengthen downstream diversification and specialty products to mitigate trade volatility, tackle sustainability challenges through traceability, emission reduction, and low-carbon pathways along the supply chain, as well as support smallholder expansion through digital extension, affordability, and adoption-focused research. The R&D focus areas synthesise the 2025 R&D advances through the lens of productivity, sustainability, resilience, and value-chain transformation.

RESEARCH AND DEVELOPMENT (R&D) FOCUS AREAS IN 2025

Methodology

This review of the R&D focus areas adopted a structured narrative approach. Literature published between the end of 2024 and 2025 was identified from databases such as Scopus,



Note: SBO - soybean oil; SFO - sunflower oil; CPO - crude palm oil; DIFF - difference between CPO and SBO prices.
Source: MPOB (2026).

Figure 4. Palm oil vs. other vegetable oil prices, 2025.

Web of Science, and Google Scholar using combinations of keywords including oil palm, palm oil, oil palm biomass, biofuel, sustainability, breeding, genomics, *Ganoderma*, greenhouse gas, biodiversity, tocotrienols, food safety, nutrition, and oleochemicals. Industry statistics were obtained from MPOB sources. Studies were prioritised based on relevance to Malaysian oil palm performance and innovation themes.

Precision Breeding: Integrating with Genomics

Field evaluation of progeny and germplasm remains critical to ensure practical breeding gains. Performance assessments of AVROS and Yangambi-derived three-way crosses demonstrated significant variations in bunch yield, oil yield and vegetative traits, allowing the identification of high-performing genotypes suitable for future breeding programs (Nor Azwani et al., 2025). Broader breeding strategies emphasise the integration of mechanisation, compactness, dwarfness, and other value-added traits to enhance yield and productivity under labour constraints, while expanding the genetic base using conserved germplasm collections from multiple countries (Mohamad et al., 2025). Recent advances in molecular genetics and genomics would aid oil palm (*Elaeis guineensis*) improvement through precise breeding, effective quality control, enhanced disease diagnostics, and informed germplasm conservation. Collectively, studies across multiple breeding programmes demonstrate how simple sequence repeat (SSR) or single-nucleotide polymorphism (SNP) markers, genome-wide analyses, and high-resolution genotyping platforms are accelerating the development of elite planting materials while strengthening the genetic foundation of the crop. Marker-assisted selection has shown strong value in improving oil quality, particularly through the identification of functional SSR markers associated with lipase activity. The mEgCIR_LIP03 marker, located in the promoter of the *EgLIP1* gene, differentiates low- and high-lipase genotypes in Ulu Remis *tenera* materials, enabling breeders to eliminate high-free fatty acid (FFA) palms and fast-track the development of low-acidity lines (Weng et al., 2025b). Genetic purity verification has also advanced with the use of SSR and SNP markers. In interspecific BC1 × BC1 populations, polymorphic SSRs successfully identified outliers and enabled full reconstruction of true parental genotypes (Serdari et al., 2025). Genomic tools also support trait improvement, particularly for canopy compactness and height, which are crucial for high-density planting and long-term harvesting efficiency. Genome-wide association studies identified significant SNPs associated with height increment (Weng et al., 2025a) and 27 SNPs linked to compact architecture traits across diverse *tenera* families (Samsudin et al., 2025). These markers and candidate genes provide a pathway towards marker-assisted breeding for more manageable, high-yielding palms. Genomic analyses continue to highlight the importance of conserving the crop's genetic base. Genotyping-by-sequencing (GBS)-based evaluation of 478 African accessions identified over 7,000 SNPs, revealed the highest diversity and private alleles in Nigerian populations and defined a core set of 96 palms for conservation (Zolkafli et al., 2025). Complementary SSR-based assessment of Tanzanian accessions revealed moderate diversity and evidence of historical gene flow, underscoring the need for broader germplasm collection to strengthen future breeding (Masanja et al., 2025).

On an industry-wide scale, deoxyribonucleic acid (DNA) testing of smallholder fields using the *Shell* gene array revealed extensive contamination with low-yielding non-*tenera* palms, showing that the systematic "screen-then-plant" DNA verification approach could dramatically increase smallholder income and mill productivity (Maskromo et al., 2025). A complementary innovation involving mixed-pollen controlled crosses was also introduced. Multiple paternal sources are applied simultaneously, and progenies are later assigned using a 108-SNP panel. This novel approach increases breeding efficiency, reduces dependency on flowering cycles, and provides accurate parentage resolution (Yaakub et al., 2025). Pollen from multiple paternal sources was combined and used to hybridise a female flower from a single maternal palm. The subsequent progenies could be assigned to specific families (paternal source) using MPOB's True-to-Type platform. This genomic platform achieved >99.4% accuracy in parentage assignment and improved legitimacy detection across diverse genetic backgrounds, offering robust quality control for breeding, seed gardens, nurseries, and tissue culture operations (Ting et al., 2025).

Somatic embryogenesis remains a cornerstone for clonal propagation, with studies showing that even virescent off-type plantlets, previously discarded due to abnormality concerns, can develop into normal fruiting palms, thereby improving the efficiency of seedling production (Karyanti et al., 2025). However, tissue culture-derived palms can still exhibit floral and fruit abnormalities such as thin/thick coat, squirrel tail, and mantling, which underscores the need for careful morphological screening and selection (Sari & Sari, 2025). Complementing traditional tissue culture, monitoring epigenetic changes such as those observed in the Karma transposable element and other heritable modifications can potentially enhance oil palm resilience and trait stability, particularly when coupled with priming strategies and epigenome editing tools (Sarpan & Ooi, 2025). Genome editing and transformation technologies are increasingly applied to accelerate oil palm improvement. The development of CRISPR/Cas9 ribonucleoprotein (RNP)-mediated editing of embryogenic calli allows transgene-free modifications with higher efficiency and reduced regeneration time (Norfaezah et al., 2025). Progress in *Agrobacterium*-mediated transformation and biolistic delivery systems, including optimised particle bombardment parameters, further supports functional genomics and targeted trait modification such as oil yield, plant height, fruit colour, and stress resistance (Hanin et al., 2025; Nurfahisza et al., 2025). These molecular approaches, combined with mixed-pollen strategies and SNP-based fingerprinting, allow breeders to assign progenies accurately to paternal sources and efficiently manage breeding populations (Nurfahisza et al., 2025). Together, these studies highlight the integration of biotechnology, genome editing, epigenetics, and traditional breeding as complementary approaches for sustainable oil palm improvement. This integrated approach is key to establishing the foundations of a modern precision breeding era for the oil palm industry.

Multi-Omics Perspective

Recent advances in functional genomics, metabolomics, and molecular breeding have substantially deepened our understanding of the biological processes governing oil palm (*E. guineensis* and *E. oleifera*) productivity, oil quality, abiotic stress resilience, and metabolic regulation. Collectively, findings from eight studies highlight the expanding role of integrative omics, promoter characterisation, and gene-function validation in accelerating genetic improvement of this globally important crop. Oleic acid biosynthesis remains a central target for improving palm oil quality due to its oxidative stability and health benefits. Multi-omics analyses comparing seedless and *tenera* palms across fruit developmental stages identified key genes including *SAD, FabD, LACS6, BC, FabB,* and *FabI*, as positive regulators of oleic acid accumulation, whereas *LACS9* showed negative associations (Xu et al., 2025). These results reveal distinct regulatory mechanisms shaping fatty acid synthesis across genetic backgrounds, providing actionable targets for high-oleic breeding. Promoter biology also continues to advance, with the MSP-C7 promoter showing tissue-specific functionality in oil palm mesocarp and transgenic tomato. A 2.1 kb promoter fragment (MSP-C7-F1) was sufficient to drive expression in monocot and dicot systems, highlighting its utility for genetic engineering strategies aimed at fruit-specific transgene deployment (Badai et al., 2025). Complementing this, functional dissection of the EgPAL5 promoter uncovered strong responsiveness to developmental cues, osmotic stress, and transcriptional regulation by AtMYB58, pinpointing essential *cis*-elements that modulate phenylpropanoid metabolism and drought induction (Yusuf et al., 2025). At the protein level, the mesocarp lipase PLA1-3 was successfully cloned, expressed, and partially purified, demonstrating notable catalytic activity (210.1 U mg^{-1}). This work enables future biochemical characterisation of lipases affecting innate oil quality and offers prospects for industrial biocatalysis (Safiudin et al., 2025).

Abiotic stress responses were further explored through transcription factor (TF) analysis. Twenty TFs belonging to MYB, HD-ZIP, NF-Y, and HSFF families exhibited coordinated induction under both drought and salinity stress. Promoter and homology studies support their conserved roles in stress regulatory networks, with qRT-PCR validating most RNA-Seq patterns. These candidates form a foundation for engineering multi-stress-tolerant cultivars, though functional validation remains essential (Salgado et al., 2025). Genetic variation in key lipid biosynthesis genes also offers future breeding opportunities. Sequence analysis of the *SAD* gene across *E. guineensis, E. oleifera*, their hybrids, and various fruit forms identified 15 SNPs across intronic, exonic, and UTR regions. Derived SNP markers exhibited diversity across 50 accessions, indicating their potential use in selecting lines with improved oleic acid profiles (Rismayanti et al., 2025).

Biodiversity in Oil Palm Landscapes

Studies on biodiversity in oil palm landscapes are pivotal for balancing the maximisation of palm oil productivity with sustainable development, with new findings providing insights into the positive development in the protection of biodiversity. For example, in oil palm ecosystems, the most diverse bird families with important ecological roles include Ardeidae, Cuculidae, Nectariniidae, and Cisticolidae. However, further studies are needed to investigate the interactions between dominant insectivorous birds and their arthropod prey within these ecosystems, to better understand their roles in regulating ecosystem balance (Amit et al., 2025). In the Central Kalimantan, Indonesia, observation of the White-throated kingfisher, *Halcyon smyrnensis*, in an oil palm plantation has proven that species exhibiting high adaptive behavioural capacity to the agricultural and urban development will increase their distribution range and colonise new areas. The species that was initially occasional visitor has now become a resident breeder and consequently, expanded its distribution range (Silmi et al., 2025).

Progress in Effective Management of Pests and Diseases

Ganoderma boninense, a basidiomycete fungus that causes basal stem rot (BSR) disease, remains a major threat to the industry, leading to substantial economic losses (Hamid et al., 2025). This has driven continued studies in addressing the concern, including the development of various methodologies for its detection and curative control. Improved detection methodologies have been developed with the potential for management of the disease. These included qPCR primer for detection in soil (Tung et al., 2025), object-based image analysis from drone for detection in oil palm (Izzuddin et al., 2025) and a recombinase polymerase amplification-lateral flow assay (RPA-LFA) for onsite and early detection at the asymptomatic phase (Lakshmi et al., 2025).

Transmission of BSR disease primarily occurs through root-to-root contact in the soil. However, the role of *Ganoderma* basidiospores in the transmission process has often been overlooked. A study conducted at five plantations in Sabah, Malaysia, found that at least 55 arthropod species were associated with *Ganoderma* basidiocarps. Among the insects identified in the study carrying the basidiospores, the handsome fungus beetle, *Eumorphus* spp., was found to have the greatest potential for transmitting the basidiospores, showing the potential sporadic spread of BSR and upper stem rot (USR) incidences in the field (Syarif et al., 2025).

Chemical control of *G. boninense* has been proven to be ineffective and thus, prompted the exploration of microbial-based biocontrol, with potential synergistic effect of fungal isolates as biocontrol agents and bioformulations in suppressing the fungus (Anuar et al., 2025). Alternatively, *Trichoderma* isolates from the rhizosphere soil of oil palm seedling plantations have shown potential as biological control of *G. boninense* (Lisnawita et al., 2025), while *Pseudomonas aeruginosa* and *T. asperellum* exhibit potential in promoting plant growth and suppression of the disease infestations in oil palm (Muniroh et al., 2025). A combination of organic fungicide, biofungicide, endophytic bacteria and biostimulant has been effective in curative control of various stages of infection (Eris et al., 2025). Interestingly, the volatile organic compounds (VOCs) from *Fusarium foetens* can inhibit *G. boninense* growth by up to 60% (Lutfia & Rupaedah, 2025). Four key VOCs were identified, with two compounds, 2,3-pyrazinedicarboximide and indolylmethyl thiohydroximate, exhibiting binding affinities similar to the standard antifungal agent hexaconazole.

Improved tools for analysing pathogenic interactions were demonstrated through pathway analysis of *G. boninense* metabolomics data (Nurazah et al., 2026). Enrichment of starch and sucrose metabolism, branched-chain amino acid degradation, and tricarboxylic acid (TCA) cycle pathways across temporal stages highlighted key metabolic shifts supporting fungal growth. These insights improve our understanding of pathogen biology and may inform integrated disease management.

Pests such as rodents and insects have impacted the productivity of the oil palm industry. Rodents cause significant financial losses in the oil palm industry, highlighting the critical need for effective pest management. Understanding the habitat requirements of rodents in oil palm plantations is crucial for effective population control (Asrif et al., 2025). The presence and genetic characterisation of *Bartonella* in small mammals, particularly rodents, were assessed to evaluate the zoonotic risk (AbdulHalim et al., 2025). The study identified the predominant rodent species, *Rattus tanezumi* (R3 mitotype) and *R. tiomanicus*, as hosts for *Bartonella phoceensis*. Although the zoonotic potential of this bacterium remains unconfirmed, these findings highlight the need to adopt precautionary measures to protect workers from potential infection. Moreover, they emphasise the need for continuous monitoring of host-pathogen interactions and the mitigation of risks associated with emerging zoonotic diseases.

Technological Advancement in Enhancing Productivity

The impact of climate change on oil palm plantations has become a critical concern. A systematic review was conducted to examine the effects of climate change on oil palm productivity and plantation physical conditions (Wulandari et al., 2025). The study provided insights into the roles of relevant stakeholders in developing effective adaptation strategies to mitigate the impacts of climate change on oil palm plantations and recommended various measures to sustain productivity and enhance industry resilience. In addition, the impacts of applying advanced technologies associated with the Fourth Industrial Revolution (IR4.0) including artificial intelligence (AI), blockchain, the Internet of Things (IoT), big data analytics and remote sensing, in the oil palm industry were assessed (Zaki et al., 2025). The findings indicated that these technologies can positively improve palm oil production by offering innovative solutions, enhancing operational efficiency and reducing environmental impacts.

Mapping of oil palm plantations and individual trees across Malaysia using the Single Tree Extraction Method-Oil Palm (STEM-OP) with sub-meter visible-light satellite imagery (RGB bands) was conducted in 2022. The study estimated that Malaysia had 5.25 million hectares of oil palm plantations, with approximately 572.6 million trees (Zhang et al., 2025). The method achieved over 90% accuracy for mapping plantation extent and 80% accuracy for counting individual trees.

A study produced a 10–30 m oil palm extent map and a pixel-wise stand-age layer using a multi-sensor remote sensing approach, which integrated 38 years (1987–2024) of Landsat data with 2024 Sentinel optical-synthetic-aperture radar (SAR) composites in a cloud-native workflow (Weerakitikul et al., 2025). This combined approach provides nationally relevant data on plantation area, supporting policy-making decisions and commercial applications. However, future work should focus on expanding field validation, refining age-detection thresholds, and integrating age maps with yield and carbon models.

A dataset of FFB images from commercial plantations in Central Kalimantan, Indonesia, was compiled, covering five different maturity stages: Unripe, underripe, ripe, flower and abnormal (Suharjito et al., 2025). The images were captured using smartphone video from multiple angles under varying conditions, then frame-extracted and annotated. The dataset is validated using a digital census approach, with the aim of supporting the development of deep learning models for FFB detection and classification. These images will support the monitoring of harvest times, predicting yield, and optimising resource allocation.

A non-destructive method combining near-infrared (NIR) spectroscopy with chemometric analyses using Empirical Wavelet Transform and Gaussian Process Regression was developed for the multi-parameter prediction of oil palm fruit quality (Nanda et al., 2025). These parameters predicted included water content, oil content and FFA, which facilitate the optimisation of harvest timing and enhancement of productivity.

Nutrient management plays a crucial role in enhancing palm oil productivity. The growing interest in the development of organic fertilisers has led to increased research on the impacts of fertilisers on palm oil production. A comprehensive assessment of various commercial fertilisers used in oil palm plantations in Indonesia was conducted (Sukarman et al., 2025). The study highlighted the prevalence of counterfeit and non-conforming fertilisers, which negatively impact the productivity and sustainability of oil palm plantations in the country. The findings emphasise the need to use authentic, high-quality fertilisers to ensure the long-term productivity and sustainability of the oil palm industry.

A combination of visible-NIR spectroscopy with advanced statistical analyses has been shown to provide enhanced precision in monitoring and detecting nutrient levels in oil palm plants, compared to traditional methods (Zahir et al., 2025). The red-edge band (660–770 nm) yielded the best results for identifying nutrient-stressed conditions. The study also highlighted that the application of 0.8 g of NPK fertiliser every two weeks significantly improves plant nutrient conditions, as indicated by the spectral index calculation.

The effect of various types of fertilisers on the growth of oil palm in Indonesia, with a focus on root morphology and nutrient uptake was investigated (Wahyudin et al., 2025). The modification of root morphology through root pruning enhances plant nutrient uptake, as root cutting can stimulate the growth of new roots at the cut ends. The study found that plants aged seven years showed the best response in terms of stem circumference and stem diameter, compared to plants aged 12 and 16 years. Additionally, an analysis of variance (ANOVA) revealed a significant difference in the normalised difference vegetation index (NDVI) across plants of different ages, with the highest NDVI value observed in plants aged seven years. No significant difference in NDVI was found between plants aged 12 and 16 years.

The application of slow-release fertiliser to promote growth in oil palm seedlings was investigated (Harahap et al., 2025). The study found that a slow-release fertiliser dose of 75% resulted in the greatest increase in oil palm seedling growth, with an agronomic effectiveness value exceeding 95%, compared to doses of 50% and 150%. Additionally, the use of organic fertilisers and biochar produced from empty fruit bunches (EFB) significantly reduces the need for synthetic fertilisers (F. R. Yusuf et al., 2025). The utilisation of organic fertilisers not only reduces GHG emissions, improving the environmental impact of the oil palm industry, but also lowers the input costs associated with synthetic fertilisers.

Sustainable Development for Smallholders

Sustainable palm oil continues to be a growing global demand. Consumers who increasingly recognise their purchasing power play an important role in increasing the demand for sustainable palm oil as they become more aware of the environmental and social impacts of palm oil. This shift in consumer behaviour underpins Malaysia's strong commitment to the MSPO certification, positioning it as a national priority for sustainability, market credibility, and industry accountability (Rahami, 2024). MSPO serves as a proven mechanism, driving the adoption of best agronomic practices and introducing shared standards that align smallholders' operations with industry expectations. In addition, in an effort to strengthen MSPO outcomes, the Social Impact

Assessment procedure was enforced in June 2025 to enhance compliance and elevate the overall certification standard.

Recent empirical studies, supported by extensive field data, highlight the challenges faced by smallholders (Charlotte & Oliver, 2025; Hong et al., 2025) and evaluate the effectiveness of MSPO certification in strengthening operational compliance and sustainability outcomes (Supriatna et al., 2025). Meanwhile, smallholders continue to face significant barriers to achieving sustainability certification such as financial strain, limited knowledge, and a high dependence on the extension services. Therefore, sustaining smallholders' participation in MSPO ultimately depends on whether independent certified smallholders receive a premium price for their produce, as a direct incentive and reward for implementing the practices (Ansah et al., 2025).

Smallholders are encouraged to adopt crop or livestock integration as a practical path toward income diversification. Recent findings show that oil palm fronds are a viable and cost-efficient substitute for conventional roughage, showing equivalent impacts on livestock performance (Mat Rodi et al., 2025). This allows smallholders to cut feed costs while extracting greater value from existing oil palm resources. Controlled grazing within farm plots naturally returns organic nutrients to the soil, reducing dependence on chemical fertilisers and improving input efficiency without sacrificing yield performance. Although nature-based land management improves soil health and sustainability over time, smallholders' adoption is primarily held back by high upfront costs and the slow impact (Sibhatu et al., 2025).

A robust traceability system is essential to ensure that sustainably produced palm oil reaches the intended consumers and provides verifiable origin claims (Lai et al., 2025). As environmental and economic pressures intensify, the oil palm industry is compelled to increase productivity, strengthen sustainability, and expand transparency throughout its supply chain. Evidence shows that AI technologies can significantly improve production, elevate supply chain visibility, and reinforce environmental compliance (Judijanto, 2025b). The Malaysian oil palm industry is undergoing a steady yet high-stakes transition toward stronger environmental sustainability, propelled by regulatory demand, market forces, and the rising awareness of long-term economic value (Hong et al., 2025).

Sustainable Palm Oil Mill Effluent (POME) Treatment and Biogas Recovery

The palm oil milling sector plays a vital role in the palm oil production and processing supply chain but faces growing challenges such as stagnant efficiency, stringent environmental regulations and increasing sustainability demands. A key focus area in the milling sector relates to the management of palm oil mill effluent (POME). Sustainable treatment of POME is crucial for preserving the environment while supporting the commitment to sustainable development.

In recent years, POME treatment technology has advanced significantly beyond just conventional ponding systems. Innovations such as the POMEVap technology (Alfa Laval Malaysia Sdn. Bhd., Malaysia) have introduced an efficient, compact and environmentally friendly alternative for managing POME. This technology applies evaporation-based separation to recover reusable water and solid concentrate, enabling zero liquid discharge and reducing raw water consumption through internal reuse. Its closed-loop, methane-free design minimises GHG emissions, while its forced-circulation plate evaporator ensures high turbulence and a self-cleaning effect, thereby attenuating fouling and maintenance needs (Nasrin et al., 2025).

Continuous adsorption system (CAS) of POME using palm kernel shell (PKS) activated carbon offers a cost-effective and high-performance approach for polishing final discharge. The system consistently achieves significant reductions for crucial pollutant indicators, including biochemical oxygen demand (BOD) (by up to 85%), suspended solids (by approximately 70%), chemical oxygen demand (COD) (by approximately 80%), and colour removal reaching zero ADMI units, thereby producing effluent that nearly meets drinking water standards. Beyond its superior pollutant-removal efficiency, the CAS is also cost-effective, which warrants a lower capital cost than membrane-based systems. In keeping with the principles of a circular economy, the CAS further generates economic value through annual water-reclamation savings and the conversion of saturated activated carbon into bio-fertilisers (N. H. Zainal et al., 2025).

Another emerging technology in POME polishing is the photocatalytic degradation of anaerobically-treated POME with green-synthesised zinc oxide-lemongrass nanoparticles. This eco-friendly approach has demonstrated remarkable reductions in pollutant metrics, such as turbidity (by 85.6%), COD (by 97.0%), and colour (by 50.9%), thus yielding treated effluents that meet the Malaysian Department of Environment's (DOE) discharge standards. The enhanced performance is attributed to the small nanoparticle size and lemongrass-based capping, which improve photocatalytic activity and stability (Sidik et al., 2025).

The use of pre-treated EFB as a co-substrate in POME anaerobic digestion has gained increasing attention due to its ability to improve biodegradability and methane generation.

Pre-treatment methods such as chemical, hydrothermal, bacterial and subcritical water-based means are used to delignify EFB by breaking down its lignocellulosic structure. This not only improves the accessibility of cellulose but also reduces the resistance of the biomass. These pre-treatment approaches therefore facilitate a greater release of fermentable compounds and improve the overall digestibility, leading to more efficient co-digestion with POME. When integrated into modern high-rate anaerobic systems, these approaches can strengthen process stability, accelerate degradation and maximise renewable energy recovery, making them promising pathways for advancing biogas production from POME (Hamzah et al., 2025).

Additionally, a conductive carbon brush (CB) has emerged as a promising material for enhancing acetoclastic methanogenesis in POME. CB promotes direct interspecies electron transfer between microorganisms, leading to faster and more stable electron flow, thus accelerating organic matter degradation and improving methane production. By improving electron transfer, CB also stabilises pH levels, mitigating typical acidification issues and leading to more consistent and efficient biogas recovery. CB can potentially be integrated into existing anaerobic POME pond or digester tanks without major structural changes (Ngatiman et al., 2025).

Another promising approach involves the synergistic impact of biochar and torrefied products from oil palm biomass to optimise biodegradability and methane production. Biochar enhances anaerobic digestion by providing a favourable microbial habitat, reducing the methanogenic lag phase, and boosting methane production, leading to improved sustainability and efficiency in biogas recovery (Yan et al., 2025). Similarly, torrefaction of oil palm frond (OPF) before its co-digestion with POME significantly increases methane yield due to structural and chemical changes that enhance biodegradability. Co-digestion with torrefied OPF also leads to high removal efficiencies for COD, volatile solids and total nitrogen of POME (Milicevic et al., 2025). Combining biochar and torrefied biomass as co-feeds offers a sustainable, energy-efficient solution to enhance POME treatment and resource recovery by the oil palm industry.

Biogas capture facilities can reduce energy costs, generate revenue by selling power to the grid, and potentially benefit from carbon credit mechanisms. In addition, biogas can be upgraded to biomethane for use in vehicles or injected into the natural gas grid. Moreover, the use of POME treatment and biogas capture technology can support the achievement of the Nationally Determined Contribution targets for GHG reduction and promote energy transition and the use of green technologies as outlined in the National Energy Transition Roadmap (NETR) and National Agricommodity Policy 2021–2030 (DAKN 2030) (Kementerian Perusahaan Perladangan dan Komoditi, 2021).

Biomass and Bioenergy Innovation

The transition towards a low-carbon economy is gaining momentum in Malaysia through innovative uses of oil palm biomass. Lignocellulosic materials of oil palm biomass predominantly contain a mixture of cellulose, hemicelluloses (polyose), and lignin (a complex phenolic polymer). This biomass holds significant potential as a sustainable source of fuels, bio-products, and chemicals due to its abundance, renewability, low cost, and non-competing food sources.

Oil palm trunk (OPT) has been estimated to constitute approximately 5% of the total oil palm biomass, with quantities reaching up to 75 t ha^{-1} during the replantation period (Norhazimah et al., 2025). Currently, only a small portion of the felled OPT is utilised for plywood manufacturing or sold to interested buyers, while the majority is typically shredded and mulched in the fields to decompose naturally for nutrient recycling (Uke et al., 2021). Thus, there is growing interest in studying alternative ways to fully exploit and tap the potential of underutilised OPT. The enzymatic hydrolysis of lignocellulose from OPT has been identified as a potential alternative for the production of fermentable sugars. Utilisation of surfactant significantly improves the enzymatic hydrolysis performance of OPT at increasing solid loadings by enhancing glucose yields, and maintaining higher conversion efficiencies compared to non-supplemented systems (Bukhari et al., 2025).

Oil palm fibres possess promising characteristics for composite applications. However, their hydrophilic nature, residual oil content, and high lignin composition present challenges in composite fabrication, affecting fibre-matrix adhesion and overall performance. To overcome these limitations, exploration of hybridisation with other synthetic or natural fibres, as well as nano-integration using nanomaterials, has been studied to enhance the structural integrity of the composite. A combination of oil palm fibres with other reinforcements has led to enhanced mechanical, thermal, and durability properties, making the composites suitable for various engineering applications (Aisyah et al., 2026). Meanwhile, due to the presence of sugars and starches in their tissues, oil palms have an exceptionally low natural resistance to fungi, making them susceptible to contamination. Therefore, palm oil-based particle board shows susceptibility to fungal attack, especially from

Aspergillus sp., *Trichoderma* sp., and *Paecilomyces variotii*. Additional treatments to control or prevent fungal growth were required to increase the commercial value of particleboard (Idris et al., 2025).

Mesocarp fibre is a by-product generated from the palm oil mill, which has been highly used as a boiler fuel, containing 5%–6% of residual oil (dry basis). However, it has a lot of potential to be used as a dietary fibre with many health benefits, especially for lowering blood sugar levels and improving gut health. The physicochemical and functional properties of mesocarp fibre suggest that it could serve as an alternative source of dietary fibre and functional ingredient for mitigating the risk of dietary fibre deficiency-related diseases (Teh & Lau, 2025).

The increasing demand for clean and renewable energy storage has driven studies into cutting-edge battery materials using oil palm biomass. EFB is identified as a potential material for producing activated carbon for battery electrodes based on its carbon properties. The production of the material was carried out by chemical activation using NaOH and KOH, followed by physical activation using varying temperatures. High activation temperature (900°C), long soaking time (18 hr) in a 2 M concentration of KOH resulted in the highest surface area, specific capacitance, and potential (Triana et al., 2025). Meanwhile, PKS has the potential to produce SiO_2/hard-carbon-like nanocomposites for applications in dual carbon sodium-ion batteries. The pyrolysis temperature has been found to have a significant effect on the structural, chemical, and electrochemical properties of the resulting materials. High-quality hard carbons are produced at 700°C with a combination of high specific capacitance, balanced defect density, graphitic ordering, and hierarchical porosity, enhancing Na^+ ion adsorption and charge transfer. The pores produced by PKS hard carbon-like have great potential as sodium ion intercalation sites for sodium batteries (Mas'udah et al., 2025). In addition, pellet-form graphitic carbon (PGC) for use in supercapacitor applications was successfully synthesised from OPF using a one-step carbonisation process without the use of chemicals for activation. High-quality graphitic carbon products were produced with characteristics such as optimal specific capacitance (334 F g^{-1}) for the PGC electrode in 1 M H_2SO_4 electrolyte, as well as the fabrication of a symmetric supercapacitor that achieved an energy density of 18.86 Wh kg^{-1} and a power density of 1,386 W kg^{-1}. The use of redox enhancers, especially hydroquinone, further improved the specific capacitance (Ullah et al., 2025).

Palm biodiesel has been introduced into the Malaysian market by blending it with petroleum diesel to produce a more environmentally friendly fuel. However, its use may be limited in cold climates and highland areas because biodiesel can thicken in cold weather due to its feedstock composition. Palm-based additives can help reduce the cold filter plugging point of biodiesel blends, making them more suitable for cold conditions. These additives improve the cold flow properties of the fuel, especially when combined with higher levels of monoglycerides (Nursyairah et al., 2025). The use of 10% biodiesel and 90% petroleum diesel (B10) and 20% biodiesel and 80% petroleum diesel (B20) palm biodiesel in heavy-duty diesel vehicles on the road showed positive effects. The two biodiesel blends did not show significant differences in fuel economy and maintenance intervals. In addition, the B20 sample showed almost the same degradation as B10. However, the total base number and iron content in the engine oil of the B20 group showed changes that had a positive effect on the condition of the engine oil (Thaddeus et al., 2025).

The SAF or biojet is a hot topic discussed by researchers recently. The use of raw materials from the palm oil industry to produce SAF is also being actively pursued. Refined palm oil was used as a feedstock to produce SAF range hydrocarbons using a hydrogen-free hydrothermolysis process and a Ni/AC-Al_2O_3 catalyst. The study conducted shows that an economical and environmentally friendly approach for the production of biojet without using hydrogen offers an alternative to traditional processes (Ahmad et al., 2025). Palm fatty acid distillate (PFAD), by-product of the physical refining of CPO, is also a suitable raw material to produce biojet due to its free fatty acids content, primarily palmitic acid (C16). PFAD through hydrogen-free and solventless deoxygenation reaction using a bimetallic NiCo/SBA-15-NH_2 catalyst has successfully produced biojet, achieving the highest hydrocarbon yield (C8–C20) of 83% and biojet selectivity of 88% (Derawi et al., 2025). In addition, PFAD can be extracted to produce squalene, one of the bioactive compounds that has various applications in the food, cosmetic, and pharmaceutical industries (Nor Faizah et al., 2025). *Table 7* presents a comprehensive overview of recent innovations in oil palm biomass utilisation, highlighting the diversity of biomass sources, conversion approaches, and value-added products developed for bioenergy, advanced materials, and industrial applications.

Food Safety and Quality Research

Adulteration in food is a widespread issue with global implications. Being a common oil used in food preparation, fraudulent activities involving

TABLE 7. SUMMARY OF RECENT INNOVATIONS IN OIL PALM BIOMASS UTILISATION FOR BIOENERGY, ADVANCED MATERIALS AND VALUE-ADDED PRODUCTS

Material/product	Biomass source	Description	Key findings	Reference
Fermentable sugars	Oil palm trunk	Enzymatic hydrolysis with surfactant	Improved glucose yield and conversion efficiency	Bukhari et al. (2025)
Hybrid composites	Oil palm fibre	Hybridisation and nano-integration	Enhanced mechanical and thermal properties	Aisyah et al. (2026)
Particleboard	Oil palm biomass	Lignocellulosic board material	Susceptible to fungi; requires treatment	Idris et al. (2025)
Dietary fibre	Mesocarp fibre	Functional food ingredient	Improves gut health and blood sugar control	Teh & Lau (2025)
Activated carbon	Empty fruit bunches	Chemical + physical activation	High surface area and capacitance	Triana et al. (2025)
Hard carbon composite	Palm kernel shell	SiO_2/hard carbon nanocomposite	Enhanced Na^+ storage for batteries	Mas'udah et al. (2025)
Graphitic carbon	Oil palm frond	One-step carbonisation	High-energy-density supercapacitor	Ullah et al. (2025)
Biodiesel additive	Palm-based	Cold flow improver	Reduced cold filter plugging point (CFPP) in biodiesel blends	Nursyairah et al. (2025)
Biojet fuel (SAF)	Palm oil/palm fatty acid distillate	Catalytic conversion	High hydrocarbon yield (C8–C20)	Ahmad et al. (2025); Derawi et al. (2025)
Squalene	Palm fatty acid distillate	Bioactive compound extraction	High-value product for pharma/cosmetics	Nor Faizah et al. (2025)

Note: SAF - sustainable aviation fuel; SiO_2 - silica.

palm oil, such as dilution with cheaper oils, addition of dyes/chemicals, and mixing with used cooking oil, pose serious health threats and economic risks. Conventional chemical tests, such as fatty acid composition, have limitations in detecting these adulterations. Therefore, newer approaches such as metabolomics in combination with chemometric tools shall offer a comprehensive method to identify fraudulent additions in cooking oil (Sulaiman et al., 2025).

Similarly, in order to address the possible negative impacts on oil quality and investigate strategies to reduce the production of pollutants across the supply chain, it is crucial to study the processing of underripe and overripe fruit. A study by Ramli et al. (2025) reported that the ripeness of FFB affects both chemical contaminants, such as 3-monochloropropane-1, 2-diol esters (3-MCPDE) and refined oil colour stability, making proper harvesting and processing crucial for ensuring high-quality and safe palm oil. FFB are classified as unripe, underripe, ripe or overripe, with optimal ripeness at 20–22 weeks after anthesis. Processing of under-ripe fruits leads to low oil content, resulting in lower OER, inferior CPO quality, and low kernel extraction rate (KER). Meanwhile, ripe fruits yield CPO with lower chlorophyll, higher deterioration of bleachability index (DOBI), and higher carotene contents, which are the indications of high-quality CPO. Therefore, in order to preserve the quality of CPO, the authors recommend that underripe fruit processing should be avoided.

Nutrition and Health

Palm oil is used globally as cooking oil, including in baked and processed food, cosmetics, as well as biofuels. However, the nutritional aspects of the oil are commonly debated. However several reviews and meta-analyses conducted in recent years have systematically analysed the literature and shown positive nutritional benefits of incorporating palm oil in diet (Judijanto, 2025a; Parveez et al., 2025; Voon et al., 2024).

A recent systematic review reported the outcomes from 22 papers published from 2020 to 2025 aimed to discuss the nutritional attributes of palm oil and the contribution of the palm bioactives in achieving beneficial outcomes (Judijanto, 2025a). The strategic predomination of palmitic acid at the *sn*-1 and *sn*-3 positions of the glycerol molecules selectively reduces the atherogenic effect of the palm oil. In addition to the saturated fatty acids, the unsaturated fatty acids composition in palm oil also contributes to cardiovascular neutrality reported in several clinical studies. Palm oil is concluded as a nutritionally rich oil if it is consumed in minimally processed conditions within the recommended dietary guidelines.

Additionally, a pilot randomised crossover study in 10 healthy young men compared the postprandial effects of high-fat meals rich in palmitic acid from palm olein and lard with virgin olive oil (Nagapan & Teng, 2025). After consuming 50 g of each test fat, blood samples were collected over 4 hr to assess endothelial dysfunction biomarkers. All three fats led to significant reductions in plasminogen activator inhibitor-1 (PAI-1) and soluble CD40 ligand (sCD40L), with no notable effects on other pro-inflammatory markers (monocyte chemoattractant protein-1 [MCP-1], E-selectin, soluble vascular cell adhesion molecule-1 [sVCAM-1]). Overall, palmitic-acid-rich diets showed minimal impact on endothelial dysfunction biomarkers postprandially, indicating the need for larger studies to confirm these findings. The authors reported that the findings may not be applicable to females due to different physiological make-up between the genders that may affect metabolic processes, while also acknowledging the sample size was relatively small to observe a significant outcome (Nagapan & Teng, 2025).

Red palm olein (RPO) is recognised as the most prolific natural botanical source of carotenoids, predominantly comprising almost equal amounts of alpha-carotene and beta-carotene. Since the 1930s, RPO has garnered scholarly interest as a therapeutic agent for xerophthalmia, and its supplementation has been studied as a preventive strategy for xerophthalmia among vitamin A-deficient (VAD) primary schoolchildren, with several trials indicating significant benefits. A recent randomised controlled trial in Malaysia found that a 6-month supplementation with RPO-enriched biscuits led to a significantly higher prevention rate of xerophthalmia and conjunctival xerosis compared to the control group. Although the findings were not significant for an improved resolution rate of xerophthalmia between the two groups, it was reported that children receiving RPO were about five times less likely to develop xerophthalmia as compared to the control group (Tan et al., 2025a). In addition, another intriguing result published by the investigators showed a positive correlation between alpha-carotene, beta-carotene, alpha-tocopherol, and haematological outcomes in children receiving RPO-enriched biscuits and significant reductions in alpha diversity indices and increased abundance of potentially beneficial gut bacteria (Tan et al., 2025b). RPO supplementation is a promising, food-based preventive solution for xerophthalmia and VAD in at-risk children, with additional benefits for iron status and inflammation. Incorporation of RPO into the nutritional programs may help to address these health issues in vulnerable populations.

Fuelled by interesting outcomes from pre-clinical studies of tocotrienols, an intervention study with tocotrienol-rich fraction (TRF) was conducted in patients with rheumatoid arthritis (RA), an autoimmune disease predominantly caused by inflammation, by Z. Zainal et al. (2025). Following a 6-month of supplementation with 400 mg of TRF day^{-1} with the conventional disease-modifying anti-rheumatic drugs (cDMARDs), a significant drop in the Disease Activity Score-28 (DAS28), a standard measure of RA activity was reported. Patients also experienced less knee pain, improved physical function, reduced joint stiffness, swelling (edema), and discomfort. Therefore, TRF was found to be a well-tolerated adjunct that can effectively lower RA disease activity and improve patients' quality of life. Meanwhile, two proceeding papers were also published on the anti-tumour potentials of tocotrienols in pre-clinical models. A study by Hafid et al. (2025) shows that combining TRF from palm oil with dendritic cell (DC) vaccines significantly improves anti-tumour immune responses and tumour control in mouse models of breast cancer. TRF acts as an adjuvant, boosting the effectiveness of DC-based cancer vaccines. Meanwhile, in acute myeloid leukaemia (AML) cell lines, the combination of TRF and cytarabine, a leukaemic drug, inhibited cell growth with a profound elevation of caspase activities, indicating the pro-apoptotic activities of the treatment (Iran et al., 2025).

Oil palm phenolics (OPP), obtained from the by-products of oil palm fruit processing, are a source of bioactive polyphenols with valuable nutraceutical properties. Numerous studies have demonstrated the physiological properties of this water-soluble bioactive. In line with this, based on a phase one clinical trial, the supplementation of encapsulated OPP in healthy individuals remarkably lowered blood pressure after 60 days and improved total antioxidant capacity following 30 days of supplementation. The authors recommended that the findings from this phase one trial serve as a strong foundation for longer intervention studies in the population with a clinical condition (Fairus et al., 2025).

Food and Feed Studies

A robust quantification method for identifying the major phenolic acids was recently developed and published using ultra-high-performance liquid chromatography with ultraviolet/ photodiode array detection (UPLC-UV/PDA). The method showed excellent specificity, linearity, accuracy, precision, and robustness. When applied to water-soluble palm fruit extract (WSPFE) samples from Malaysian and Mexican facilities, the quantified phenolic contents were comparable and aligned with results from Folin-Ciocalteu and 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assays. Overall, the validated method provides a reliable tool for consistent quality control of WSPFE in supplement and functional food industries (Leow et al., 2025).

Growing concerns on food sustainability, animal welfare, and the increasing global population shifted attention towards plant-based meat analogues (PBMA), with palm oil emerging as a noteworthy potential. Approximately 6% of saturated fat was required to achieve realistic texture and palatability in PBMA. Incorporation of palm oil in PBMA has indeed shown promising outcomes in achieving the taste while having lesser saturated fats, unlike meat products (Wazir et al., 2025). Apart from this, the usage of RPO was further explored in RPO-based candies, which are easy to consume and to cater to the health-conscious consumer population. The formulation was optimised using RPO powder, erythritol as the sweetener, and gum arabic as the binding agent (Tang et al., 2025). The resulting candies were found to achieve the desired quality with moderate hardness and reduced friability.

In recent times, there has been an increased interest in organogelation techniques using vegetable oils. Despite showing increased hardness, the palm-based monoacylglycerol oleogels showed a complicated pattern of crystalline and amorphous behaviour with big crystals and voids, which led to reduced stability. This information improves our comprehension of superolein oleogel behaviour, which is crucial for their use in a variety of sectors (Saw et al., 2024).

Apart from this, in the broiler feed industry, agricultural by-products present an affordable way to lessen dependency on traditional feed grains. The effect of newly developed feed formulation incorporated with CPO and palm kernel meal in comparison with commercial feed in mixed gender Ross 308 broiler chicks fed for 35 days was evaluated (Wan Mohamed et al., 2025). The broilers fed with newly formulated feed had higher weight gain despite lower intake of feed as compared to the broilers fed with conventional feed. The higher feed conversion ratio was another promising output from this study, with the authors recommending partial substitution of imported feed ingredients with CPO and PKO to achieve comparable meat quality. Similarly, another feeding intervention was carried out in beef cattle in Malaysia. This study compared an oil palm by-product (OPB) feed pellet with a commercial pellet in 42 beef cattle over 90 days. Both pellets had similar protein and energy content. Cattle fed with OPB pellets showed comparable growth to those on the commercial pellet, although the OPB group had a slightly higher average daily gain and a marginally better feed conversion ratio (9.59 vs. 9.98). Meat from OPB-fed cattle also had significantly higher redness and yellowness, suggesting better visual quality. Overall, the OPB feed pellet provided complete nutrition and performed on par with commercial pellets in supporting cattle growth (Ibrahim et al., 2025).

Oleochemical Innovations in Non-Food Products

In 2025, the Malaysian oleochemical industry is categorised by steady growth, strong global demand, and a strategic focus on sustainability and value-added products. The key trends include a rise in eco-conscious consumption that drives demand for natural products. Oleochemical innovations focused on developing sustainable alternatives and green technologies over petrochemical products. The key innovations include the use of oleochemicals derived from CPO and CPKO into bio-based non-food products such as bio-lubricants, specialty chemicals, surfactants, and agrochemicals, to meet the rising demand for eco-friendly solutions.

One of the highlighted innovations is the development of bio-lubricants derived from palm-based oleochemicals. A high-performance estolide ester (2-ethylhexyl ester of lauric acid-esterified estolide [2EHLaPE]), a lubricant base oil produced from PFAD and oleic acid (OA), was developed, with its lubricating properties comparable to those of commercial bio-lubricants (Hoong et al., 2025). During the reaction, peracetic acid was generated *in situ* through the reaction between hydrogen peroxide and acetic acid. This peracetic acid subsequently reacted with the alkene groups in the PFAD-OA mixtures, converting them into epoxide groups. The newly formed epoxide groups were then ring-opened by the carboxylic acid groups of the fatty acids, resulting in the formation of the desired estolide products. A higher proportion of OA in the PFAD-OA blend used as the starting material increased the kinematic viscosity, and the incorporation of OA also improved the cold flow properties of the 2EHLaPE. This innovation has emerged as a promising alternative to mineral-based lubricants due to its non-toxic nature and high biodegradability. Optimisation of the types and compositions of base stocks and additives was involved in the development of lubricant formulation. Tribology additives are one of the crucial elements in ensuring optimum lubrication performance, and investigating the tribological behaviours of these additives is essential.

A comprehensive thermodynamic modelling approach was used to determine the optimal lubrication and synergistic additives combination, even when the evaluated lubricants exhibited comparable outcomes in tribological assessments (Chan et al., 2026). The model employs a dissipative coefficient of the lubrication as a reliable assessment parameter, providing insights into the lubrication ability to minimise wear and sustain tribological operations effectively.

Drilling fluid used in the oil and gas industry acts as a lubricant, reducing friction and heat between the drill bit, drill string, and borehole wall. A newly developed ester blend, combining

2-ethylhexyl palm fatty acid distillate (2EH-PFAD) and commercial esters that meets the requirements for drilling fluid base oils and is formulated into an ester-blended drilling fluid (EBDF) (Pauzi et al., 2025). PFAD, used in the EBDF, is a cost-effective and sustainable resource. The developed EBDF demonstrated enhanced rheological properties, reduced fluid loss, and excellent electrical stability.

The R&D innovations for oleochemical derivatives also extended to the development of environmentally friendly, cost-effective, and high-performance bio-based corrosion inhibitors. Mohd et al. (2025) had synthesised imine-based compounds from palm-based fatty hydrazide and evaluated the inhibition efficiency of different alkyl chain lengths of the imine-based organic inhibitors on carbon steel in hydrochloric acid solution. Performance test results revealed that these compounds significantly reduced corrosion, with the highest inhibition effectiveness observed at C12 chain length. The alkyl chain length affects solubility in the acid solution, whereby longer chain length prevents further improvements in inhibition efficiency. The study also reported steric hindrance associated with longer alkyl chains, which may reduce the accessibility of polar head groups to the steel surface, thereby lowering inhibitor performance.

The demand for renewable and sustainable chemical innovations extends to insecticide formulations. A new water-based insecticide formulation, comprising solvents and surfactants derived from palm-based materials, with deltamethrin as the active ingredient, was developed to control dengue outbreaks and minimise the environmental impact of vector control (Mohsin et al., 2025). The formulation is naphtha-free and uses palm oil as feedstock, resulting in a cost-effective product. It offers comparable efficacy to the commercial naphtha-based formulation. Field trials using both cold and thermal fogging methods showed no significant differences in droplet size between the two formulations, although cold fogging generally performed better in terms of knockdown and mortality rates. This innovation highlights the potential of biodegradable palm-based ingredients, particularly palm methyl ester solvent and palm-based surfactants, leveraging the locally abundant renewable raw material, palm oil.

Innovations in analytical methods are also important for safeguarding the quality of oleochemical products. A method for detecting and quantifying unreacted dimethyl sulphate (DMS) in fabric softeners containing vegetable and tallow-based esterquats has been developed (Ping & Aziz, 2025). Active ingredients in fabric softener formulations, such as esterquats, have been widely used to replace the conventional distearyldimethylammonium chloride (DSDMAC) due to biodegradability concerns. However, higher incorporation of these active compounds increases the risk of residual DMS. Given its toxicological concerns and classification as a potential human carcinogen, detecting DMS in fabric softeners, even at trace levels, is crucial. The simple direct injection method followed by gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring analysis was validated according to International Council for Harmonisation (ICH) guidelines and demonstrated good selectivity, sensitivity, repeatability, inter-day precision, and accuracy. This analytical monitoring can serve as an effective quality control tool for quantifying unreacted DMS that may be present in fabric softeners.

Environmental sustainability and safety are the key factors to the development and application of palm-based oleochemicals. The lifespan of the developed bio-based products must adhere to the stringent ecological and health standards. To address this concern, studies have also focused on evaluating the potential risks posed by environmental contaminants to human and aquatic organism health, providing critical data to guide sustainable production practices and ensure consumer safety. The effects of plasticisers and additives in plastics on oysters were evaluated to assess their impact on marine ecosystems and the safety of seafood harvested from contaminated waters (Ishak et al., 2025). The widespread use of plasticisers, prevalent chemical contaminants that leach from plastics into aquatic ecosystems, poses potential risks to human and aquatic organism health. Both phthalate and non-phthalate-based plasticisers, including epoxidised methyl oleate (EMO), derived from palm-based sources, were evaluated, and the palm-based plasticiser was found to be less toxic than conventional petroleum-based phthalate plasticisers, making them a safer and more environmentally friendly alternative.

CONCLUSION

In summary, the Malaysian oil palm sector in 2025 showed signs of recovery and adaptation, although many of these gains remain conditional and uneven. The most strategically important R&D priorities are those that can improve yield recovery after replanting, reduce labour dependence, strengthen sustainability verification, and increase downstream value capture. Future progress will depend not only on scientific advances but also on effective technology deployment, smallholder inclusion, and market acceptance. The oil palm industry remains a vital pillar of socioeconomic development, contributing positively to the growth of the Malaysian economy. Although the global commodity market continues to be affected by volatility arising from ongoing geopolitical tensions, fiscal uncertainties, and policy challenges, the oil palm industry has demonstrated resilience, as reflected in its strong performance in 2025. Continued demand from key markets, including domestic consumption, together with favourable prices, has supported the industry's competitiveness. The oil palm industry continues to progress, driven by the transition towards a low-carbon and circular economy. Robust R&D progress and achievement are being further strengthened and accelerated by the technological advancements, ensuring that the industry remains sustainable, resilient, and competitive within the global vegetable oils industry.

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PARADIGMS AND KNOWLEDGE GAPS IN OIL PALM STEM ROTS CAUSED BY *Ganoderma*

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ABSTRACT

Stem rots of oil palms caused by Ganoderma boninense *(basal stem rot and upper stem rot) were first reported in Southeast Asia some 90 years ago. Despite considerable observation and research since that date and the construction of various paradigms, they remain the biggest threat to sustainable oil palm production in SE Asia and Oceania. In this article, we discuss some of the paradigms developed in* Ganoderma *research over many decades and identify some "knowledge gaps" that may be significant for developing improved disease control. Fourteen, specific recommendations on several different aspects of the disease, its control, and palm husbandry are provided and we believe, these should be considered by researchers who continue to study these economically important diseases.*

Keywords: *Ganoderma boninense* research, knowledge gaps, oil palm, recommendations.

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INTRODUCTION

Stem rots caused by *Ganoderma boninense* remain the greatest challenge to sustainable oil palm production in Southeast Asia and the Pacific (Flood et al., 2022). In Malaysia, fresh fruit bunches (FFB) yield reduction at the rate of 0.04 to 4.34 t/ha at 10 to 22 years of planting respectively was reported by Roslan and Idris (2012) with economic losses reported of up to USD500 million (Bharudin et al., 2022; Zakaria, 2023). In North Sumatra, by the time of replanting (25 years), 40%–50% of palms in some fields were lost with the majority of those remaining showing disease symptoms (Rees et al., 2007). The level of basal stem rot (BSR) in Papua New Guinea (PNG) is not as high as in Southeast (SE) Asia although 50% has been recorded (Pilotti et al., 2018).

In recent years, there have been numerous reviews about *Ganoderma*-induced stem rots in oil palm (e.g., Bharudin et al., 2022; Chong et al., 2017a; 2017b; Mercière et al., 2017; Siddiqui et al., 2021; Supramani et al., 2022; Zakaria, 2023). Although these publications have, to varying degrees, considered both historical perspectives and new research, several basic questions remain to be answered before much of the existing research can be fully utilised for possible control strategies. Siddiqui et al. (2021) and Flood et al. (2022) both published updated reviews of the current knowledge on BSR of oil palm and also briefly highlighted some of the key challenges that remain for researchers and the oil palm industry in general. This approach was expanded by Pilotti and Bridge (2023) who reviewed *Ganoderma* diseases of several tropical crops and also identified several fundamental issues and areas where further research and interpretation are required.

In this article, we have attempted to discuss the paradigms that have impacted *Ganoderma* research over many decades and have identified the "knowledge gaps" that we believe may be significant for the development of robust monitoring and husbandry required to improve disease control. We have considered the historical and current information in two sections that are essentially "What do we know?" (Current Knowledge) and "What are we unsure of?" (Uncertainties). We have then attempted to identify what activities may be required to clarify the uncertainties that remain in *Ganoderma* research that require further investigation. These are provided as several specific recommendations in different aspects of

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Ganoderma research that we believe should be considered by researchers who continue to study this economically important disease.

CURRENT KNOWLEDGE

The Fungus

Historically, various species of *Ganoderma* were suggested as responsible for BSR in oil palms. In 1985, Ho and Nawawi determined that all occurrences of BSR in Malaysia were due to the single species *G. boninense* (Ho & Nawawi, 1985). Subsequently, all instances of BSR on oil palm in Southeast Asia and Oceania have been identified as *G. boninense*. Although the names *G. miniatocinctum* and *G. zonatum* have been used for some collections in Malaysia, molecular and mating studies have repeatedly confirmed that these isolates have been misidentified and that they are examples of *G. boninense* (Flood et al., 2022; Fryssouli et al., 2020; Midot et al., 2019; Pilotti & Bridge, 2023). *Ganoderma boninense* was first described in 1888 and has been recorded from a wide range of palm hosts throughout SE Asia and Oceania (Patouillard, 1889; Pilotti & Bridge, 2023; Steyaert, 1975). The species was therefore present in the region and probably widely distributed on native palms before the commercial introduction of oil palm (Mercière et al., 2017; Midot et al., 2019).

Systematically, *G. boninense* has been placed together with a small number of other species in a distinct clade within the genus (Fryssouli et al., 2020). This clade was originally termed the "palm clade" by Moncalvo (2000) and includes *G. zonatum* and *G. ryvardenii* which occur on palms in the Americas and Africa respectively. Modern species within *Ganoderma* are thought to be relatively young in evolutionary terms and probably diverged around 30 million years ago (MYA). This is after the origin of palms (around 100 MYA) and the formation of the modern continents (around 60 MYA (Flood et al., 2022). *Ganoderma* species are largely associated with soil and plant debris and recent studies suggest that there may be significant biogeographic distributions within the genus (Fryssouli et al., 2020; Moncalvo & Buchanan, 2008). It is therefore likely that the modern palm pathogens have evolved independently on each continent from a common ancestor (Loyd et al., 2019; Pilotti & Bridge, 2023).

The fungus is typical of many basidiomycetes in that the fruiting body (bracket) produces monokaryotic spores. These basidiospores germinate to produce monokaryotic mycelium and this then fuses with mycelium from another compatible spore to produce dikaryotic mycelium that ultimately, produces a new fruiting body. *Ganoderma boninense* has a tetrapolar mating system and this favours outbreeding between spores from different fruiting bodies (Pilotti et al., 2002). [Bharudin et al., (2022) and Pilotti & Bridge, (2023)] described the detailed life cycle of *G. boninense*.

The Disease

Ganoderma diseases are generally confined to woody plants and palms. A small number of species can infect many tropical and sub-tropical crops including rubber, tea, other beverage crops, citrus, and *Acacia* spp. as well as betel, coconut and oil palms (Pilotti & Bridge, 2023). The fungi occur as decomposers of dead wood in natural ecosystems and no doubt have evolved with specific enzyme systems capable of degrading a variety of materials (Papp, 2019; Pilotti & Bridge, 2023). This is evident in the 'palm specific' species that appear to have become specialised and pathogenic on palms viz. *G. boninense, G. ryvardenii* and *G. zonatum*.

Basal stem rot (BSR). Most instances of *Ganoderma* diseases on palms manifest as a basal rot of the bole tissue which may subsequently move into the roots. Initially, infection in oil palms was thought to begin in root tissue and move into the bole area, but it is believed that the inoculum potential in single palm roots is inadequate to initiate infection in basal tissue and multiple infections would be needed (Rees et al., 2009). Anecdotal evidence indicates that the infection may begin in the base of the palm, *via* pruned frond butts, a route first suggested some 90 years ago by Thompson (1931). Panchal and Bridge (2005) detected the pathogen's DNA in the tissue behind pruned frond bases while *G. boninense* has also been detected in inoculated cut fronds (Rees et al., 2009). These findings were from palms before disease symptoms were apparent and so suggest early colonisation in the frond bases might occur. Unlike other palms such as coconut, it is necessary to prune the green fronds of oil palm to allow access to the fruit bunches during harvest. Pruning typically begins two to two and a half years after planting and continues throughout the economic life of the palm. It is possible that mechanical injury at the bole-root interface of palms caused by strong winds could also provide an avenue for the entry of fungal inoculum in the form of basidiospores. Mechanical weeding could also cause wounds to the base of the palms and the root ring providing an avenue of infection.

The decay is typically a tan-coloured dry rot with or without darker 'zone' lines and an advancing yellow margin. The latter area is believed to be composed of extracellular fungal enzymes that begin the decay process, possibly by removing hemicellulases and starting to expose the target constituents of cellulose and lignin (Ariffin et al., 2000).

The duration of the disease cycle may depend on the susceptibility of the breeding line and the time of infection. Diseased palms have been known to survive after early symptom expression from as little as six months to as long as two to three years (Ariffin et al., 2000; Zakaria, 2023). However, this field-based evidence does not take into account the incubation period which is as yet unknown but appears to be variable, possibly relating to the genetics or nutritional status of the palm. Some evidence from field trials in PNG shows that the application of Muriate of Potash (MoP) may play a role in reducing *Ganoderma* disease incidence (Papua New Guinea Oil Palm Research Association [PNG OPRA], 2002). It is generally believed that the disease cycle will be faster and disease incidence will increase with increasing replantings. This is generally true for areas with relatively high initial disease incidence (<10%>) but there may be some exceptions to this general assumption, especially in areas where oil palms have been planted into primary or secondary forested areas. Under this scenario, the build-up of disease inoculum may take longer to manifest since the initial inoculum source is likely to be basidiospores liberated from naturally decaying native palms or distant fields of coconut or oil palm (Sanderson, 2005).

The association of previous plantings of coconut with *Ganoderma* disease of oil palms is well established (Abdullah, 2000; Ariffin, 2000; Sanderson et al., 2000) and isolates from both hosts are conspecific (Pilotti et al., 2002). Although the disease incidence in individual plantation blocks is variable, the incidence of basal stem rot is generally higher in plantings after coconut (Ariffin, 2000; Pilotti, 2005; Singh, 1991). Stem rots of coconut have not been of consequence in most countries except India where the disease has a different and as yet unresolved etiology (Pilotti & Bridge, 2023). The fungus does however, grow saprophytically on dead coconut trunks in most countries where oil palm is planted and these are believed to be an initial source of infection for newly planted oil palms, either through basidiospores liberated from basidioma, or mycelium. A similar situation exists for ornamental palms attacked by *G. zonatum* in North America where initial infections are believed to be initiated by basidiospores (Elliot & Broschat, 2000; Loyd et al., 2018; Pilotti & Bridge, 2023).

Expression of symptoms is similar among palm species with progressive wilting and yellowing of fronds as the disease advances throughout the basal elements (Pilotti & Bridge, 2023). Usually when symptoms are observed in oil palms, it is not possible to apply remedial treatments and the palm will eventually succumb to the infection. Basidioma of *Ganoderma* will be evident when symptoms (and assumed bole decay) are at an advanced stage in infected palms. It has been observed in PNG and Solomon Islands (SI) that about 10% of palms expressing varying levels of disease symptoms do not produce basidioma on the exterior of the trunk (PNG OPRA, 2007). The reasons for this are unknown but could relate to the state of the fungal mycelium (monokaryotic, see *The Fungus* section) or secondary infections by other fungi or bacteria that may out-compete the initial infection by *G. boninense.*

The spread of *Ganoderma* differs among crops; particularly between palms and hardwood trees. However, molecular evidence suggests that both basidiospores and mycelium play a role in the persistence and spread of stem rots (Flood et al., 2022; Pilotti & Bridge, 2023). In a plantation environment such as for oil palm, disease spread will differ for different stages of the planting cycle. For example, in a first-generation planting, basidiospores will largely be responsible for disease initiation, and spread away from initial foci will become evident as palms reach the end of the first planting cycle of normally, 25 years. Under this scenario, disease patterns will generally be random in the first half of the planting cycle and become more aggregated in the latter half due to hypothetical spread between palms, although the overall disease pattern will still be considered random (Pilotti, personal communication, 2023; Pilotti & Bridge, personal communication, 2023). Evidence for this has been obtained from molecular and somatic incompatibility studies between isolates from field palms (Miller et al., 2000; Pilotti, 2018; Pilotti *et al.,* 2018). As the planting cycles increase, it is considered that the fungal mycelium will feature more prominently in disease spread. This is by way of residual inoculum in the debris remaining in the soil from the previous planting as well as increased spread between palms with readily available routes for mycelium to follow (e.g. dead and severed roots) from the previous planting. Such a scenario leads to more aggregated disease patterns but this does not rule out the continuing establishment of new infection foci by basidiospores in secondary infections. Sanderson (2005) estimated that around 2 million spores could be released from a 100 x 50 mm bracket every minute (equating to 2.9 x 10–12 spores per day) and although the great majority of these will fall to the ground, Rees et al. (2012) reported 2–11,000 spores/m^{-3} in oil palm plantation air samples.

Upper stem rot (USR). The incidence of USR in oil palms tends to increase towards the end of the planting cycle. Low incidences of USR were observed in the first-generation oil palm plantings (Pilotti, 2005) and may increase in subsequent plantings, although this may be correlated to the expected increase in the incidence of basal stem rot. Current thinking is that USR is initiated from secondary infections by basidiospores liberated from basidioma produced from palms with basal stem rot in the same field or plantation. It is known that basidiospores can travel and survive long distances (Pilotti et al., 2003) and that mating between compatible monokaryons (to form dikaryons) takes place readily in a plantation environment (Pilotti, 2005; Pilotti & Bridge, 2023). Therefore, chance infections in the upper portion of palm trunks are possible and do occur. Data collected on USR incidences in oil palm seed gardens in PNG and Indonesia indicate that higher levels of USR are observed, probably due to mechanical injury to palm trunks with regular climbing of mother palms for hand pollination. Insects and small mammals may also play a role in disseminating basidiospores but obtaining proof of this would require careful observation and study.

Early Detection

One major barrier to the management of BSR is early detection. The earlier the disease is detected, the quicker management options can be considered. Traditionally, this was done using a six-month census of the estates by trained staff based on external symptoms or detection through culturing of the fungus; both are time-consuming and labour-intensive so new technologies have been investigated. These have been reviewed in detail by Flood et al. (2022) and Pilotti and Bridge (2023) and only broad details of the main remote detection methods and laboratory methods will be provided here.

Remote detection methods. Geospatial technologies have been increasingly used with varying degrees of success. These include hyperspectral remote sensing, multispectral remote sensing, terrestrial laser scanning tomography, intelligent electronic nose, micro-focus X-ray fluorescence (XRF), spatial maps, and the increased use of machine learning methods. These methods are discussed in detail in Flood et al. (2022).

Khosrokhani et al. (2016) concluded that hyperspectral remote sensing (HRS) was capable of detecting BSR in oil palm estates using either aerial or ground-based sensors and that these technologies could also determine disease severity, but differentiation between healthy and slightly infected palms was more difficult. HRS sensors work well in combination with other data sets and models. Modified red edge simple ratio (MRS) sensors can discriminate between healthy and infected palms but can less differentiate infection severity. The intelligent nose system (Abdullah et al., 2011, 2012a, 2012b) can also distinguish infected and non-infected using artificial neural network algorithms (ANN) and multivariate statistics but more research is needed to determine BSR severity. Electrical capacitance volume tomography is a quick and non-destructive method and sonic tomography combined with expert knowledge can detect BSR and determine severity levels. Micro XRF (μXRF) sensors have some potential but distinguishing BSR severity is difficult. Santoso et al. (2011; 2017) used spatial maps to determine BSR distribution and using the Kriging interpolation method, the distribution appeared as random. However, QuickBird imaging-derived maps, showed that disease distribution was sporadic in older palms and dendritic in young palms which may be linked to different methods of disease spread (see *Basal Stem Rot* section). Izzuddin et al. (2020) analysed BSR in the field using unmanned aerial vehicles linked to field-based ground truthing. A three-band combination of the multispectral images plus object-based analysis did detect moderately and severely infected palms. However, as with many of these technologies, detection of slightly infected palms from healthy ones was problematic and this distinction is key for early detection.

Light detection and ranging (LiDAR) works well in forestry applications (Lefsky et al., 2002) and in oil palm (Shafri et al., 2012). LiDAR technologies have shown promise for early detection of BSR. Khairunniza-Bejo and Vong (2014) used a ground-based LiDAR technique, known as Terrestrial Laser Scanning (TLS) and their results indicated correlations between the oil palm trunk's perimeter, Diameter-Based Height (DBH) and canopy area, with BSR disease although *Ganoderma* incidence is known to increase with age. Subsequently, Husin et al. (2020) used TLS as a means of early detection of BSR in nine-year-old palms. Palms were categorised into four health levels – T0, T1, T2 and T3, representing healthy, mildly infected, moderately infected and severely infected, respectively. Statistical analysis revealed that frond number was the best single parameter to detect BSR disease as early as T1 (slightly affected). In classification models, although a linear model with a combination of parameters, ABD – A (frond), B (frond angle) and D (S200 – canopy strata at 200 cm from the top) delivered the highest average accuracy (86.7%) for classification of healthy/ unhealthy trees.

Much research effort has been undertaken into remote sensing but problems remain including differentiating between slightly infected palms and healthy palms in the field which is vital for early detection.

Laboratory methods. Over the last 50 years, numerous researchers have looked at methods to detect *Ganoderma* in palm tissues and their potential to provide early diagnostic tests. Essentially, the methods can be grouped under the four headings culture, physiology, immunology, and molecular (Pilotti & Bridge, 2023).

Culturing. The ability to isolate and culture a viable fungus from plant material may indicate a disease, but this approach has many complications. Failure to isolate an organism may not indicate its absence as the isolation methods and the culture medium need to be optimal and the location of viable fungal material in the plant sample needs to be considered. Similarly, positive isolation may be due to fungal propagules on the surface of the sample or in soil associated with the sample, so considerable care has to be taken with any isolate and culture method including appropriate and thorough surface sterilisation of the sample. A significant barrier to identifying *Ganoderma* by isolation and culture is that the mycelial form of the fungus has few diagnostic characteristics to differentiate it from many other basidiomycetes. A selective culture medium (GSM) (Ariffin & Idris, 1992) has been developed but this is only semi-selective and it will also support the growth of other genera of wood-rotting fungi (Farid et al., 2018). Unfortunately, some components of GSM are either not widely available or considered environmentally unacceptable and so a replacement medium is needed (Amanda & Prakoso, 2018; Pilotti & Bridge, 2023).

Physiology. Various workers have attempted to detect *Ganoderma* in palm material by identifying or assaying different fungus components or the biochemical activity associated with infection. Probably the earliest of these were the studies of *Ganoderma* in coconut palms which measured some physiological properties and developed colorimetric and spectroscopic assays for some chemical properties (Natarajan et al., 1986; Pilotti & Bridge, 2023) and reviewed by Raju et al. (2015). In oil palm, several chemical components of either the fungus or of the plant defenses have been considered, and these are detailed in Pilotti and Bridge (2023), but to our knowledge, none of these methods has been widely adopted in practice.

Immunology. The first attempts to obtain specific antibodies to *Ganoderma* and to use them to detect the fungus in palm tissue were with betel nut and coconut palms (Ananthanarayanan & Reddy, 1984; Reddy & Ananthanarayanan, 1984). Subsequently, Indonesian researchers developed both polyclonal and monoclonal antibodies and they were able to detect *Ganoderma* in diverse oil palm tissues (including leaves) at various stages of the disease (Darmono, 2000; Utomo & Niepold, 2000a). These antibodies can be linked to various markers to provide ELISA and dot blot-based diagnostic tests for plant tissue (Darmono, 2000; Rajendran et al., 2009). Although some questions have been raised regarding specificity, and these need to be considered alongside what other fungi might be expected in the samples, immunological tests can be easily automated to allow for bulk screening of multiple tissue samples (Hushiarian et al., 2013; Pilotti & Bridge, 2023; Utomo & Niepold, 2000b).

Molecular. DNA sequence data is now a fundamental character for classifying and identifying fungi (Borman & Johnson, 2020; May, 2020). Although DNA sequences obtained from numerous genes have been used by various workers for both classification and identification, the internal spacer regions (ITS) of the nuclear ribosomal RNA gene cluster have been adopted as a universal marker for fungi (Schoch et al., 2012). ITS sequences have been widely considered in the classification and identification of *Ganoderma* species (e.g., Frysouli et al., 2020; Moncalvo, 2000). A benefit of ITS sequences is that they contain various variable regions that can be correlated with species identity and so can be used to develop primers that can then be used to selectively amplify DNA from individual species by the Polymerase Chain Reaction (Atkins & Clarke, 2004). This approach was developed to allow specific detection of *Ganoderma* in oil palm tissues in the late 1990s and several specific primers and associated methodologies have been published (Bridge et al., 2000; Pilotti & Bridge, 2023; Utomo & Niepold, 2000a, 2000b). ITS-PCR methodology has been used for palm samples obtained in surveys and has shown the presence of *Ganoderma* in frond bases and root material of young palms that subsequently developed BSR (Panchal & Bridge, 2005; Utomo et al., 2005).

More recently ribosomal nucleic acid (RNA) markers that occur during early disease stages have been investigated as potential diagnostic markers (Faizah et al., 2020). There are indications that the detection of these markers in palm tissue through reverse transcriptase (RT) PCR could also provide a potential early diagnostic test (e.g. Permatasari et al., 2023). However, RNA can be somewhat transient in samples.

Management of Basal Stem Rot (BSR)

Management of BSR is currently based on a combination of cultural, biological, and breeding approaches; chemical control has also been attempted. These aspects have been discussed in depth in Flood et al. (2022) and Pilotti and Bridge (2023). What follows are short summaries of various aspects of BSR management in SE Asia and the Pacific.

Cultural control. When BSR emerged as a serious problem of oil palm in SE Asia, cultural control was all that was available. The disease incidence was low and cultural approaches were confined to treating individual palms. Initially, the removal of the decayed part of the bole and trunk sometimes accompanied by the application of chemicals was tried, as was mounding of palms using soil around the palm base and isolation of symptomatic palms, by digging trenches to prevent the possible mycelial spread of the pathogen to neighbouring healthy palms or root contact between diseased and healthy palms; all were generally ineffective. Measures were only applied to visibly diseased palms; untreated symptomless palms remained a potential source of infection. Also, this strategy assumed that infection occurred by mycelial spread or root-to-root between neighbouring palms. As disease incidence increased in first-generation plantings, the recommendation to isolate individual palms was changed to the removal of these palms. However, after 10 years of growth, it is not feasible to continue to resupply and any management is left to replant. Economically, the labour costs needed became prohibitive for many oil palm plantations as well as for smallholders and many infected palms remained *in situ* (Chung, 2011; Flood et al., 2022).

As the industry in SE Asia faced replanting of the first generation of oil palm, it was recommended that preparation of the site at replanting time (clean clearing) offered the best practical opportunity to remove infected material (Ariffin et al., 2000; Singh, 1991). Such practices have been subsequently widely adopted throughout the region, to different degrees, dependent on BSR incidence in previous plantings, labour availability, and costs involved. These practices have continued as more generations of oil palm have been planted. Removing stumps and large pieces of debris will reduce viable residual inoculum in the form of mycelium from the field before planting the next crop. Whilst "clean-clearing" practices generally result in lower disease incidence in replanted oil palms (in comparison with other replanting techniques), the incidence of the disease may still be unacceptably high (Pilotti & Bridge, 2023). In cases where debris from the first generation remained buried (or partially buried) and seedlings were planted near this material, disease incidence was observed earlier in the replanted field (e.g., within 2 years of planting) (Flood, personal communication, 2023). Windrowing was practised, where all palm material (trunks, boles and roots) was placed unshredded in rows on the soil surface. Later, shredding of oil palm material was advocated to allow natural microorganisms to colonise the material to out-compete the pathogen, and this method is widely used in Malaysia. In Sumatra, Virdiana et al. (2012) reported 13 years of field observation and suggested seedlings be planted at least 2 m away from the edge of windrows to delay infection in the next generation. Fallowing for one year also significantly reduced *Ganoderma* infection in the subsequent planting and shredding reduced infection in the next generation (Virdiana et al., 2012).

Fallowing has major economic implications for estates (Virdiana et al., 2019) but fallowing can be a useful option for smallholders and is already recommended to them in PNG where half of the block is felled and fallowed with the other half still under production. For replanting in larger plantations, field preparation includes machine-felling, windrowing of trunks and removal of all bole tissue remaining in the planting holes when palms are felled. Holes created by felling are immediately back-filled and the boles of infected palms exposed during felling are chipped with an excavator bucket. Shredding is not practiced due to the high cost of the machinery required and its questionable benefit compared to mechanical chipping. Burial of whole palm trunks is strongly discouraged unless they can be buried at depths >3 m from the surface, a practice that would incur considerable cost.

Monitoring through regular surveys and removal of BSR palms in PNG and SI begins at six years of age and is a continuous annual activity until replanting at 23–25 years. The rationale behind the recommended sanitation program in these countries was based on both field evidence and later, on scientific evidence obtained from studies on the population biology of *G. boninense* (Pilotti et al., 2003; Sanderson, 2000; Sanderson & Pilotti, 1997, 1998). Emphasis was placed on the presence of *G. boninense* fruiting bodies and infected boles as potential sources of inoculum with little or no regard for subsurface roots and higher portions of the trunk, unless they were infected with upper stem rots. Palm removal was critical as the recommended procedure also prevented the regrowth of *Ganoderma* brackets, considered a high risk for new infections by basidiospores (Sanderson et al., 2000). At low levels of disease (<5%), manual removal of palms identified in biannual disease surveys was manageable and cost-effective (Griffiths et al., 2001; Marshall et al., 2004).

In addition, it is thought that continuous and regular removal of diseased palms potentially reduces the inoculum levels remaining from the previous crop at replanting. Sanitation practices continue to be implemented in PNG and SI although much of the plantations have undergone replanting in the last 10 years. The true effects of the recommended disease management strategies in PNG and SI should be seen in 10–15 years although recommendations in both PNG and SI, now include early replanting in blocks severely affected by BSR disease (Pilotti, 2019). The uptake of this recommendation remains to be seen.

Management of USR is similar to that of BSR. USR is usually only detected when palms fracture after severe wind events or when heavily laden with fruit bunches (Sanderson, 2000). Diseased palms are removed following the same procedures for palms with BSR where diseased portions are manually chipped with harvesting chisels and scattered on the plantation floor (Pilotti, personal communication, 2023).

Chemical control. Early studies indicated some success with the use of various chemicals and application methods including pressure injection of fungicides (Ariffin et al., 2000) but Chung (2011) stated that while chemicals such as hexaconazole had been investigated, the approach was rarely practiced as it did not provide effective control. Additional difficulties include the effective placement of fungicides where they are needed and significantly, food safety problems as concentrations of fungicide residues in palm oil may prevent adoption within the industry.

More recently, novel chemical methodologies have been undertaken. Maluin et al. (2020) reported more than 74% disease reduction in seedlings compared to untreated seedlings where hexaconazole and/or dazomet were encapsulated into chitosan nanoparticles for the formulation of chitosan-based agro-nanofungicides. Further work on the effect of surfactants on the fungitoxicity of a dazomet-micelle nano delivery system against *G. boninense* has also been reported (Mustafa et al., 2023). Whilst interesting research, field-orientated work is needed to demonstrate the effectiveness of these new methodologies at the field level. Currently, for smallholders and plantations alike, chemical control remains an expensive option and is not recommended at all in PNG/SI due to RSPO guidelines.

Biological control. Biological control refers to the use of one organism to limit the growth and multiplication of another and the degree of disease suppression achieved with biological agents can be comparable to that achieved with chemicals (O'Brien, 2017). Modes of action include antibiosis, hyper-parasitism, induced resistance, competition, and toxin inactivation (Kohl et al., 2019). Usually, Biological Control Agents (BCA) are isolated by screening organisms from the rhizosphere or endophyte populations in host plants, and control is based on the inhibition of growth of the target pathogen *in vitro*. The use of BCA to control BSR on oil palm has been extensively studied in both Malaysia and Indonesia (Sariah & Zakaria, 2000; Susanto et al., 2005). Various microorganisms are antagonistic to *Ganoderma* including fungi such as *Penicillium, Aspergillus, Hendersonia,* and *Trichoderma* along with various mycorrhizal fungi, and bacteria largely from the genera *Bacillus, Burkholderia* and *Pseudomonas* as well as Actinomycetes. Combinations of various amendments such as calcium nitrate have been recommended (Sariah & Zakaria, 2000) and mixed antagonists have also been studied (Sundram et al., 2015). Different application methods such as soil augmentation, in combination with mulches, as seed coatings, and in conjunction with composting have been proposed (Jawak et al., 2018).

An alternative biocontrol approach is removing nutrients available to the pathogen through degrading the diseased material. *Ganoderma* is known to be a poor competitor in soil (Rees et al., 2007), and increasing the rates of decay of oil palm material in the field would be an attractive solution for estates - reducing both *Ganoderma* inoculum and *Oryctes* spp. nesting sites, reducing nutrient loss and so reducing fertiliser application. Rees (2006) identified several basidiomycetes that successfully degraded oil palm wood blocks *in vitr*o and then trialed candidates in the field in Sumatra but with only limited success. Early unpublished studies in PNG using field-isolated basidiomycetes arrived at the same conclusion as decay was slow in inoculated oil palm trunks. Studies using *Thielaviopsis paradoxa* were promising and rapid degradation of the upper portions of the mostly hemicelluose-containing oil palm trunks was achieved (50% degradation within 3 weeks). However, due to the apparent implication of *T. paradoxa* in other palm diseases, this avenue of control was not pursued (PNG OPRA, 2000). More recently, Naidu et al. (2017) reported *in vitro* degradation of oil palm wood blocks but degradation was mainly of wood polysaccharides with only minimal degradation of lignin. Nevertheless, these authors considered the work to be a first step toward developing a solution to degrading palm waste.

Host resistance. Breeding programs for resistant planting material have been set up across SE Asia (Wening et al., 2020). Many companies and institutes involved have extensive germplasm collections from diverse origins to allow screening and selection. Sources of resistance within existing *Elaeis guineensis* commercial germplasm include material from the centre of origin of *E. guineensis* (West Africa) and *E. oleifera* (South American oil palm).

Breeding for resistance/tolerance in oil palm is not easy. Durand-Gasselin et al. (2005) reported field observations on a series of planting materials of *E. guineensis* and *E. oleifera* of known origins which showed differences in disease susceptibility. *E. guineensis* material was highly variable, and material of Deli origin (used as female parental palms for seed production) was highly susceptible to *Ganoderma* compared to material of African origin. Differences in reactions between parents and between crosses from within a given origin were observed. Durand-Gasselin et al. (2005) also reported variable resistance in clones derived from palms of the same origin and concluded that an early selection test in the nursery was needed. Due to space and time constraints, it is essential to screen out very susceptible material and allow only the most promising material to be screened in the field. Screening at the nursery stage with artificial inoculation with rubber wood blocks is a common approach (Breton et al., 2006). Purba et al. (2012) suggested that the inheritance of *Ganoderma* tolerance is generally additive and therefore, the performance of crosses can be predicted if parental genotypes have been tested for susceptibility/ tolerance or the Deli *dura* material is introgressed with *Ganoderma* tolerant material.

Tisné et al. (2017) identified four *Ganoderma* resistance loci in oil palm from data obtained from 25 years of field observations. Two loci control the development of the first *Ganoderma* symptoms while the other two are related to the death of the palms. Favourable haplotypes were detected among a major gene pool from the ongoing breeding programs. This provided important information for the selection and improvement of resistant varieties. However, the long-term nature of breeding for resistance to *Ganoderma* is very costly and requires reliable access to the field so, many researchers have started to examine alternative approaches to aid breeding programs.

Daval et al. (2021) reported the use of computer modelling using extensive data sets derived from an ongoing breeding program. A pedigree-based Quantitative Trait Loci (QTL) mapping approach was applied to more than 10 years of data collected during pre-nursery tests. The study revealed the quantitative nature of *Ganoderma* resistance and identified underlying loci segregating which were relevant to the breeding program. To assess the reliability of QTL effects between the pre-nursery and the field, data was also collected on the disease status of specific individuals planted in the field and modelled with the pre-nursery-based QTL genotypes. Results indicated that in the field, individuals were less likely to be infected with *Ganoderma* when they carried more favourable alleles at the pre-nursery QTL. Daval et al. (2021) suggested that their studies provided proof of concept for an approach that was both efficient and cost-effective and could be applied to other breeding programs.

Another alternative to traditional breeding is mutation breeding (Nur et al., 2018) which is faster than traditional approaches but is not a genetic modification (GM) technology. However, for oil palm, the length of time needed to develop mutant varieties as well as the need for field access to allow populations to mature does mean that constraints are similar to those of traditional breeding. New tissue culture approaches may allow more efficient methods for the selection and development of mutant lines. Genomic screening in early generations could provide a means to short-cut generations and accelerate mutation breeding in perennial crops. Haploid production methods have been developed for oil palm (Dunwell et al., 2010), and these now offer a new target for mutation induction, as homozygous mutants can be produced instantly by the conversion of haploids to double haploids. This is significant, as the vast majority of induced mutations are recessive, and therefore their phenotype cannot be seen until the mutant alleles are made homozygous. Ithnin and Kushari (2020) provided a comprehensive review of breeding and genetic improvement in oil palm while Martin et al. (2022) summarised biotechnological methods that can transform traditional breeding including genomics, marker-assisted breeding, genetic engineering and genome editing techniques; these authors also address the concerns connected to these techniques and their applications in practical breeding.

Breeding for resistance to *Ganoderma* is a major goal in the management of BSR strategy. Such improved material would benefit both the commercial sector and smallholders, and could reduce financial investment in sanitation practices. However, Purba et al. (2012) considered it essential that the SE Asian oil palm industry continues to investigate alternative management options as breeding is such a long-term enterprise. An integrated approach to BST management is expected for the foreseeable future.

THE UNCERTAINTIES

Having summarised the current knowledge of *Ganoderma* disease in oil palms, we now examine the uncertainties within the study of this pathogen and its effects. Many of these uncertainties relate to the lack of fundamental research that remains to be done including the gaps in knowledge that exist around the infection process, disease spread, identification of the pathogen, mechanisms of resistance, *etc*. While management practices are being conducted, without greater knowledge of these fundamental gaps, such management practices will continue to fail.

The Fungus

Although *G. boninense* has been studied extensively, there are some areas and aspects where some uncertainties remain.

Systematics. Modern mycological systematics is determined by phylogenetic analyses. The rDNA ITS sequence has been adopted as a universal "barcode" for fungi. Still, there are some limitations in the use of this sequence and so it is desirable also to consider DNA sequences obtained from additional genes (Nilsson et al., 2008; Schoch et al., 2012, 2014; Simon & Weiss, 2008). Wide-scale taxonomic studies of the genus or specific sections of it, generally use only a few representatives of each species and so although other gene sequences have been used for *G. boninense*, they are available for only a few isolates (Hapuarachchi et al., 2015; Tchoumi et al., 2019). Another concern with *Ganoderma* species is that reference DNA sequences are frequently not linked to type material. In the case of *G. boninense*, two sequences from later collections from Bonin Island are generally used although the type collection is available in at least two public herbaria (Kew & Paris, see Pilotti & Bridge, 2023).

Many of the molecular studies of *Ganoderma* have included only a small number of ITS sequences from *G. boninense* but Fryssouli et al. (2020) included over 80 ITS sequences obtained from different isolates, and Flood et al. (2022) considered some 143 sequences. Both of these studies demonstrated around 2% variation within the sequences, and Fryssouli et al. (2020) suggested that some of these could be considered as new "cryptic" species. High sequence variability and multiple ITS forms are not uncommon in basidiomycete fungi (Chen et al., 2016; Lindner & Banik, 2011; Simon & Weiss, 2008), but have not been widely considered in *G. boninense*.

Wang and Yao (2005) recovered multiple ITS sequences from single strains of four *Ganoderma* species. Most molecular studies with *G. boninense* have used either fruiting bodies or mycelial cultures derived from these. Both types of material are dikaryotic and have resulted from a previous mating between monokaryotic basidiospores. To our knowledge, there have not been any studies that have considered ITS sequence variation or inheritance during this process in *G. boninense.*

Ecology/disease initiation and spread. *Ganoderma* species are prevalent on oil palm throughout Southern Asia and Oceania. They also occur and cause disease on other perennials to varying extents from limited (e.g., *Casuarina* spp.) to extensive (e.g., *Acacia* spp.) (Pilotti & Bridge, 2023). The differences in prevalence may be largely attributed to the level at which different crops are cultivated with more intensively cultivated crops having the highest incidences of stem and butt rots caused by *Ganoderma.* The species identified on oil palm appear to be very closely related and have probably originated from other naturally occurring palms (Mercière et al., 2017). Although *G. boninense* has been implicated in Thanjuvar wilt of coconut, this identification has not been confirmed and further investigations on this disease in coconuts are warranted, particularly since this species occurs prolifically as a saprophyte on dead coconut in SE Asia but does not appear to cause disease in living palms outside the Indian subcontinent.

The widespread occurrence of BSR on new oil palm plantings suggests that potential inoculum is relatively common in the surrounding environment. In a "new" planting, the first occurrence of *G. boninense* will probably originate from either debris from previous vegetation, or the surrounding vegetation. There are however relatively few reports of *G. boninense* on native palms and we are not aware of any surveys for the pathogen that have been conducted before planting. This information could be important as planting in an area where the pathogen was already present, would likely lead to earlier and greater infection levels compared to areas where the pathogen was either absent or only present at low levels.

The relatively high level of variability in ITS sequences seen in *G. boninense* is unusual when compared to the other *Ganoderma* palm pathogens. Midot et al. (2019) identified eight ITS haplotypes for *G. boninense* from oil palm in Sarawak, Malaysia and Flood et al. (2022) found some 59 distinct ITS sequence types among all sequences available from multiple countries and hosts. Fryssouli et al. (2020) suggested that the ITS sequences from some oil palm isolates from Indonesia were sufficiently different to possibly constitute a separate "cryptic" species. In contrast, Elliott et al. (2018) reported very little ITS sequence variation in *G. zonatum* isolates from multiple palm hosts in the US, and the same is true for the Thanjuvar wilt pathogen of coconut in Southern India, which could be a single homothallic population (Rolph et al., 2000).

It is unclear if the ITS sequence variation seen in *G. boninense* is the result of the huge numbers of individuals present, segregation or crossover from multiple mating events, or if there are multiple species involved. Some recent research has suggested that both non-random mating and bottlenecks occur in *G. boninense* in oil palm plantations (Wong et al., 2022). Although there have been some studies linking mating events and some microsatellite markers (Pilotti et al., 2021) it would be useful to ascertain what effect mating has on ITS sequences and if isolates from the different ITS sequence groups are all interfertile.

For disease initiation in the field, it is well established that in the absence of larger mycelium-bearing inoculum sources such as palm trunks or root debris, the fungus primarily establishes in the field as basidiospores (Miller et al., 1999; Pilotti et al., 2003, 2018). Although a large number of isolates derived from spore inoculum have been observed in seedlings growing near *Ganoderma*-infected oil palms (Pilotti et al., 2018), it is not clear how, when, or if, the disease manifests in young seedlings and the conditions necessary for disease initiation *via* either spores or mycelia in young palms are uncertain. Nursery testing currently requires a large mycelial inoculum source to initiate infections in young seedlings (Flood et al., 2022).

The survival of both basidiospores and fungal mycelia under natural conditions has not been investigated fully. It is known that *G. boninense* can survive for several years in oil palm and coconut trunks but the fate of liberated basidiospores and movement and translocation away from infected trunks is still unclear. Unlike fungi such as *Armillaria*, *Ganoderma* does not form rhizomorphs although it does form mycelial cords in sterilised soil (Rees, 2006). The fungus is considered to be a poor soil competitor (Rees et al., 2007) and requires plant material to survive and persist within the soil thus forming inoculum for subsequent planting.

Evidence for both mycelial spread and spore spread between mature oil palms has been obtained from several studies using vegetative compatibility and mating studies on field isolates of *G. boninense.* It is clear that disease is spread between neighbouring mature palms but what is not clear is when and how palms become infected after replanting as well as during the entire planting cycle. In a mature plantation, disease foci do not necessarily expand continuously, and in fields with high disease incidence, 'hotspots' tend to develop in confined areas with continuous initiation of new foci and subsequent expansion of these to varying extents (Pilotti, personal communication, 2023). Both anecdotal and scientific evidence for disease spread between mature oil palms is available but clear spatial and space-time studies of these relationships are rare.

Infection. To date, artificial inoculation has only been achieved using inoculated rubber wood blocks attached to roots or by coating roots in mycelial suspensions. While these are useful tools for inducing infections for detailed laboratory-based studies (resistance mechanisms etc.) and for nursery screening in association with breeding trials, they are unlikely to represent the natural infection process in the field. One significant feature of BSR in oil palm is the high level of infection, which is much greater than is seen in other crops such as rubber and tea where infections are usually restricted to local clusters of plants. *Ganoderma* species are widely regarded as "wound pathogens" and cut frond bases could provide a "natural" infection route. However, to date, artificial inoculation of stems, peduncles, frond bases, and cut fronds with a range of inocula has failed to initiate infection, although *G. boninense* has been detected at these locations in both symptomatic and asymptomatic palms in the field (Panchal & Bridge, 2005; Pilotti & Bridge, 2002; Rees et al., 2007).

Oil palm is largely planted commercially from seeds produced through sexual mating, therefore plantings consist of groups of genetically different individuals. Similarly, *G. boninense* is an outbreeding dikaryotic fungus and so also represents a genetically heterogeneous population. Alleles associated with resistance have been identified in some plants (see earlier) and it is also possible that individual strains of *G. boninense* may show differing levels of vigour or pathogenicity. A molecular survey has shown that not all young palms shown to carry *G. boninense* subsequently went on to develop BSR (Panchal & Bridge, 2005; Pilotti & Bridge, 2002) and this will have implications for both developing pathogenicity tests and early detection methods.

Early Detection

Remote sensing. Despite investment in various forms of remote sensing methods, detection of slightly infected palms (as compared to healthy palms) has remained a challenge and this is crucial for early detection. TLS has shown some promise in the detection of slightly infected palms and may be developed further using a combination of techniques but challenges remain. These technologies are costly in terms of equipment and the technical analysis needed and skilled technical advice would be needed to advise producers. These costs may put these remote sensing technologies beyond the budget of smallholders and even plantations.

Laboratory tests. At present, most laboratory-based detection methods have been developed with artificially inoculated plants or environmental samples from single time points. Costs for simple molecular and immunological tests have decreased in recent years, particularly with the wider adoption of laboratory automation. Although immunological and molecular methods for detecting *G. boninense* have been available for over 20 years, we are only aware of one published study that has used any of these (specific ITS primers) to follow infection in the field as an oil palm block becomes established (Panchal & Bridge, 2005; Pilotti & Bridge, 2002). As a result, it is not possible to compare the comparative predictive performance of these methods. As mentioned above early detection of the pathogen needs to be shown to give rise to subsequent disease.

Management. Many studies of biocontrol of BSR, concentrate on *in vitro* work or nursery trials on seedlings. While these are useful for understanding the host/pathogen interaction and help with the selection of effective candidates, more field studies are needed (Sundram et al., 2015, Virdiana et al., 2019). These trials need to include those where BCAs in the oil palm ecosystem are quantified over time so that persistence and activity in the field are more fully understood. Ease of mass production of the putative BCA is needed and *Trichoderma* is easily mass produced on many estates throughout SE Asia and is consequentially routinely used as a soil amendment both at replanting and after diseased palms are removed. Many of the larger estates manufacture their formulations and basic techniques are available for the multiplication and application of *Trichoderma* (Virdiana et al., 2019). Commercial formulations containing a range of microorganisms are also available (Idris et al., 2014) for use by both estates and smallholders but independent comparisons of the different commercially available formulations are lacking.

Mature oil palms planted in soil are known to support an extensive mycorrhizal community (Kaonongbua, 2018; Phosri et al., 2010). It is generally assumed that this community is acquired naturally from the planting environment, largely after the nursery stage (Phosri et al., 2010). The natural acquisition of a mycorrhizal community will depend on the levels of suitable inoculum available in the soil and the species diversity will vary depending on soil type, location, and fertiliser usage (Auliana & Kaonongbua, 2018; Phosri et al., 2010). Early inoculation with various mycorrhizae has been proposed on many occasions to help increase the growth and vigour in young plants on planting out (Galindo-Castañeda & Romero, 2013; Phosri et al., 2010; Widiastuti & Tahardi, 1993). There have also been some reports that suggest the presence of mycorrhizae may reduce the incidence of basal stem rot (Rini et al., 2022). Commercial formulations such as MycoGold are available but at the current time there is probably insufficient detailed information on natural mycorrhizal species diversity at the various sites (and soils) where oil palm is grown and the full range of interactions that may occur. For example, how do added mycorrhizal products interact with natural indigenous populations?

One of the main uncertainties for the management of BSR is access to the field where cultural approaches, putative BCA, and resistant/tolerant material can be tested (and independently) verified as part of an integrated approach. Many approaches to control are undertaken *in vitro* or in glasshouses as there is little access to the field for students and researchers alike and this inevitably leads to a lack of what is practical to the industry. Whilst the development of resistant/tolerant material would be very beneficial to estates and smallholders alike, the development of such material is long term even with the development of novel approaches such as QTLs, potential use of biomarkers, and mutant breeding. It is essential that the use and efficacy of BCA (whilst interesting in the laboratory or glasshouse as the first steps in a selection process) can be verified in the field. Understanding the ecology of any BCA in different environments and under different management practices is essential. Similarly, the long-term effects of cultural controls such as delayed replanting (fallowing), windrowing, shredding and removal of fruit bodies need to be investigated as does the potential for the use of improving the degradation of palm waste at replanting to deny the pathogen a base for colonisation, survival and subsequent infection of the next generation.

RECOMMENDATION

Given the uncertainties listed above, we have listed some specific recommendations but in general, it would be sensible for the public and private funded sectors of the oil palm industry to work more closely together. This could include sites for long-term field access for the testing of new management options but also would allow increased dialogue of the practicalities that producers (estates and small-holders) are facing. This is particularly true of the costs of some high-tech approaches and the need for highly skilled technical support for the producers. In addition, some fundamental questions remain to be answered and some of the paradigms that have been developed over decades need to be challenged and tested further. We have recommended some specific areas for further research and development.

Systematics

Recommendation 1. When compared to many other species of *Ganoderma, G. boninense* is relatively well-defined. The ITS sequences that have been proposed as reference sequences are from later collections made from the Type location and are "typical" for the species (Pilotti & Bridge, 2023). Further gene sequences have been obtained from these collections (Zhou et al., 2015) and are also considered to be "typical" of the species (Ćilerdžić et al., 2018; Nguyen et al., 2023). However, the species name is tied to the historic Type collection, and material from this is available in at least two herbaria. It would appear sensible to obtain further reference sequences from the Type material to fully validate the species.

Recommendation 2. The ITS sequence range discussed above is relatively wide for a single species and further investigation with additional gene sequences is required to fully resolve any subgroups or possible cryptic species (e.g., the "d3" group identified by Fryssouli et al. [2020]). In addition, relationships between ITS sequence variation and mating events need to be considered. For example, if monokaryons with different ITS sequences mate, do a single or both ITS sequence types occur in the subsequent dikaryon? This needs to be undertaken in conjunction with the species/ cryptic species delineation to determine if any "molecular" species are interfertile or if there is a level of sequence variation above which mating will not occur.

Ecology/ Disease Initiation and Spread

Recommendation 3. There is a need to determine further, where and at what levels, the fungus occurs in the natural environment as done by Pilotti et al. (2018). Ideally, this would require surveys of both the planting area and the surrounding environment before planting or replanting. Historically, *Ganoderma* colonisation of debris and nearby non-oil palm plants has often been reported in single studies at a single time point. We need to ensure a timeline can be established to determine the direction of transfer between oil palm and the surrounding environment as well as understand *Ganoderma* colonisation in the previous vegetation before planting e.g. in native palms.

Recommendation 4. The origins of fungal inoculum in the field remain an important question to answer. Progress has been made and although it may not be fully resolved, new insights may be gained by understanding the relationships between saprophytic and pathogenic isolates. The collection of *Ganoderma* from natural environments and research into their genetic and host relationships could provide added insight into what is already known (and assumed) regarding the opportunistic nature of *Ganoderma* infections. Such studies, while useful from a taxonomic and evolutionary perspective would also begin to establish the foundation for disease initiation in monoculture crops growing in the vicinity of natural fungal populations.

Recommendation 5. Once established, the spread of BSR requires long-term monitoring through accurate data collection and is one reason why long-term access to the field is required. This is necessary to determine spatial patterns in the spread of BSR and assess the survival and infection potential of infected palms in the field (over several generations). Such studies would allow management decisions to be made on the value of roguing in fields with varying disease incidence levels. Where successive generations of oil palm are planted, and even at first plantings, it may be useful to attempt to quantify the levels of inoculum when fields are being prepared for planting (Recommendation 3). This would provide some idea of the potential disease incidences that could be expected within a given plantation.

Recommendation 6. Assessing levels of inoculum should include quantifying spore loads in the air as well as inoculum in the soil. The value of such an exercise, on its own, would be questionable without continuous monitoring following planting and other management interventions during the planting cycle. The application of such techniques might be more applicable where fallowing is an option and inoculum levels above a determined threshold at planting could trigger decisions on the need for a fallow period.

Recommendation 7. From experience, the long-term control of *Ganoderma* largely depends on reducing inoculum levels. This can only be achieved by continuous vigilance, monitoring, and application of practices to reduce soil and airborne inocula throughout the growing cycle. Given the large numbers of basidiospores produced into the environment by mature fruiting bodies, it would seem likely that the removal of fruiting bodies from the plantings and windrows will help to reduce the inoculum potential. Aside from the roguing of diseased palms, novel ways that can be applied in the future to reduce inoculum levels could be through the use of tolerant planting material and replanting at an earlier palm age, for example between 16–20 years. The use of more tolerant material may be some years away but early replanting is a good option provided plantations can sustain yields at suitable levels to remain profitable. Other management techniques such as the application of biological control agents, require further investigation into their mechanism and effectiveness in reducing *Ganoderma* inoculum resident in the soil.

Infection

Recommendation 8. Given that the only successful inoculation method involves artificially inoculated blocks, closely attached to roots, a greater understanding of how the fungus becomes established in the oil palm in the field would be useful. This should include inoculation of recently cut fronds and inoculation of the root ring. Thompson (1931) suggested that infection was possible via cut and broken frond bases, which would be in keeping with the view that *Ganoderma* is generally a wound pathogen. Although sealing pruned frond bases to prevent subsequent infection (as is practiced with various other tree crops) has been suggested as a possible control method (Hushiarian et al., 2013; Paterson et al., 2009; Pilotti & Bridge, 2023) we are not aware of any extended field trials for this. Given that cut and open frond bases are a continuing feature of both new and established plantings, they could provide a route to the bole tissue in young palms and the upper trunk tissue in older palms. We suggest that this is a significant gap in the knowledge for infection control and some initial trials with wound dressings/coverings should be established for both newly planted and established palms to determine their effect on BSR and USR.

Early Detection Methods - Remote Sensing

Recommendation 9. Some methods such as TLS which have shown promise in differentiation of slightly affected palms (Husin et al., 2020) could be developed further. Specifically, about answering practical questions eg early detection to identify individual palms that could receive specific management measures early in the planting cycle or for assessing disease severity to guide the timing of replanting the estate or specific areas of the estate where more intensive management activities could be undertaken.

Early Detection Methods - Laboratory Methods

Recommendation 10. A single study in PNG combined molecular detection of the pathogen in the tissue of young palms with continued monitoring of the palms for disease symptoms over some years (Panchal & Bridge, 2005; Pilotti & Bridge, 2002). We are not aware of any similar long-term published studies linked to either molecular or immunological detection methods. When first developed, the molecular and immunological methods could potentially be expensive and time-consuming but the wider availability of reagents, equipment and increased laboratory automation will have substantially reduced those costs. All such methods are highly dependent on tissue handling and extraction again these have been extensively examined in the subsequent years (Karthikeyan et al., 2006; Rajendran et al., 2009). The PNG study showed that while early detection could identify the fungus in palms in the field, not all of these palms went on to develop significant BSR. We recommend that there should be a wider assessment of the accuracy and outcomes available from existing methodology before further methods for "early detection" are developed.

Management

Recommendation 11. There are many commercial formulations available that claim efficacy to BSR but they need to be tested and verified independently through field trials. This is also true of many BCA formulations (e.g., *Trichoderma)*. Do they actually reduce *Ganoderma* infection in the field? This needs to be demonstrated over several years. Linked to this there is a need for an improved understanding of the interactions of the BCA with the host plant and other members of the oil palm microbiome to fully achieve the potential of biocontrol for BSR. Trials with mixtures of organisms, application methods, testing the longevity in different soils and the effect of environmental conditions are also needed. In short, a more ecological approach is needed. In addition, improved molecular approaches could allow the identification of effective BCA based on a genetic profile and could allow a more directed approach (Benítez & McSpadden-Gardener, 2009).

Recommendation 12. The role of mycorrhizae needs to be considered in both the infection and establishment stages of basal stem rot. Current pathogenicity tests with rubber wood blocks and exposed roots will probably not include any naturally occurring mycorrhizae and so we have no information on any competitive effects that may reduce pathogenicity. As with disease progression, mycorrhizal effects are likely to occur over relatively long periods. Phosri et al. (2010) suggested that the natural mycorrhizae on newly planted palms become established after around six months, and beneficial effects are unlikely to be seen before this. Similarly, any "added" mycorrhiza will need to be able to compete with the natural microbiota and the interactions of these two populations are not understood.

Recommendation 13. Rees (2006) suggested that targeting windrowed material at replanting with fungi that had enhanced degradative abilities (as he had attempted) was unrealistic and an alternative approach was to target individual palms that succumbed to *Ganoderma* in the first year after replanting. To our knowledge, this has not been attempted but could be and linked to the early detection of palms in the early year after replanting. Longer-term field trials targeting the degradation of oil palm material at replanting combined with shredding or other replanting practices are also needed.

Recommendation 14. Breeding for disease resistance will always be an important part of the management of diseases and breeding oil palm for resistance to *Ganoderma* is no exception. Daval et al. (2021) combined the use of data sets derived from the breeding programme with QTLs described from pre-nursery tests. More of this approach of combining computing methods with long-term breeding trials would be very beneficial. The uses of biomarkers for evaluating the resistance of oil palm progenies in the field should also be investigated further.

CONCLUSION

Considerable studies has been undertaken into *Ganoderma* diseases of oil palm and although this has resulted in many publications and reviews, the disease continues to be a major concern for the industry. It is clear that there are gaps in our knowledge and that some of these relate to fundamental issues. These need to be investigated before we can fully understand and accurately interpret the various processes occurring and treatments suggested.

Some gaps, such as the variability of some DNA sequences and the incorporation of current taxonomic concepts and validated reference materials can be met from existing methodologies and a wider incorporation of existing literature. Many of the others relate to the use and performance of laboratory-derived tests and processes in a field environment. It is perhaps surprising that we have not found any instances of the quantitative measurement of sealing cut fronds, or the effects of biological control agents, in the field over a planting cycle. Similarly, while early detection methodologies such as molecular and immunological tools, and remote sensing methods, can provide an initial insight into disease occurrence, this relates only to the single point in time when the work was undertaken. For the predictive performance of these methods to be demonstrated they need to be considered in the long term. Probably the most basic question for such methods is - do all early detections result in subsequent disease? and do interventions provide a positive benefit over the life of the crop?

There are considerable practical problems with assessing laboratory-derived research in the field. These include costs and logistics in obtaining and transporting samples, the availability of training and instrumentation, and the available personnel. A further complication is that much scientific research is funded through short-term grants with sponsors expecting a "result" within the grant period. There may therefore be a need to consider how a long-term monitoring and survey programme could be established for the industry.

In 1994, the failures of morphological taxonomy for *Ganoderma* were summarised by Ryvarden who proposed that "no new species be described in *Ganoderma* in the decade to 2005 and those authors breaking this law be sentenced to do taxonomic work on at least 10 old names" (Ryvarden, 1995). The application of molecular methods has now provided a lot more clarity in the taxonomy of the genus but this in turn did require considerable re-examination of older names. It may be appropriate to consider a similar moratorium on the introduction of further methods for *Ganoderma* disease detection and control until the existing knowledge has been clarified and tested in context.

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ESTIMATING INSECT PEST CONSUMPTION BY THE ORIENTAL MAGPIE-ROBIN AND YELLOW-VENTED BULBUL IN OIL PALM PLANTATIONS FROM A FEEDING EXPERIMENT

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ABSTRACT

*Insectivorous birds contribute to the provision of ecosystem services to humans in both human-dominated and natural ecosystems around the world. Natural predation of invertebrate populations is a prime example of one of the key ecosystem services provided by farmland birds in agricultural landscapes. Yet, it is unknown how many invertebrates are consumed by common farmland bird species such as Oriental Magpie-Robin (*Copsychus saularis*) and Yellow-vented Bulbul (*Pycnonotus goiavier*), which are abundant in oil palm plantations of Southeast Asia. Therefore, this study estimated the amount of insects consumed by these birds through a feeding experiment. From an assessment of 50 captured birds (25 individuals for each species), the average daily insect consumption of Oriental Magpie-Robin was estimated at 15.03 g/bird, which is slightly higher than Yellow-vented Bulbul (11.67 g/bird). These findings indicate that the potential of the two focal species to suppress insect populations might be limited by body weight and prey preferences. This study demonstrates the advantages of maintaining biodiversity, particularly farmland birds as biological control agents. Future estimates of pest insect consumption in oil palm plantations could potentially be based on the daily insect consumption by both bird species.*

Keywords: biodiversity, biological agent, birds, conservation, ecosystem services.

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INTRODUCTION

Birds play a significant role as biological agents for controlling insect populations in both natural and human-modified landscapes (Díaz-Siefer et al., 2021; Garcia et al., 2020; Taylor et al., 2022). On a global scale, birds around the world are estimated to consume 400–500 million tonnes of invertebrates (e.g., beetles, crickets, grasshoppers) per year (Nyffeler et al., 2018). High in protein, 80% of bird species included insects as part of their diet in certain stages of their lifecycle (Mwansat et al., 2015). For

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some species, insects are the prime food resource for feeding nestlings as they provide adequate protein to support healthy growth (Capinera, 2010). Although there is a global estimate of insect consumption, little is known about insect eaten by birds and the amount of insect consumption in individual habitats, notably agroecosystems.

In agroecosystems, farmland birds provide various ecosystem services including insect pest suppression (Díaz-Siefer et al., 2021; Kamarudin et al., 2019; Milligan et al., 2016; Sow et al., 2020; Tela et al., 2021). It is estimated that 72%–89% of bird species found in oil palm plantations feed on insects (De Chenon & Susanto, 2006). In a study in Peninsular Malaysia, Yahya et al. (2023) shown the evidence of a positive correlation between the diversity of birds (both in terms of species richness and abundance) and the richness of insects in tropical farmlands, including oil palm plantations. According to their general ecological and feeding behaviour, some of the recorded bird species in agroecosystems reduce foliage and crop damages by suppressing insect and rodent pest populations resulting in an increase in crop yield (Díaz-Siefer et al., 2021; Yahya et al., 2024). In the case of oil palm cultivation, Koh (2008) highlighted the important role of insectivorous birds as natural pest control agents. However, previous studies have suggested that birds play a much smaller role as insect pest suppression agents compared to other taxa (e.g., arthropods) (Denan et al., 2019; 2023; Denmead et al., 2017; Nobilly et al., 2023). Nonetheless, Razak et al. (2020) found that oil palm smallholdings with high bird species richness and feeding guild diversity, but low bird abundance, produced higher yields, suggesting a significant role for birds as pest supressing agents in oil palm plantations.

Farmland birds are threatened by agriculture intensification, particularly by applying synthetic pesticides (Geiger et al., 2010). In industrial monoculture agroecosystems such as oil palm plantations, both chemical herbicides and insecticides are commonly used to control competing weeds and pest insects (Azhar et al., 2021; Razak et al., 2020; Tohiran et al., 2017, 2019; Wibawa et al., 2010). Prolonged application of such agrochemicals caused significant negative impacts on the ecosystems (Tohiran et al., 2019). For instance, heavy application of herbicides reduces understory vegetation complexity and structure (Nobilly et al., 2022), thus decimating one of the important habitats for biodiversity in oil palm agroecosystems (Ashton-Butt et al., 2018; Azhar et al., 2015). Understory vegetation provides valuable resources for farmland birds (i.e., nesting sites and food resources) including Oriental Magpie-Robin, *Copsychus saularis* (OMR) and Yellow-vented Bulbul, *Pycnonotus goiavier* (YVB) in oil palm plantations (Jambari et al., 2012).

OMR and YVB are common birds in oil palm plantations of Peninsular Malaysia, and they have been reported to consume insects including pest species in the plantations (Amit et al., 2015; Atiqah et al., 2019; Azhar et al., 2013; 2014; Azman et al., 2011; De Chenon & Susanto, 2006; Nursyamin et al., 2023; Tohiran *et at.*, 2019) (*Figure 1*). Both species were also recorded (often in high abundance) in oil palm plantations in other parts of Southeast Asia including Borneo (Amit et al., 2021; Gervais et al., 2012; Koh, 2008; Mohd-Azlan et al., 2019; Sheldon et al., 2010; Yudea and Santosa, 2019), Sumatra (De Chenon & Susanto, 2006), and the Philippines (Achondo et al., 2011; Cagod and Nuñeza, 2012). Compared to the introduced Barn Owl (*Tyto alba*), these understory resident passerine bird species are undervalued in terms of the ecosystem services they provide. To date, measures for promoting natural predation of pests in oil palm plantations are solely limited to Barn Owl (Atikah et al., 2020; Kamarudin et al., 2019; Yahya et al., 2016; 2020; Zainal-Abidin et al., 2021). For instance, providing nest boxes for Barn Owl has been commonly implemented in oil palm agroecosystems (Yahya et al., 2020).

(a)

(b)

Figure 1. In Southeast Asia, (a) Oriental Magpie-Robin and (b) Yellow-vented Bulbul are common farmland birds in oil palm plantations.

Oil palm stakeholders and growers have yet to consider the potential of farmland birds such as OMR and YVB to provide pest control services. Unlike the Barn Owl, exactly how many invertebrates are consumed by both OMR and YVB in oil palm plantations or other habitats (e.g., urban parks and forest edges) is not known. OMR exclusively feeds on insects (Collar et al., 2020), while YVB is a generalist that feeds on both plants and invertebrates, and it consumes 10%–35% of various invertebrates as its diet (Fishpool et al., 2020; Okosodo et al., 2016). Both local passerine species are likely to enhance natural pest control services in oil palm plantations as they are commonly seen in industrial palm plantations and smallholdings in Southeast Asia (Yahya et al., 2017, 2022, 2023). As changes in agricultural management may affect bird predation services by altering the resource base for natural enemies (Liere et al., 2017), there is an urgent need to understand pest insect control services provided by OMR and YVB in oil palm plantations.

This study aimed to assess the potential of two common farmland bird species (i.e., OMR and YVB) in suppressing insect populations in oil palm plantations via a feeding experiment. Mealworms (*Tenebrio molitor*) were used as substitutes for pest insects predated by the bird species. It was assumed that the captured birds would consume a similar amount of food insects as in the wild. Specifically, the objectives were to: 1) Estimate the amount of insect food intake by OMR and YVB; 2) compare the amount of insect food intake between OMR and YVB; and 3) examine the association between bird weight and insect food intake. To understand the potential pest control services by those bird species, two hypotheses were proposed. First, it was predicted that OMR consumes more insects than YVB. Second, it was predicted that insect consumption is positively related to body size. Findings from this study will improve the understanding of oil palm industry stakeholders regarding the conservation value of farmland birds, as well as their important roles in controlling insect populations in oil palm agroecosystems. This will help stakeholders to make more informed conservation decisions and improve their agricultural policies by incorporating land sharing or bird-friendly farming strategies.

MATERIALS AND METHODS

Study Area and Bird Trapping

The study was conducted in Lahad Datu District, Sabah, East Malaysia. Lahad Datu has a typical equatorial climate; hot and humid year-round with a daily mean temperature of 26.9°C and an average annual rainfall of 2,063 mm. Throughout the study, a total of 50 wild birds comprising OMR and YVB (25 birds for each species) were captured using mist-nets (6 x 3 m, 15 mm mesh-size) in oil palm smallholdings located at Kampung Kongsi (05°01′12″ N, 118°25′07″ E) and Kampung Bikang (05°00′56″ N, 118°27′11″ E). Ten mist-nets were deployed during each session to increase the chance of capturing focal species. Deployed mist-nets were checked frequently (every 20 min). Only adult OMR and YVB were kept for the feeding experiment. All bird trapping sessions were conducted between August and September 2021.

Feeding Experiment

Prior to the experiment, birds were first weighed using a portable digital weighing scale on the day of capture. Each bird was individually kept in a cage (55 x 55 x 55 cm) inside a well-ventilated aviary throughout the experiment (10 consecutive days). Each cage was carefully placed inside the aviary to avoid direct exposure to sunlight. Only 10 captured birds were attended at a time. Birds captured were fed with fine food pellets and plain water was provided in separate containers for the night. To avoid acute stress on the birds, each occupied cage was covered with a cloth throughout the experiment. The feeding experiment was conducted the following morning at 06:00 by providing 30 g of live mealworms in a container. Mealworms were used for this experiment due to their morphological similarity to lepidopteran larvae.

After a 24 hr period, the leftover mealworms were weighed with a portable digital spoon scale. The leftover mealworms were replaced with a new 30 g of live mealworms, and this process was repeated for the next nine consecutive days. Water containers were cleaned and refilled daily. Prior to the release, all birds were weighed and tagged with a leg band before being released at the location where they were captured. The tag was to ensure no same individual bird was repeatedly used for the experiment. To safeguard animal welfare and avoid causing them unnecessary physical injuries, a qualified veterinary doctor monitored the trapping procedures and feeding experiment.

Data Analysis

Generalised linear mixed models (GLMMs) that included both fixed and random effects were performed to assess the relationship between mealworms consumed, body weight, and bird species (Bolker et al., 2009; Schall, 1991). A normal distribution with an identity-link function was used for the models. A predictive model of the average consumed weight of mealworms was developed. The body weight of birds and species (i.e., OMR and YVB) were fitted as fixed effects.

Plot location was included in the model as a random factor (Bolker et al., 2009; Piepho et al., 2003). The final model was selected by sequentially adding explanatory variables to the initial model. The conventional and adjusted coefficients of regression, as well as the r^2 for the model, were reported. Paired t-tests were conducted to compare the body weight of each species before and after the feeding experiment. A one-way ANOVA was performed to test the effects of different birds and days on mealworm consumption. Post hoc Tukey's test was used to compare the means of mealworm consumption across different birds and days. All analyses were conducted in GenStat (VSN International, UK).

RESULTS AND DISCUSSION

The results revealed that mealworm consumption was positively related to body weight (coefficient = 0.034, Wald = 13.82, $p < 0.001$). Irrespective of species, birds with heavier body weight tend to consume more insect food in comparison to lighter birds. The body weights of OMR (back-transformed mean ± SD = 41.67 ± 6.46 g per bird, df = 24, t = 0.77, $p = 0.446$) and YVB (back-transformed mean ± SD = 26.00 ± 5.10 g per bird, df = 24, t = 0.20, $p = 0.846$) did not significantly change before and after the feeding experiment. It was observed that the mean weight of OMR was higher than that of YVB both prior to and after the feeding procedures (*Table 1*). In daily mealworm consumption, OMR (mean = 15.03 g per bird) was significantly greater (YVB, coefficient = –3.352, Wald = 4.07, $p = 0.044$) than YVB (mean = 11.67 g per bird) (*Figure 2*). On a daily basis, OMR is likely to eat more insects than YVB in oil palm plantations. The statistical model explained 27.57% (adjusted r^2 = 24.49%) of the variation in mealworm consumption by both bird species. It was found that mealworm consumption by OMR and YVB varied significantly in different birds (OMR: Variance ratio = 7.22, $p<0.001$; YVB: Variance ratio = 1.86, $p = 0.011$). Only insect consumption by OMR varied significantly on different days, but YVB did not (OMR: Variance ratio = 3.25, $p < 0.001$; YVB: Variance ratio = 1.74, $p = 0.080$). Post hoc test results indicate that mealworm consumption varied among birds and days (*Table 2* and *3*).

TABLE 1. SUMMARY STATISTICS FOR MEASUREMENTS OF ORIENTAL MAGPIE-ROBIN AND YELLOW-VENTED BULBUL BODY WEIGHT BEFORE/AFTER THE FEEDING EXPERIMENT

Species	Value	Before feeding experiment (g)	After feeding experiment (g)
Oriental Magpie-Robin (n = 25)	Mean ± SD	54.4 ± 7.3	53.4 ± 7.2
	Median	55	50
	Minimum	40	45
	Maximum	65	65
Yellow-vented Bulbul (n = 25)	Mean ± SD	37.6 ± 3.9	37.4 ± 3.9
	Median	40	35
	Minimum	30	30
	Maximum	45	45

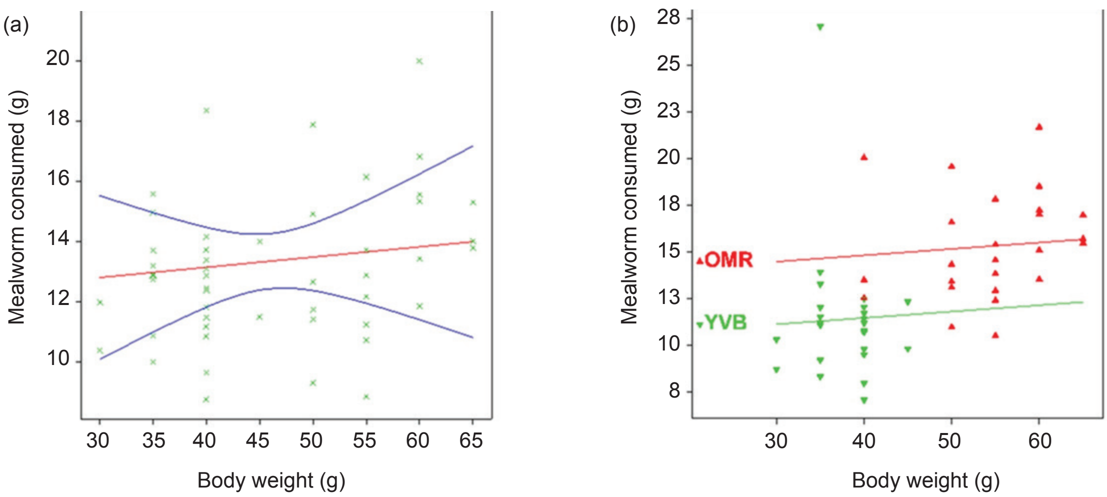

Figure 2. (a) Scatter plot with a regression line (red) and 95% confidence intervals (blue) demonstrating a positive relationship between mealworm consumption and body weight in both bird species, and (b) scatter plot illustrating mealworm consumption by OMR and YVB, proportionate to body weight.

TABLE 2. TUKEY'S HONEST SIGNIFICANCE DIFFERENCE TEST RESULTS SHOWING THE COMPARISON OF MEALWORM CONSUMPTION IN DIFFERENT BIRDS. MEALWORM CONSUMPTION VARIED SIGNIFICANTLY BETWEEN CERTAIN INDIVIDUALS

Species	Bird	Mean (g)	Tukey
Oriental Magpie-Robin	OMR18	10.52	a
	OMR8	10.98	a
	OMR7	12.41	ab
	OMR6	12.53	ab
	OMR11	12.93	ab
	OMR17	13.10	abc
	OMR1	13.42	abc
	OMR10	13.50	abc
	OMR21	13.54	abc
	OMR9	13.84	abc
	OMR15	14.34	abcd
	OMR13	14.56	abcde
	OMR24	15.10	abcde
	OMR14	15.39	abcde
	OMR22	15.47	abcde
	OMR20	15.70	abcde
	OMR2	16.59	bcdef
	OMR25	16.98	bcdef
	OMR5	17.02	bcdef
	OMR23	17.24	bcdef
	OMR19	17.83	bcdef
	OMR4	18.51	cdef
	OMR16	19.57	def
	OMR12	20.04	ef
	OMR3	21.68	f
Yellow-vented Bulbul	YVB22	7.08	a
	YVB14	7.98	a
	YVB15	8.33	a
	YVB9	8.71	a
	YVB11	9.21	a
	YVB17	9.50	a
	YVB20	9.80	a
	YVB24	9.82	a
	YVB12	10.31	a
	YVB16	10.70	a
	YVB8	10.78	a
	YVB6	11.08	a
	YVB1	11.19	a
	YVB10	11.19	a
	YVB5	11.22	a
	YVB25	11.42	a
	YVB3	11.52	a
	YVB23	11.71	a
	YVB18	12.03	a
	YVB19	12.05	a

TABLE 2. TUKEY'S HONEST SIGNIFICANCE DIFFERENCE TEST RESULTS SHOWING THE COMPARISON OF MEALWORM CONSUMPTION IN DIFFERENT BIRDS. MEALWORM CONSUMPTION VARIED SIGNIFICANTLY BETWEEN CERTAIN INDIVIDUALS (continued)

Species	Bird	Mean (g)	Tukey
	YVB2	12.33	a
	YVB7	12.48	a
	YVB4	13.28	ab
	YVB21	13.92	ab
	YVB13	27.07	b
	YVB22	7.08	a
	YVB14	7.98	a
	YVB15	8.33	a

TABLE 3. TUKEY'S HONEST SIGNIFICANCE DIFFERENCE TEST RESULTS SHOWING THE COMPARISON OF MEALWORM CONSUMPTION ON DIFFERENT DAYS. MEALWORM CONSUMPTION DIFFERED BETWEEN DAYS, BUT VARIED SIGNIFICANTLY IN SPECIFIC DAYS

Species	Bird	Mean (g)	Tukey
Oriental Magpie-Robin	Day10	12.67	a
	Day9	13.84	ab
	Day8	13.90	ab
	Day4	15.25	ab
	Day7	15.36	ab
	Day6	15.44	ab
	Day5	16.07	ab
	Day1	16.57	b
Yellow-vented Bulbul	Day1	9.35	a
	Day10	9.48	a
	Day8	10.31	ab
	Day6	10.46	ab
	Day9	10.69	ab
	Day7	10.76	ab
	Day2	11.34	ab
	Day4	11.80	ab
	Day3	12.29	ab
	Day5	17.39	b

By estimating the daily insect consumption by both bird species from the feeding experiment, the findings highlight the importance of undervalued common farmland birds as potential providers for insect pest regulation in oil palm production landscapes. The prediction made in this study is considered conservative due to the tendency for both bird species to exhibit a greater consumption of different insects within oil palm plantations, as compared to the bird specimens obtained in the feeding experiment. The daily insect intake of both bird species might be used in the future to estimate pest insect control services in oil palm operations. In the future, the density of both bird species in oil palm plantations should be assessed to estimate insect consumption. Such data on bird density in oil palm fields is currently lacking since many published bird studies have focused on species diversity (Atiqah et al., 2019; Azhar et al., 2011, 2013, 2014; Azman et al., 2011; Tohiran et al., 2019; Yahya et al., 2017, 2022).

In general, it was found that OMR consumed a larger amount of mealworms than YVB. Such finding was expected, although considered as generalist and opportunist, small fruits and berries make up a large proportion of the adult YVB diet (Fishpool et al., 2020). YVB has also been observed to feed more on invertebrates than plants when feeding its chicks. Wee (2009) also noted that YVB feeds its chicks with invertebrates as its primary source of food. On the other hand, OMR feeds mainly on termites, ants and black soldier fly larvae

(Ashitha & Seedikkoya, 2020). Though OMR is considered insectivorous, it has also been observed feeding on geckos and other small lizards.

The results suggested that mealworm consumption varied between individuals. Some individual birds are likely to consume more insects compared to others. Although the ability of OMR and YVB to suppress insect populations may vary depending on the individuals, both species are likely to be valuable biological agents to control and suppress insect populations to a certain threshold given the immediate presence of both species in oil palm plantations, particularly in the study region. Both species are considered common and can be found in high abundance in Malaysia (Amit et al., 2021; Atiqah et al., 2019; Azhar et al., 2013, 2014; Mohd-Azlan et al., 2019; Yahya et al., 2017).

Mealworm consumption was associated with bird weight, with larger individuals consuming significantly more mealworms (*Figure 2a*). Our results also indicate that this relationship is proportionate to body weight for both OMR and YVB (*Figure 2b*). It is generally assumed that insectivorous birds consume half of their body weight per day to meet the daily energy demand (Railsback & Johnson, 2011). While it is likely that larger-bodied birds consume a greater amount of insect prey per day, oil palm is less favourable to most of the large-bodied insectivorous species (e.g., trogons, malkohas, drongos) which are commonly associated with closed habitats (e.g., lowland dipterocarp forests) (Billerman et al., 2022; Jeyarajasingam, 2012; Wells, 1999, 2010). Oil palm agriculture is commonly associated with various small to medium-bodied insectivorous birds (both diurnal and nocturnal birds) with various foraging specialisations (e.g., bark gleaning, foliage gleaning, sallying, terrestrial) (Azhar et al., 2013, 2014). For example, in Sarawak, Malaysia, Amit et al. (2021) found insectivorous birds constituted almost 50% of recorded bird species in oil palm plantations. These common insectivorous bird species are likely to consume a range of invertebrates including those which may cause damage to crops (e.g., herbivory insects).

There is a range of invertebrate species consumed in oil palm by insectivorous birds. Amit et al. (2015) reported that YVB's invertebrate food preferences mainly comprised of beetles (Coleoptera), followed by various other arthropod orders including true bugs (Hemiptera), flies and mosquitoes (Diptera), as well as wasps, bees and ants (Hymenoptera). Amit et al. (2015) also reported that dragonflies and damselflies (Odonata), grasshoppers and crickets (Orthoptera), termites and cockroaches (Blattodea), as well as butterflies and moths (Lepidoptera) were absent in stomach content analysis conducted on 45 individual YVB (Amit et al., 2015). As a generalist, YVB tends to consume insects, but dietary preferences may vary depending on life stages, as well as the availability of certain prey within their habitat.

The results suggested that YVB consumed a similar weight of mealworms throughout the experiment, indicating that predation pressure on arthropods provided by YVB is likely to be consistent in the wild. In other locations bulbul species have been found to have similar roles as in Fiji, the invasive bulbul species, Red-vented Bulbul (*Pycnonotus cafer*) has been identified as an effective insect control agent in agricultural landscapes (Thibault et al., 2018). In India, the Red-vented Bulbul has been shown to protect crops from the invasive cotton bollworm (*Helicoverpa armigera*). Contrary to the Red-vented Bulbul species, little information is known regarding the effectiveness of YVB in suppressing insect populations in agricultural landscapes.

It is acknowledged that the present study has several limitations concerning its experimental components. While this study involved assessing the daily consumption of insects by captured wild birds, the mealworm consumption data may not be completely representative of daily insect consumption in the wild. Nonetheless, this is the first study to provide baseline data on how much insects are devoured daily by typical farmland birds in oil palm plantations. Feeding behaviours may vary considerably between captive and natural environments due to the large energy expenditure needed to obtain food in the wild. In addition, the feeding behaviour of OMR and YVB may be influenced by various factors including insect diversity and abundance, habitat structure, breeding season, provisioning of nestlings, foraging strategy and territorial behaviour of a species (Hawa et al., 2016; Sethi & Bhatt, 2007; Singh et al., 2016; Wee, 2009), all of which cannot be accounted for by the experiment design. In addition, this study did not partition the estimated consumption data according to male or female birds as the sex of the YVB could not be morphologically determined. However, this study provides a good starting point for determining the important contribution of avian fauna, particularly OMR and YVB, to pest control ecosystem services (Nyffeler et al., 2018; Razak et al., 2020; Yahya et al., 2024).

The data suggests that associated biodiversity, particularly farmland birds and their pest control services can be crucial to maintaining high oil palm yields providing further evidence of the importance of undertaking land sharing or bird-friendly farming strategy. The implementation of land sharing strategy can benefit birds such as OMR and YVB by protecting their habitats and reducing the application of synthetic pesticides in oil palm landscapes. In addition to the benefits for the focal species, such strategies can also protect numerous animal species that inhabit or visit oil palm plantations. Land sharing therefore needs to be an integral part of oil palm certification schemes (e.g., Roundtable on Sustainable Palm Oil [RSPO] and Malaysian Sustainable Palm Oil [MSPO]) and Integrated Pest Management (IPM).

CONCLUSION

In the future, the daily insect consumption by both bird species can be potentially used to estimate pest insect control services in oil palm plantations. The estimates presented in this paper emphasise the ecological and economic significance of common farmland birds in controlling insect populations, especially in tropical agricultural landscapes. The findings demonstrate the capabilities of common farmland bird species in suppressing insect populations might be limited by their food intake patterns which are influenced by various factors including species, body weight and prey preferences. The industry stakeholders should maintain both bird species' habitats inside oil palm plantations since they are potential biological control agents.

Minimising the use of agrochemicals and promoting natural processes (e.g., natural predation) are viable ways to improve sustainability in large-scale oil palm agroecosystems. Oil palm stakeholders, both smallholders and plantation companies, should consider associated biodiversity (i.e., farmland wildlife) as much as forest biodiversity in their conservation and landscape management. The conservation of beneficial birds such as OMR and YVB can be enhanced through land sharing or bird-friendly farming strategies in oil palm landscapes. Future work should investigate the role of associated biodiversity including farmland birds and other animal taxa, in oil palm production landscapes (i.e., smallholdings and industrial plantations) and their contribution to ecosystem services and disservices.

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In Planta EFFICACY OF LOCAL *Trichoderma* ISOLATES AND SELECTED COMMERCIAL BIOLOGICAL AGENTS AGAINST *Ganoderma boninense* IN OIL PALM

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ABSTRACT

Trichoderma *species are well-known biological control agents with significant antagonistic activity against various fungal phytopathogens. This study evaluated the* in planta *efficacy of local* Trichoderma *isolates (*Trichoderma virens *and* Trichoderma asperellum*) in comparison to commercial biological control agent (BCA) products in controlling* Ganoderma *disease in oil palm seedlings. The local* Trichoderma *isolates were applied either singly or as a mixture to the soils of both transplanted and* Ganoderma*-inoculated oil palm seedlings. Additionally, two commercial BCA products were tested as benchmarks, with a negative and a positive control. It was found that the local* Trichoderma *isolate, either applied singly or as a mixture, could reduce disease by 41.82%–57.73%, and be on par with the commercial BCA products. Untreated positive control showed a significant loss in physiological integrity, in terms of chlorophyll content, plant height, bole diameter, and the number of fronds, due to the* G. boninense *infection, meanwhile, the treated seedlings with local* Trichoderma *isolates and commercial BCAs were able to resist the infection significantly to a certain degree. These isolates are promising BCAs for the future management of* G. boninense *in oil palm.*

Keywords: biological control agents, *Ganoderma boninense*, oil palm, *Trichoderma asperellum*, *Trichoderma virens*.

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INTRODUCTION

The African oil palm (*Elaeis guineensis* Jacq.) is a major vegetable oil-producing crop contributing to 40% of the global vegetable oil supplies (United States Department of Agriculture [USDA], 2025). In Malaysia, oil palm is grown on more than 5.65 million hectares and produces more than 18 million tonnes of palm oil per year (Malaysian Palm Oil Board [MPOB], 2023). One of the major disease challenges associated with large-scale oil palm plantations in Malaysia is the threat of basal stem rot (BSR), also known as *Ganoderma* disease due to the pathogenic fungus, *Ganoderma boninense*. It has been estimated that the economic losses due to this disease can be as high as 68% (Assis et al., 2021). Nevertheless, integrated disease management is strongly recommended to minimise economic losses, such as by integrating biological control agents (BCAs), cultural practices, and chemical treatments. In this context, BCAs are considered a sustainable method to control plant diseases, as they are non-toxic and environmentally friendly (TariqJaveed, 2021).

To date, *Trichoderma* species are known for their effectiveness as a promising BCA for suppressing the growth of various soilborne phytopathogens, such as *Rhizoctonia solani*, *Fusarium oxysporum*, *G. boninense* and *Sclerotium rolfsii*, due to its ideal properties such as rapid growth, wide adaptation to different soil types and temperature ranges, good root colonisation and survivability under

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extreme conditions (Meena et al., 2017). In addition, *Trichoderma* species have various direct and indirect mechanisms of action for controlling phytopathogenic fungi (Ghorbanpour et al., 2018). *Trichoderma* species suppress the growth of pathogens directly through competition, antibiosis, the production of cellulases and other hydrolytic enzymes and mycoparasitism. *Trichoderma* species can suppress pathogens indirectly by inducing plant resistance (Saravanakumar et al., 2016). In numerous studies, *Trichoderma* species have been tested individually or in combination in nursery trials and have been effective in controlling *Ganoderma* disease in oil palm (Musa et al., 2017). However, the use of a single BCA often leads to inadequate performance in agriculture (Harshita et al., 2018), as BCAs tend to be highly disease-specific. Researchers are increasingly interested in combining BCAs with different mechanisms to improve the success of biological control (Rajeela et al., 2018). Compatible combinations of BCAs may have synergistic effects and significantly increase their efficacy. On the other hand, there are many commercial BCA products available in the market that are effective in controlling *Ganoderma* disease (e.g. combinations of *Trichoderma* species, mycorrhizal fungi and *Bacillus* species). Most of these commercial products are not locally sourced, so the introduction of foreign microorganisms can affect local agroecology (Myers & Cory, 2017). Importing products containing foreign microorganisms requires approval from the local authority, such as the Sabah Department of Agriculture, and also requires assistance with transportation costs. The use of local *Trichoderma* isolates makes the extraction and production of BCAs comparatively easier. It also helps to preserve the local ecosystem without harming some beneficial microorganisms found in natural habitats. Benchmarking the local *Trichoderma* isolates with commercial BCAs can provide useful information on the possibility of proposing the potential local *Trichoderma* isolates for commercial mass production.

Therefore, the study was conducted to evaluate the *in planta* efficacy of local *Trichoderma* isolates (*Trichoderma virens* and *Trichoderma asperellum*) in comparison to commercial BCA products in controlling *Ganoderma* disease in oil palm seedlings, in terms of disease and plant physiology assessments and soil and plant chemical properties.

MATERIALS AND METHODS

Preparation of Planting Materials

Oil palm seedlings used in an *in planta* study were 3-month-old, hybrid (*Dura* × *Pisifera*) grown in a nursery facility at MPOB Research Station, Lahad Datu, Sabah. A germinated seed was sown in a small polyethylene planting bag (15 × 23 cm) containing a soil mixture in a ratio of 2:1 (non-sterile soil: compost) for three months. Then, the same soil mixture was filled into a bigger polyethylene planting bag (30 x 38 cm) before being used for transplanting.

Preparation of *Ganoderma* Inoculum

Ganoderma inoculum was prepared on a 6 × 6 × 6 cm rubber wood block (RWB). The RWB were soaked in tap water overnight, and inserted into a heat-resistant polypropylene plastic bag. Later, 10 mL of potato dextrose broth (PDB) was added and autoclaved at 121°C for 25 min. Additionally, the RWB was supplemented with an additional 10 mL of sterile molten potato dextrose agar (PDA) and allowed to solidify. Six mycelia plugs (5 mm diameter) from a 7-day-old culture of *G. boninense* were placed on each side of the RWB aseptically, and the plastic was sealed to avoid contamination. The inoculated RWBs were incubated in the dark at room temperature (28°C ± 2°C) for 10 weeks to allow the fungal mycelium to fully colonise the block. The fully colonised and uncontaminated RWBs were applied as inoculum to oil palm seedlings (Nusaibah et al., 2016).

Artificial Inoculation

Inoculation was performed upon transplanting of the oil palm seedlings using the RWB sitting technique according to Rakib et al. (2015). The RWB inoculum was placed in the middle of the polybag containing soil mixture, and then the 3-month-old oil palm seedling was transplanted on top of the RWB inoculum to ensure direct contact between the roots and the inoculum.

Preparation of *Trichoderma* Conidial Suspension

The local *T. virens* and *T. asperellum* were previously isolated from the rhizosphere of the roots of healthy oil palm trees from a local oil palm estate in Sandakan, Sabah, Malaysia and both *Trichoderma* isolates were identified based on the internal transcribed spacer sequence (Darlis et al., 2023). The local *Trichoderma* isolates used as treatments were sub-cultured by plating an 8 mm of a 7-day-old mycelia plug onto a PDA plate and incubating at 25°C temperature for seven days. The culture was harvested with sterilised distilled water (10 mL) by carefully separating it with an L-shaped glass rod. The conidial suspension was filtered through a filter paper with 11 μm pore size and 9 cm diameter to remove mycelial fragments. The concentration of conidia suspension was adjusted to 1×10^6 conidia mL^{-1} using a hemocytometer.

Application of *Trichoderma* Species and Experimental Design

Two local *Trichoderma* isolates, and two commercial BCA products (Product X and Y) were tested for their efficacy as a treatment to suppress *G. boninense* infection in oil palm seedlings. All seven treatments consisted of 10 replications (*Table 1*). The experimental units were arranged in a complete randomised design (CRD) in a nursery at MPOB Research Station, Lahad Datu, Sabah. Regular weeding and watering (twice a day) were performed and fertiliser was applied according to standard commercial practices (MPOB, 2016).

Disease Assessment

The data presented in this study were according to the records at nine months after inoculation. The disease incidence (DI) and the disease severity index (DSI) were based on the foliar symptoms and disease class (*Table 2*), the area under the disease progress curve (AUDPC) and percentage of necrotic primary roots were calculated according to Rakib et al. (2015). The DI, DSI, AUDPC, percentage of necrotic primary roots and percentage of disease reduction were calculated using Equations 1, 2, 3, 4 and 5, respectively. To complete Koch's postulates, the presence of *Ganoderma* was either confirmed visually (*Ganoderma* basidiocarp) or by employing *Ganoderma* selective media (GSM) plating of necrotic primary roots for non-visible *Ganoderma* presence (Rakib et al., 2015).

TABLE 2. NUMERICAL DISEASE CLASSES AND THEIR CORRESPONDING FOLIAR SYMPTOMS ON OIL PALM SEEDLINGS

Class	Symptoms
0	Healthy plants with green leaves and no fungal mass development on any part of the plant.
1	Appearance of 1–3 chlorotic leaves with no fungal mass development on any part of the plant.
2	Appearance of fungal mass with or without chlorotic leaves.
3	Appearance of >3 chlorotic leaves, necrotic leaves (dead leaves) with or without fungal mass development on any part of the plant.
4	At least 50% of the total leaf number shows severe chlorosis or necrosis with or without fungal mass.
5	Dead plants with or without fungal mass.

Source: Rakib et al. (2015).

$$\text{Disease incidence (\%)} = \frac{\text{Number of infected seedlings}}{\text{Total number of seedlings}} \times 100 \quad (1)$$

$$\text{Disease severity index (\%)} = \frac{\sum (A \times B)}{\sum n \times 5} \times 100 \quad (2)$$

where, A is the disease class (0 to 5), B is the number of seedlings showing the disease class per treatment, n is the total number of replications, and 5 is the constant representing the highest class of assessment.

$$\text{Area under disease progress curve unit} = \sum_{i}^{n-1} \left(\frac{Y_i + Y_{i+1}}{2} \right) (t_{i+1} - t_i) \quad (3)$$

TABLE 1. EXPERIMENTAL TREATMENTS AND DESCRIPTION

Code	Treatment	Descriptions
T1	Untreated (negative control)	Non-inoculated seedlings and without any treatment.
T2	Untreated (positive control)	*Ganoderma*-inoculated seedlings, and without any treatment.
T3	*Trichoderma virens*	*Ganoderma*-inoculated seedlings and treated with locally isolated *T. virens*. Conidial suspension of 10^6 was applied once by drenching 100 mL onto the soil after transplanting.
T4	*Trichoderma asperellum*	*Ganoderma*-inoculated seedlings and treated with locally isolated *T. asperellum*. Conidial suspension of 10^6 was applied once by drenching 100 mL onto the soil after transplanting.
T5	*T. virens* and *T. asperellum*	*Ganoderma*-inoculated seedlings and treated with a mixture of locally isolated *T. virens* and *T. asperellum*. Conidial suspension of 10^6 was applied once by drenching 100 mL onto the soil after transplanting.
T6	Commercial product X	*Ganoderma*-inoculated seedlings and treated with a commercial product containing a mixture of 12 species of arbuscular mycorrhizal fungi (250–300 spore/10 g product). Applied by broadcasting 40 g of the product once into the planting hole upon transplanting.
T7	Commercial product Y	*Ganoderma*-inoculated seedlings and treated with a commercial product containing a mixture of *T. viride*, *T. harzianum*, *Paecilomyces lilacinus*, *Pseudomonas fluorfecens* and *Bacillus subtilis*. Applied by broadcasting 5 g of the product once into the planting hole upon transplanting.

where, n is the number of assessment times, Y is the disease incidence, and t is the observation time.

$$\text{Necrotic primary roots (\%)} = \frac{\text{Number of necrotic primary roots}}{\text{Total number of roots per seedling}} \times 100 \quad (4)$$

$$\text{Disease reduction (\%)} = \frac{X1 - X2}{X1} \times 100 \quad (5)$$

where, X1 is the AUDPC of positive control (T2), and X2 is the AUDPC of the treated seedlings.

Plant Physiology Assessment

Chlorophyll content was recorded using a SPAD chlorophyll device (Rakib et al., 2019). Plant height was measured from the ground to the tip of the highest leaf by placing a steel tape measure next to the plant. The stem diameter was also measured using a digital caliper and the number of fronds was counted.

Soil Sampling and Chemical Properties Analysis

Soil samples were collected after nine months of nursery trials. Soil samples at a depth of 0–15 cm were collected from the growing medium in the planting bags. A total of 10 samples from each replication were bulked into one sample, homogenised, air-dried, ground and sieved through a 2 mm mesh. The dried and ground samples were stored in an air-tight polyethylene container for further analysis.

Soil pH was determined at a soil-to-distilled water ratio of 1:2.5 (w/v) using an Orion combination electrode pH (Eckert & Sims, 2013). The pH meter was calibrated before being used to read the pH value. Available nutrient elements were determined by leaching the soil sample with 100 mL of ammonium acetate (Castilho & Rix, 1993). The leachate was used for measuring the concentration of nutrients such as phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), zinc (Zn) and copper (Cu) using an Inductively Coupled Plasma Optical Emission spectroscopy (ICP-OES).

Plant Sampling and Chemical Properties Analysis

Leaf samples were collected by identifying the frond 3, at 12 months of age in young palms (Sabri et al., 2019). The leaf samples were oven-dried at 80°C for 24 hr (constant weight), pulverised using an electrical grinder, and kept for further analysis. The leaf samples were analysed for N, P, K, Ca, Mg, Zn and Cu. The samples were extracted using the dry ashing method except for N (Enders & Lehmann, 2012). The concentration of these elements was measured using an ICP-OES, while N was measured using a CHN analyser.

Statistical Analysis

Analysis of variance (ANOVA) was performed and the means were compared using Tukey's test at the significance level of $p \leq 0.05$. Pearson's correlation was performed between the variables. All statistical analysis was performed using the SPSS statistical software (SPSS version 24).

RESULTS AND DISCUSSION

Disease Suppression in Oil Palm Seedlings

The results of the *in planta* nursery experiment showed that treatments by the local *Trichoderma* isolates and the selected commercial BCAs significantly reduced disease signs and symptoms in the infected oil palm seedlings with *G. boninense* compared to the untreated positive seedlings (T2). The local *Trichoderma* species (T3, T4 and T5) and the commercial BCAs (T6 and T7) showed similar efficacy in terms of the DSI (8.67%–21.33%), AUDPC (21.67 to 45.00 units) and percentage of necrotic primary roots (6.76%–17.73%). Application of the local *Trichoderma* species and commercial BCAs has resulted in a significant disease reduction ranging from 50.91%–76.36%. In terms of the DI, the positive control recorded 100%, while there was a significant reduction in the treated oil palm seedlings, where the local *T. virens* (T3), Product X (T6) and Product Y (T7) recorded the lowest DI. However, the DI was not significantly different among the local *T. virens* (T3), local *T. asperellum* (T4) and the mixture of both local isolates (T5) (*Figure 1*).

The application of *Trichoderma* isolates and arbuscular mycorrhizal fungi (AMF) can suppress *G. boninense*. The AMF mainly improves plant nutrition, altering the morphological structure of plant roots, regulating the synthesis of secondary metabolism, improving the microenvironment in the rhizosphere of plants, directly competing with pathogenic microorganisms for invasion sites and nutrients, and inducing resistance to plant diseases and the formation of a defense system (Tatsumi et al., 2021). *Trichoderma,* on the other hand, can control plant disease due to diverse antifungal activities such as mycoparasitism, antibiosis, competition for nutrients and induced systemic resistance in plants (Druzhinina et al., 2011).

It is noteworthy that the single application of *T. asperellum* and *T. virens* resulted in disease suppression against *G. boninense* with 41.82% and 57.73% disease reduction, respectively. The combination of these *Trichoderma* species did not

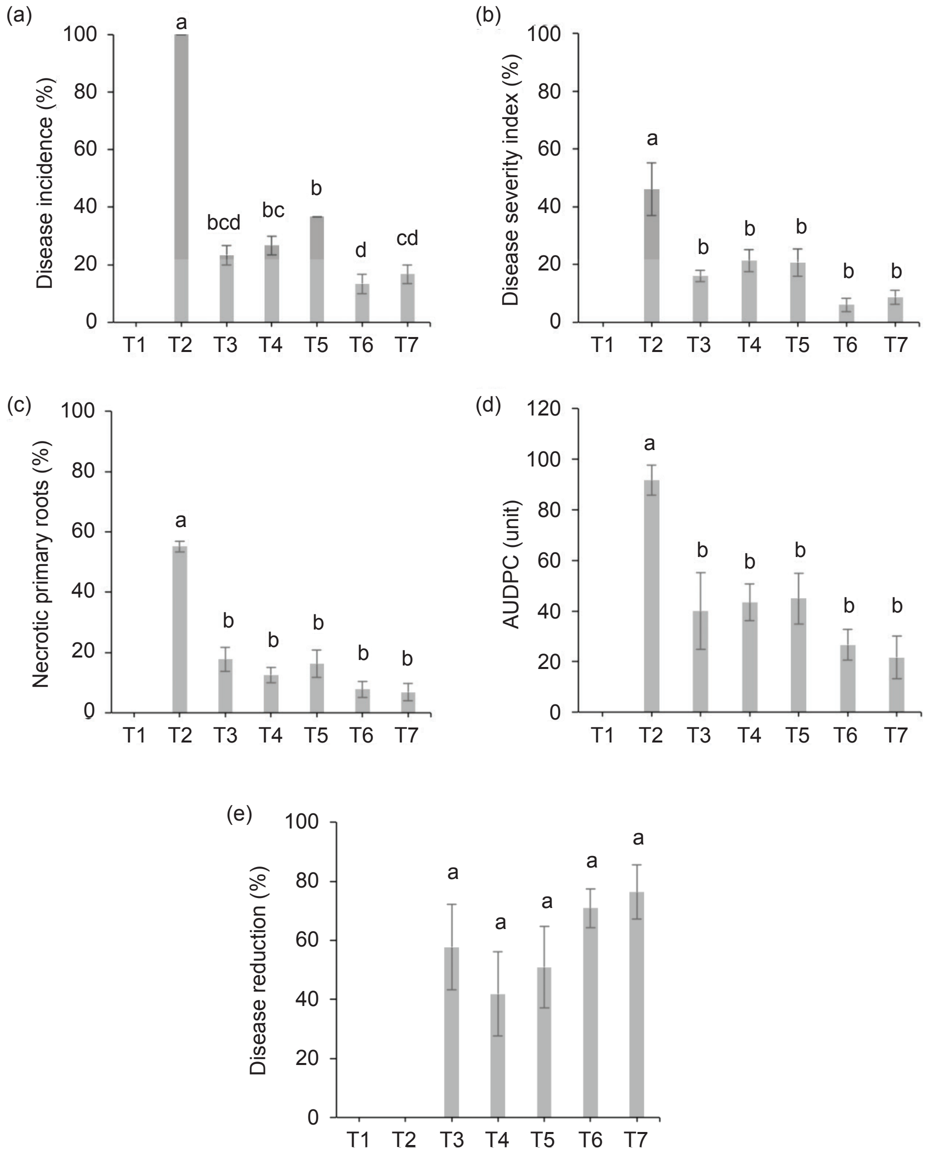

Figure 1. Effect of local Trichoderma *isolates and commercial BCAs against* G. boninense, *nine months after inoculation. (a) Disease incidence; (b) disease severity index; (c) area under progress curve (AUDPC); (d) percentage necrotic roots and (e) disease reduction. T1, Untreated (negative control); T2, Untreated (positive control); T3,* T. virens; *T4,* T. asperellum; *T5,* T. virens *and* T. asperellum; *T6, Commercial product X; and T7, Commercial product Y. Means (± standard error) followed with different letters were significantly different at p ≤ 0.05 by Tukey's test.*

show better results either. This could be due to some incompatibility as the *Trichoderma* species were selected for their antagonistic behaviour against *G. boninense in vitro*, and not for their combined efficacy. Similar to the findings in the current study, Shamala (2013) also reported these species as potential BCAs against *G. boninense* infection in oil palm nursery experiments. These *Trichoderma* species produce chitinase, cellulase, protease and β-glucanase, which play a crucial role in mycoparasitism in fungi (Musa, 2017) and reduce the integrity of the pathogen's cell wall by breaking the polysaccharides chitin and β-glucan (Howell, 2003). Furthermore, *Trichoderma* species outperform phytopathogens in terms of nutritional competition, and release siderophores to mobilise iron in the soil, which may have efficiently inhibited the growth of *G. boninense*, thus suppressing *Ganoderma* disease in oil palm seedlings due to iron uptake (Saha et al., 2013). Moreover, these *Trichoderma* species are also reported to produce volatile organic compounds, which may contribute to reducing the hyphal growth of *G. boninense* (Inayati et al., 2020).

In this study, commercial Product X also showed a disease reduction of 70.91%. Product X (T6) contains a mixture of 12 species of AMF, including fungi from the genera *Glomus*, *Sclerocystis*, *Acaulospora*, *Gigaspora* and *Scutellospora*. Weng et al. (2022) described the direct and indirect mechanisms of AMF as BCA, including enhancing lignification of the cell wall, and root branching. Furthermore, mycelial networks of the AMFs work as a barrier in the root epidermis, improving soil structure, trapping pathogens, killing pathogens by root exudates, enhancing the proliferation of beneficial microorganisms and improving plant nutrition and water absorption (Weng et al., 2022). Meanwhile, the commercial Product Y (T7) has a mixture of *T. harzianum*, *T. viride* and a few other BCAs, which resulted in a 76.36% disease reduction. The disease suppression achieved with Product Y can be due to the compatible combination of mixed strains, which can show synergy and significantly increase the effectiveness of the product. In addition, this could be due to diverse toxic substances (gliotoxins) produced by the different *Trichoderma* species, which can aid in the growth of *Trichoderma* in the soil.

Effects on Physiology of Oil Palm Seedlings

The infection of *G. boninense* in oil palm seedlings has caused a significant loss in chlorophyll content, plant height, bole diameter and frond production. However, the application of the local *Trichoderma* isolates, either singly (T3 and T4) or as a mixture (T5) has provided significant disease resistance in the oil palm seedlings towards *G. boninense* infection, as attributed to the physiological responses, compared to the untreated positive control. Moreover, there was no significant difference among all BCA-treated oil palm seedlings (T3, T4, T5, T6 and T7), indicating the local *Trichoderma* isolates have similar efficacy in resisting the loss of plant physiological integrity. The percentage of reduction in terms of chlorophyll content, plant height and bole diameter in the treated oil palm seedlings ranged between 9.49%–19.40%, 11.41%–18.87% and 4.26%–18.82%, respectively, as compared to the untreated negative control (*Figure 2*). These findings were further validated by Pearson's correlation analysis which revealed a negative correlation between the disease variables and physiological responses as shown in *Table 3*, while there was only a positive correlation with disease reduction, suggesting that disease reduction by these BCAs in oil palm seedlings was directly correlated with the promotion of plant physiology.

These were consistent with other studies reported by Syafiq et al. (2021), in which they observed an enhanced growth response induced by *Trichoderma* species in infected *Ganoderma* oil palm seedlings. The finding supports the hypothesis that numerous antagonistic microbes,

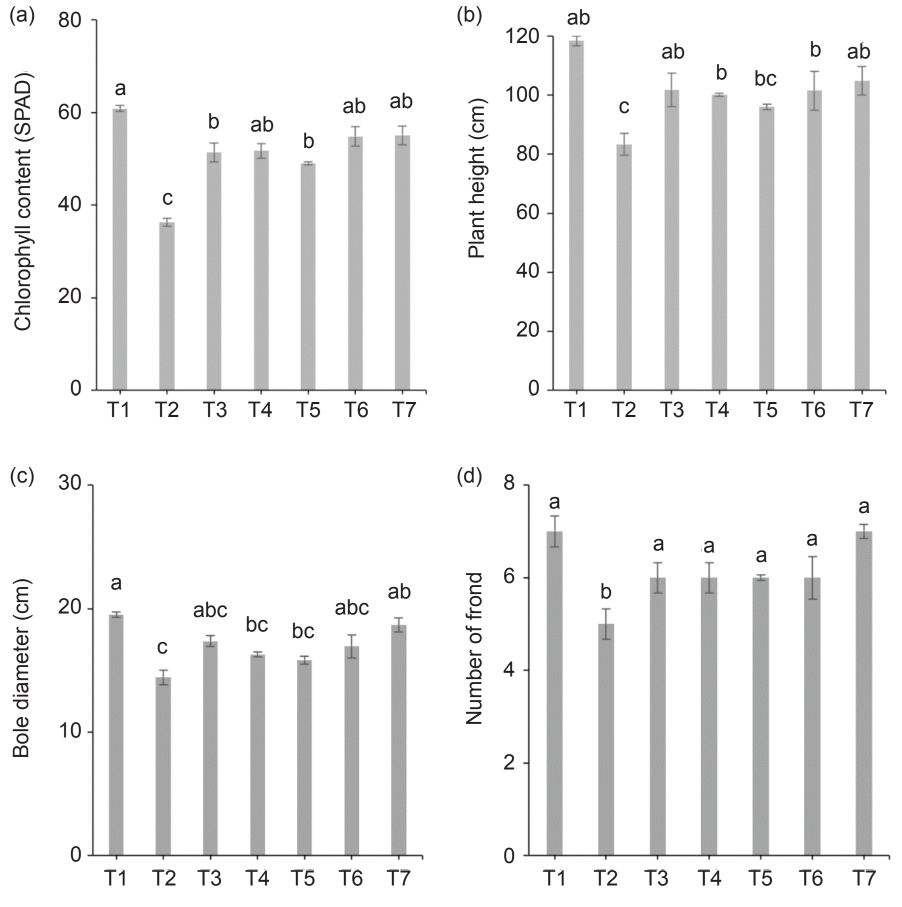

Figure 2. Effect of local Trichoderma *isolates and commercial BCAs on plant growth, nine months after inoculation. (a) Chlorophyll content; (b) plant height; (c) bole diameter and (d) number of fronds. T1, Untreated (negative control); T2, Untreated (positive control); T3,* T. virens*; T4,* T. asperellum*; T5,* T. virens *and* T. asperellum*; T6, Commercial product X; and T7, Commercial product Y. Means (± standard error) followed with different letters were significantly different at p ≤ 0.05 by Tukey's test.*

like *Trichoderma*, indirectly promote plant growth by mitigating the impacts of pathogens and reducing disease severity (Beneduzi et al., 2012). Interestingly, *T. virens* and *T. asperellum* have been reported to produce a phytohormone-like auxin, which significantly increased plant height, frond number and chlorophyll content in this study. This phytohormone promotes plant growth directly and indirectly by balancing other hormones like gibberellic acid (GA3). It also promotes plants by expanding lateral and adventitious roots, improving nutrient access and enhancing root exudation which offers more resources for soil microbe and root interactions (Spaepen & Vanderleyden, 2011). According to Brummel and Hall (1987), even at lower levels, this hormone can promote plant growth. This may contribute to increasing plant performance through *Trichoderma* treatment in nursery trials.

Effect on Soil and Plant Nutrients Uptake

This study also investigated the effect of the *Trichoderma* isolates and the commercial BCAs on soil nutrient availability and total nutrients in the oil palm seedlings by the end of the nursery trials. As compared to the untreated negative control, the treated soils in T3, T4, T5, T6 and T7 resulted in significantly higher availability of P (0.025–0.029 g kg^{-1}), K (0.128–0.131 g kg^{-1}), Ca (1.36–1.41 g kg^{-1}), Mg (0.119–0.124 g kg^{-1}) and Zn (6.60–7.55 mg kg^{-1}) (*Table 4*). This is consistent with a study by Khan et al. (2017) showed that the breakdown and adsorption of P, K, Ca, Mg, Cu, Fe, Mn and Zn in the soil can be greatly enhanced by BCAs such as *Trichoderma* species.

Furthermore, the higher availability of nutrients in the soil was directly attributed to the higher total nutrients in the leaves of oil palm seedlings. Generally, significantly higher P (1.85–1.87 g kg^{-1}), K (12.53–13.43 g kg^{-1}), Ca (17.51–18.60 g kg^{-1}), Mg (3.07–3.15 g kg^{-1}) and Zn (18.27–18.60 mg kg^{-1}) were recorded in the treated oil palm seedlings, as compared to the untreated negative control (*Table 5*).

Certain nutrients in the soil are present in a poorly soluble or insoluble form, limiting the usual nutrient cycle in the soil. *Trichoderma* species may secrete organic acids that alter the pH of the soil rhizosphere, thus improving the plants' uptake of macronutrients such as P and micronutrients such as Fe, Mn and Zn (Li et al., 2015). *Trichoderma* species also have a strong colonisation ability, which

TABLE 3. PEARSON'S CORRELATION ANALYSIS BETWEEN VARIABLES MEASURED FOR DISEASE AND PLANT PHYSIOLOGICAL RESPONSES

Variable	DI	DSI	AUDPC	PR	DR	CHL	HT	BD
DSI	0.96**	-	-	-	-	-	-	-
AUDPC	0.93**	0.98**	-	-	-	-	-	-
PR	0.99**	0.96**	0.93**	-	-	-	-	-
DR	-0.93**	-0.98**	-1.00**	-0.93**	-	-	-	-
CHL	-0.98**	-0.98**	-0.97**	-0.98**	0.96**	-	-	-
HT	-0.89**	-0.92**	-0.96**	-0.88**	0.95**	0.96 **	-	-
BD	-0.83*	-0.90**	-0.93**	-0.82*	0.93**	0.90**	0.95**	-
NF	-0.84*	-0.89**	-0.92**	-0.87*	0.92**	0.90**	0.90**	0.95**

Note: DI - disease incidence; DSI - disease severity index; AUDPC - area under disease progress curve; PR - percentage of necrotic primary roots; DR - percentage of disease reduction; CHL - chlorophyll content; HT - plant height; BD - bole diameter; NF - number of fronds. *Significantly correlated at $p \leq 0.05$. **Significantly correlated at $p \leq 0.01$.

TABLE 4. EFFECT OF LOCAL *Trichoderma* ISOLATES AND COMMERCIAL BIOLOGICAL CONTROL AGENTS ON SOIL NUTRIENT AVAILABILITY, NINE MONTHS POST INOCULATION

Treatment	pH	Available P (g kg^{-1})	Available K (g kg^{-1})	Available Ca (g kg^{-1})	Available Mg (g k^{g-1})	Available Zn (mg kg^{-1})	Available Cu (mg kg^{-1})
T1	6.61 ± 0.23^{a}	0.023 ± 0.00^{c}	0.125 ± 0.00^{c}	1.24 ± 0.00^{c}	0.119 ± 0.00^{b}	6.16 ± 0.04^{b}	1.22 ± 0.01^{a}
T2	5.23 ± 0.06^{b}	0.018 ± 0.00^{d}	0.111 ± 0.00^{d}	1.15 ± 0.00^{d}	0.111 ± 0.00^{c}	3.62 ± 0.13^{c}	0.93 ± 0.00^{b}
T3	7.53 ± 0.67^{a}	0.029 ± 0.00^{a}	0.130 ± 0.00^{a}	1.36 ± 0.00^{b}	0.124 ± 0.00^{a}	7.51 ± 0.20^{a}	1.25 ± 0.00^{a}
T4	6.68 ± 0.94^{a}	0.029 ± 0.00^{a}	0.130 ± 0.00^{a}	1.37 ± 0.01^{b}	0.124 ± 0.00^{a}	7.70 ± 0.09^{a}	1.25 ± 0.00^{a}
T5	6.37 ± 0.06^{a}	0.029 ± 0.00^{a}	0.131 ± 0.00^{a}	1.36 ± 0.01^{b}	0.123 ± 0.00^{a}	7.55 ± 0.22^{a}	1.25 ± 0.02^{a}
T6	7.01 ± 0.09^{a}	0.025 ± 0.00^{b}	0.128 ± 0.00^{b}	1.38 ± 0.00^{ab}	0.119 ± 0.00^{b}	6.60 ± 0.15^{b}	1.25 ± 0.00^{a}
T7	6.94 ± 0.06^{a}	0.027 ± 0.00^{ab}	0.129 ± 0.00^{ab}	1.41 ± 0.00^{a}	0.120 ± 0.00^{b}	6.74 ± 0.01^{b}	1.25 ± 0.00^{a}

Note: P - posphorus; K - potassium; Ca - calcium; Mg - magnesium; Zn - zinc; Cu - copper; T1 - Untreated (negative control); T2 - Untreated (positive control); T3 - *T. virens*; T4 - *T. asperellum*; T5 - *T. virens* and *T. asperellum*; T6 - Commercial product X; T7 - Commercial product Y. Means (± standard error) followed with different letters within a column were significantly different at $p \leq 0.05$ by Tukey's test.

TABLE 5. EFFECT OF LOCAL *Trichoderma* ISOLATES AND COMMERCIAL BIOLOGICAL CONTROL AGENT ON OIL PALM SEEDLING NUTRIENT CONTENT NINE MONTHS POST INOCULATION

Treatment	N (%)	P ($g\ kg^{-1}$)	K ($g\ kg^{-1}$)	Ca ($g\ kg^{-1}$)	Mg ($g\ kg^{-1}$)	Zn ($g\ kg^{-1}$)	Cu ($g\ kg^{-1}$)
T1	7.04 ± 0.32^{a}	1.75 ± 0.03^{b}	12.16 ± 0.10^{b}	15.52 ± 0.10^{d}	3.03 ± 0.01^{b}	16.40 ± 0.26^{b}	7.20 ± 0.06^{a}
T2	6.54 ± 0.01^{b}	1.16 ± 0.20^{c}	8.66 ± 0.30^{c}	14.67 ± 0.12^{e}	2.25 ± 0.00^{c}	11.17 ± 0.09c	3.37 ± 0.09^{b}
T3	7.12 ± 0.01^{a}	1.85 ± 0.02^{a}	12.85 ± 0.00^{ab}	18.60 ± 0.23^{a}	3.08 ± 0.01^{b}	18.50 ± 0.23^{a}	7.50 ± 0.06^{a}
T4	7.04 ± 0.07^{a}	1.87 ± 0.01^{a}	13.43 ± 0.19^{a}	17.54 ± 0.01^{c}	3.15 ± 0.02^{a}	18.60 ± 0.06^{a}	7.43 ± 0.24^{a}
T5	7.02 ± 0.08^{a}	1.86 ± 0.01^{a}	12.66 ± 0.01^{ab}	17.74 ± 0.07^{bc}	3.08 ± 0.01^{b}	18.60 ± 0.11^{a}	7.67 ± 0.15^{a}
T8	7.01 ± 0.01^{a}	1.86 ± 0.00^{a}	12.53 ± 0.24^{b}	17.51 ± 0.13^{c}	3.05 ± 0.01^{b}	18.27 ± 0.12^{a}	7.23 ± 0.09^{a}
T9	7.01 ± 0.07^{a}	1.86 ± 0.01^{a}	12.58 ± 0.26^{ab}	18.29 ± 0.08^{ab}	3.07 ± 0.02^{b}	18.37 ± 0.09^{a}	7.30 ± 0.06^{a}

Note: N - nitrogen; P - posphorus; K - potassium; Ca - calcium; Mg - magnesium; Zn - zinc; Cu - copper; T1 - Untreated (negative control); T2 - Untreated (positive control); T3 - *T. virens*; T4 - *T. asperellum*; T5 - *T. virens* and *T. asperellum*; T6 - Commercial product X; T7 - Commercial product Y. Means (± standard error) followed with different letters within a column were significantly different at $p \leq 0.05$ by Tukey's test.

can increase the root-soil contact area and improve access to nutrients as the root system grows and expands. In addition, several isolates of *Trichoderma* have been shown to produce bio-stimulant and hormone-like compounds that may promote plant nutrient uptake. However, this study did not evaluate the plant-promoting traits of *T. viren* and *T. asperellum*. These *Trichoderma* species have been shown to have various plant growth-promoting properties, including the ability to solubilise phosphate, and produce indole-3-acetic acid (IAA) and siderophere (Inayati et al., 2020; Muniroh et al., 2019). *Trichoderma* species can colonise and live endophytically in plant roots, similar to those of mycorrhizal fungi (Kleifeld & Chet, 1992), and IAA synthesis by these fungi can enhance plant root mass (López-Bucio et al., 2015). *Trichoderma* species' ability to dissolve phosphate and produce siderophores may have increased nutrient release for plant root uptake (Rudresh et al., 2005). Consequently, these *Trichoderma* species could significantly improve the physiological status of plants as well as their nutritional status.

CONCLUSION

Both local *T. virens* and *T. asperellum*, either applied singly or as a mixture, were able to reduce disease by 41.82%–57.73%, and at par with the commercial BCA products. The untreated oil palm seedlings (positive control) showed a significant loss in physiological integrity in terms of chlorophyll content, plant height, bole diameter, and number of fronds due to the *G. boninense* infection, meanwhile, the treated seedlings with local *Trichoderma* isolates and commercial BCAs were able to resist the infection significantly to a certain degree. The application of the local *Trichoderma* species and both commercial BCAs also generally improves the availability of macronutrients in the soil, as well as the nutrient content in the plants. Therefore, it was suggested to further study the synergy of the local *Trichoderma* isolates with other BCAs to further improve their efficiency in suppressing phytopathogens, as well as improving soil health, crop growth and yield. The performance of the local *Trichoderma* isolates is considered similar to that of commercial BCAs and can be further exploited for mass production and commercialisation.

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APPLICATION OF OLD OIL PALM TRUNKS AFFECTS THE GROWTH PERFORMANCE OF OIL PALM SEEDLINGS

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ABSTRACT

*Oil palm plantations generate substantial biomass waste, primarily old oil palm trunks (OPT), during replanting. As part of a sustainable plantation management program, old OPT are returned to the plantation and released nutrients into the soil for new oil palm seedlings. However, whether this method improves soil nutrient levels is unclear. In this study, we investigated the effects of OPT on oil palm seedling growth and soil microbial communities. The plant height, chlorophyll content, leaf area, and biomass weight were low in seedlings grown in soil containing OPT (44.3 cm, 44,278.9 cm^2, 19.6 g [dry shoot], and 14.9 g [dry root]). Similar results were obtained for seedlings grown in soil containing cellulose or soil containing OPT and fertiliser. Leaf nitrogen, phosphorus, and potassium contents were similar in seedlings grown in soil amended with OPT and control seedlings. However, the calcium content was significantly lower in seedlings grown in soil containing OPT (0.424 ± 0.004%) than in control seedlings (0.496 ± 0.006%). Metagenomic analysis of soils showed that three lignocellulose-degrading fungal genera (*Chaetomium*, *Mortierella*, *and* Staphylotrichum*) were abundant in soil containing OPT. Thus, the return of OPT promotes the growth of lignocellulose-degrading microorganisms and decreases fertiliser nutrient availability.*

Keywords: *Chaetomium*, lignocellulose-degrading microorganisms, oil palm trunk, plant growth performance, soil metagenomics.

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INTRODUCTION

Palm oil is regarded as one of Malaysia's primary sector industries and is a major agricultural product exported globally. Oil palm productivity decreases every 20 to 25 years, and new oil palm seedlings are replanted (Corley & Tinker, 2015). The old oil palm trees are felled to clear the land, and are left in the plantation to decompose. Some plantations use the chip and windrow method (e.g., Guan Soon Plantation, Alor Pongsu, Perak, Malaysia), whereas some use the pulverisation technique, such as Kuala Lumpur Kepong Berhad (2018), whereby entire old oil palm trunks (OPT) are pulverised into smaller pieces that are then spread on the cleared land (Pulingam et al., 2022). OPT left using the windrow method takes at least two years to decompose completely, whereas those that are pulverised and spread onto soil degrade within approximately one year, which helps to shorten the fallow period. The decomposing old OPT is considered to serve as mulch (Sung, 2016) while simultaneously replenishing soil nutrients as they break down (Pulingam et al., 2022).

However, during the decomposition period, various environmental issues may arise. For example, OPT eventually become a breeding site for pests, such as *Oryctes rhinoceros* (Manjeri et al., 2014), and the fungus *Ganoderma boninense*, which causes basal stem rot disease (Gorea et al., 2019).

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Additionally, the high carbon-to-nitrogen (C:N) ratio (Lin et al., 2019) lengthens the decomposition period (Akratos et al., 2017) and deprives the soil of nitrogen, which is needed for plant growth (Lin et al., 2019). Usually, a C:N ratio between 25 and 35 is optimal for agricultural applications; however, OPT has a high C:N ratio of 155 (Loh et al., 2013) and it takes a long time for this ratio to decrease. Therefore, the substantial OPT biomass waste generated during replanting and left in oil palm plantations to decompose may negatively affect plant growth. The application of OPT results in decreased growth performance compared with empty fruit bunches (Mohammed et al., 2014). It also alters the soil microflora and decreases the availability of nutrients, such as nitrogen, calcium and magnesium, for plant uptake (Uke et al., 2021). Nitrogen promotes leaf growth. The leaf is one of the most important plant organs because it is a nitrogen storage site that synthesises amino acids. Calcium is an important component of cell walls and membranes, thereby contributing to the structural integrity of cells. This helps to maintain the physical barriers that protect plants from pathogens, leading to enhanced immunity. Magnesium is a component of chlorophyll, which is essential for photosynthesis. A magnesium deficiency may cause chlorosis.

The soil type and quality of organic matter affect the diversity and abundance of soil microflora (Li et al., 2018; Savy et al., 2020). The soil and rhizosphere microorganisms are bio-indicators of soil quality because they are sensitive to the smallest changes in abiotic conditions such as environmental stress and soil perturbation. In addition, land fertility and environmental biodiversity are affected by the cultivation of oil palm (Ashton-Butt et al., 2018; McGuire et al., 2014).

To understand how OPT breakdown affects soil health and microbial diversity within a plantation, it is important to determine the effect of applying OPT fibre directly to the soil, mimicking the pulverisation technique, on oil palm seedling growth and the soil microbial community. In this study, we explored the effects of OPT fibre and the contrasting effects of fertiliser applications on the growth of oil palm seedlings. We experimented with various soil amendments: Unamended soil (T1), soil + OPT fibre (T2), soil + cellulose (T3), soil + fertiliser (T4), soil + OPT fibre + fertiliser (T5), and soil + cellulose + fertiliser (T6). Soil metagenomics analyses were conducted to explore the diversity and abundance of soil microflora. Thus, this study provides valuable insights into the sustainability and productivity of oil palm plantations by determining the effects of OPT biomass waste on the growth of oil palm seedlings and the soil microbial community.

MATERIALS AND METHODS

Polybag Assays

To establish the different treatments, dried OPT fibre, cellulose powder as cellulosic material, and fertiliser was added to the soil that was collected from an Malaysian Palm Oil Board (MPOB)-certified oil palm nursery in Alor Pongsu, Perak, Malaysia. Fresh OPT fibre (>20 years old) was air-dried under the sun until the moisture content reached <10% (w/w) to prevent any microbial growth (Lai et al., 2014). Fifty-five 4-month-old oil palm seedlings (*Elaeis guineensis*) were used for the polybag assay, which was conducted in an open space at an oil palm seedling distributor in Alor Pongsu (5°04′37.8″ N, 100°35′52.4″ E). Twin Arrow Fertiliser (TAF) (15:15:6:4/N:P:K:Mg+TE), a commercial fertiliser from TAF Sdn. Bhd., Selangor, Malaysia and cellulose fibre powder (Arbocel®, Germany) were used. The polybag assay was further modified according to our previous study (Uke et al., 2021). The four-month-old seedlings were transplanted into polybags (33 × 35 cm) containing 10 kg soil. The six treatments were prepared as shown in *Table 1*. In total, 55 polybags of oil palm seedlings were prepared, with 10 replicates for Treatment 1, 2, 4 and 5; seven replicates for Treatment 3; and eight replicates for Treatment 6. All polybags containing seedlings were arranged in rows with spacing of 0.75 × 0.75 × 0.75 m following the "Code of Good Nursery Practice for Oil Palm Nurseries" published by the MPOB (2016). A standardised average of at least seven replicates of oil palm seedlings per treatment was used to validate the results. The treatments were applied using a factorial and randomised design. The amount of OPT fibre added to each polybag (200 g) was determined using Equation (1). The OPT fibre was added to the soil and mixed thoroughly. This was based on an initial dry weight of OPT of approximately 1,315 kg. However, the amount was reduced by half to ensure that the OPT fibre could fit in each polybag. Seedlings were grown under a natural photoperiod and were watered daily. Fertiliser was added to certain treatments of oil palm seedlings (Treatment 4, 5 and 6) at the dose recommended by the manufacturer at the start of the experiment.

$$\begin{array}{c}\text{Dry weight}\\ \text{of OPT}\end{array} \times \begin{array}{c}\text{Number of oil palm}\\ \text{trees per hectare}\end{array} \times \begin{array}{c}\text{Area of}\\ \text{polybag}\end{array} \qquad (1)$$

Plant Measurements

The plant height, relative leaf area, relative chlorophyll content (as determined using a Soil

TABLE 1. TREATMENTS APPLIED TO OIL PALM SEEDLINGS OVER A FOUR-MONTH GROWTH PERIOD

Treatment label	Treatments	Amounts of treatments applied (g/polybag)
T1	Unamended soil (control)	No amendments
T2	Oil palm trunk (OPT) fibre	200 g
T3	Cellulose powder	200 g
T4	Fertiliser	7 g
T5	OPT fibre + Fertiliser	200 g of OPT fibre + 7 g fertiliser
T6	Cellulose + Fertiliser	200 g of cellulose + 7 g fertiliser

Plant Analysis Development [SPAD] meter), soil pH, and biomass weight were measured to determine the growth performance of oil palm seedlings in the various treatments (Uke et al., 2021). The plant height was measured from the ground level up to the top shoot (Adip et al., 2022). The relative leaf area was determined by multiplying the length and width of the fully extended leaves (length × width). The relative chlorophyll content was measured using a Minolta SPAD-520 Plus meter (Konica Minolta Inc., Japan). A soil pH and moisture tester (Model DM-15, Takemura Electric Works Ltd., Japan) was used to determine the soil pH. Measurements were taken at the start of the experiment and every month for four consecutive months. The biomass dry weight was determined at the end of the 4-month growth period by oven-drying the shoots and roots to a constant weight at 70°C.

Leaf Nutrient Analysis

The leaf samples were analysed by Applied Agricultural Resources Sdn. Bhd., Malaysia. The nitrogen, phosphorus, potassium, calcium, and magnesium contents of oil palm seedling leaves were determined. Kjeldahl's method was used to analyse the total nitrogen content and the acid digestion method was used to analyse the phosphorus content. The potassium content was determined using the ammonium acetate leaching method followed by atomic absorption spectrophotometry using a Perkin Elmer Analyst 100 Atomic Absorption Spectrometer (USA).

High-throughput Sequencing

DNA extraction. Soil from each treatment was used for DNA extraction. Before soil samples were collected, 1 to 2 cm of the top soil layer was removed. For each treatment, soil was collected through mixing. The samples were immediately stored at –20°C until DNA extraction was conducted. Total genomic DNA was extracted from 250 mg soil using the DNeasy PowerSoil Pro Kit (Qiagen, , Germany) according to the manufacturer's instructions. The DNA concentration was determined using a NanoDrop One Microvolume UV-VIS Spectrophotometer (Thermo Fisher Scientific, USA). A polymerase chain reaction (PCR) targeting the internal transcribed spacer 1 (ITS) region of the fungal rRNA gene was conducted using the primers ITS1F_KYO1 (CTHGGTCATTTAGAGGAASTAA) and ITS2_KYO2 (TTYRCTRCGTTCTTCATC) as well as Tks Gflex DNA Polymerase Low DNA (TaKaRa, Japan). The DNA amplification conditions were as follows: Initial denaturation at 94°C for 1 min; 35 cycles of 98°C for 10 s, 55°C for 15 s, and 68°C for 1 min; and final extension at 68°C for 5 min. The reaction products were separated on a 2% (w/v) agarose gel, stained with ethidium bromide, and purified (Ungkulpasvich et al., 2021). After diluting to 25 pM, all sample libraries were combined for an automated emulsion PCR and bead enrichment and then loaded onto 510™, 520™, and 530™ chips for sequencing using an Ion Chef™ instrument (Thermo Fisher Scientific). The libraries were sequenced using the Ion GeneStudio™ S5 System with 850 flow cycles.

Operational taxonomic unit clustering and community analyses. The raw sequences generated by the Ion Chef™ instrument were subjected to further filtering and trimming to obtain sequences >150 bp and <300 bp using CLC Genomics Workbench v.23.0.4 and CLC Microbial Genomics Module v.20.1 (Qiagen, USA). The UNITE database was used to identify the operational taxonomic units (OTUs) with 97% nucleotide identity (Uke et al., 2021). The software automatically identified and discarded chimeric sequences. The filtered read sets were then grouped into OTUs with 0.02 distance unit cut-offs according to the BLASTN algorithm in the National Center for Biotechnology Information GenBank database. The abundance and diversity of the fungi present in the soil were analysed.

Statistical analysis. Statistical analyses were conducted using SPSS (version 24.0) (IBM Inc., USA). Data were subjected to a one-way ANOVA, with means separated using the least significant difference test. Differences were considered significant at $P \leq 0.05$

RESULTS AND DISCUSSION

Effects of Adding Oil Palm Trunk (OPT) Fibre to Soil on Plant Growth Performance

The effects of adding OPT fibre and other substances to soil on plant growth performance (i.e., plant height, relative leaf area, chlorophyll content and biomass) were determined after four months of growth (*Figure 1*). At the end of the 4th month after application (MAA), the height of oil palm seedlings was significantly lower in the OPT fibre (T2) and cellulose treatments (T3) than in the unamended (T1) and fertiliser treatments (T4). Moreover, the growth indexes of seedlings in T2 and T3 were significantly different from those of seedlings in the T1, T4, OPT + fertiliser (T5) and cellulose + fertiliser (T6) treatments. This implied that fertiliser nutrient availability decreased in the presence of OPT fibre and cellulose. Interestingly, the comparison with the T1 revealed plant growth and development were significantly inhibited in the T2 and T3 treatments. Similarly, the relative leaf area and the chlorophyll content of the oil palm seedlings were significantly lower in T2 and T3 treatments than in the other treatments and T1 (*Figure 1b* and *1c*).

The small relative leaf area, homogenous yellowing of leaves, and stunted growth observed in T2 and T3 are symptoms of chlorosis. The lower height of the oil palm seedlings in these treatments, compared with that in T1, was associated with decreases in the chlorophyll content and leaf area. The chlorophyll content (Wen et al., 2019), total nitrogen content, soluble protein content in leaves, and the net photosynthetic rate are all affected by nitrogen availability (Qu et al., 2022). Plants under nitrogen deficiency stress have a reduced photosynthetic capacity (Qu et al., 2022), which eventually leads to poor growth and root development (Kang et al., 2023). Our results also show that the seedlings in T2 and T3 had less fibrous roots than T1 (*Figure 2*). It has been reported that the morphological development of oil palm roots (length, surface area, and volume) is restricted under nitrogen-deficient conditions (De la Peña et al., 2024).

The reduced plant biomass (both shoot and root) in T2 and T3 may be because of microbial nitrogen immobilisation (Uke et al., 2021). The carbon content affects the amount of nitrogen immobilised by the soil microbial community. Microbial biomass and respiration are relatively low under high C:N conditions because of the limited availability of nitrogen for microorganisms, leading to delayed degradation of plant fibre. Crop residues with high C:N ratios include OPT fibre (155) (Loh et al., 2013) and sugarcane bagasse (213) (Bhat et al., 2015). In another study, the application of sugarcane straw affected the growth and development of sugarcane plants, although the yield and quality of sugarcane juice were unaffected (Souza et al., 2020). Our results show that OPT fibre left in the soil eventually decreased the efficiency of soil microbe-mediated degradation of cellulosic biomass waste because of its high C:N ratio. Additionally, it takes approximately two years for OPT to decompose and the high C:N ratio of OPT is expected to decrease during the degradation period. This finding highlights the effects of OPT degradation in soil. Although this study was conducted using polybags, similar effects may be observed in oil palm plantations.

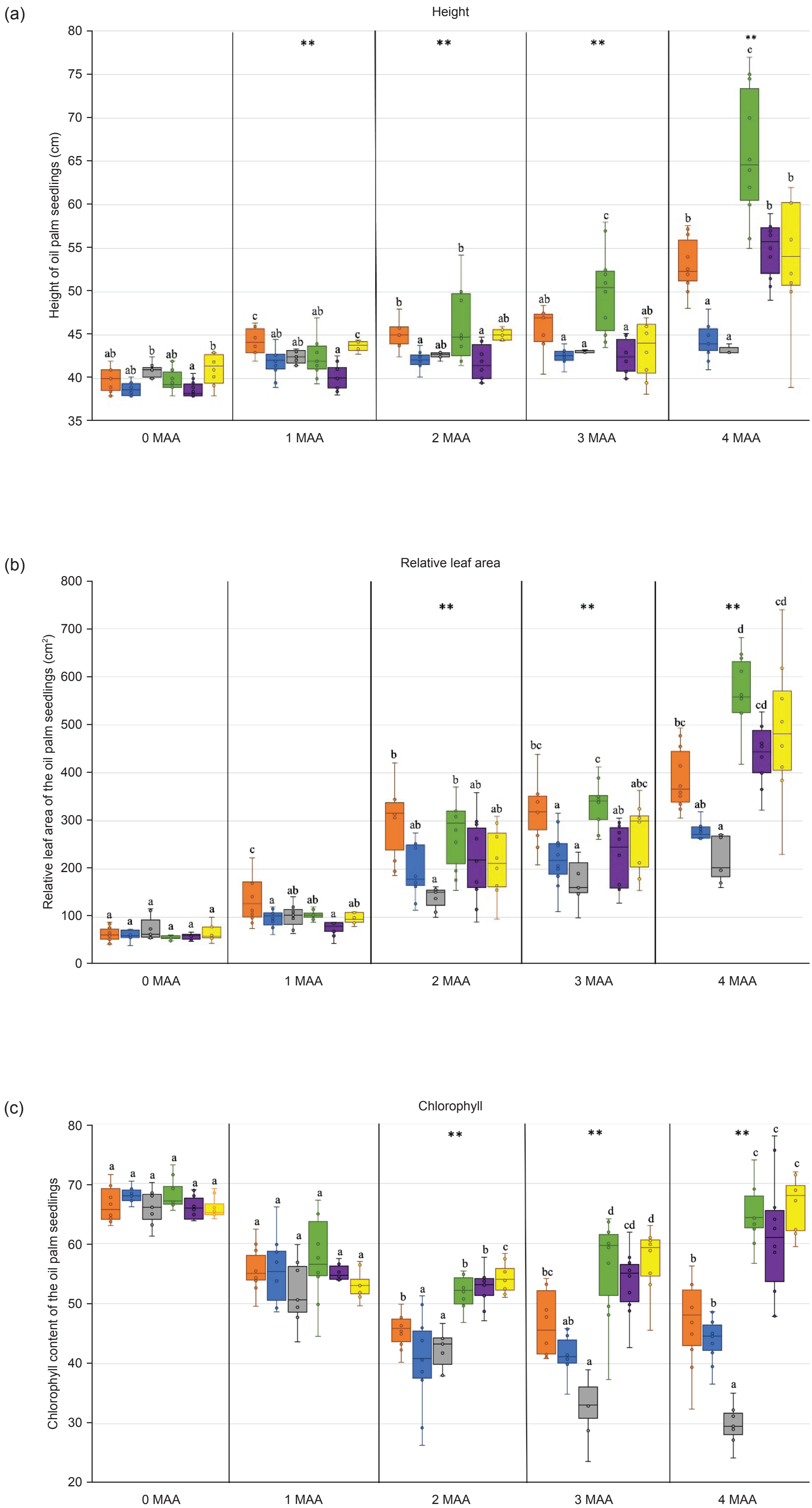
Effects of Various Treatments on Nutrient Contents in Oil Palm Seedling Leaves

The nitrogen content in oil palm seedling leaves was higher in T2 (2.19 ± 0.01) and T5 (3.41 ± 0.008) than in T1 (1.94 ± 0.01). The calcium content in oil palm seedling leaves was higher in T1 (0.50 ± 0.006) than T2 and T5 (0.42 ± 0.004 and 0.32 ± 0.002, respectively) and the magnesium content was also higher in T1 (0.46 ± 0.004) than in T2 and T5 (0.43 ± 0.005 and 0.38 ± 0.002, respectively) (*Table 2*). These results show that the addition of fertiliser to the soil amended with OPT fibre did not increase the availability of nutrients for new oil palm seedlings.

According to the results of the foliar analysis of oil palm seedlings, we speculated that the OPT fibre may have negatively affected leaf growth because of the low contents of certain nutrients, including nitrogen, magnesium, and calcium. This is supported by the overall plant growth parameters (i.e., height, chlorophyll content, relative leaf area, and shoot and root biomass) that were adversely affected. Similarly, a previous study showed the addition of wheat residue to soil (applied to the soil surface or mixed with the soil) delayed winter wheat seed emergence; the resulting seedlings were shorter than normal and exhibited an abnormal geotropic response, which may reflect the phytotoxic effect of the residue (Wuest et al., 2000). The application of winter wheat residue is similar to the application of OPT in oil palm plantations using the chip and windrow method and the pulverisation technique. Thus, to replenish nitrogen losses in soil, larger amounts of nitrogen fertiliser were required for the new plants to grow and achieve a satisfactory yield.

Effects of Various Treatments on the Soil Microbial Community

Plant growth is affected by soil fertility, which in turn is affected by the soil microbial population. The structure of soil microbial communities determines the quality of soil in oil palm plantations because microbes function as carbon recycling agents


(a)
Height
Height of oil palm seedlings (cm)
0 MAA
1 MAA
2 MAA
3 MAA
4 MAA
(b)
Relative leaf area
Relative leaf area of the oil palm seedlings (cm2)
0 MAA
1 MAA
2 MAA
3 MAA
4 MAA
(c)
Chlorophyll
Chlorophyll content of the oil palm seedlings
0 MAA
1 MAA
2 MAA
3 MAA
4 MAA

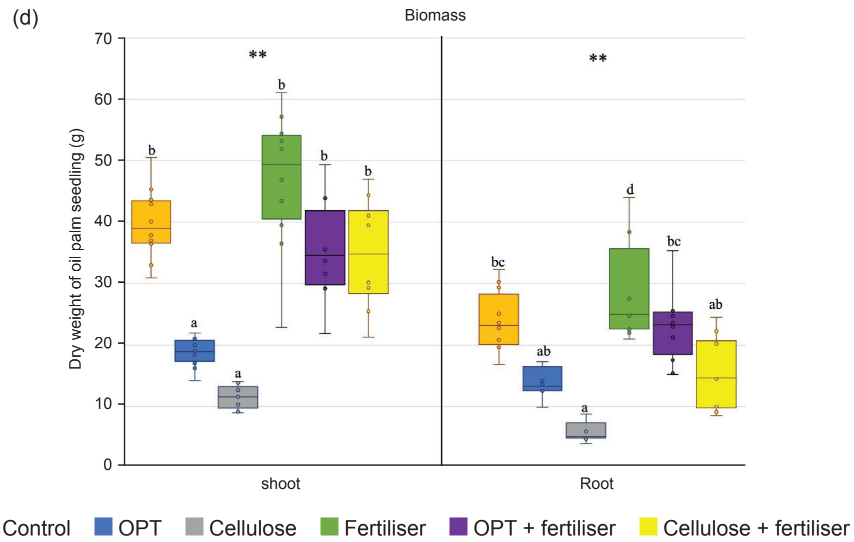


*Figure 1. Growth performance of oil palm seedlings in soil containing OPT fibre, cellulose, fertiliser, OPT fibre + fertiliser, and cellulose + fertiliser. (a) Height, (b) leaf area, (c) chlorophyll content, and (d) dry weight of the oil palm seedling shoot and root. Significant differences were observed among the treatments at 4 MAA (ANOVA; F = 22.8; P < 0.001 for height, ANOVA; F = 16.9; P < 0.001 for relative leaf area, ANOVA; F = 41.1; P < 0.001 for chlorophyll content, ANOVA; F = 16.8; P < 0.001 for shoot dry weight, ANOVA; F = 8.591; P < 0.001 for root dry weight). N = 10 for control, OPT fibre, fertiliser, and OPT fibre + fertiliser, N = 7 for cellulose only, and N = 8 for cellulose + fertiliser. Significant differences among treatments at specific time points are indicated with asterisks (** P < 0.001).*

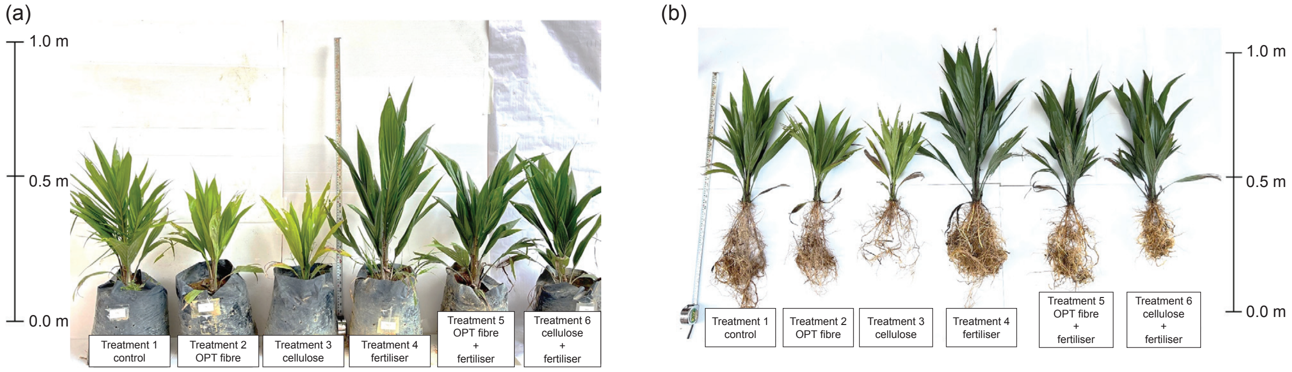


Figure 2. (a) Growth of oil palm seedlings in various treatments and (b) roots and above-ground parts of oil palm seedlings. Treatment 1: Unamended soil (control), Treatment 2: OPT fibre (200 g), Treatment 3: Cellulose (200 g), Treatment 4: Fertiliser (7 g), Treatment 5: OPT fibre (200 g) + fertiliser (7 g), and Treatment 6: Cellulose (200 g) + fertiliser (7 g).

TABLE 2. NUTRIENT CONTENTS IN OIL PALM LEAVES

Elements	Unamended soil (T1) (%)	OPT fibre (T2) (%)	OPT fibre + fertiliser (T5) (%)
Nitrogen (N)	1.942 ± 0.010^{e}	2.188 ± 0.010^{d}	3.410 ± 0.008^{c}
Phosphorus (P)	0.205 ± 0.001^{c}	0.227 ± 0.001^{b}	0.241 ± 0.0003^{a}
Potassium (K)	1.918 ± 0.008^{e}	2.398 ± 0.030^{ab}	2.294 ± 0.010^{c}
Calcium (Ca)	0.496 ± 0.006^{a}	0.424 ± 0.004^{b}	0.316 ± 0.002^{d}
Magnesium (Mg)	0.460 ± 0.004^{d}	0.432 ± 0.005^{d}	0.382 ± 0.002^{b}

Note: OPT - oil palm trunk.

and as a source of nutrients (Situmorang et al., 2016). Microbes that function as carbon recycling agents include bacteria and fungi. Bacteria are active decomposers in soil and are responsible for decomposing dead plants and animals. Fungi are the predominant microbes in the soil microbial community (in terms of biomass) and they metabolise carbon-rich substrates (Wu et al., 2024). Therefore, we analysed the eukaryotic microbial community structure in all the soil treatments (T1–T6). We focused on eukaryotic microbes because analyses of the ITS1 sequence data suggested that fungal OTUs showed the widest variation among treatments. The fungal genera *Chaetomium*, *Mortierella*, and *Staphylotrichum* were most abundant in T2 and T5 (*Table 3*).

TABLE 3. RELATIVE ABUNDANCE OF MICROBIAL SEQUENCES IN DIFFERENT SOIL AMENDMENT TREATMENTS

Abundance	T1.1	T1.2	T2	T3	T4	T5	T6
Chaetomium spp.	484	1, 222	6,050	111	109	23,095	2,465
Mortierella spp.	4,756	4,224	1,445	296	2,549	1,076	91
Staphylotrichum spp.	0	2	4,497	0	0	601	0

Note: T1.1 - unamended soil at 0 MAA; T1.2 - unamended soil at 4 MAA; T2 - oil palm trunk (OPT) only; T3 - cellulose only; T4 - fertiliser only; T5 - OPT + fertiliser; T6 - cellulose + fertiliser.

This observation implies the application of OPT fibre results in a unique soil microflora community composition.

Chaetomium spp. in the Chaetomiaceae family has been found in soils amended with compost (Zhang et al., 2017). Members of this genus contain genes encoding enzymes that degrade lignocellulose biomass (Banerjee et al., 2016). *Chaetomium* spp. thrive and reproduce in the presence of lignin (Dicko et al., 2020), which is one of the major components of OPT. Additionally, *Mortierella* spp. accumulate lipids that are produced from sugars that are used as a carbon source (Ruan et al., 2012). OPT (70% moisture) have a high sugar content, especially during its decomposition period (Hanis et al., 2024; Yamada et al., 2010). Therefore, it can be inferred that the high relative abundance of these genera in the soil microflora was because the decaying OPT served as their main carbon source. Despite the abundance of *Staphylotrichum* spp. in the microflora in soil amended with OPT, to date, few studies have explored their role as lignocellulose-degrading fungi. However, one of the species in this genus, *Staphylotrichum longicolleum*, can degrade chitin in wood, sugarcane bagasse, and maize leaves (Ali et al., 2021).

During the decomposition of OPT, the starch in parenchyma cells is degraded and further fermented into sugars by wild endophytic fungi within the trunk (Abdul-Hamid et al., 2015). The high starch and sugar contents (i.e., glucose, fructose, and sucrose) of OPT (Hanis et al., 2024; Yamada et al., 2010) serve as the energy source for the growth and metabolism of microorganisms. Additionally, OPT has a high C:N ratio (Loh et al., 2013) and contains a considerable amount of xylan and cellulose (Uke et al., 2021), which influence and promote the accumulation of lignocellulose-degrading fungi. Thus, the composition of the soil microbial community may be affected by OPT degradation. All of the events that occur in the presence of OPT (De Lima Brossi et al., 2016) increase the abundance and diversity of certain soil microflora; this may result in the decreased availability of nutrients for plants, which was previously reported for corn plants grown in soil amended with OPT residue (Uke et al., 2021). According to soil metagenomics data, *Chaetomium* spp., *Mortierella* spp., and *Staphylotrichum* spp. substantially affect nutrient dynamics. These microorganisms were highly effective at capturing and using available soil nutrients for their growth processes, which further decreased the availability of these nutrients for plants. The ability of these microorganisms to immobilise nutrients suggests that their presence and abundance may significantly influence soil nutrient profiles and, subsequently, plant productivity. These findings were results of previous studies, but they provide specific insights into the microbial species that predominantly affect nutrient availability in the current study.

CONCLUSION

Based on our results, we conclude that leaving the old OPT fibre residue in the plantation soil negatively affects the growth of oil palm seedlings because of soil nutrient deficiencies. Our results indicate that new oil palm seedlings were directly affected and grew relatively poorly (i.e., decreased height, leaf area, chlorophyll content and biomass) because of competition for nutrients with abundant lignocellulose-degrading fungi. In addition, fertiliser nutrient availability decreased in the presence of OPT. Thus, it is highly recommended that OPT be removed from oil palm plantations, even in small quantities, to decrease the amount of fertiliser required and to reduce fertiliser waste. The current practice of leaving OPT in the plantation requires a supplemental fertiliser application to compensate for the deficiency in available nutrients during the decomposition of OPT. To ensure a sustainable oil palm industry, the large amount of biomass in OPT should be used to its utmost potential for the production of value-added products (i.e., bioethanol, bio-pellets, and bioplastics).

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SOIL CHARACTERISTICS INFLUENCE THE DISTRIBUTION OF BORON FRACTIONS IN SOILS OF OIL PALM PLANTATIONS

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ABSTRACT

In oil palm growing countries like India, nutrient constraints were found to be the major factor limiting oil palm productivity. Despite the regular application of recommended Borax at 100 g palm^{-1} yr^{-1}, boron (B) was reported to be among the deficient nutrients observed in the oil palm plantations. There is a need to study the B fractions and their correlation with the soil characteristics to understand the fate and transformation of the applied fertiliser and their efficient management. The four types of soils (alfisol, entisol, inceptisol, and vertisol) at 0–60 cm depth were collected and analysed for textural class, pH, conductivity, organic carbon, calcium carbonate ($CaCO_3$), sesquioxides, nutrient content and B fractions. The predominant B fraction was residual B accounting for approximately 71.4%–99.3% of total B, whereas only less than 2.0% of total content accounted for plant available B fractions which included fractions that were soluble readily and adsorbed specifically in the soil surfaces. The oxide bound and organic bound fractions varied between 0.97 and 9.12 mg kg^{-1}, and 2.92 and 9.47 mg kg^{-1}, respectively. Soil characteristics like organic carbon content and soil reaction influenced the plant available B fractions positively. Readily soluble B exhibited a positive association with specifically adsorbed and organic bound, suggesting the role of both in replenishing the accessible soil B. The study shows that rather than B application, management strategies should be formulated to improve its availability from the total content.

Keywords: boron fractions, distribution, oil palm plantations, soil characteristics.

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INTRODUCTION

Oil palm is a highly nutrient demanding crop which requires adequate and balanced fertilisation for its growth and bunch yield. However, in oil palm growing countries like India, nutrient constraints were found to be the major factor limiting oil palm productivity. The micronutrient that is very much essential for the metabolic activities of plants is boron (B) (Aftab et al., 2022). The plants absorb 96% of B as boric acid molecules and the rest 4% as borate anions (Brdar-Jokanovic, 2020). Having greater mobility in the soil, this nutrient could be available to the plants easily but it shows widespread deficiencies in the soils next to zinc (Zn). About 132 crops from more than 80 countries were reported to be B deficient (Das et al., 2019), representing a significant impediment to crop production. The analytical results of 242,827 soils taken from agricultural regions of 615 districts across 28 Indian states showed B deficiency of 44.7% (Shukla et al., 2021). Rather than B as a neglected micronutrient, it has been overlooked in recent times, especially in oil palm growing countries like India. Despite the regular application of recommended Borax at 100 g palm^{-1} yr^{-1}, it was reported to be among the deficient nutrients found in the oil palm plantations. Studies (Behera et al., 2016a, 2016b, 2016c, 2017, 2019) on Diagnosis and Recommendation Integrated System (DRIS) indices found that soils of oil palm plantations in states like Goa, Gujarat, Andhra Pradesh, Karnataka and Mizoram of India had B as limiting nutrient. In Malaysia, under severe B deficient conditions, a yield loss of 83% was

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recorded in oil palm (Rajaratnam, 1973). Thus, B supply in sufficient levels is essential for obtaining higher yields in oil palm. It is very much essential to understand the fate and transformation of the applied borax for efficient B management.

The presence of B in the solid or solution phase depends on adsorption and desorption reactions in the soil (Premalatha et al., 2024). It has been reported that B gets adsorbed to clay minerals, organic matter, and iron (Fe) and aluminium (Al) oxides (Eynde et al., 2020). Most soil research solely concentrated on the B form that is dissolved in hot water, with sporadic attempts on B fractions. The experiments on B fractions were also carried out in soils lacking micronutrient management. This study on B fractions in different soil types of oil palm plantations managed with recommended B applications will provide basic information on B availability, and the factors affecting it. Knowledge on total B of soils is not adequate to know the chemical behaviour and its availability. The total B present in soils was quantified through various fractions like readily soluble fraction, specifically adsorbed fraction, organically bound fraction, oxide bound fraction, and residual fraction (Datta et al., 2002; Kasture et al., 2019). Readily soluble B is the B which remains in the solution phase or is adsorbed to the soil particles by weak force (Padbhushan & Kumar, 2017). Specifically adsorbed B includes the B which is specifically adsorbed to the organic matter or clay (Padbhushan & Kumar, 2015a). The readily soluble and specifically adsorbed B forms are available to the plants. B adsorbed to the oxides and hydroxides of Al and Fe forms oxide bound B, whereas B bound to different forms of organic matter are organically bound B (Kasture et al., 2020). Residual B depends on the primary and secondary minerals that make up the crystal structure (Datta et al., 2002).

Although these B forms coexist in soils in a state of dynamic equilibrium (Das et al., 2019), they differ in mobility and chemical behaviour (Javed et al., 2021). Depending on the soil type, the quantity of each B fraction differs significantly (Raza et al., 2002). The total B concentration in soils under Indian conditions varied from 7.0 to 630.0 mg kg^{-1} (Prasad et al., 2014), but only a small proportion (less than 12.2 mg kg^{-1}) of that is in a form that plants may use. The presence of B in the soil's liquid phase is greatly impacted by the adsorption reactions, which greatly impacts the fertilisation efficiency in the soil-plant system. The B in the liquid phase is available to the plants, whereas the B adsorbed onto the soil surfaces remains unavailable (Communar & Keren, 2006). Various factors like the amount of sesquioxides (R_2O_3), clay content, clay mineralogy, Calcium carbonate ($CaCO_3$) content, moisture, organic matter content and solution pH decide the degree of B availability and its adsorption in soils (Sidhu & Kumar, 2018; Padbhushan & Kumar, 2017). Soils with low organic matter, light texture, salinity and high content $CaCO_3$ express a high degree of B deficiency (Boparai & Machanda, 2017). This study aimed to understand the B fractions in the soils of different types (entisol, alfisol, inceptisol and vertisol) in oil palm plantations of India and to determine their association with the soil properties. We hypothesised that soil properties significantly influence the distribution of B fractions in soils of oil palm plantations. Therefore, data obtained on B distribution in varied soil types is crucial for efficient B management.

MATERIALS AND METHODS

Soil Sampling

In this study, soil samples were collected from oil palm plantations of the two Indian states: Andhra Pradesh and Gujarat. The location, geo coordinates, climate, average annual precipitation, age, soil texture and soil types of the abstracted samples are presented in *Table 1*. The soil samples were collected between November and December 2020. Oil palm is grown as an irrigated crop in these states in equilateral triangular planting with a spacing of 9 x 9 x 9 m. The recommended dose of fertilisers (1200:600:1200:500:100 g of $N:P_2O_5:K_2O:MgSO_4$:borax palm^{-1}) was applied in four equal splits annually and they were applied in the weeded palm basins of 3.0 m radius. Immediately after fertiliser application, the palm basins were irrigated. Since it is a shallow rooted crop and most roots are present within 60 cm soil depth, the soils were collected from 0–20, 20–40, and 40–60 cm depths. The samples were collected 1.0 m away from the palm trunk and within the weeded palm basins. From each sampling point, three sub samples were collected and mixed to get a representative soil sample. A total of 90 soil samples (30 each from 0–20, 20–40 and 40–60 cm) were collected from 30 sampling points in each soil type. Altogether 360 soil samples were collected from four soil types. The debris and roots present in the soil were removed, and the soils were processed as per the standard procedure given by the Food and Agriculture Organization of United Nations (2019). The samples, after processing, were kept in clean airtight containers for further analysis.

Soil Analysis

The method given by Piper (1966) was followed to determine the soil textural class. Soil pH and soluble salt content were measured using a buffer calibrated pH electrode (Model-361, Systronics) and conductivity bridge (Model-306, Systronics) in soil suspension (2.5:1.0 water to soil ratios) following 30 min equilibrium (Jackson, 1973). The organic carbon content of the soil was assessed through the chromic acid wet oxidation technique (Walkley & Black, 1934). Soil available nitrogen (N) was estimated in Kelplus Ultima Duo (Pelican Equipment, India) distillation system by following the alkaline $KMnO_4$ method given by Subbiah and Asija (1956). The available phosphorus (P) was estimated by spectrophotometry at 660 nm (UV 1900i, Shimadzu Corp., Japan) following extraction with Olsen's reagent (0.5 M $NaHCO_3$, pH at 8.5 and soil to extractant ratio of 1:10) (Olsen, 1954). Ammonium acetate (1.0 N, pH 7.0) extraction given by Hanway and Heidal (1952) was done for available potassium (K), exchangeable form of calcium (Ca) and magnesium (Mg) estimation with the soil to extractant ratio of 1:5. Available K was analysed through flame photometer (Model-128, Systronics), whereas the exchangeable Ca and Mg were estimated by Versenate titration method (Jackson, 1973). The micronutrients like copper (Cu), Fe, manganese (Mn) and Zn were extracted by extractant consisting of 0.005 M Diethylene Triamine Penta Acetic Acid (DTPA), 0.01M $CaCl_2$. $2H_2O$ and 0.1 M Triethanolamine as outlined by Lindsay and Norvell (1978). The pH of the extractant was adjusted to 7.3 and added to the soils in the ratio of 1:2 for micronutrient extraction. Following that, the extracted micronutrients were estimated by Atomic Absorption Spectrophotometer (Model-AA7000, Shimadzu) with the analytical wavelengths of 324.8, 248.3, 279.5 and 213.9 nm for Cu, Fe, Mn and Zn, respectively. Iron (Fe_2O_3) and aluminium (Al_2O_3) oxides were estimated by the process outlined by Piper (1966). The $CaCO_3$ content was assessed by the titrimetric procedure given by Rowell (1994). The B fractions were extracted sequentially and determined by the colorimetric method using UV 1900i described by Datta et al. (2002). The readily soluble B and specifically adsorbed B were extracted by 0.01 M $CaCl_2$ and 0.05 M KH_2PO_4, respectively and estimated by the Azomethine-H method at 420 nm wavelength (Gupta, 1967). The oxide bound B (0.175 M $[NH_4]_2C_2O_4$ [pH 3.25] extractable), organically bound B (0.5 M NaOH extractable) and residual B (extracted by a mixture of H_2SO_4, HF, $HClO_4$) were estimated by carmine method at the wavelength of 585 nm outlined by Bingham (1982). The summation of these five B fractions contributes to the soil's total B content.

Statistical Analysis

Descriptive analysis like minimum, maximum, standard deviation, and mean were computed for all soil characteristics (*Table 2*). Version 16 of the SPSS programme was utilised to perform all

TABLE 1. DETAILS OF EXPERIMENTAL SOIL SAMPLES

Location	Latitude	Longitude	Climate	Average annual rainfall (mm)	Plantation age (yr)	Soil texture	Taxonomical class[a]
Polavaram mandal, Andhra Pradesh	17°11′18″ to 17°14′27″ N	81°31′1″ to 81°37′18″ E	Hot and humid tropical climate	1,687	5–15	Sandy clay loam to sandy loam	Alfisol
IIOPR research farm, Andhra Pradesh	16°48′29″ to 16°48′57″ N	81°7′37″ to 81°8′20″ E		1,698	7–11	Sandy loam	Entisol
Pedapadu mandal, Andhra Pradesh	16°40′9″ to 16°43′43″ N	80°59′21″ to 81°2′33″ E		1,597	5–15	Sandy clay loam to sandy loam	Inceptisol
Surat, Gujarat	20°51′81″ to 21°22′54″ N	72°48′72″ to 73°34′48″ E	Tropical	1,846	2–7	Clayey to clay loam	Vertisol

Note: [a] - Soil taxonomy (Soil survey staff, 2014).

statistical computations. The significance of the data was examined by one way ANOVA method. To document the differences in B fractions among soil types, the Duncan test with a 5.0% probability was employed (Duncan, 1955). The association among B fractions and soil characteristics was computed using Pearson's correlation analysis.

RESULTS AND DISCUSSION

The soils showed considerable variability, as seen by the wide range of values for pH, EC, organic carbon, $CaCO_3$, Al_2O_3, Fe_2O_3, clay, silt, sand, major and micronutrients (*Table 2*). The variations in the contents of different B fractions were observed in different soil types considered in the study (*Figure 1*). The readily soluble B is the B available in the soil's liquid phase and it accounts for 0.08%–1.86% of total B (*Table 3*). Similar findings were observed by Padbhushan and Kumar (2015a; 2015b) who studied the B fractions in Ca rich soils of Punjab. In all soil types, increasing soil depth resulted in a drop in readily soluble B concentration, which may be possible because of changes in organic carbon contents and pH. Higher soil organic matter in 0–20 cm soil depths of all experimental soils resulted in the accumulation of B rather than leaching (Chaudhary & Shukla, 2004). The average pH of the soils decreased with higher soil depths. The readily soluble B exhibited a strong positive association with the soil pH (alfisol, entisol, inceptisol) and organic carbon (entisol, inceptisol) (*Table 4* and *Figure 2*). An increase in soil pH increases the surface negative charges and thereby increases the availability of soluble B (Datta et al., 2002). A comparatively higher proportion of readily soluble B in entisol might be ascribed to greater organic carbon. In soil depths of 0–20 and 20–40 cm, lower contents of readily soluble B in alfisol were due to higher Al_2O_3 and Fe_2O_3 content which exhibited a negative relationship. Though the average organic carbon was higher in vertisol, the lower readily soluble B in soil layers of 20–40 and 40–60 cm was due to the presence of clay fractions in large quantities (52.3%–58.4%) (*Table 2*). This fraction had a significant negative relationship with clay content in vertisol. A positive correlation between readily soluble B and major nutrients like available N (entisol, inceptisol), P (entisol, vertisol), K (entisol) and micronutrients like Cu and Zn (entisol, inceptisol, vertisol) was also observed. Considering the relationship between B fractions, readily soluble B was positively correlated with specifically adsorbed B (entisol and inceptisol) and organically bound B (alfisol, inceptisol and vertisol) (*Table 5*). This supports the conclusions of Gurel et al. (2019), who studied soil B fractions and their accessibility to olive trees.

TABLE 2. SOIL PROPERTIES IN EXPERIMENTAL SOIL

Parameters	Alfisol												Entisol											
	Minimum			Maximum			Mean			Standard deviation			Minimum			Maximum			Mean			Standard deviation		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
pH	6.28	6.15	6.13	7.92	7.70	7.52	7.43	7.34	7.19	0.46	0.45	0.39	6.62	6.56	5.96	7.89	7.57	7.24	7.39	7.19	6.91	0.35	0.28	0.37
EC (dS m^{-1})	0.14	0.13	0.18	0.60	0.65	0.62	0.32	0.37	0.36	0.15	0.17	0.13	0.15	0.08	0.09	0.41	0.43	0.48	0.21	0.18	0.19	0.08	0.10	0.11
Organic carbon (%)	0.33	0.19	0.12	0.82	0.62	0.51	0.55	0.37	0.29	0.12	0.15	0.12	0.47	0.31	0.20	0.98	0.82	0.52	0.68	0.53	0.36	0.15	0.17	0.10
$CaCO_3$ (%)	0.00	0.00	0.00	0.00	0.25	1.48	0.00	0.05	0.15	0.00	0.11	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R_2O_3 (%)	8.65	10.80	11.40	15.20	20.80	23.70	11.80	14.10	17.30	2.31	2.94	3.68	9.60	4.00	4.11	16.50	6.70	8.26	11.96	5.47	6.12	1.89	0.90	1.28
Al_2O_3 (%)	5.55	3.36	7.56	8.90	17.60	14.40	7.42	8.44	10.60	1.23	4.10	2.10	4.29	1.52	1.73	10.60	3.84	5.54	6.18	3.01	3.40	1.83	0.80	1.14
Fe_2O_3 (%)	3.00	3.10	3.24	6.30	9.80	11.10	4.41	5.64	6.63	1.12	2.22	2.56	5.22	1.92	2.10	8.43	2.86	3.20	5.89	2.46	2.72	1.03	0.33	0.34
Clay (%)	21.80	20.00	17.00	31.60	28.00	24.00	28.10	25.10	19.50	2.90	2.79	2.62	11.30	11.80	10.20	16.30	18.00	13.50	13.60	14.60	12.20	1.54	1.73	1.00
Silt (%)	13.00	11.00	14.00	16.00	15.00	16.20	14.40	12.40	15.30	0.99	1.15	0.71	17.50	19.00	17.00	25.00	28.00	21.00	20.90	23.40	18.20	2.33	2.91	1.41
Sand (%)	60.40	58.00	54.10	69.50	68.20	62.80	66.00	62.20	56.50	2.87	3.43	2.82	59.10	55.80	66.50	69.20	66.90	71.70	65.50	62.00	69.60	2.87	3.50	1.68
N (mg kg^{-1})	140.00	112.00	112.00	260.00	182.00	154.00	172.00	147.00	128.00	39.80	24.10	16.80	98.10	84.10	56.00	252.00	182.00	168.00	159.00	130.00	109.00	44.50	31.00	31.50
P (mg kg^{-1})	11.40	10.00	10.00	57.10	25.70	18.10	21.40	15.50	12.40	13.00	4.28	2.43	9.15	7.59	7.59	50.00	34.50	29.10	23.10	18.30	16.00	11.40	8.67	7.10

TABLE 2. SOIL PROPERTIES IN EXPERIMENTAL SOIL (continued)

Parameters	Alfisol												Entisol											
	Minimum			Maximum			Mean			Standard deviation			Minimum			Maximum			Mean			Standard deviation		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
K (mg kg^{-1})	77.70	76.30	74.50	238.00	210.00	164.00	150.00	131.00	114.00	62.30	49.50	39.20	84.40	80.80	48.20	224.00	143.00	104.00	117.00	102.00	89.20	35.80	17.40	16.20
Ca (meq 100 g^{-1})	2.50	2.40	2.40	6.10	6.50	6.50	4.14	4.26	4.40	1.29	1.43	1.42	2.00	2.00	2.40	4.00	3.56	4.00	3.23	2.92	3.20	0.63	0.59	0.55
Mg (meq 100 g^{-1})	1.40	1.10	1.40	4.10	4.20	4.30	2.50	2.53	2.60	1.00	1.06	0.97	1.20	1.20	0.30	2.17	2.27	2.40	1.74	1.77	1.58	0.39	0.43	0.68
Fe (mg kg^{-1})	5.44	4.84	4.05	13.44	8.85	6.70	9.42	7.00	5.17	2.10	1.34	0.92	3.62	3.46	3.48	6.69	5.69	5.71	4.91	4.23	4.45	0.93	0.71	0.76
Mn (mg kg^{-1})	3.10	2.04	2.05	4.26	3.16	3.06	3.67	2.81	2.17	0.58	0.51	0.31	2.77	2.59	2.46	7.79	6.62	6.61	5.78	4.84	4.17	1.80	1.36	1.31
Zn (mg kg^{-1})	0.93	0.64	0.63	1.45	1.05	0.88	1.11	0.80	0.72	0.16	0.12	0.07	0.80	0.83	0.80	1.30	1.00	0.94	1.06	0.91	0.87	0.15	0.06	0.05
Cu (mg kg^{-1})	0.40	0.45	0.31	0.61	0.63	0.70	0.49	0.54	0.47	0.08	0.06	0.11	0.09	0.06	0.06	0.18	0.10	0.09	0.12	0.08	0.08	0.03	0.01	0.01

Parameters	Vertisol												Inceptisol											
	Minimum			Maximum			Mean			Standard deviation			Minimum			Maximum			Mean			Standard deviation		
	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III	I	II	III
pH	7.31	7.26	7.20	8.28	8.04	7.84	7.66	7.50	7.39	0.34	0.26	0.21	7.40	7.33	7.22	8.24	8.17	7.98	7.90	7.78	7.63	0.28	0.27	0.22
EC (dS m^{-1})	0.45	0.21	0.32	1.16	1.02	1.11	0.73	0.60	0.55	0.28	0.26	0.23	0.24	0.19	0.30	0.70	0.71	0.70	0.44	0.45	0.47	0.16	0.18	0.16
Organic carbon (%)	0.45	0.31	0.27	1.01	0.82	0.51	0.59	0.46	0.36	0.17	0.14	0.08	0.27	0.16	0.12	0.70	0.47	0.31	0.49	0.32	0.23	0.13	0.10	0.06
$CaCO_3$ (%)	0.13	0.32	0.25	3.00	4.10	4.88	1.71	1.89	1.97	1.01	1.11	1.30	0.00	0.00	0.00	0.00	0.50	1.30	0.00	0.10	0.30	0.00	0.17	0.50
R_2O_3 (%)	5.35	5.10	5.20	8.60	8.93	8.76	6.47	7.17	7.48	0.88	1.16	1.03	2.58	2.50	2.72	9.30	7.94	7.10	6.32	5.74	5.05	2.38	1.97	1.51
Al_2O_3 (%)	2.24	2.20	1.30	4.45	4.40	5.00	3.21	3.53	3.76	0.79	0.83	1.09	0.71	0.33	0.21	6.38	3.72	3.67	2.64	2.16	1.73	1.80	1.25	1.15
Fe_2O_3 (%)	2.40	2.10	2.30	4.15	4.69	4.92	3.25	3.64	3.72	0.61	0.80	0.76	3.43	3.00	2.86	5.92	4.83	4.37	4.45	3.94	3.53	0.82	0.63	0.56
Clay (%)	32.00	48.50	54.70	44.50	57.00	62.50	37.90	52.30	58.40	3.64	2.79	2.94	14.50	20.50	12.00	19.00	29.00	15.00	16.80	24.40	13.30	1.62	2.97	1.00
Silt (%)	27.00	20.00	15.00	38.50	27.00	25.00	34.40	23.20	19.90	3.35	2.14	3.24	24.50	20.00	30.50	31.00	24.00	35.00	28.40	21.80	32.60	2.20	1.58	1.95
Sand (%)	25.40	20.30	17.50	29.50	30.30	27.40	27.70	24.50	21.80	1.62	3.70	2.96	50.20	48.00	52.30	59.50	59.50	56.70	54.80	53.80	54.10	3.14	3.66	1.55
N (mg kg^{-1})	84.00	84.00	58.90	180.80	168.00	140.10	130.10	116.20	96.70	28.60	26.50	22.90	84.00	70.00	56.00	196.10	154.10	126.10	138.70	112.00	88.20	37.10	32.4	22.00
P (mg kg^{-1})	9.11	5.98	7.72	30.60	25.00	19.80	20.80	15.50	13.40	8.53	5.70	3.80	14.00	12.80	11.00	31.30	26.50	26.70	22.20	18.10	14.80	5.83	4.53	4.90
K (mg kg^{-1})	65.90	60.60	61.80	304.80	185.70	152.20	169.40	129.80	101.90	80.90	44.00	28.00	74.10	77.00	48.20	204.30	184.80	182.10	137.50	120.00	111.30	47.60	35.90	39.90
Ca (meq 100 g^{-1})	8.30	6.50	6.50	12.50	12.20	11.00	10.40	8.80	8.40	1.30	2.19	1.53	2.10	2.00	2.20	6.50	6.30	6.60	4.32	4.12	4.33	1.70	1.65	1.68
Mg (meq 100 g^{-1})	6.00	4.70	5.20	10.40	9.30	8.8.	7.60	6.30	6.40	1.35	1.83	1.09	1.00	1.00	1.10	4.00	4.30	4.10	2.45	2.43	2.52	1.15	1.22	1.17
Fe (mg kg^{-1})	4.17	3.46	3.51	7.74	6.41	5.52	5.33	4.88	4.58	1.10	0.85	0.56	3.14	2.54	2.53	5.06	4.50	4.46	4.08	3.69	3.63	0.72	0.76	0.74
Mn (mg kg^{-1})	3.10	2.07	2.05	6.11	4.08	5.06	3.87	3.28	3.12	1.09	0.65	0.89	2.08	2.19	2.05	5.10	4.13	4.04	3.36	3.02	2.87	0.93	0.53	0.52
Zn (mg kg^{-1})	0.94	0.85	0.81	1.14	1.00	0.91	1.04	0.93	0.87	0.06	0.04	0.03	0.90	0.61	0.59	1.06	0.82	0.77	0.99	0.73	0.68	0.06	0.06	0.05
Cu (mg kg^{-1})	0.74	0.58	0.41	0.91	0.83	0.61	0.83	0.66	0.49	0.06	0.08	0.06	0.14	0.17	0.16	0.40	0.30	0.24	0.30	0.24	0.20	0.09	0.04	0.03

Note: EC - electrical conductivity; $CaCO_3$ - calcium carbonate; R_2O_3 - sesquioxides; Al_2O_3 - aluminium oxide; Fe_2O_3 - iron oxide; N - nitrogen; P - phosphorus; K - potassium; Ca - calcium; Mg - magnesium; Fe - iron; Mn - manganese; Zn - zinc; Cu - copper.

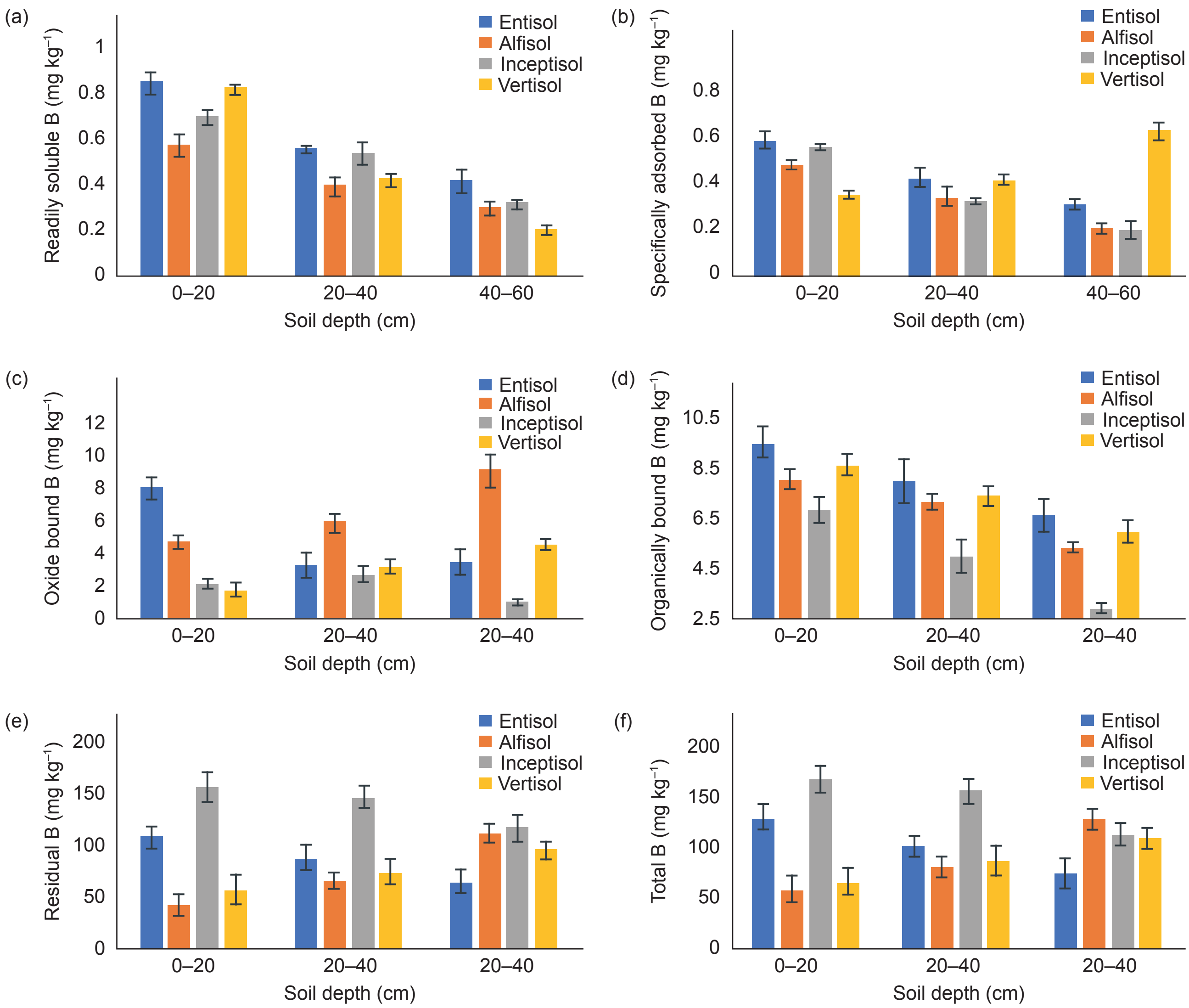

Figure 1. Depth wise distribution of (a) readily soluble B, (b) specifically adsorbed B, (c) oxide bound B, (d) organically bound B, (e) residual B and (f) total B in different soil types.

TABLE 3. RELATIVE DISTRIBUTION OF BORON (B) FRACTIONS IN DIFFERENT SOIL TYPES

Soil type	B fractions as a percentage of total B (%)										Average total B (mg kg^{-1})
	Readily soluble B		Specifically adsorbed B		Oxide bound B		Organically bound B		Residual B		
	Min	Max	Min	Max	Min	Max	Min	Max	Min	Max	
0–20 cm											
Alfisol	0.49	1.76	0.62	1.94	5.13	13.09	8.05	17.00	71.40	85.66	57.5 ± 13.7
Entisol	0.23	0.66	0.17	0.51	4.22	8.79	3.40	8.55	82.19	91.38	127.9 ± 12.6
Inceptisol	0.20	0.97	0.19	0.75	0.43	2.15	3.01	7.55	89.04	96.15	173.9 ± 13.6
Vertisol	0.28	1.86	0.24	0.83	2.08	3.60	7.16	18.16	77.12	89.20	65.8 ± 13.5
20–40 cm											
Alfisol	0.18	1.28	0.22	0.65	3.35	12.15	3.91	18.01	77.64	88.43	80.2 ± 10.1
Entisol	0.47	1.33	0.22	0.97	3.56	5.83	7.35	12.75	80.25	88.11	101.6 ± 10.5
Inceptisol	0.14	0.69	0.09	0.44	1.01	4.34	0.83	7.18	89.34	96.89	156.3 ± 12.9
Vertisol	0.10	0.98	0.13	0.96	2.62	5.10	3.09	15.77	80.14	93.66	86.6 ± 15.1
40–60 cm											
Alfisol	0.08	0.40	0.41	1.20	4.67	10.57	2.20	6.66	82.09	91.94	128.3 ± 10.1
Entisol	0.30	0.80	0.14	0.82	2.19	6.48	3.94	13.63	80.16	91.32	75.0 ± 15.2
Inceptisol	0.11	0.65	0.07	0.43	0.13	2.68	0.31	6.77	90.93	99.30	113.1 ± 10.6
Vertisol	0.08	0.33	0.22	1.23	2.02	6.69	3.30	12.45	83.98	91.16	109.8 ± 10.4

Note: Min - minimum; Max - maximum.

TABLE 4. CORRELATION AMONG SOIL CHARACTERISTICS AND BORON (B) FRACTIONS

	pH	EC	Organic carbon	$CaCO_3$	R_2O_3	Al_2O_3	Fe_2O_3	Clay	Silt	Sand	N	P	K	Ca	Mg	Fe	Mn	Zn	Cu
Alfisol																			
Readily soluble B	0.385*	-0.033	0.180	-0.205	-0.470**	-0.353	-0.312	0.353	0.152	-0.436*	0.197	0.037	0.188	-0.318	-0.340	-0.073	0.131	0.027	0.035
Specifically adsorbed B	0.038	0.131	0.478**	-0.153	-0.246	-0.337	-0.201	0.584**	0.111	-0.504*	-0.309	0.437**	0.250	0.008	0.044	-0.009	-0.059	-0.256	0.155
Oxide bound B	-0.238	0.320	-0.324	0.015	0.407*	0.129	0.510**	0.219	0.287	-0.318	-0.372*	-0.258	-0.432*	-0.064	-0.222	0.100	0.414*	-0.307	-0.365*
Organically bound B	0.273	-0.139	0.253	-0.038	-0.461*	-0.264	-0.418*	0.342	0.219	0.399**	0.406*	0.160	0.279	0.084	0.031	0.290	0.315	0.186	0.090
Residual B	-0.263	0.200	-0.491**	-0.083	0.367*	0.265	0.258	0.250	0.303	-0.267	0.376*	-0.190	-0.275	0.075	-0.077	-0.356	-0.047	-0.170	-0.039
Total B	-0.254	0.208	-0.481*	-0.078	0.358	0.251	0.261	0.243	0.305	0.317	-0.366*	-0.194	-0.279	0.071	-0.089	-0.170	-0.235	-0.057	-0.067
Entisol																			
Readily soluble B	0.411*	-0.110	0.542**	-	-0.142	-0.090	-0.159	0.379*	0.342	-0.413*	0.407*	0.617**	0.616**	-0.154	-0.006	0.132	0.257	0.739**	0.542**
Specifically adsorbed B	0.663**	-0.142	0.303	-	0.008	-0.001	0.012	0.323	0.298	-0.357	0.516**	0.448**	0.462**	0.129	0.174	0.138	0.298	0.601**	0.387*
Oxide bound B	0.105	0.039	0.194	-	0.842**	0.646**	0.849**	0.443*	0.601**	-0.633**	-0.021	0.035	0.144	-0.290	-0.047	-0.125	0.123	0.033	-0.011
Organically bound B	0.103	0.190	0.675**	-	0.042	0.099	-0.017	0.038	0.112	-0.100	0.159	-0.091	0.284	0.025	-0.034	0.079	0.202	0.338	0.500**
Residual B	0.114	-0.168	0.196	-	0.588**	0.451**	0.594**	0.248	0.580**	-0.537**	-0.003	0.223	0.092	-0.482**	-0.014	0.103	0.189	0.293	0.136
Total B	0.128	0.142	0.245	-	0.612**	0.474**	0.614**	0.273	0.596**	-0.559**	0.013	0.211	0.123	-0.469**	-0.018	0.092	0.203	0.304	0.163
Vertisol																			
Readily soluble B	0.147	0.187	0.161	-0.074	-0.579**	-0.331	-0.445*	-0.612**	0.612**	0.380*	0.294	0.701**	0.183	0.218	-0.050	0.005	0.190	0.505**	0.683**
Specifically adsorbed B	-0.111	-0.232	-0.168	-0.097	-0.140	0.039	-0.254	0.488**	-0.481**	-0.315	-0.435*	0.185	-0.330	0.285	0.127	-0.239	-0.071	-0.186	-0.235
Oxide bound B	0.088	0.176	-0.299	0.709**	-0.018	0.073	-0.116	0.721**	-0.610**	-0.654**	0.040	-0.228	-0.007	-0.065	-0.194	-0.265	-0.232	-0.032	-0.314
Organically bound B	0.211	0.137	0.513**	-0.196	-0.217	-0.251	-0.009	-0.708**	0.637**	0.571**	0.350	0.044	0.265	0.294	0.161	0.245	0.192	0.598**	0.656**
Residual B	0.151	0.160	-0.217	0.807**	0.126	0.204	-0.067	0.651**	-0.535**	-0.620**	0.128	-0.276	0.161	0.072	-0.002	-0.329	-0.202	-0.459*	-0.198
Total B	0.160	0.172	-0.209	0.816**	0.104	0.188	-0.079	0.642**	-0.526**	-0.615**	0.141	-0.270	0.166	0.077	-0.011	-0.330	-0.199	-0.449*	-0.184
Inceptisol																			
Readily soluble B	0.606**	-0.382*	0.738**	-0.321	0.248	0.216	0.203	0.280	-0.253	-0.069	0.469**	0.168	0.070	-0.299	-0.292	0.261	-0.145	0.494**	0.475**
Specifically adsorbed B	0.459*	0.108	0.429*	-0.228	0.281	0.149	0.347	0.095	-0.227	0.217	0.084	0.316	0.362*	0.334	0.230	0.208	0.199	0.607**	0.450*
Oxide bound B	0.212	-0.087	0.201	-0.027	-0.003	0.043	0.068	0.797**	-0.716**	-0.202	0.190	0.394*	0.075	-0.239	-0.193	-0.008	-0.014	0.051	-0.069
Organically bound B	0.066	-0.247	0.493**	-0.228	0.347	0.302	0.238	0.214	-0.179	-0.078	0.652**	0.208	0.198	-0.329	-0.264	0.212	0.276	0.507**	0.249
Residual B	-0.196	0.168	-0.068	-0.302	0.207	-0.049	0.330	0.160	-0.246	0.135	0.066	0.031	-0.033	0.306	0.270	0.165	0.341	0.309	0.168
Total B	-0.180	0.150	-0.034	-0.311	0.223	-0.027	0.340	0.189	-0.271	0.125	0.103	0.053	-0.020	0.277	0.144	0.174	0.344	0.334	0.180

Note: *- $p < 0.05$; **- $p < 0.01$; EC - electrical conductivity; $CaCO_3$ - calcium carbonate; R_2O_3 - sesquioxides; Al_2O_3 - aluminium oxide; Fe_2O_3 - iron oxide; N - nitrogen; P - phosphorus; K - potassium; Ca - calcium; Mg - magnesium; Fe - iron; Mn - manganese; Zn - zinc; Cu - copper..

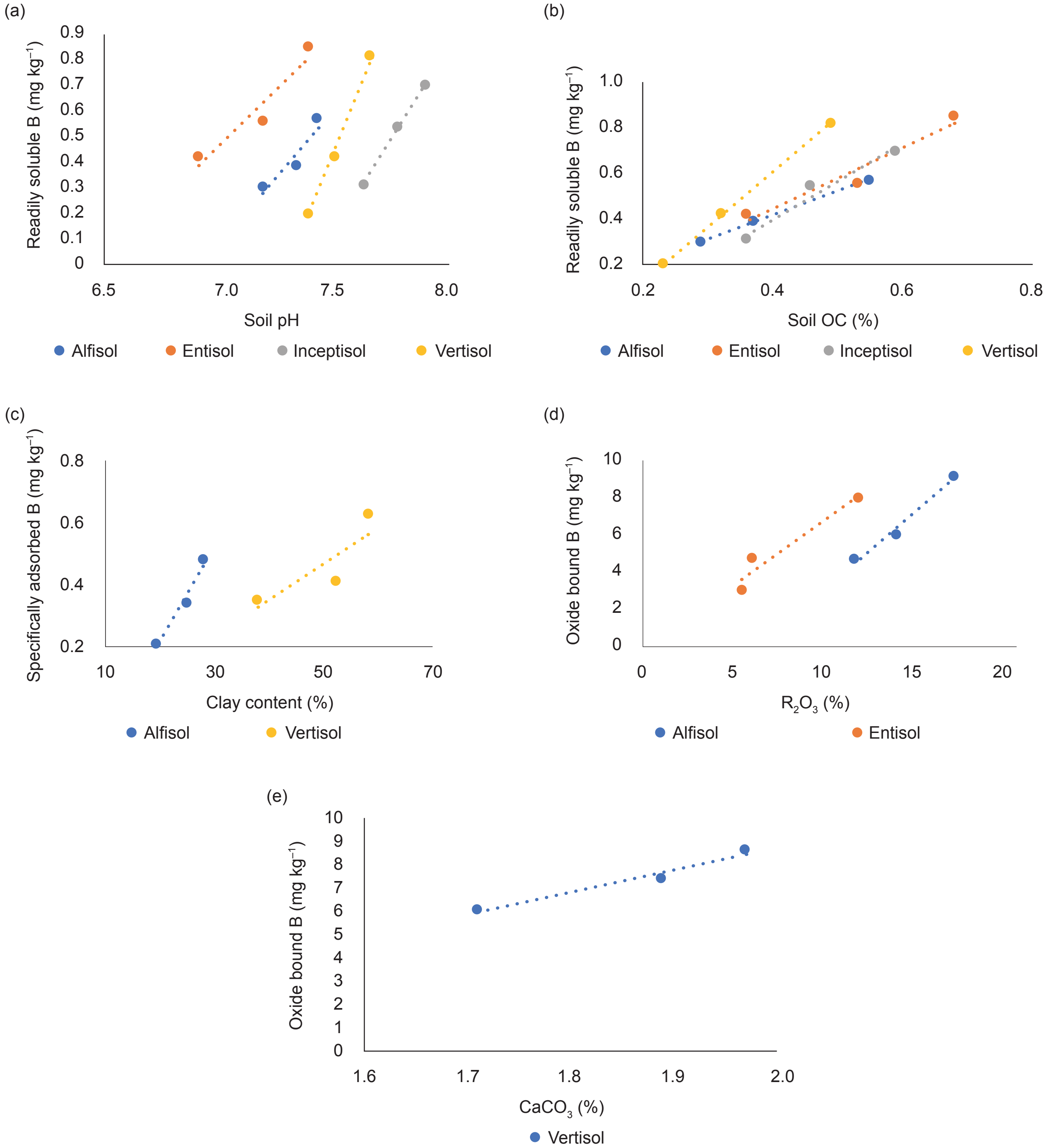

Figure 2. Effect of (a) soil pH, (b) soil organic carbon (OC), (c) clay content, (d) R_2O_3, and (e) $CaCO_3$ on various B fractions.

The specifically adsorbed B constitutes 0.07%–1.94% of total B (*Table 3*). Comparable amounts of these pool were reported by Padbhushan and Kumar (2015a). Like readily soluble B, the specifically adsorbed B content also dropped as soil depth increased in all soil types except vertisol. Decline in absorbing surfaces with soil depth could be the cause for such a reduction. The presence of organic matter in higher quantities accounts for relatively higher quantities of specifically adsorbed B in entisol. The role of organic matter as B adsorbing surface was reported by Padbhushan and Kumar (2017). Higher clay content in vertisol is attributed to the higher contents of specifically adsorbed B at 40–60 cm (*Table 2*). Diana and Beni (2006) reported the dominating role of clay on specifically adsorbed B. The specifically adsorbed B had a positive relation with clay content (in alfisol, and vertisol), pH (in entisol, and inceptisol), organic carbon (in alfisol, and inceptisol), available N (in entisol), P (in alfisol, and entisol), and K and micronutrients like Cu and Zn (in entisol, and

TABLE 5. CORRELATION AMONG THE BORON (B) FRACTIONS STUDIED IN SOILS

Item	RDS B	SAD B	OXD B	ORG B	RSL B	TOT B
Alfisol						
Readily soluble B (RDS B)	1					
Specifically adsorbed B (SAD B)	0.340	1				
Oxide bound B (OXD B)	-0.223	0.306	1			
Organically bound B (ORG B)	0.440*	0.407*	-0.349	1		
Residual B (RSL B)	-0.210	0.340	0.781**	-0.352	1	
Total B (TOT B)	-0.189	0.337	0.802**	-0.307	0.998**	1
Entisol						
Readily soluble B (RDS B)	1					
Specifically adsorbed B (SAD B)	0.718**	1				
Oxide bound B (OXD B)	-0.047	0.136	1			
Organically bound B (ORG B)	0.303	0.156	-0.301	1		
Residual B (RSL B)	-0.229	0.198	0.696*	0.207	1	
Total B (TOT B)	-0.247	0.216	0.744**	0.282	0.995*	1
Vertisol						
Readily soluble B (RDS B)	1					
Specifically adsorbed B (SAD B)	0.358	1				
Oxide bound B (OXD B)	-0.153	0.242	1			
Organically bound B (ORG B)	0.454*	0.299	-0.504**	1		
Residual B (RSL B)	-0.013	0.220	0.872**	-0.448*	1	
Total B (TOT B)	0.029	0.210	0.882**	-0.428*	0.999**	1
Inceptisol						
Readily soluble B (RDS B)	1					
Specifically adsorbed B (SAD B)	0.418*	1				
Oxide bound B (OXD B)	0.267	0.068	1			
Organically bound B (ORG B)	0.461*	0.177	0.251	1		
Residual B (RSL B)	-0.057	0.280	0.048	0.245	1	
Total B (TOT B)	-0.022	0.292	0.086	0.297	0.998**	1

inceptisol) (*Table 4* and *Figure 2*). Supporting this, Gurel et al. (2019) established a significant positive association between specifically adsorbed B and soil parameters (pH, EC, $CaCO_3$, organic matter, clay, exchangeable K and Mg). Lower contents of specifically adsorbed B in inceptisol may be because of lower adsorbing surfaces (*Table 2*) especially organic carbon (0.23%–0.49%), sesquioxides (5.05%–6.32%) and Fe_2O_3 (3.53%–4.45%). Dey et al. (2017) reported lower contents of specifically adsorbed B in soils with low organic carbon. A strong positive association was found between specifically adsorbed B and organically bound B in alfisol (*Table 5*).

Of the total B content, the oxide bound B accounted for 0.13%–13.09% (*Table 3*). Comparable outcome was stated by Bhupenchandra et al. (2024) in acidic inceptisol of Eastern Himalaya. Pachauri et al. (2024) reported that yearly application of B resulted in a significant increase in oxide bound B fraction in *Typic hapludolls*. In comparison to the readily soluble B and specifically adsorbed B, the oxide bound B was larger suggesting that oxides and oxyhydroxides contribute significantly to fixation of B by isomorphous exchange and complex formation (Bhupenchandra et al., 2020). These contents were notably higher in entisol at 0–20 cm and in alfisol at 20–40 and 40–60 cm soil depths (*Figure 1*). This is linked to the higher content of oxides and hydroxides of iron and aluminium (*Table 2*), which may have offered plenty of sites for the B [$B(OH)_4^-$, $B(OH)_3$] adsorption through ligand exchange (Datta et al., 2002; Kaundal et al., 2014). Lower oxide bound B content was found in vertisol at 0–20 cm, whereas at soil depths of 20–40 and 40–60 cm inceptisol recorded significantly lower oxide bound B (*Figure 2*). This was the result of lower mean contents of Fe_2O_3 (3.25%–4.45%) and

Al_2O_3 (2.03%–3.76%) in specified soil types (vertisol and inceptisol) than others (*Table 2*). The oxide bound B exhibited a strong positive association with the amount of clay and Al_2O_3, Fe_2O_3 in entisol, whereas this fraction was positively associated with the clay content and $CaCO_3$ in vertisol (*Table 4* and *Figure 2*). Diana et al. (2008) reported a positive correlation between oxide bound B and Fe oxides in alluvial soils of Italy. Similarly, Barman et al. (2014) reported that $CaCO_3$ makes B unavailable by adsorption, co-precipitation, or occlusion. Also, this fraction had a negative association with available N, K, DTPA extractable Cu and a positive association with DTPA extractable Mn in alfisol (*Table 4*). In light of its association with other B fractions, a positive relationship was found with residual B and total B in alfisol, entisol and vertisol (*Table 5*). Supporting this, Kaundal et al. (2014) observed a positive association among oxide bound B and total B in the acid alfisol of Northwestern Himalayas.

About 0.31%–18.16% of total B was made up of organically bound B (*Table 3*) and were unavailable for uptake by plants (Bhupenchandra et al., 2024). This fraction's presence in the soil could be related to the organic matter, which adsorbed the B through ligand exchange (Bolan et al., 2023). Depending on the soil's level of organic carbon content, the organically bound B varied under different soil types. Here, the entisol showed higher organically bound B content followed by vertisol (*Figure 1*), which is a result of higher organic carbon status of the soils (*Table 2*). Chaudhary and Shukla (2004) stated organic carbon as the major contributor of organically bound B. The organically bound B had significant positive association with organic carbon content in entisol, vertisol and inceptisol (*Table 4*). Supporting this, Datta and co-workers found a significant positive relationship among organically bound B and soil parameters like clay, ammonium oxalate extractable Al, Fe and organic carbon (Datta et al., 2002). Also, the organically bound B was positively correlated with DTPA extractable Zn (in inceptisol, and vertisol) and Cu (in entisol, and vertisol). This fraction had a positive and significant correlation with readily soluble B in alfisol, inceptisol, and vertisol (*Table 5*). To meet the plant's B demand, organically bound B may have transformed into a readily soluble B. This fraction is viewed as primary sink of plant available B (Bhupenchandra et al., 2020), released on decomposition of organic matter (Kumar et al., 2023). In vertisol, organically bound B was negatively correlated with oxide bound B, residual B and total B (*Table 5*).

The residual B constituted the most prominent B pool, accounting for approximately 71.4%–99.3% of the total B (*Table 3*). They were present within the atomic structure of the minerals, and were improbable to be liberated in the medium and long terms (Padbhushan & Kumar, 2017; Rahman & Schoenau, 2020). Regardless of the soil, crops, fertilisation practice, and climate, a higher percentage of residual B fraction (even above 80%) has been observed by numerous workers (Das et al., 2023; Datta et al., 2002; Gurel et al., 2019; Kaundal et al., 2014; Kumari et al., 2017; Xu et al., 2001). The soils of inceptisol had relatively higher residual B, since B fertilisers were continuously added (100 g palm^{-1} yr^{-1}) for 5–15 years over the oil palm plantation's age. The findings of Jegadeeswari and Muthumanickam (2017) reported significant variations in residual B under varying dose and frequencies of B application in maize-sunflower cropping system. Datta et al. (2002) reported that residual B is the structural component of R_2O_3 and clay minerals. The residual B had a notable positive association with R_2O_3, Al_2O_3 and Fe_2O_3 content in entisol, whereas it was positively associated with clay and $CaCO_3$ content in vertisol (*Table 4*). This is consistent with the observations of Kaundal et al. (2014). Residual B was positively correlated with oxide bound B and total B, whereas it had strong negative relationship with organically bound B in vertisol (*Table 4*).

The total of all the extracted soil B fractions was added up to determine total B and it is not a trustworthy measure of B that is accessible for plant absorption (Kasture et al., 2020). Their concentration varied between 65.8 and 173.9 mg kg^{-1} (*Table 3*). This was consistent with the results of Diana and Beni (2006). Significant differences in concentration of total B in different soils might be due to parent material, their level of weathering, and soil management (Das & Purkait, 2020). The inceptisol recorded high total B at 0–20 and 20–40 cm, whereas alfisol recorded higher contents at 40–60 cm soil depth (*Figure 2*). A marked trend in total B at different soil depths was not noticed. The total B showed a positive association with Al_2O_3, and Fe_2O_3 content in entisol, whereas it was positively associated with clay and $CaCO_3$ content in vertisol (*Table 4*). Irrespective of soil types, total B had strong positive association with oxide bound B and residual B (*Table 5*).

CONCLUSION

The qualitative and quantitative relevance of soil B is better defined by their fractionation in the soils. This study offers better insights of different B fractions and their association with soil characteristics under different soil types. The residual B accounted for 71.4%–99.3% of total B, and it was the predominant fraction. The plant available B fractions (readily soluble B and specifically adsorbed B) accounted for less than 2% of total B. Soil properties like pH and organic carbon had positive relationship with readily soluble B and specifically adsorbed B, while Fe_2O_3 and Al_2O_3, clay content had positive association with oxide bound B. The association among different fractions of B indicated their interdependence. Readily soluble B showed a positive correlation with specifically adsorbed B and organically bound B, suggesting their role in replenishing the accessible soil B. This study suggests that rather than B application, management strategies should be formulated to improve its availability from the total content.

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TRUE-TO-TYPE VERSION 2 – HIGH RESOLUTION GENOTYPING PLATFORM FOR PARENTAL IDENTIFICATION IN OIL PALM

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ABSTRACT

This study reports on developing the TRUE-TO-TYPE Version 2 DNA genotyping platform. Relative to SureSawit™ TRUE-TO-TYPE (Version 1), the new platform has improved genotyping resolution for high-precision parental identification in Elaeis guineensis*, allowing accurate assignment of a palm to their actual parents even if the parentage information is not available. The feature was developed by increasing the marker-set from 24 (in Version 1) to 108 stably inherited SNPs. The optimal performance of TRUE-TO-TYPE Version 2 was confirmed by analysing the genotypes of 2,301 offspring from three genetic backgrounds. A total of 17 panels with different numbers of SNP markers were evaluated for this purpose. The finalised panel was validated in 460 offspring and 41 candidate parents from 20 families of diverse genetic backgrounds. Our data demonstrated >99.4% accuracy in predicting the true parents for the palm materials analysed using TRUE-TO-TYPE Version 2. The platform retains the ability to detect illegitimates and track genetic lineage accurately. It is a quality control tool for managing palm materials in seed-gardens, breeding programs, commercial nurseries and tissue-culture laboratories. The genetic information obtained will also facilitate the establishment of a comprehensive DNA fingerprint database for the palm materials.*

Keywords: advanced breeding lines, germplasm, parentage analysis, purity testing, SNP panel.

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INTRODUCTION

Oil palm is an important commodity crop, especially in Malaysia and Indonesia, contributing to approximately 2.5% and 4.5% of the Gross Domestic Product (GDP) in 2022. Its economic importance has resulted in the wide-scale cultivation of oil palm in these two countries. In Malaysia alone, the planting areas across the Peninsular, Sabah and Sarawak were estimated at about 5.7 million hectares (Parveez et al., 2023). The hectarage consists of >770 million standing palms at a modest planting density of 136 palms/ha, not including seedlings in the nurseries and ramets produced in the tissue culture laboratories.

Therefore, a reliable and efficient management system is required for the large-scale production of good quality planting materials. Most oil palm plantation companies have established their best-practice system in managing the germplasm (Germ), advanced breeding lines (ABL) and materials produced for commercial planting in the production fields. The system is also in place to manage seedlings in nurseries and ramets produced via tissue culture. However, the management systems practised currently mostly rely on paper-trail which involves physically tracking the movement of materials, starting from the crossing of selected

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palms to the seed production facility, followed by tracking seedlings in nurseries and planting in the breeding or commercial fields. A similar exercise is carried out throughout the tissue culture process where plantlets (ramets) are moved from laboratories to nurseries and transferred to the field (Rohani et al., 2000; Soh et al., 2011). Undeniably, as thousands of breeding crosses are made yearly and dozens of ortets are cloned (each giving several lines), mislabelling and other mistakes in tracking are likely common.

A significant concern is the presence of illegitimates in the controlled crosses and mix-up of clones which, cannot be identified based on their physical appearance, especially at the early stages. The occurrence of such incidents has been well documented (Corley, 2005; Hama-Ali et al., 2014; Teh et al., 2019). There are also potential yield losses if illegitimates or unintended clones are embedded in the commercial fields, as demonstrated in the large-scale screening of 1,150,827 palm materials by Malaysian Palm Oil Board (MPOB) (Ooi et al., 2016, 2023; Singh et al., 2021), using the first available oil palm deoxyribonucleic acid (DNA) diagnostic test. The survey revealed unexpectedly high levels of contamination (ranging from 10.7%–2.8%) in the *dura* x *pisifera* controlled crosses, causing losses in billions of Malaysian ringgit to the industry. The single nucleotide polymorphism (SNP) markers used by Ooi et al. (2019) and Singh et al. (2018) targeted a particular gene – *SHELL* – that differentiates the commercially acceptable fruit form (*tenera*) from the undesired planting materials (non-*tenera*). Other studies using SSR markers have detected illegitimates ranging from 8.3%–98.0% (Zolkafli et al., 2021) and recommended different numbers of SSR markers that could be utilised for each of the genetic backgrounds screened. The limitation in the SSR studies mentioned above is that, the known parental pair is required, and if an illegitimate is detected, it cannot be accurately assigned to the correct parental pair with high confidence, even when a set of possible parents are genotyped.

Hence, it is important to develop a marker panel that has sufficient discriminating power and is amenable to high-throughput genotyping. Exploring the genomic resources available at MPOB namely, the resequencing of selected Germ (unpublished data) anchored to the oil palm genome (Singh et al., 2013), SureSawit™ TRUE-TO-TYPE genotyping platform (Version 1) consisting of 24 genome-wide SNP markers was developed in 2018 (Ooi et al., 2019; Singh et al., 2018). The Version 1 genotyping platform proved useful in detecting illegitimates, discriminating individual palms according to their genetic lineage, and allowing parentage assignment when the true parents are known. In such a guided-analysis, Version 1 was used to effectively validate the legitimacy of controlled crosses across a broad range of genetic backgrounds. However, in cases where the parentage information was unavailable, reduced accuracy was observed in assigning the individuals to their biological parents.

As Version 1 had limitations, an advanced platform for parental identification was thus needed. This study describes the steps taken in selecting, optimising, and subsequently, validating the SNP panel for that purpose. For fish and mammals, parentage analysis has been well established where many studies reported a range of 50–150 (Abadía-Cardoso et al., 2013; Beacham et al., 2018; Dussault & Boulding 2018; Liu et al., 2016; Tong et al., 2023) and 84–700 (Bell et al., 2013; Calvo et al., 2020; Clarke et al., 2014; Gebrehiwot et al., 2021; Heaton et al., 2014; Holl et al., 2017; Strucken et al., 2015; Tortereau et al., 2017) SNP markers can reliably resolve parentage assignment. For oil palm, accurately assigning every individual to their biological parents, especially in the breeding programs, is very important to ensure that the parental combining ability is accurately accessed. As making controlled crosses is an expensive and laborious exercise, the ability to assign the individual palms accurately to their respective parental pair will improve efficiency in breeding programs. Since the palms in such trials are the selected high-yielding breeding stocks, establishing a DNA database for these materials will be especially beneficial, as it will be valuable for the overall management system of important breeding lines as well as for protecting possible intellectual property (IP) related to the palm materials.

MATERIALS AND METHODS

Plant Materials

In this study, a total of 2,301 confirmed offspring resulting from 22 crosses involving 25 parental palms (18 maternal and seven paternal palms) was used for developing a suitable SNP marker panel. They were categorised under seven progeny groups based on the common paternal palm (*Table 1*). For the validation experiment, the sample panel consisted of 460 offspring palms derived from 21 crosses, where the 41 candidate parental palms were of diverse genetic backgrounds, including ABL and Germ (*Table 2*). The number of offspring for the individual crosses was relatively small, ranging from 10–30 palms.

Development of SNP Panel

Information on a total of 2,280 SNP markers, previously genotyped in the 78K and 92K high-density SNP arrays (Ting et al., 2023a, 2023b) on the families listed in *Table 1*, was extracted from MPOB's in-house database. Selection of these markers was based on three parameters: 1) Evenly distributed across the 16 pseudo-chromosomes in the *E. guineensis* genome; 2) showed the Mendelian segregation profiles across the three genetic backgrounds and; 3) had high minor allele frequencies ranging from 10%–50% (average 40%) across the three genetic backgrounds.

For determining the optimum number of SNP markers, 17 panels with increasing number of SNP markers ranging from 24 (of Version 1) to 50, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 180, 200, 500, 1,000 and 2,280 SNPs were evaluated. For each panel, the selection of SNP markers was carried out in six independent repetitions where, the first round only picked markers that were evenly spaced and the following five rounds involved random sampling.

TABLE 1. SAMPLES USED FOR DEVELOPING AN OPTIMAL SIZE OF SNP MARKER PANEL

Family type	Genetic background	Progeny group	Number of offspring	Number of parent(s) involved
Full-sib	Deli *dura* x AVROS *pisifera*	1	995	2
Full-sib	Nigeria *tenera* selfed	2	223	1
Linked half-sib	Deli *dura* x Nigeria *pisifera*	3	275	6
		4	112	3
		5	452	9
		6	112	3
		7	157	4
Total			**2,326***	**25**

Note: * - Including 25 suspicious palms.

TABLE 2. DETECTION OF ILLEGITIMATE PALMS IN VARIOUS CROSSES INVOLVING ABL AND GERM MATERIALS USING TRUE-TO-TYPE VERSION 2

No.	Material	Genetic background	Parental fruit form	Number of offspring	Number of illegitimate
1	ABL x ABL	Banting x Ulu Remis	D X D	23	0
2	ABL x ABL	Pamol x Ulu Remis	D X D	23	0
3	ABL x ABL	Johor Labis x AVROS	D X P	23	0
4	ABL x ABL	Ulu Remis x Yangambi	D X P	20	0
5	ABL x ABL	Deli x AVROS	D X P	10	1
6	ABL x ABL	Deli x AVROS	D X P	10	0
7	ABL x ABL	Serdang x Banting	D X D	23	1
8	ABL x ABL	Deli x Serdang	D X D	23	2
9	ABL x ABL	Marihat/Klanang/Ulu Remis x Yangambi	D X P	20	1
10	ABL x Germ	Ulu Remis x Nigeria	D X P	30	0
11	ABL x Germ	Ulu Remis x Nigeria	D X P	29	0
12	ABL x Germ	Klanang/Marihat x Yocoboue/IRHO	D X P	20	3
13	Germ x ABL	Zaire x AVROS	D X P	23	1
14	Germ x ABL	Angola x AVROS	D X P	22	2
15	Germ x ABL	Tanzania x AVROS	D X P	23	5
16	Germ x Germ	Sierra Leone x Sierra Leone	D X D	23	0
17	Germ x Germ	Nigeria	T selfed	29	0
18	Germ x Germ	Nigeria	D X D	17	8
19	Germ x Germ	Guinea	D X D	23	1
20	Germ x ABL	Cameroon x AVROS	D X T	23	4*
21	Germ x Germ	Nigeria x Nigeria	D X D	23	3*
Total				**460**	**32**

Note: * - Including both parents; ABL - advanced breeding lines; Germ - germplasm.

Parental prediction via Cervus 3.0 (Marshall et al., 1998) was first performed for the five progeny groups in Deli *dura* × Nigeria *pisifera* to evaluate the efficiency of the markers in identifying the true parents when no information was provided on the identity of the parental pair (maternal-unfixed mode) in comparison to when only paternal was made unknown (maternal-fixed mode). The subsequent parental blinded analyses aimed to predict maternal and paternal parents.

Validation of TRUE-TO-TYPE Version 2

Primer-pairs were designed for the optimised SNP panel using a proprietary primer selection pipeline, similar to Primer3 (Koressaar et al., 2018) and subsequently, used to genotype the 501 samples derived from diverse genetic backgrounds (*Table 2*), using an in-house amplicon sequencing platform similar to Multiplex PCR targeted amplicon sequencing (Onda et al., 2018). Reproducibility of genotyping was determined by genotyping a subset of samples in two to four replicates and parentage assignment was carried out as described above.

RESULTS AND DISCUSSION

Optimal SNP Panel Size for TRUE-TO-TYPE Version 2

The main objective in designing the present assay was to identify the true parents for palms analysed, especially when both parents are unknown. The maternal-unfixed mode (available in Cervus 3.0) is an ideal option for such analysis; hence, the 13 marker panels' efficiency in assigning the true parental pair of the five Deli *dura* × Nigeria *pisifera* progeny groups was evaluated. The test revealed consistently high levels of prediction accuracy in the marker panels containing ≥110 SNPs, similar to the analyses performed with informed maternal palm (maternal-fixed mode). However, for smaller panels especially those containing ≤70 SNP markers, the prediction accuracy was reduced drastically (*Figure 1*), suggesting why Version 1 which had only 24 SNP markers was ineffective in identifying the true parents from a panel of possible parental pairs.

Using the maternal-unfixed mode, the blinded parentage analysis was extended to 17 marker panels involving all seven progeny groups (*Table 1*). The result showed that the panel with 110 markers was optimal for high accuracy (99.4%) parental prediction (*Figure 2*). Increasing the number of markers beyond 110 provided minimal additional parental identification benefits.

TRUE-TO-TYPE Version 2 Validation

Genotyping assay design was attempted for the 110 selected SNPs and primer-pairs could be designed for 109 SNPs. The 109 primer-pairs were used for genotyping 501 palms (and their replicates) derived from various genetic backgrounds of which, 99.6% of samples generated amplicons. Of the 109 markers genotyped, one completely failed to yield genotype data whereas, 99.0% (108 markers) were successfully called (99.0% call rate) and were 100.0% reproduced across replicates, indicating that they were high-performing SNPs. These 108 SNP markers were evenly distributed across the 16 pseudo-chromosomes of the latest oil palm (EG11) reference genome build (Low et al., 2024) (*Figure 3*). The genotype data obtained was subsequently examined for legitimacy and identification of true parents in the individual families.

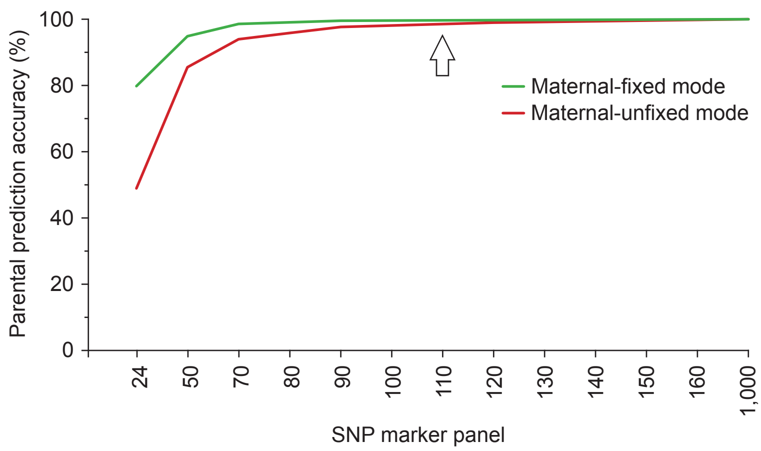

Figure 1. Comparing the prediction accuracy between the maternal-fixed and -unfixed modes in parentage analysis. The respective one- and both-parent blinded-analyses for five progeny groups under the Deli dura *x Nigeria* pisifera *background were performed across 13 panels containing different numbers of SNP markers.*

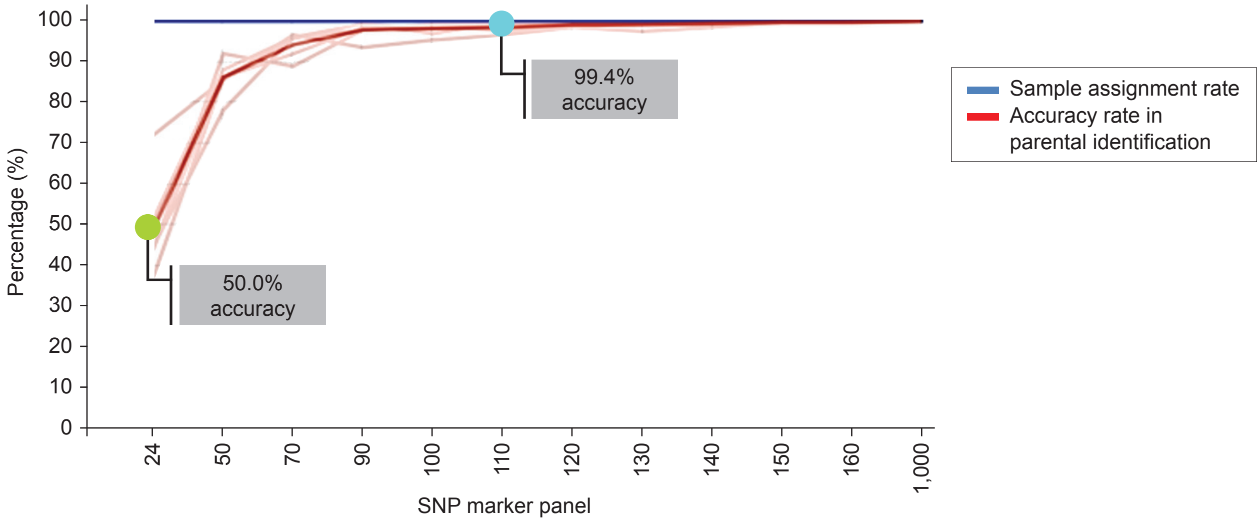

Figure 2. Determining the optimal SNP panel size for TRUE-TO-TYPE Version 2 by repeating blinded-analyses. The marker panel with 110 SNPs (indicated by the fluorescent blue dot) was selected as the optimal size for high confident (>95.0%) parental prediction while the 24-SNP panel of Version 1 (indicated by the fluorescent green dot) only encountered ~50.0% accuracy.

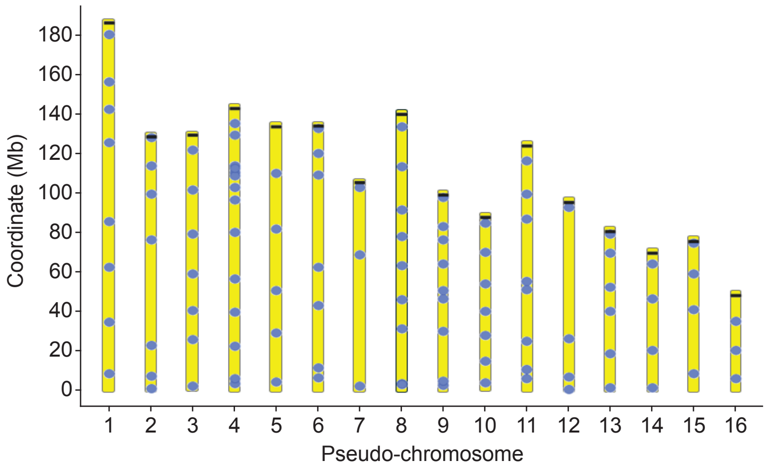

Figure 3. Distributions of TRUE-TO-TYPE Version 2 SNPs on the oil palm genome assembly. Yellow vertical bars represent the pseudo-chromosomes of EG11. Blue circles represent the positions of 108 SNP markers, except three SNPs that mapped to the unplaced scaffolds. Black horizontal bars represent the uppermost coordinate of each pseudo-chromosome.

Detection of illegitimates. Marker data for the 21 full-sib crosses involving a total of 460 offspring (*Table* 2) was used to construct a weighted Neighbor-Joining dendrogram using DARwin version 6 (Perrier & Jacquemoud-Collet, 2006), to visualise the genetic relatedness among the samples (*Figure* 4). The samples were clustered into 20 genetic groups, instead of the 21 families that were expected. Crosses No. 5 and 6 (*Table* 2) were obtained from the same parental pair. The dendrogram also showed the presence of several outliers that are possibly illegitimate. The subsequent legitimacy check using a pool of 41 candidate parents confirmed the observation. A total of 32 illegitimate palms (with a triad score ≥0.05) were identified where one to eight offspring were contaminants in the first 19 crosses. None of the assumed parental palms were the true parents for Crosses No. 20 and 21 (*Table* 2), suggesting a possible mix-up when samples were moved from the seed production facility to the nursery and subsequently, to the field. This demonstrates that the TRUE-TO-TYPE Version 2 can screen illegitimate palms efficiently at both offspring and parent levels, across a wide range of genetic backgrounds.

Parental identification efficiency. The 389 confirmed offspring palms from Crosses No. 1–19 (*Table* 2) were subjected to the predictions of both parents (maternal-unfixed mode). The result showed 100.0% accuracy in all the predictions made across the offspring of different genetic backgrounds, demonstrating that the TRUE-TO-TYPE Version 2 is highly reliable for parental identification even when the number of palms analysed was as few

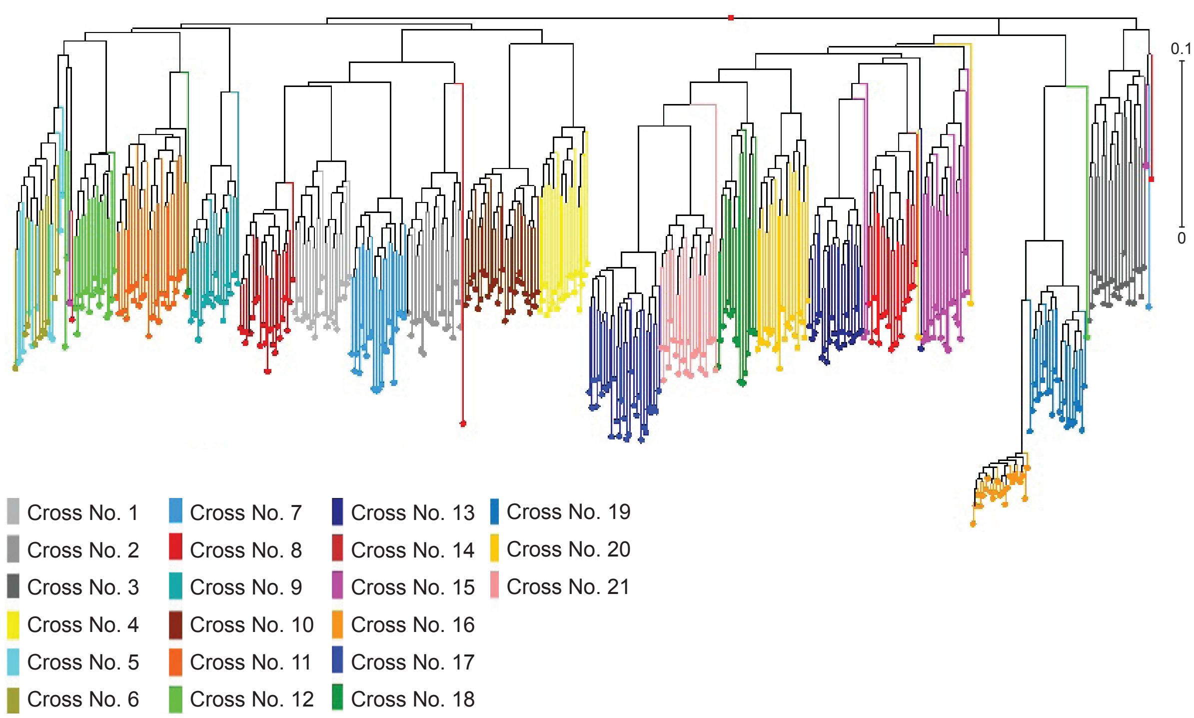

Figure 4. Genetic tree for 460 offspring collected from 21 crosses. Each dot represents an offspring and each family is given in different colour.

as nine. The accuracy rate for the similar parental prediction using the 24 SNP markers of Version 1 was only 79.0%.

For a more detailed examination, the estimated LOD scores for the individual offspring in a representative cross – Ulu Remis *dura* x Yangambi *pisifera* (Cross No. 4 in *Table 2*) were compared between Version 1 and 2 (*Figure 5*). Using the present 108 SNP markers, both parents of all 20 offspring were accurately predicted and a large gap in LOD scores was observed between the true and false parental pairs in the blinded analysis. A similar pattern was not observed when Version 1 was used. In contrast, the LOD scores observed for the samples analysed with the 24-SNP panel were at much lower levels (LOD ≤12) and failed to separate the true from the predicted false parental pairs. This explains the lower rate of identifying the significant parental pair and subtly, lower accuracy of the parental prediction in Version 1 if the actual parental pair was unknown.

Feasibility of Establishing a DNA Fingerprint Database Using TRUE-TO-TYPE Version 2

The TRUE-TO-TYPE Version 2 platform, which uniquely identifies individual palms and assigns them to specific parents across a wide range of genetic backgrounds, represents a core set of 108 universal SNP markers that can help establish an oil palm DNA fingerprint database. The database captures unambiguous genotype data for the individual palms and if generated for their parental palms, could help establish the DNA fingerprint of an invaluable asset. This will be useful for integrating into a company's management system to protect the IP related to key breeding lines used for commercial seed production. Although morphological traits are widely used to determine distinctness, uniformity, and stability (DUS) for plant variety protection (Plant Varieties Board Malaysia, http://pvpbkkt.doa.gov.my), the narrow genetic pool of commercially produced oil palm materials in Malaysia and other oil palm producing countries (Hartley, 1967; Kushairi & Nookiah, 2000), severely limits the ability to use phenotypes for this purpose. Therefore, a DNA database can protect the existing key breeding lines and new varieties being developed by a particular company. Such DNA fingerprint databases have been established for specific plant species such as maize, where an SSR-based DNA fingerprint database was established to better manage the maize varieties in China (Wang et al., 2017). More recently, SNP markers have been the popular choice which, a core set ranging from 18–200 markers has been developed for setting-up DNA fingerprint databases for variety protection and to overcome potential intellectual disputes in maize (Jiang et al., 2020; Tian et al., 2021; Zhao et al., 2021), tobacco (Wang et al., 2021), cauliflower (Yang et al., 2022) and tomato (Zhang et al., 2023).

The DNA fingerprint database generated using the TRUE-TO-TYPE Version 2 platform will also be very useful in deciphering genetic information for important breeding lines which can help characterise the level of diversity within an organisation's seed garden. The various genetic parameters that can be obtained include the genetic diversity or relatedness of the materials, which can be easily visualised as demonstrated for the samples analysed in this study (*Figure 6*). In addition, the allelic information including the number of alleles per locus (k), observed heterozygosity (H_{Obs}), expected heterozygosity (H_{Exp}), polymorphic information content (PIC) and frequency of null alleles (F_{null}) (*Figure 7*), are important parameters to help evaluate the diversity of the materials in the seed garden. Interestingly for all crosses analysed in this study, the H_{Obs} on average were higher than H_{Exp}, suggesting high genetic variability and low levels of inbreeding, contributed by the fact that the crosses involved two parents, with one exception, Cross 17 (selfed cross).

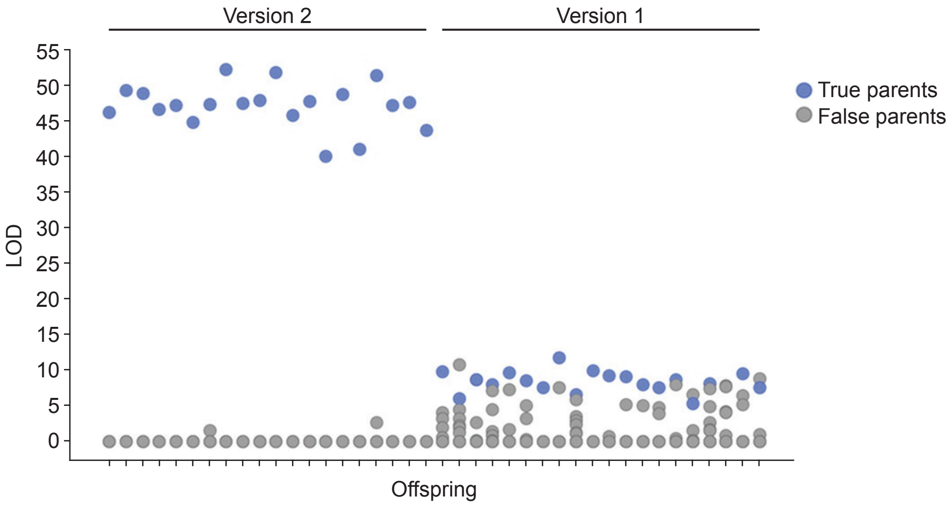

Figure 5. Blinded parental analysis for 20 confirmed offspring in a representative full-sib cross. Positive LOD scores for the true (blue dots) and false parent pairs (grey dots) are plotted for each offspring palm when tested with 24 (Version 1) and 108 (Version 2) SNP markers.

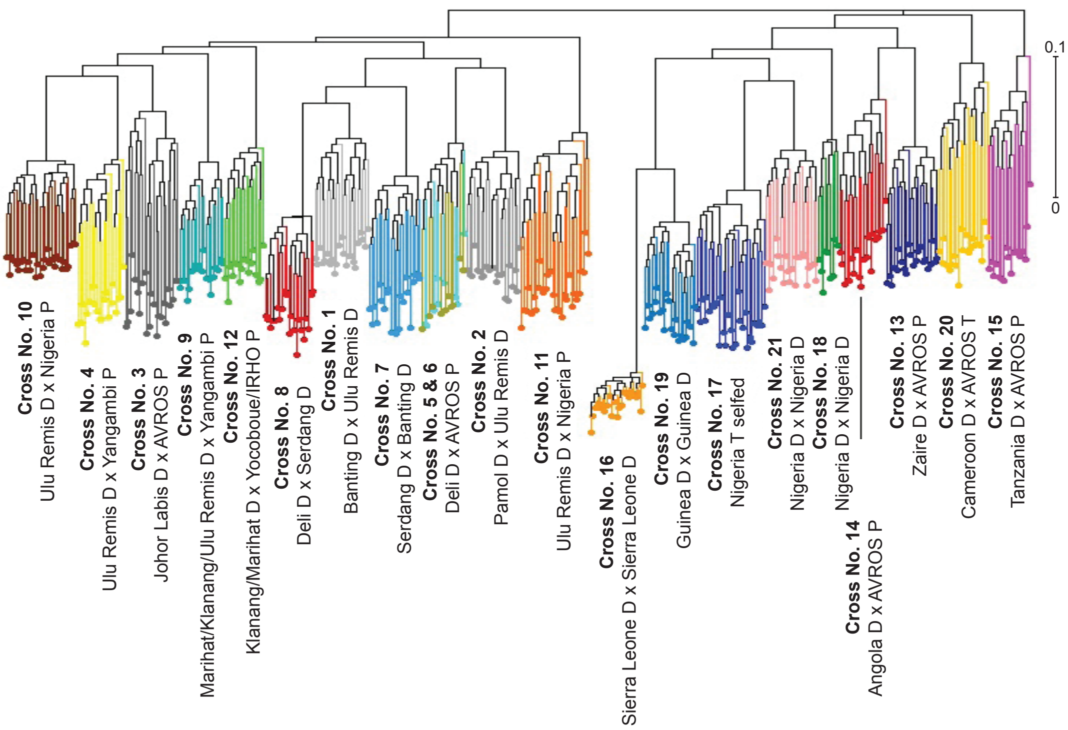

Figure 6. Genetic distances and diversity among legitimate palms of 20 different families validated by TRUE-TO-TYPE Version 2.

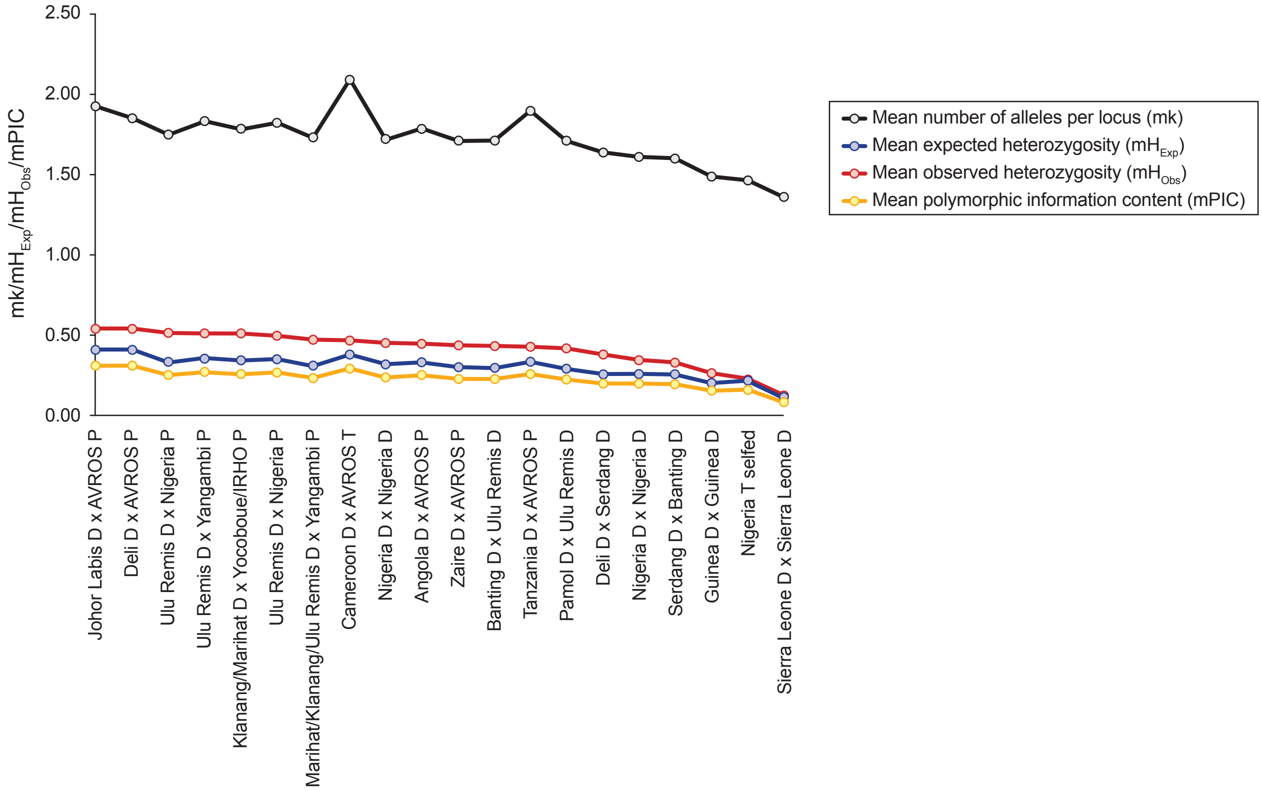

Figure 7. Summary of the allelic information for palm families genotyped by TRUE-TO-TYPE Version 2.

CONCLUSION

The genotyping resolution of TRUE-TO-TYPE Version 2 has been maximised with a powerful set of 108 SNP markers, four times more relative to Version 1 introduced six years ago. This study has demonstrated the efficiency of TRUE-TO-TYPE Version 2 in assigning legitimate palms to their true parental pair in crosses of various genetic backgrounds. This improvement is significant to ensure the parental lines for various palm materials are genetically traceable, particularly in breeding and improvement programs. As a result, other features originally designed for Version 1 such as detecting illegitimates and tracking genetic lineage are also further improved in this new genotyping platform. The new genotyping platform as such, can be utilised for better quality control and to significantly improve breeding and tissue culture procedures' efficiency, which will help accelerate the development of new varieties. More importantly, the new genotyping platform will facilitate the establishment of an unambiguous DNA fingerprint database for the palm materials owned by an organisation. This is useful to avoid infringement of their respective company brand name and fraud concerning the illegal use of a particular company name to sell inferior oil palm seeds and seedlings.

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IMPROVING OIL PALM BREEDING EFFICIENCY VIA MIXED POLLINATION AND PATERNITY DETERMINATION USING SINGLE NUCLEOTIDE POLYMORPHISM (SNP) PANEL

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ABSTRACT

This study introduces a novel approach in oil palm breeding, where pollen from different sources is mixed and utilised in a single controlled cross, and the seedlings generated are subsequently separated based on their paternal source. Mixes of up to four pollen sources were created and crossed each time with a single dura *maternal palm, generating seedlings for fingerprinting analysis. A total of 12 crosses were generated to produce 1,811 progenies (43–415 progeny/cross) from a pool of 16 candidate parental palms. DNA fingerprinting analysis was carried out using 24 and 108 SNP panels. The assignment found that the 108 SNP panel is more effective in tracking genetic lineage and assigning seedlings to their true parental combination. The dominant contribution of certain pollen sources was obvious, suggesting that even though the pollen viability is similar, other factors may also be a contributing factor such as genetic background of pollen source, storage period and the age of pollen-producing palms. Nevertheless, the present approach effectively improves breeding efficiency by reducing the number of individual crosses required, making it cost-effective and importantly minimises the impact of depending on the flower cycle to make the necessary crosses in a breeding programme.*

Keywords: breeding, crossing, fingerprinting, mixed pollination.

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INTRODUCTION

The oil palm is a monoecious species as it bears both male and female inflorescences on the same palm in an alternating cycle. The reported period of each floral cycle is around four to six months, largely influenced by genetic and environmental factors (Purseglove, 1972). Male and female inflorescences can have more than 100 spikelets each with the female inflorescence consisting of about 30 flowers per spikelet compared to the male inflorescence which can carry up to 1,200 flowers per spikelet (Rajanaidu et al., 2000). The male inflorescence produces up to 40 g of pollen which remains viable for at least six days. The pollen can be stored for up to one year at –5°C for subsequent use in controlled pollination (Hardon & Davies, 1969). The female flowers are usually receptive to the pollen for about 36–48 hr after anthesis (Latiff, 2000). Oil palm open pollination is carried out by wind and weevil *Elaeidobius kamerunicus,* the main pollinating insect in Malaysia, introduced from West Africa in 1981 (Syed et al., 1982).

Oil palm yields the highest amount of oil per unit area (more than 4 t/ha) and has the largest market share among the major oil crops. Malaysia with

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5.65 million hectares of land under oil palm cultivation; produced 18.55 million tonnes of palm oil and 2.12 million tonnes of palm kernel oil, making it the second most significant producer of the commodity (Parveez et al., 2024). However, as arable land is limited, the only feasible option to improve production to meet the ever-growing demand is the development of new and improved planting materials. In line with this, oil palm breeders have long realised the need for continuous production of improved commercial *dura* x *pisifera*, D×P (*tenera*) planting materials, which in turn requires the development of elite *dura* and *pisifera* lines with good combining ability. Oil palm breeders have made considerable improvement, where average yield increments of about 1.5%/yr have been estimated (Soh et al., 2002). Oil palm genetic improvement has been realised due to carefully designed breeding and selection strategies. Generally, in oil palm breeding, pollen from a selected palm (usually *pisifera*) is crossed to a few mother palms (*dura*s) using the North Carolina Model 1 breeding design (Rajanaidu et al., 2000). On average, the female flowers grow in large inflorescence and mature in 5–6 months after pollination. The combining ability of the parental palms can be evaluated by the performance of the progenies produced in the breeding scheme. A minimum of four years of yield recording at maturity (around six years after planting) is required before a decision can be made to select specific palms for seed production and/or further improvement via subsequent rounds of crossing. In general, an oil palm selection cycle in a conventional breeding programme can take at least 10 years. It is also difficult to fix desirable genes in a population, which often requires numerous breeding and selection cycles (Rajanaidu et al., 1999).

The above factors make conducting breeding trials for oil palm an extremely slow, laborious and expensive exercise. As such, to alleviate some of these constraints, this study describes a novel approach, where pollen sourced from several intended parental palms can be mixed and used for controlled pollination on a selected maternal palm. Since only a single pollen grain in the mix contributes to the fertilisation of the individual flower in the inflorescence, the resulting seeds and seedlings derived from the bunch can be differentiated based on the unique DNA profile of the paternal palms. A single nucleotide polymorphism (SNP) panel that can facilitate such an approach has been reported for oil palm (Leslie et al., 2019; Ting et al., 2023). This approach improves breeding efficiency by reducing the number of individual crosses required and minimises delays caused by waiting for the female flower cycle, if crossing is to be carried out separately for each pollen source.

MATERIALS AND METHODS

Parental Palms and Crossing Programme

In total, 12 crosses were generated to verify the mixed pollen strategy in breeding programmes, and these involved nine *dura* (maternal) and seven *pisifera* (pollen source) palms. *Dura* palms were of the Deli *dura* lineage, while the *pisifera* pollen parents were of the AVROS, Lame, Yangambi and Nigerian sources. The pollens were harvested carefully from the individual paternal palms and stored separately in sealed and labelled containers at a temperature of –20°C to maintain their viability for extended periods, ranging from months to years. The viability of the selected pollens exceeded 75% (with one exception) to avoid selection bias, which may affect the number of progenies derived from a particular pollen source (*Table 1*). An independent mix of two, three and four pollen sources were carried out and each source of mixed pollens was used to pollinate the designated female flower of a single *dura* palm. The amount of pollen used was 0.1 g/pollen source and mixed together before puffing on the anthesising female inflorescences in one application.

The young inflorescences were initially bagged at least a week before anthesis, and hand-pollinated at the first sign of anthesis by injecting pollen through a small hole in the bag. The resulting progenies were labelled and carefully tracked for fingerprinting analysis in the nursery. The crossing scheme is shown in *Table 2*.

The resulting bunches from the specific crosses were harvested five months after pollination, labelled and processed for seed production as described by Rao and Choong (2014). For germination, the seeds were kept in a hot room with a temperature of 40ºC for 60 days, to allow germination. Subsequently, the germinated seeds were transferred to a small polybag in the pre-nursery, with proper tracking. After three months, leaf samples were collected from individual seedlings using the leaf sampling kit (Singh et al., 2007) and genotyped with the TRUE-TO-TYPE Version 1 – 24 SNP panel (Leslie et al., 2019) and TRUE-TO-TYPE Version 2 – 108 SNP panel (Ting et al., 2023).

Genotyping of Samples with the TRUE-TO-TYPE SNP Panel Version 1 and 2

The SNP assay for both versions of the SNP panel was carried out as described previously (Leslie et al., 2019). The parental DNA were all assayed in duplicate, to ensure consistency of the SNP calls and SNP genotype for the parental palms and each seedling assayed was recorded in an Excel Sheet. Data analysis was carried out using Cervus 3.0

TABLE 1. THE LIST OF *Pisifera* PALMS USED AS POLLEN SOURCES AND THE VIABILITY OF RESPECTIVE POLLENS

No.	Pollen	Viability (%)	Genetic background	Date collected
1	0.243/43	80.0	La Me	17.03.16
2	0.395/421	80.0	AVROS	04.12.15
3	0.395/175	77.6	Yangambi	25.10.13
4	0.395/324	60.0	Nigeria	15.04.13
5	0.174/480	82.2	AVROS	09.12.16
6	0.174/655	80.0	AVROS	11.02.18
7	0.395/182	80.0	AVROS	14.01.16

TABLE 2. LIST OF CROSSES GENERATED TO EVALUATE THE MIXED POLLEN APPROACH. THE NUMBER AND SPECIFIC *Pisifera* PALMS USED AS THE PATERNAL SOURCE IN THE POLLEN MIX ARE DESCRIBED

No.	Progeny	Female parent	Male parents			
1	PK7422	0.484/411	0.243/43	0.395/421		
2	PK7401	0.484/997	0.243/43	0.395/421		
3	PK7558	0.484/1,003	0.243/43	0.395/421		
4	PK7569	0.484/351	0.243/43	0.395/421		
5	PK7570	0.484/1,003	0.243/43	0.395/421	0.395/175	
6	PK7425	0.484/875	0.243/43	0.395/421	0.395/175	
7	PK7446	0.484/857	0.243/43	0.395/421	0.395/175	
8	PK7453	0.484/518	0.243/43	0.395/421	0.395/175	
9	PK7464	0.484/518	0.243/43	0.395/421	0.395/175	0.395/324
10	PK7471	0.484/993	0.174/480	0.174/655	0.395/421	0.395/182
11	PK7468	0.484/875	0.174/480	0.174/655	0.395/421	0.395/182
12	PK7476	0.484/871	0.174/480	0.174/655	0.395/421	0.395/182

software (Marshall et al., 1998) for the assignment of individual seedlings to their respective parental pairs in each cross, without any prior information. The analysis was then repeated by fixing the female parent for each progeny, and data was only considered acceptable if both analyses agreed. In the Cervus parent analysis, a logarithm of the odds (LOD) score exceeding the threshold established in the simulation phase was used to indicate that the candidate was the true parent. To account for genotype errors, a typing error of one among the 108 loci tested was considered acceptable, if the LOD score was positive. Further analysis to discriminate individual progenies to their related pedigrees was also carried out to confidently assign each seedling to the respective parental pair.

RESULTS AND DISCUSSION

Fruit bunches were harvested for all 12 crosses, approximately five months after pollination, and seeds from these were successfully germinated from all the experimental crosses. The germination rate exceeded 50%, thus sufficient number of samples were obtained for analysis. The two versions of the SNP panel, the first consisting of a set of 24 SNP markers and a second version consisting of 108 SNP panels, were utilised for DNA fingerprinting in this study. The first version (TRUE-TO-TYPE SNP Panel version 1), which is the 24 SNP panel, consists of SNPs that had been selected based on physical genomic location to minimise genetic linkage and minor allele frequency and maximise genetic informativity within and between populations (Leslie et al., 2019). Version 1 SNP panel is efficient for assigning palms to known parents and weed out illegitimates. However, a larger SNP panel is required for correct parental identification from a pool of possible candidates. A 108 SNP panel (Version 2) proved optimal for high accuracy (99.4 %) parental prediction (Ting et al., 2023). These two panels were developed from a range of diverse materials consisting of germplasm and advanced breeding lines to ensure that only palms with the desired genetic lineage are planted in breeding trials and commercial plantations. The availability of these SNP panels and their amenability to high throughput processing provides the required platform to evaluate the strategy of discriminating seedlings generated from mixed pollen crosses to their respective paternal source and hence assign each seedling to the correct pedigree, which is essential to make informed decisions in breeding programmes.

All 1,811 seedlings (43–415 progeny/cross) generated from the 12 DxP crosses and the pool of 16 candidate parental palms (*Table 3*), were genotyped with both SNP panels. The result was analysed using Cervus 3.0 software (Marshall et al., 1998) for the assignment of individual palms to their respective parental pairs in each cross. The result obtained for both panels is shown in *Figure 1* and it is clear that the 108 SNP panels (Version 2) contained the optimal number of SNPs to correctly assign seedlings to their respective parental pairs. Data obtained from the 108 SNP panel assigned 99.0% of the progeny with high confidence to a paternal palm in the "pollen- mix", compared to 88.0% of the progeny palms obtained using the 24 SNP panel. The analysis further revealed that only a small number of samples could not be assigned to any parental palms in both 108 and 24 SNP panels, which is about 0.4% and 0.5%, respectively. A limitation of using a low number of SNP markers was also evident where more than 11.0% of the progenies were assigned to incorrect males (paternal palms that were not represented in the pollen mix) when using the 24 SNP panel. This was reduced to only about 0.7% with the 108 SNP panel. The major limitation of the 24 SNP panel is that the number of SNPs employed, which are biallelic, is not sufficient to discriminate parental palms used in the crossing scheme based on the genotype profiles. The 108 SNP panel can discriminate more effectively closely related paternal palms, albeit with some mismatch. To achieve higher discriminative power a panel consisting of a larger number of SNP markers is needed (Ting et al., 2023). However, the slight gain achieved needs to be balanced with the increasing cost of genotyping with additional SNP markers. In a breeding programme, the seedlings produced are often in excess of the numbers required in a breeding plot for a particular cross. As such, discarding the less than 1.0% of samples that could not be assigned with confidence may be more cost-effective than achieving near 100.0% accuracy using the mixed pollen strategy with DNA profiling to track and assign genetic lineage.

TABLE 3. NUMBER OF SAMPLES ANALYSED FROM 12 PROGENIES DEVELOPED USING THE POLLEN MIX APPROACH

No.	Progeny	No. of samples for SNP analysis
1	PK7422	43
2	PK7401	88
3	PK7558	141
4	PK7569	195
5	PK7425	88
6	PK7570	200
7	PK7446	89
8	PK7453	124
9	PK7464	122
10	PK7471	415
11	PK7468	101
12	PK7476	175
Total		**1,811**

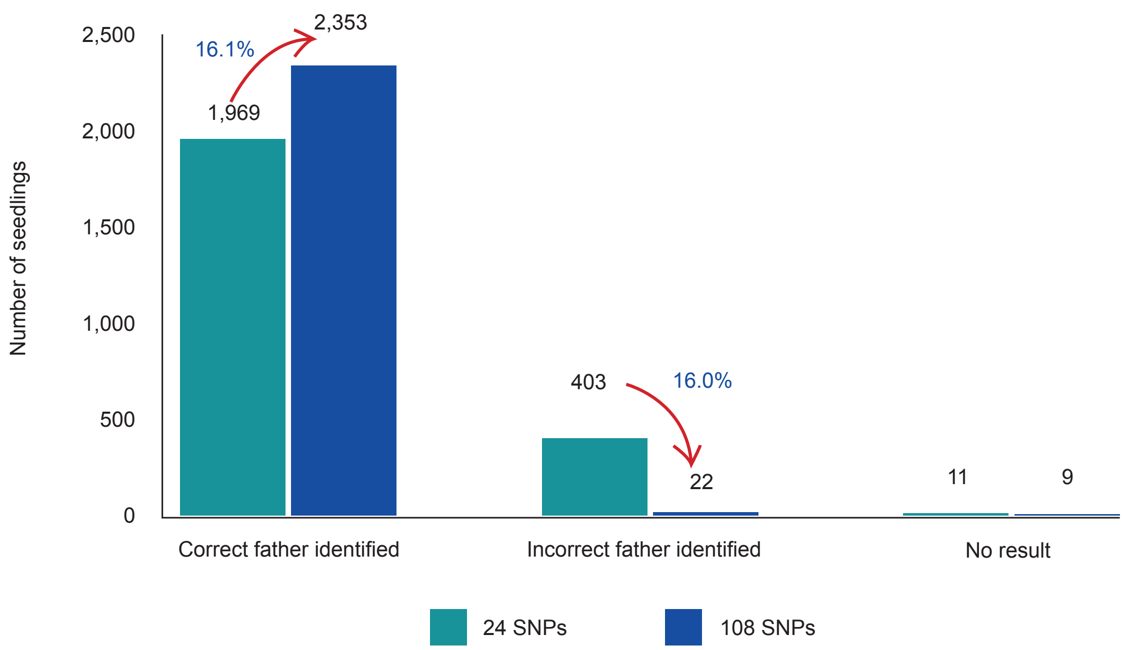

Figure 1. The comparison of fingerprinting output between 24 and 108 SNP panels.

Subsequent analysis was carried out to evaluate the efficiency of mixing two, three and four pollen sources to generate the respective controlled crosses. Only results obtained from the 108 SNP panel are discussed in detail in this study. In the "two pollen mix", four independent crosses were created using different maternal palms (*Table 1*). The two paternal palms were 0.243/43 and 0.395/421 of La Me and AVROS genetic backgrounds, respectively. The viability of pollens from both sources was about 80% and the "two pollen mix" was used to generate progenies PK7422, PK7401, PK7558 and PK7569. The number of seedlings used for DNA fingerprinting with the two SNP panels ranged from 43–194/progeny. The uneven number of seedlings genotyped from each progeny was a factor in the germination rate observed for each cross and damage by rodents to some seedlings in the nursery before sampling. The assignment of individual seedlings to their respective parent is summarised in *Figure 2*. The percentage of seedlings assigned to paternal palms in each cross ranged between 17%–39% for 0.243/43 and 61%–83% for 0.395/421. Seedlings were successfully discriminated and assigned with high confidence to the respective pollen source, although a larger number of seedlings appear to be contributed by pollen from 0.395/421. This suggests that even though the pollen viability and storage period for both sources are similar, other factors such as genetic background and the age of pollen-producing palm could have contributed to the results observed. The results further suggest that in a "two pollen mix" system, genotyping 200 seedlings should provide sufficient palms for each pedigree to plant in a breeding plot, where the minimum requirement is generally about 32 palms/progeny (planted in two replicates).

A similar independent exercise was also carried out for the "three pollen mix", where four progenies namely PK7570, PK7425, PK7446, and PK7453 were generated. The three pollen sources included the two described above and with the addition of pollen from the Yangambi *pisifera* palm 0.395/175. As described in *Table 1*, the viability of 0.395/175 pollen at 77.6% was slightly lower compared to the other two pollen sources in the mix. The distribution of individual seedlings to their respective parent is shown in *Figure 3*. As expected, the assignment of seedlings to 0.395/175 (range from 5%–8%) is lower compared to the other two pollen sources, likely caused by lower pollen viability and longer storage period (there was a difference of two years in the storage facility). Similar to results in the two-pollen mix, a higher number of seedlings were assigned to pollen 0.395/421 at a range of 63%–80%, while the percentage of seedlings assigned to pollen 0.243/43 is within the range of 12%–30%. The results suggest that even if three different sources of pollen are mixed, seedlings can still be assigned to all three paternal sources, albeit at different frequencies. Based on the results above if three pollen mixes were utilised, about 400 seedlings need to be tested to get sufficient numbers for each pedigree to meet the minimum requirement of 32 palms for the standard breeding programme.

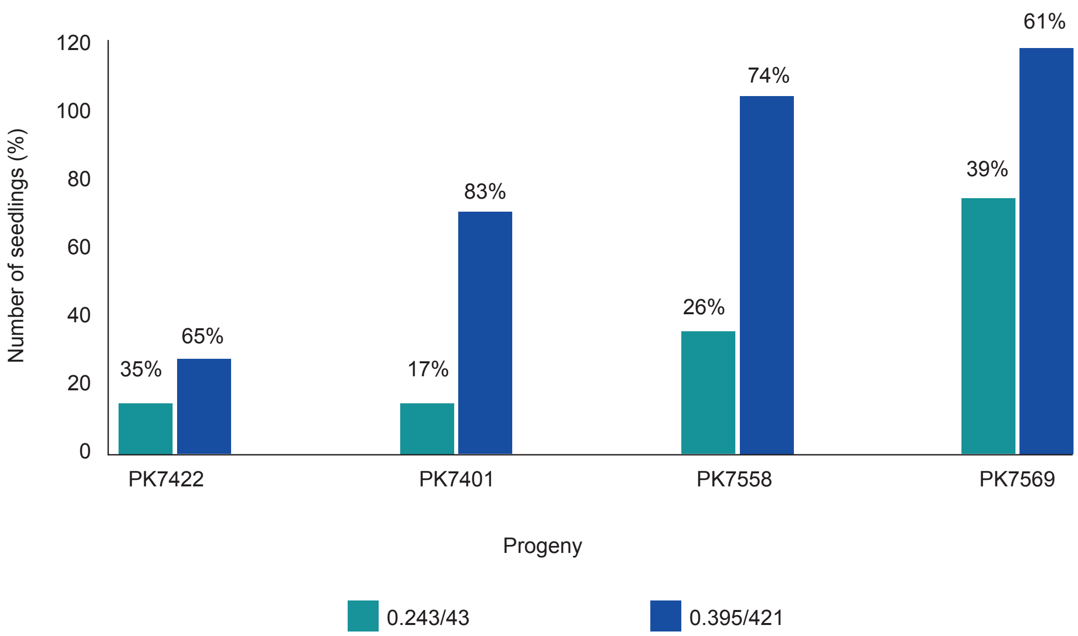

Figure 2. The assignment of individual seedlings to their respective parent for a mix of two pollen crosses using 108 SNPs.

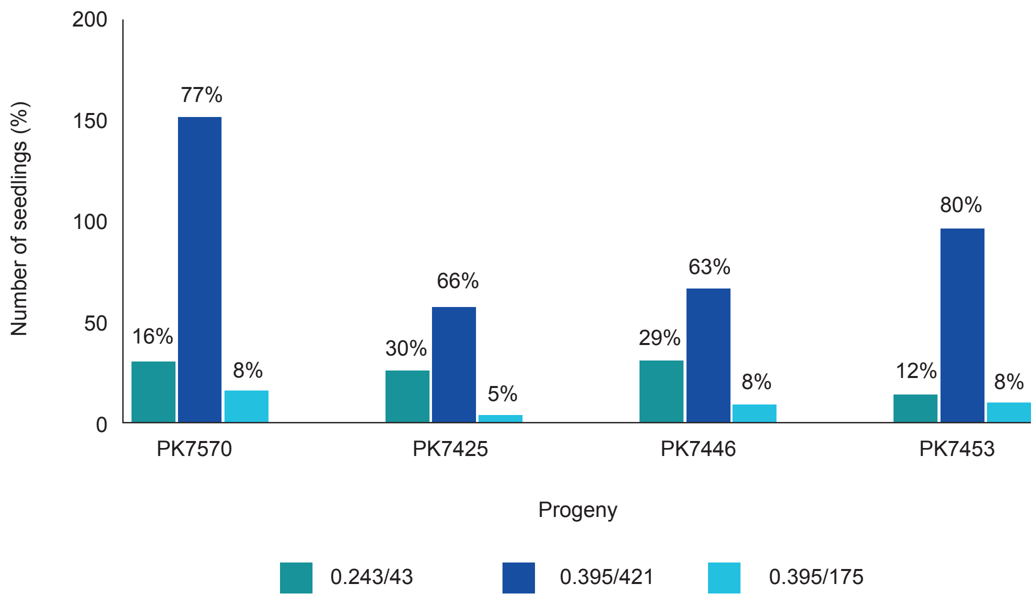

Figure 3. The assignment of individual seedlings to their respective parent for a mix of three pollen crosses using 108 SNPs.

The analysis was further extended to the "four pollen mix", where an additional four independent crosses were generated. Progeny PK7464 was obtained using the pollen sourced from the three palms described above with an additional pollen coming from palm 0.395/324 (*Table 1*). The pollen sourced from 0.395/324 had a lower viability (60%), and was included to obtain a general idea of the effect of low viability pollen in the study. As expected, of the 112 seedlings genotyped, only one seedling (1%) was assigned to this specific paternal palm. The assignment of seedlings to the other three pollen sources, namely 0.243/43, 0.395/421 and 0.395/175 pollens, was 19%, 74% and 7%, respectively (*Figure 4*). Interestingly, the percentage of seedlings assigned to the three-pollen source was similar to that obtained in the "three pollen mix" study above. The data obtained here and from the "three pollen mix" study clearly suggests that pollen with lower viability is unable to compete for pollination and produces less offspring in the mixed pollen study.

The other three crosses generated in the "four pollen mix" study involved a different set of paternal palms namely 0.174/480, 0.174/655, 0.395/421 and 0.395/182. The viability of all selected pollen exceeded 80%, with the difference being that pollen 0.174/655 was a more recent collection compared to other pollen which was harvested and stored 15 months earlier. The DNA fingerprinting result revealed that the majority of the seedlings were assigned to the paternal palm 0.174/655 in the range of 53%–66% (*Figure 4*). The percentage of seedlings attributed to the other pollen source was in the range of 2%–27%, with the largest number of seedlings obtained contributed by 0.395/182 followed by 0.395/421 and 0.174/480. However, the association between the length of pollen storage and their ability to compete to produce offspring in mixed pollen study needs further verification as other factors (genetic background and storage conditions) also need to be factored in to make a definitive conclusion. The fact that in a mixed pollination experiment, one donor is favoured for pollination over the other has been documented, likely due to pre-zygotic selection (Björkman et al., 1995). The analysis further revealed that a maximum mix of four pollen different pollen sources may be the limit to get offspring for each pedigree if implemented in a breeding programme. The number of seedlings that need to be generated and genotyped to assign to each of the four pedigrees also has to be relatively high (>1,600) if sufficient numbers are to be obtained for a breeding trial.

It is clear that the 108 SNP panel described by Ting et al. (2023) has sufficient discriminative power as a DNA fingerprinting tool to assign samples in a mixed pollen study to their respective lineage with high confidence. The fact that seedlings of different crosses were easily distinguished, suggests that the SNP panel is also an excellent tool to facilitate identification and subsequent removal of undesired seedlings in breeding and commercial nurseries. More importantly, the strategy of using mixed pollen with DNA profiling to segregate seedlings to their pedigree, not only saves cost and time in conventional breeding but also ensures the fidelity of the controlled crosses, which is

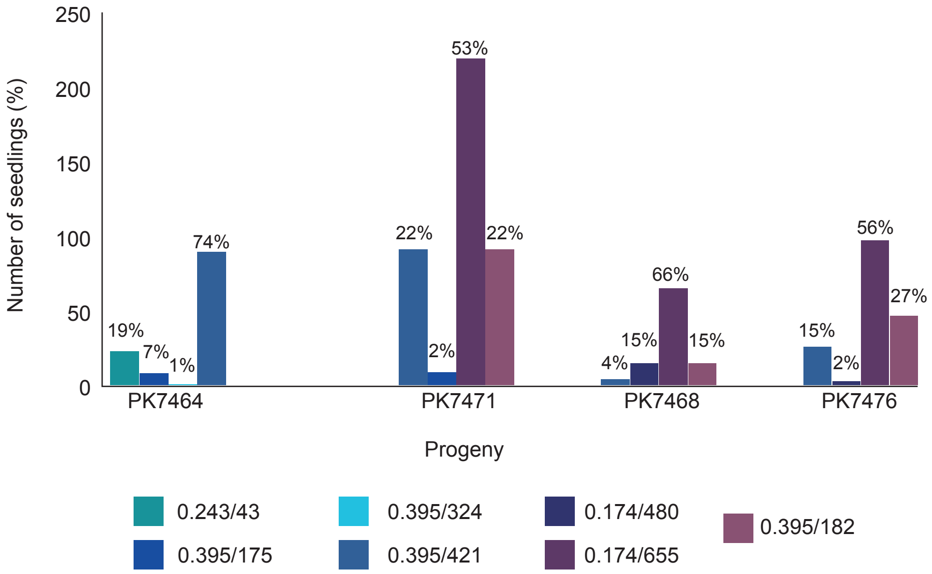

Figure 4. The assignment of individual seedlings to their respective parent for a mix of four pollen crosses using 108 SNPs.

important to make sure the correct conclusions and selections are made in breeding programmes (Corley, 2005). Interestingly, the platform also provides an interesting opportunity to establish a DNA database of all breeding materials, which collectively can improve the management and quality control of propagated materials in breeding and commercial nurseries as well as in tissue culture laboratories.

The strategy of mixing pollen from different paternal sources for making a controlled cross and subsequently assigning seedlings to the paternal source is described here for the first time in oil palm which is a perennial crop with a very laborious and costly breeding programme (Zolkafli et al., 2021). In other crops, the use of mixed pollen strategy has been mostly used to decipher the evolution of flowers and plant mating (Pannell & Labouche, 2013). It has also been described to investigate the effects of self-pollen contamination on fruit set (Matsumoto et al., 2022), evaluating the effects of pollen load composition and deposition pattern on pollen performance (Németh & Smith-Huerta, 2002), and studies focussed on fertilisation study of incongruous pollen for interspecific crosses of lilies (Proscevičius et al., 2012). The current approach described here further complements existing studies that have described an optimum set of SSR markers required for the general fingerprinting of oil palm (Sarimana et al., 2021; Singh et al., 2007; Zolkafli et al., 2021). The strategy described here can be potentially expanded to other cross-pollinating crops such as pine (Eliott et al., 2005), olive (Díaz et al., 2007), coconut (Azevedo et al., 2018) and maize (Xu et al., 2017), where an optimal set of SSR and SNP have already been developed for routine DNA fingerprinting.

Undeniably the study has shown that the mixed pollen strategy with DNA profiling is an effective approach and can expedite the breeding programme by reducing the number of crosses required and minimising the time required to wait for the female flower cycle. However, to ensure success and to obtain a sufficient number of seedlings attributed to a particular pedigree, issues such as low germination rate and destruction of seedlings in nurseries by rodents or other factors have to be minimised. Limited sample size could lead to a downward bias of the mixed pollen approach and limit the potential of this approach to improve the efficiency of the breeding exercise.

CONCLUSION

The prospect of implementing a mixed pollen approach is very promising and it offers a promising new strategy to implement in oil palm breeding programmes. The vast majority (>99%) of the progenies in all mixed pollen crosses were successfully discriminated to their related pedigrees using a panel of 108 SNP markers in the fingerprinting analysis. The number of seedlings contributed by a particular pollen source in the mix appears to be influenced by pollen viability and to some extent length of pollen storage as well as the genetic background, and possibly the age of pollen-producing palms. All these factors may need to be considered in implementing the pollen mix approach. There is no denying that the adoption of molecular marker technology is a viable option in the breeding programmes of a perennial crop like oil palm. In line with this, the present study provides a novel strategy for using genomics tools to help develop new and improved oil palm varieties cost-effectively and efficiently.

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CONSERVATION OF PREDATORY ANTS *Myopopone castanea* SMITH (HYMENOPTERA: FORMICIDAE) IN AN OIL PALM PLANTATION IN NORTH SUMATERA

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ABSTRACT

The predatory ant, Myopopone castanea *Smith, F.* (*Hymenoptera: Formicidae*) *has been reported as a natural enemy for the oil palm pest,* Oryctes rhinoceros *Linnaeus* (*Coleoptera: Scarabaeidae*)*. This study aims to determine the distribution of the predatory ants among selected oil palm* Elaeis guineensis *Jacq.* (*Arecales: Arecaceae*) *plantations of North Sumatra Province, Indonesia. The presence of* M. castanea *colonies was conducted by checking several fallen and rotting oil palm trunks. Conservation efforts to ensure a sustainable ant population were made by inserting* M. castanea *colonies into each of the two deteriorating palm trunks, which were identified as sustainable habitats for the ants. The palm trunks were placed at the frond stacks and trunk heaps and kept in the open field for five days. It was discovered that* M. castanea *colonies, with variable ant counts in each colony, are present on several oil palm plantations. The results indicated that the frond stacks contained more dead prey and ants than the trunk heaps. Since ants do not have a preferred habitat for nest-building, rearing the ant colonies in the frond stacks should provide a higher population of ants compared to the trunk heaps.*

Keywords: conservation, *Myopopone castanea*, oil palm plantation, *Oryctes rhinoceros*.

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INTRODUCTION

The African oil palm *Elaeis guineensis* Jacq. (Arecales: Arecaceae) is planted in the tropics of Southeast Asia, the African continent, and the Americas (Brazil, Columbia, Costa Rica) (Cheng et al., 2019; Danylo et al., 2021; Tan et al., 2009). Oil palm plantations are mainly found in Indonesia, with an area reaching 16.883 million hectares in 2021 and crude palm oil (CPO) production at 45.10 million tonnes (Badan Pusat Statistik, 2022). Currently, Indonesia is the largest CPO producer in the world. It is imperative to manage oil palm cultivation in compliance with the principles set forth by the Roundtable on Sustainable Palm Oil (RSPO) to attain maximum yields in oil palm production (Köhne, 2014; Webber & Atchanah, 2015). This comprehensive management approach includes addressing various factors such as reducing the use of chemical fertilisers and pesticides (Wood & Norman, 2019a, 2019b) utilising empty oil palm fruit bunches to improve soil structure, and incorporating flowering plant cultivation to increase arthropod diversity; practices that exemplify a sound agroecosystem management in oil palm plantations (Bessou et al., 2017).

The management of ecosystems within the oil palm plantations includes a variety of conservation initiatives, with a focus on preserving

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the natural enemies which are native to these environments. According to Nurdiansyah et al. (2016), conservation of natural enemies is a critical biological control technique used to regulate pests in oil palm plantations. According to Rizali et al. (2019), effective habitat management practices are critical for ensuring the viability and persistence of these natural enemies. One notable habitat management strategy involves the strategic planting of diverse flowering plants alongside oil palm plantation thoroughfares. This intentional inclusion of flowering plants serves the dual purpose of providing nectar as a sustenance source for natural enemies, particularly parasitoids, as demonstrated by Dislich et al. (2017) and Tawakkal et al. (2019), and also as refugia plants (Mokoginta & Mohamad, 2022).

Biological control is the best and most environmental friendly alternative method to control pests and plant diseases. Many biological control agents that are being widely carried out for *Oryctes rhinoceros* pest, include the use of entomopathogens, such as *Metarhizium anisopliae* (Fauzana et al., 2020; Indriyanti et al., 2017; Suryanto, 2020), *Bacillus thuringiensis* (Pujiastuti et al., 2022), *Beauveria bassiana* (Indriyanti et al., 2021; Nasution et al., 2018), and *Baculovirus oryctes* (Rahayuwati et al., 2020).

Ants have long been recognised as one of the predators of many plant pests. *Oecophylla smaragdina* Smith. (Hymenoptera: Formicidae) is also a potential predator in controlling oil palm leaf-eating pests (Exélis et al., 2024). Falahudin (2013) stated that this ant can prey on the nettle caterpillar pest (*Setora nitens*) while Pierre and Idris (2013) explained that this ant is also a predator of the bagworm pest *Pteroma pendula*. In addition, other ant species that can be predators in oil palm plantations are *M. castanea* ants. This ant is a predator of *O. rhinoceros* larvae (Widihastuty, 2020). While these ant colonies are commonly observed in the smallholder plantations in the Binjai region, their prevalence in various private or state-owned plantations (PTPN) has not been thoroughly investigated (Susanti et al., 2017; Widihastuty et al., 2019). *M. castanea* ants typically establish colonies in decaying logs or fallen oil palm trunks infected with *Ganoderma*. The larvae and pupae of *O. rhinoceros* also live in piles of rotting organic material including fallen rotting oil palm trunks. The goal of this study is to look into the presence of the predatory ant *M. castanea* in oil palm plantations, towards implementing conservation measures to protect its presence within the plantation areas.

MATERIALS AND METHODS

Study Sampling Site

This investigation was carried out across various oil palm plantations located in various districts and cities throughout the Indonesian province of North Sumatra. The study focused on rotting oil palm trunks as the ants' nest within these plantations (*Figure 1*), and the exploratory method to detect the presence of *M. castanea* ants involved chopping, sectioning and dismantling the oil palm trunks. The validity of the *M. castanea* species was confirmed and identified at the Biological Research Center LIPI Bogor.

Figure 1. (a) The palm trunk stump as a nest, (b) colonies M. castanea *in the nest and (c) ants feeding hemolymph of* O. rhinoceros *larvae.*

When an ant colony was identified within the oil palm trunk, a specialised measuring device was used to assess the abiotic conditions within the ant nest, which included the temperature, humidity and pH levels. The abiotic environment data were described and analysed using the descriptive statistical analysis method. Following the discovery of existing ant colonies, specimens were carefully transferred to plastic containers and were transported and kept together in the laboratory.

Experimental Study Design

The study of *M. castanea* ants was conducted at the Bandar Khalifah PTPN 2 plantation in Deli Serdang Regency, North Sumatra (3°39′14.9″ N 98°48′14.0″ E). As part of the conservation efforts, a colony of *M. castanea* ants was introduced into two decaying oil palm trunks, each measuring 35 cm in diameter and 25 cm in thickness. In one of the palm trunks, a hole with a diameter of 15 cm and a depth of 5 cm was created to house 50 *M. castanea* ants and five *O. rhinoceros* second instar larvae (*Figure 2*). The experiment was repeated five times. The hole was then filled with another trunk. The trunk was strategically placed in two locations: One at the frond stack, and another in the trunk heap. These trunks were exposed in the field for five days. The trunks were then unsealed to assess the presence of *M. castanea* ants, with subsequent quantification and recording of the remaining ant population and prey mortality. The data observed was analysed statistically using t-test.

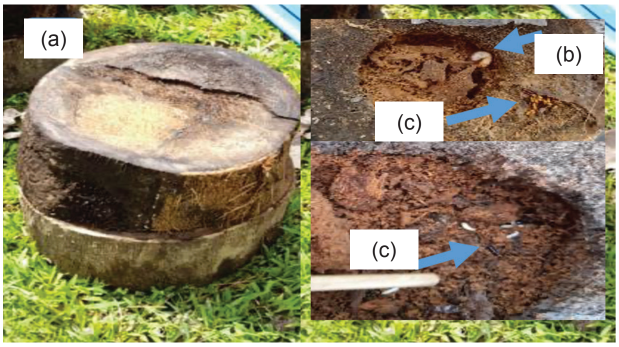

Figure 2. (a) Palm trunks conservation, (b) O. rhinoceros *larvae as a prey and (c) colonies* M. castanea.

The Exploration to Detect the Presence of *M. castanea*

The presence of *M. castanea* colonies (*Figure 3*) in various oil palm plantations in several districts and cities of North Sumatra is shown in *Figure 4*. *M. castanea* belongs to the primitive ant group of the Amblyoponinae family, which is usually found in the forest and commonly lives on rotting logs (Wilson, 1971). *M. castanea* ant nests were rarely found in plantations with young palms because rotting trunks are rare.

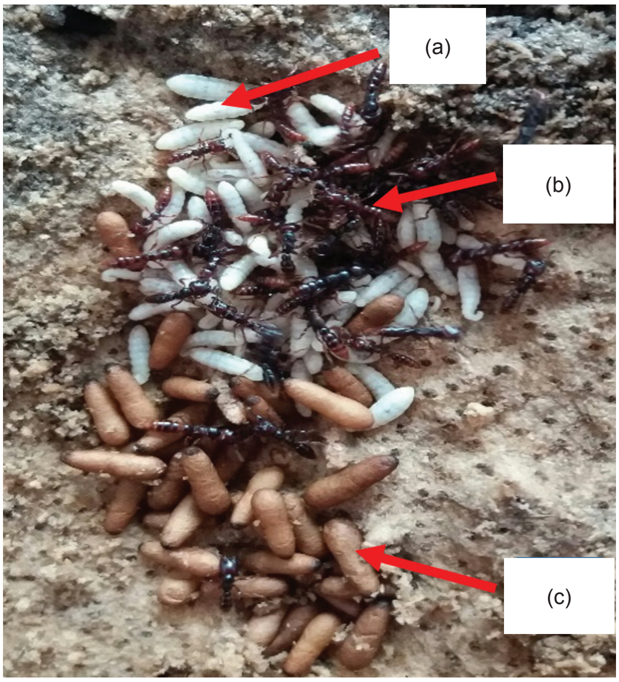

Figure 3. M. castanea *colonies; (a) larvae; (b) ant worker and (c) pupae.*

Environmental factors strongly influence the presence of ants in an ecosystem. The abiotic environment within the colony of *M. castanea* ants in this exploratory study is shown in *Table 1*. The mean temperature obtained ranged from 28.77°C–30.44°C, while the mean humidity was 69.75%–76.33%. The mean pH found was 6.05–6.20. The abiotic environment factors such as temperature, humidity and light conditions affect the foraging patterns, distribution and activity of the ant species (Philip et al., 2018).

Land use changes affect the availability of suitable habitats for ants, leading to shifts in ant species composition and a decrease in ant diversity (Johari et al., 2021; Philip et al., 2018). The ecological roles of plants in oil palm plantations, which provide ground cover to regulate soil moisture and producing litter for nutrient formation, can impact the availability of resources for ant species that nest in decaying logs (Nahlunnisa & Kwatrina, 2023; Syarif et al., 2025). According to Widihastuty et al. (2019), the average temperature within *M. castanea* nests was around 29°C, while the average ground surface temperature in immature plantations was generally higher than in mature plantations. This is because the palm crowns in the mature plantations have overlapped with each other, hence lowering the temperature of the ground surface.

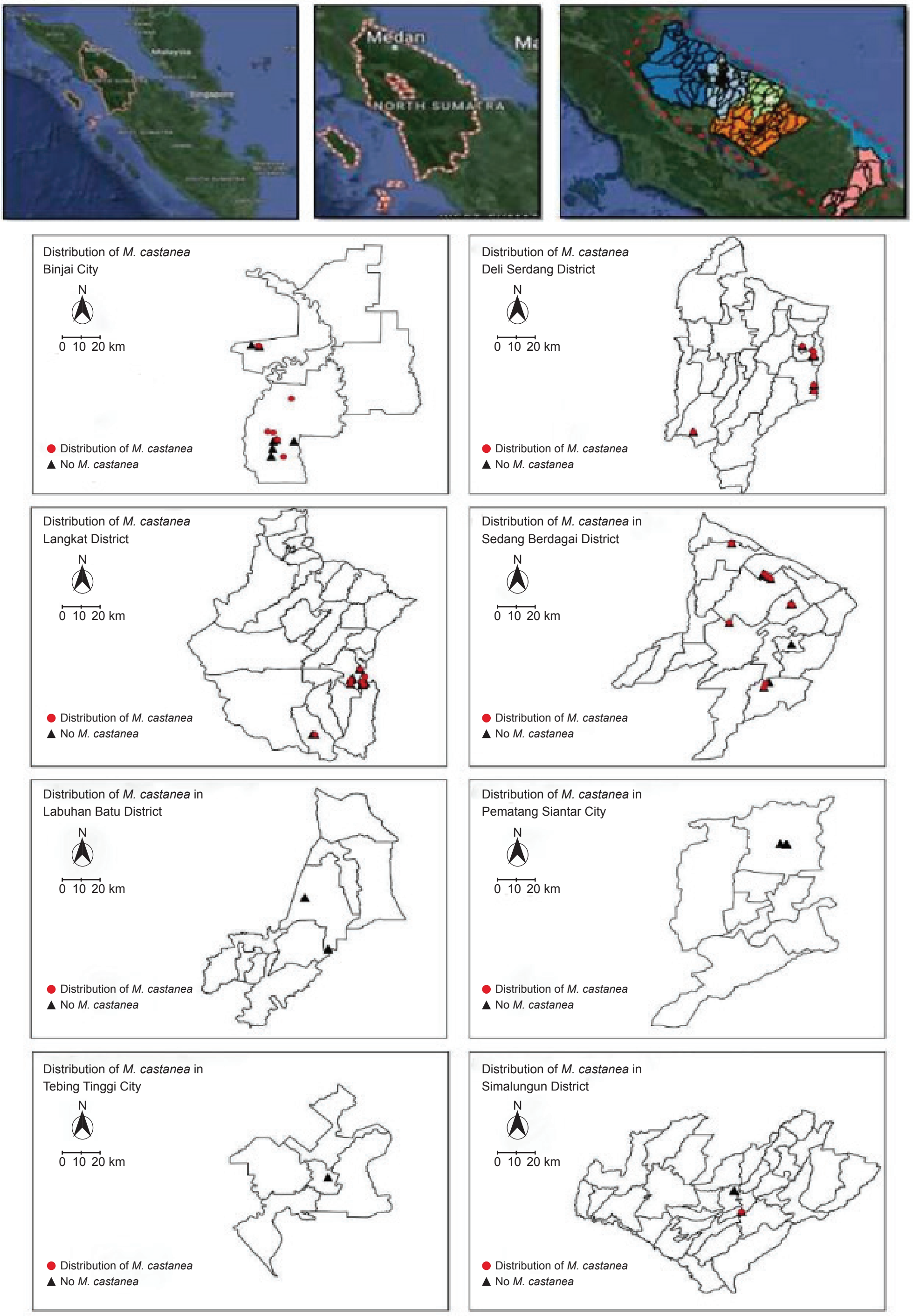

Figure 4. Distribution of M. castanea *ants in several oil palm plantations in several districts/cities in North Sumatra Province.*

TABLE 1. THE AVERAGE ABIOTIC ENVIRONMENTAL CONDITIONS OF *Myopopone castanea* NEST IN SEVERAL OIL PALM PLANTATIONS

Regency	Temperature (°C)	Humidity (%)	pH
Binjai	29.55 ± 0.63	74.75 ± 2.38	6.05 ± 0.21
Langkat	30.16 ± 0.47	70.20 ± 3.53	6.12 ± 0.18
Deli Serdang	30.44 ± 0.45	69.75 ± 0.88	6.08 ± 0.20
Serdang Bedagai	29.81 ± 0.63	70.06 ± 0.97	6.20 ± 0.23
Simalungun	28.77 ± 0.36	76.33 ± 1.78	6.07 ± 0.09

There are very few fallen oil palm trunks in private plantations, particularly in the Labuhan Batu Regency, causing fewer chances for the *M. castanea* to build their nests. Private plantations are typically very strict in keeping their plantations free of decaying oil palm trunks, as it is feared that they will become breeding sites for *O. rhinoceros* beetles (Egonyu et al., 2022). The oil palm plantations in the Labuhan Batu district were planted on peat soil, with an average pH of 4.28. The presence of organisms and microorganism activity is also determined by acidic soil pH conditions. Many microorganisms, including fungi and bacteria, are involved in the weathering of wood. These microorganisms have less activity in decomposing cellulose compounds in acidic soil than in neutral soil pH conditions (Hafif et al., 2022). Furthermore, not every oil palm trunk site investigated will have the *M. castanea* ant colony. There are many abiotic factors which influence ants to build nests or colonies in the decaying trunks. Temperature, humidity and the degree of weathering in the wood will all influence the nesting of the ants. Widihastuty et al. (2019) discovered that the C/N ratio of oil palm trunks that have weathered between 66%–69% can become conducive for *M. castanea* ants to build their nest.

The larvae of *O. rhinoceros* are not always found in every rotting oil palm trunk. If no *O. rhinoceros* larvae were discovered in the fallen trunk, most likely, there would be no *M. castanea* ant colony defected. The absence of the prey in an ecosystem will undoubtedly affect the higher trophic level (natural enemies), hence reducing the chances of finding their population in the ecosystem (Suman et al., 2023). *M. castanea* ants are predatory ants with a narrow prey range, as opposed to other predators in general, which typically have a broad prey range. *M. castanea* is a predator that feeds on the hemolymph fluid of Coleoptera larvae (Ito, 2010).

Myopopone castanea Ant Colonies

The *M. castanea* ant colonies which are mainly found in several oil palm plantations are mostly occurring without the alate queen. As shown in *Table 2*, only a small number of colonies have a comprehensive caste system. *M. castanea* ants only have two castes in their colony, namely the worker caste and the reproductive caste, which consist of winged male and female ants. If the female ant has mated with a male ant, they will shed their wings and function as a queen in the colony (Widihastuty et al., 2020).

Most ant colonies found in exploratory research were colonies that did not have an alate queen. This phenomenon is called gamergate. The gamergate phenomenon, which is characterised by the absence of reproductive castes but with the presence of groups of eggs, was observed in several ant colonies. The gamergate phenomenon describes a scenario in which the worker ants gain the ability to reproduce sexually after mating. Most ant species have sterile female worker ants; however, the gamergate phenomenon has been observed in taxa where the worker ants have sperm reservoirs that function similarly to a spermatheca (Peeters & Fisher, 2016; Schmidt & Shattuck, 2014).

According to Ito (2010), the *M. castanea* worker ants have ovarioles that are similar to those of the queen ants. This factor strengthens the suggestion that these ants may also exhibit the gamergate phenomenon, although there was no previous study to explain why and how the gamergate phenomenon occurs in *M. castanea* ants.

Conservation of the Ant *M. castanea*

The conservation efforts for the *M. castanea* ant were carried out in two distinct locations: The frond stack and sites where there were abundant decomposing oil palm trunks heaps resulting from the previous replanting activities. During the five day field experiment, the predation rate of *M. castanea* ants had reached up to 80% in frond stack, and 68% in trunk heap areas. Notably, the number of individual ants remaining after the exposure period was higher in the frond stack than in trunk heap locations (*Figure 5*). The t-test analysis for the number of remaining ants revealed a significant correlation effect ($R = 0.3202$, $p = 0.00125$), however, the t-test analysis for predation on the frond stack and the trunk heap showed no significant effect ($R = -0.1648$, $p = 0.52913$).

The increased predation rate in the frond stack was likely attributed to a large number of ant colonies that remained in the conservation trunks, sustaining the predation process for as long as the colonies remained. In contrast, fewer individual ants were observed in the trunk heaps locations, raising the possibility that *M. castanea* ants may have emigrated to other decomposing oil palm trunks. Widihastuty et al. (2021) discovered that when given the choice of odour between rotting palm oil trunks and *O. rhinoceros* prey, *M. castanea* ants prefer the odour of decomposing palm oil trunks over that of their prey. This preference can be explained by the idea that ants prioritise habitat or niche selection to protect their colony. Eventually, they would engage in predation to meet the nutritional needs of their colony. Foraging requirements, resource availability, the presence of similar species, and physiological considerations are all factors that influence their habitat selection (Stahlschmidt & Johnson, 2018).

TABLE 2. *Myopopone castanea* ANT COLONIES FOUND IN SEVERAL OIL PALM PLANTATIONS

Regency/city	Point sample	Number of ants	Ants colonies			
			Eggs	Larva	Pupa	Reproductive castes
Binjai	1	87	0	65	0	
	2	263	123	144	86	Female = 3 Male = 6
	3	135	0	82	0	
	4	87	0	66	54	
	5	112	0	80	24	
	6	76	0	0	77	
	7	134	0	88	65	
	8	65	0	77	54	
Langkat	1	98	67	53	34	-
	2	156	0	78	30	
	3	185	0	88	57	
	4	68	0	45	23	
	5	72	0	0	69	
Deli Serdang	1	238	147	98	113	Female = 4 Male = 6
	2	146	0	75	10	
	3	62	0	43	65	
	4	167	0	78	49	
	5	138	87	98	43	-
	6	96	0	42	38	
	7	120	0	65	45	
	8	97	0	0	64	
Serdang Bedagai	1	190	0	95	86	
	2	95	0	89	38	
	3	178	0	60	49	
	4	243	110	147	124	Female = 4 Male = 8
	5	124	0	152	0	-
	6	112	0	78	97	
	7	90	78	65	0	
	8	92	0	77	60	
	9	256	167	190	178	Female = 5 Male = 10
	10	88	0	89	77	
	11	102	0	75	64	
	12	99	0	57	66	
	13	198	87	102	48	
	14	77	0	68	54	
	15	89	0	69	77	
	16	105	0	79	65	
Simalungun	1	245	118	238	174	Female = 8 Male = 18
	2	176	0	189	123	-
	3	104	0	97	62	-

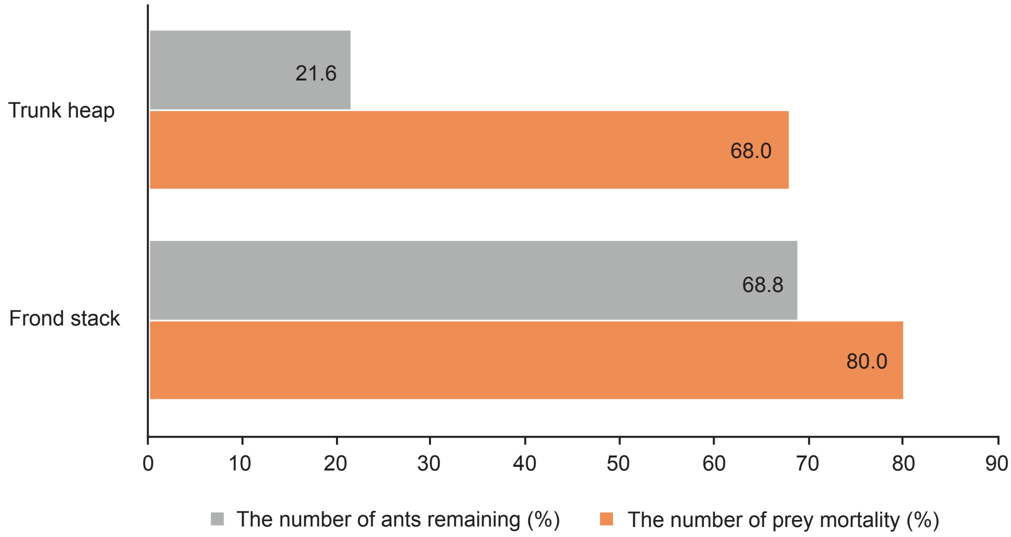

Figure 4. Percentage of the number of dead prey and the number of ants remaining in the conservation log.

The number of ants left in the conservation trunk heaps was found to be lower than at the frond stacks. This disparity was attributed to the possibility that the ant colonies in the trunk heaps may have shifted to the other decomposing heaps of oil palm trunks, as the volatile odour emanating from the rotting palm oil trunks is especially appealing to the ants.

Decomposing oil palm trunks emit a wide range of volatile compounds, the majority of which are hydrocarbons. The results of GC-MS analysis conducted by Widihastuty et al. (2021) on weathered oil palm trunks found that the dominant volatile compound was naphthalene, 2-methyl-(CAS)2-methylnaphthalene. Ants frequently communicate using hydrocarbon compounds (Chomicki & Renner, 2017; Sprenger & Menzel, 2020). Wooden logs that have decayed extensively emit a strong odour of volatile hydrocarbons, making them more appealing to ants (Hailini et al., 2020). Huber and Knaden (2018) also discovered that ants, such as *Cataglyphis fortis*, are adept at distinguishing the odour of their nest from the smell of the food provided to them.

CONCLUSION

The presence of *M. castanea* ants in oil palm plantations can still be found, but requires many explorations as their colonies are rare. As a result, immediate conservation measures are required to ensure the continued existence of these ants as effective biological agents, especially for *O. rhinoceros*. Ant conservation can be accomplished by strategically placing oil palm trunks in the frond stack areas and other locations that do not interfere with plantation operations. This proactive approach aims to provide suitable habitats for *M. castanea* ants, fostering their colonies and contributing to their role as important contributors to the ecological balance of the oil palm ecosystem.

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STUDY OF THE ANATOMICAL STRUCTURE ALONG THE OIL PALM FRUIT BUNCH STALK AND ITS IMPACT ON CUTTING PRESSURE

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ABSTRACT

The variation in anatomical characteristics of 16-year-old African oil palm fruit bunch stalk and its effect on mechanical cutting were investigated. Variations in vascular bundle (VB) density, VB size, parenchyma tissue area ratio, and total cross-sectional area along the length of the fruit bunch stalk were analysed. The correlation between cutting pressure and the corresponding anatomical structures was then established. Results show that vascular bundle density decreases from the proximal to distal positions of the fruit bunch stalk, whereas total cross-sectional area and vascular bundle size increase from the proximal to distal positions. The variation of the parenchyma tissue area ratio does not vary significantly. Multivariate regression analysis shows a positive correlation between VB density and VB size with cutting pressure ($R^2 = 0.741$), with the influence of VB density on cutting pressure being much larger than the effect of VB size. From the results of complete fruit bunch stalk cutting, it is found that the required cutting pressure is lowest when cutting at the middle section of the stalk, halfway between the stalk ring and the fruit bunch rachis.

Keywords: anatomical structure, cutting resistance, fruit bunch stalk, oil palm, vascular bundle.

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INTRODUCTION

The oil palm is one of the most valuable crops in the agriculture industry. A total of 5.67 million hectares of land in Malaysia will be utilised for oil palm plantations in 2022 (Malaysia Palm Oil Board [MPOB], 2022). Globally, the palm oil industry had a market size of USD70.44 billion in 2023 and is forecast to reach close to USD100.00 billion per year by 2030 (Grand View Research, 2024). Palm oil is ubiquitous – used to make a range of everyday products such as foods, cosmetics, soaps, lubricants and biodiesel (Basiron & Weng, 2004). There has also been extensive research on the use of palm oil by-products to produce furniture, animal feed, paper and biomass fuel to eliminate waste from the palm oil industry (Basiron & Weng, 2004; Suhaily et al., 2012).

The common type of oil palm cultivated in Southeast Asia is the species *Elaeis guineensis*. It is a monocotyledon and its stems generally comprise parenchyma tissue (PT) and vascular bundle (VB) fibres (Parthasarathy & Klotz, 1976). Monocotyledons and their VBs appear to be distributed randomly across the transverse section of the trunk/stem. However, study has since shown that the VBs actually follow a systematic pathway

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through the stems of the plants (Zimmermann & Tomlinson, 1972). VBs of monocotyledons function as the primary supporting structure and the transport vessels of the plant. They consist of the xylem and the phloem, which transport water and nutrients respectively (Corley & Tinker, 2016; Dickison, 2000; Tomlinson, 1961). PTs on the other hand are the site for metabolic processes like glucose conversion into starch as well as its storage (Bakar et al., 2008; Lim & Gan, 2005). Crucially, VBs can be visibly distinguished from the surrounding PT.

Though efforts have been put into the mechanisation of oil palm harvesting (Hao & Lai, 2024; Qun & Ripin, 2025) the harvesting of the oil palm fruit bunch stalks (FBS) has relied solely on manual labour using chisels and sickles, which is labour intensive and physically demanding. To improve the harvesting efficiency of oil palm FBS, Jelani et al. (1998) investigated the effect of cutter design, cutting angle and frond age on cutting force using conventional sickles and a mechanical claw. Similarly, Intara et al. (2013) investigated the physical and mechanical properties of oil palm FBS and fronds for use in designing mechanical pruners and harvesters. It has been shared by harvesters that the difficulty of cutting FBS depends on which section along the FBS is being cut. Therefore, the variation in the FBS's anatomical structure from the base (proximal) to the top (distal) of the FBS may be the reason for the difference in cutting difficulty.

Darwis et al. (2013) examined the correlation between VB distribution and the mechanical properties of the African oil palm (*E. guineensis*) trunks (OPT). Their results showed that the modulus of rupture (MoR) and the shearing resistance parallel to the grain of the OPT increased as the VB density increased from bark to pith, indicating a directly proportional relationship between VB density and these mechanical properties. In the same study, however, they also found that the trunk taken from the top segment of the OPT has lower mechanical strength compared with the bottom segments despite having more VBs. The authors attributed this observation to the age of the trunk section, which is younger at the top segment than at the bottom segment.

Fathi and Frühwald (2014) investigated the effect of VB dimensions and distribution on the mechanical properties of coconut palm (*Cocos nucifera*) trunks. The obtained results showed that the mechanical strength of a coconut trunk decreases as the VB density decreases from the bark to the pith section. A similar study on date palm (*Phoenix dactylifera*) trunks, on the other hand, yielded the opposite result. Fathi et al. (2017) discovered that the tensile strength and modulus of elasticity of trunk samples increased with decreasing VB density and the number of VBs from bark to pith of the date palm, which is the opposite correlation to their initial study on coconut palm.

According to studies on the vasculature of monocotyledonous plants, VBs indeed have a greater influence on the mechanical properties of the stem/stalk/trunk than the surrounding tissue. In previous studies, however, shearing resistance perpendicular to grain and its relationship to VB density, have not been studied. Furthermore, there are no systematic studies on this relationship with oil palm stalk. Since oil palm bunch harvesting involves the cutting of a large volume of FBS, this information is essential in improving the harvesting efficiency of oil palm FBS.

In this article, the variations in cross-sectional anatomical properties i.e., VB density, VB size, PT area ratio and total cross-sectional area along the proximal to distal sections of the FBS are determined. The effect of the anatomical properties on the cutting pressure is analysed. Multivariate regression analysis is used to establish the relationships between the anatomical properties and cutting pressure.

MATERIALS AND METHODS

The type of oil palm used in this study is cultivated by SD Guthrie Bhd. (Malaysia). It is of the species *E. guineensis* of the Deli *dura* x AVROS *pisifera* variant. All FBS samples used in this study were taken from different palm plants within the same plantation group of 16 years old. Furthermore, the FBS samples are of the same stage of ripeness corresponding to the age of harvesting to reduce the differences in development stages between different FBS samples. Freshly harvested FBS are transported to the lab and processed on the same day.

The region of stalk cut during harvesting is along the stalk peduncle, between the stalk ring and the fruit bunch rachis. So anatomical structure and force measurements are made within this region. The experiment is divided into two parts. In the first part, the anatomical feature measurements and mechanical cutting are conducted on small, consistently dimensioned sub-sections of the stalk cross-section. This method avoids additional forces e.g., the wedging effect and surface contact friction during the cutting process, isolating the cutting interaction between the blade and FBS's anatomical features. This section of the experiment will be referred to as "sub-section cutting". The second part involves the analysis of anatomical variations along the oil palm FBS and the study of the cutting force required during actual harvesting. This section will be referred to as "complete FBS cutting".

Sample Preparation

For the preparation of sub-section stalk samples, an FBS was first cut into five sections along its proximal to distal length with the most proximal position assigned as No. 1 and the most distal position assigned as No. 5. The most proximal position is between 2 and 4 cm from the stalk ring and each subsequent position is 3 cm apart. Each cross-section was further divided into sub-sections of approximately 2 × 1 × 1 cm as shown in *Figure 1c*. The sub-sections at the periphery of the cross-section have their rinds removed to eliminate their contribution to cutting resistance. A total of 21 sub-sections were prepared and analysed. In addition, a separate set of 23 sub-sections samples were prepared for validation purposes.

On the other hand, a total of 11 FBS were analysed for their anatomical variation along the stalk length. Cutting pressure measurement was also carried out on 7 of the FBSs. Each FBS was assigned a letter from A to K. Similarly, the cross-sections were numbered according to their positions along the FBS. The most proximal position was assigned as No. 1 and the most distal position was assigned the highest number according to the stalk length. Each section was approximately equidistant from its neighbouring sections. Stalks E and H were divided into seven and six sections, respectively, as these two stalks are longer in length. The remaining nine stalks were divided into three sections. An example of an FBS cut into three transverse cross-sections along the length of the FBS is shown in *Figure 2*.

In addition, the moisture content of FBS at different sections along the FBS stalks has been measured by comparing their weight before and after drying at 105°C for 24 hr (Mandang et al., 2018). The average moisture content is measured to be 81 ± 1%. Therefore, the contribution of moisture content to the variation in cutting characteristics along the FBS stalk is not significant.

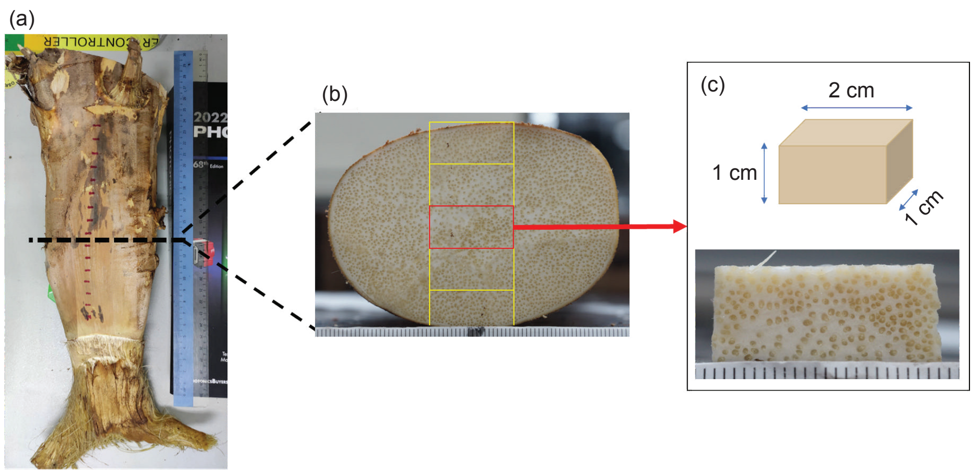

Figure 1. Division of cross-sections into smaller sub-sections for cutting. (a) Whole FBS, (b) cross-section of FBS and (c) sub-section with dimensions of approximately 2 × 1 × 1 cm.

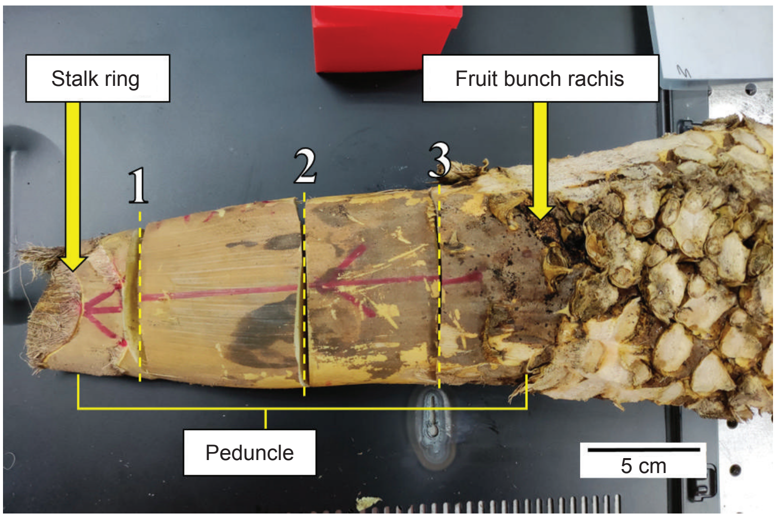

Figure 2. Stalk B is cut into three sections.

Image Analysis

Cross-section image analysis of small sub-sections was carried out using image processing software (ImageJ) and its features were measured manually. An example of a sub-section image analysed can be seen in *Figure 1c*. Three variables were measured for the sub-section image analysis which are the VB density, VB size and PT area ratio along with their corresponding uncertainties.

For the analysis of the complete FBS cross-section, an image of the entire cross-section was first captured. Then, close-up images of different parts of the cross-section were taken for image analysis. VB density, VB size and PT area ratio were then measured using ImageJ. All images were taken using a digital single lens translucent camera (Sony A77) with appropriate lighting. The images were first adjusted to improve the contrast between the VBs and the surrounding PTs as shown in *Figure 3a* and *3b*. The image was then converted to 8-bit greyscale and subsequently to binary image via binary thresholding as shown in *Figure 3c* and *3d*, respectively. The conversion to a binary image must fulfil the following two conditions: (1) Distinguishable VB silhouettes with minimal VB overlap, and (2) comparable VB silhouette size to source.

A region with clearly distinguishable VBs is required for accurate counting. An example of the region with distinguishable VBs is enclosed in a square shown in *Figure 3d*. *Figure 3e* shows the zoomed-in image of the enclosed square. Noise reduction was conducted followed by watershedding to produce an image with more well-defined VBs as shown in *Figure 3f*. The processed image is then compared with the initial, unedited cross-sectional image (*Figure 3a*) via visual inspection and manual VB size measurements. Any mismatch detected between the features in the two images is manually corrected (e.g., by drawing lines to separate VBs that were not watershed by software, filling holes in the VBs for an accurate measurement of VB size and manual removal of noise elements). A blue boarder around a feature indicates that the software identifies it as a particle as shown in *Figure 3g*. This process is repeated until the software accurately identifies all appropriate elements.

VB density was obtained by dividing the number of VBs counted by the area being analysed. The uncertainty in VB density is based on the estimated number of miscounted VBs. The value of the uncertainty was obtained by comparing the processed image with the actual image of a specific cross-section and identifying VBs that were missed or split. The average VB density uncertainty was measured to be around ± 3.5%.

The VB size was calculated by the software at the step shown in *Figure 3*. To verify the accuracy of

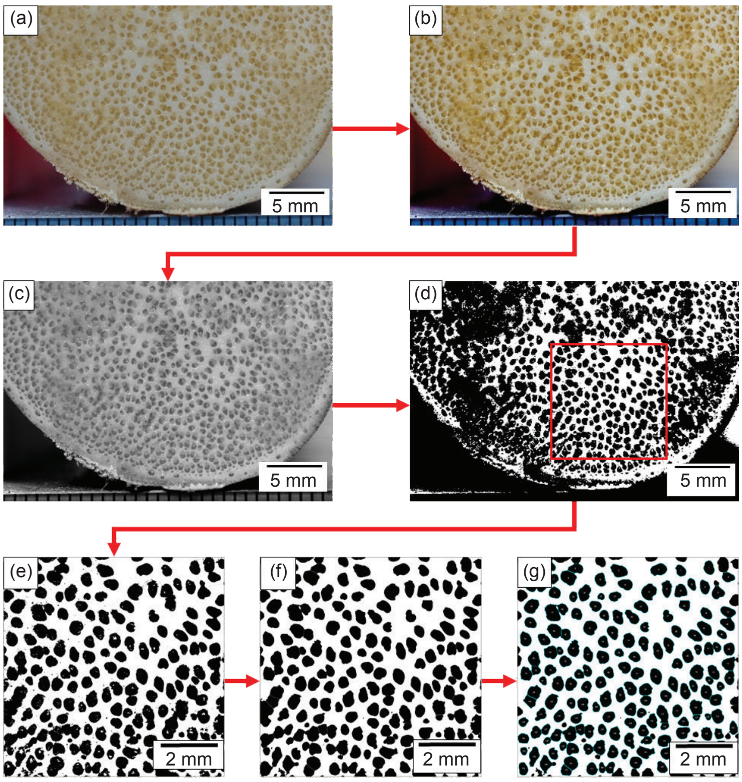

Figure 3. Image processing steps and the resultant images. (a) Original image, (b) contrast adjusted image, (c) conversion to 8-bit greyscale, (d) binary thresholding, (e) high distinguishability region, (f) noise reduction, watershedding, manual correction, and (g) particle counting and size measurements.

VB size, 30 selected VBs from the contrast adjusted image (*Figure 3*) were manually measured and compared with the VB sizes obtained from software analysis. The average VB size was obtained by averaging all measured VB sizes. The measurement uncertainty in average VB size is approximately ± 6%.

Finally, the PT area ratio is derived from both VB density and VB size and was calculated using the following Equation (1):

$$\text{PT area ratio (\%)} = (1 - [\text{average VB size} \times \text{average VB density}]) \times 100\% \quad (1)$$

Cutting Force Measurement

Cutting was carried out using a cut force measurement apparatus. The apparatus is set up according to the Universal Testing Machine reported by Intara et al. (2013). A schematic diagram and a photo of the apparatus are shown in *Figure 4*. It consists of the stalk cutter, an aluminium frame, a weighing scale and a jack. The stalk cutter uses blades with a height and thickness of 25.00 and 0.65 mm, respectively. The blade edge is tapered at an angle of 17° with a tapering length of 2.00 mm. A blade edge width of 0.30 mm was used to calculate the cutting pressure. The stalk cutter frame that holds the blade is constructed of solid wood and is guided by aluminium rods and slide bearings. It is set up to apply a force perpendicular to the cutting surface of the blade.

An aluminium frame is erected over the stalk cutter and a jack is placed between the frame and the top of the stalk cutter. To measure the weight exerted during cutting, a weighing scale was placed under the stalk cutter. The weighing scale has a sensitivity of 50 g.

Typically, cutting force is measured in the form of a specific cutting force (Ahmad et al., 2020; Boydaş et al., 2019; Jelani et al., 1998; Zhang et al., 2018) which is analogous to shear force. The Equation (2) for specific cutting force (SCF) is,

$$SCF = \frac{F_{max}}{A} \quad (2)$$

where F_{max} is the maximum force exerted during the cutting and A is the cross-sectional area of the cut plane.

However, it is more relevant to calculate the pressure required to cut through the stalk using the relationship in Equation (3):

$$\text{Pressure (N.mm}^{-2}\text{)} = \frac{\text{Weight measured (kg)} \times \text{g (10 m.s}^{-2}\text{)}}{\text{Blade edge width (0.3 mm)} \times \text{stalk-blade contact length (mm)}} \quad (3)$$

When cutting sub-section samples, the samples are placed flat on the cutter bed with the fibres oriented perpendicular to the cutter blade. The cut force and its corresponding cross-sectional anatomy for each sub-section cut were measured at the mid-section of the sample.

When cutting the complete FBS stalk, the orientation of the fibres relative to the blade is similar to sub-section cutting. The FBS stalk is first cut into smaller sections for ease of handling and placed flat on the cutter bed. Due to the irregular shape of the stalk segments, wedges were placed underneath the stalk segment to avoid tilting during cutting. The force is measured at the instance when the blade is fully embedded into the

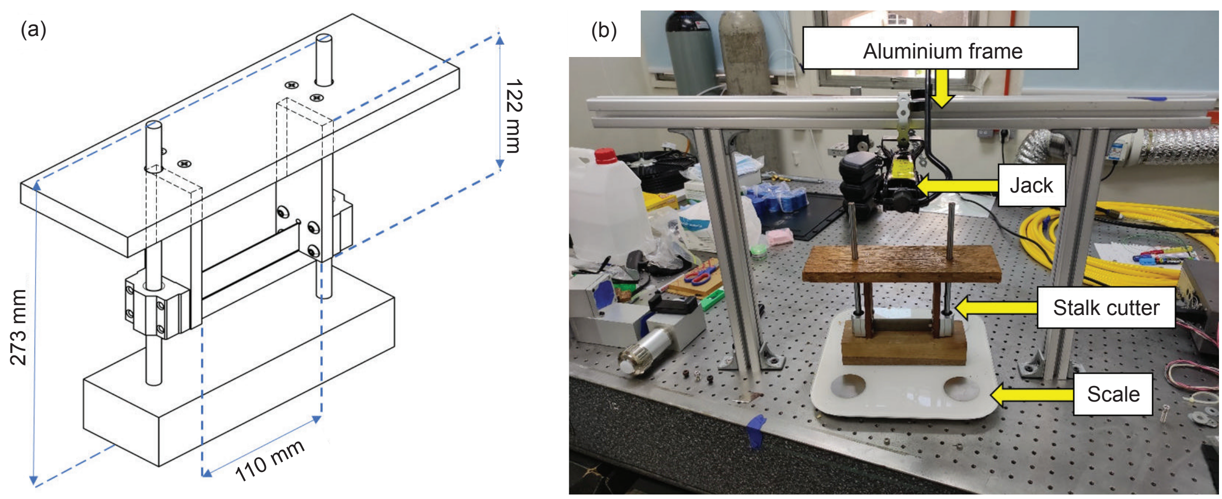

Figure 4. (a) Schematic drawing of stem cutter and (b) photo of the complete setup.

FBS. This position is selected primarily to minimise the wedging effect or friction on the blade that occurs as it further embeds into the stalk's tissue. This method also standardises the measurement process across all samples. Calculating pressure normalises the force exerted on a unit of area, which takes into account the contribution of varying sample width to the cutting force required to cut through. This is especially important when cutting complete FBSs as their widths vary along the cutting direction.

RESULTS AND DISCUSSION

Vascular Bundle Distribution Across the Fruit Bunch Stalks (FBS) Cross-Section

Figure 5 shows three cross-sections taken from three different proximal to distal positions along stalk D. Using ImageJ to mark the radial distance, the distribution of VBs was qualitatively assessed. It is found that the VB distribution changes along the length of the FBS. The proximal section (No. 1) has the highest density of VBs at the centre of the FBS as shown in *Figure 5a* and *5b*. A reduction in VB density begins at 0.4 R to 0.7 R of the FBS (R is the radial distance from the centre to the edge of the FBS cross-section) before increasing again towards the circumference of the FBS. The middle section (No. 2) of the FBS and its close-up image are shown in *Figure 5c* and *5d*, respectively. It shows an evenly distributed VB density over the entire cross-section. The distal section (No. 3) of the FBS and its close-up image are shown in *Figure 5e* and *5f*. Similar to the proximal section, the distal section has a denser distribution of VBs at the centre of the cross-section. This distribution extends to approximately 0.7 R. Beyond this radial position, the VB density drastically reduces before increasing again towards the circumference of the FBS. In all of the three positions, the cross-sections show a radial symmetry for VB distribution.

Sub-section Cutting

A total of 21 sub-section samples have been used in this study. It was found that the VB density ranges from 97–187 cm^{-2}, while the VB size ranges from 0.00175–0.00347 cm^2. The PT area ratio, which is a product of VB density and VB size, ranges from 64.2%–77.3%. It is observed that higher VB density corresponds to smaller VB size and vice versa. The minimum cutting pressure measured was 12.0 $N.mm^{-2}$, which corresponds to the lowest VB density. Whereas the maximum cutting pressure of 22.9 $N.mm^{-2}$ was measured for the sample with the second highest VB density. The trends between cutting pressure and VB size as well as the PT area ratio are not obvious. However, the correlation between anatomical features and cutting pressure requires a more detailed statistical analysis to be established. As the cutting pressure may depend on more than one anatomical parameter, multivariate regression analysis was used to investigate their correlations.

Correlation Between Vascular Bundle (VB) Density, Size and Cutting Pressure

Multivariate regression analysis using the Generalised Reduced Gradient Method was carried out to investigate the correlation between the anatomical features and cutting pressure. Two anatomical features, namely VB density and VB size,

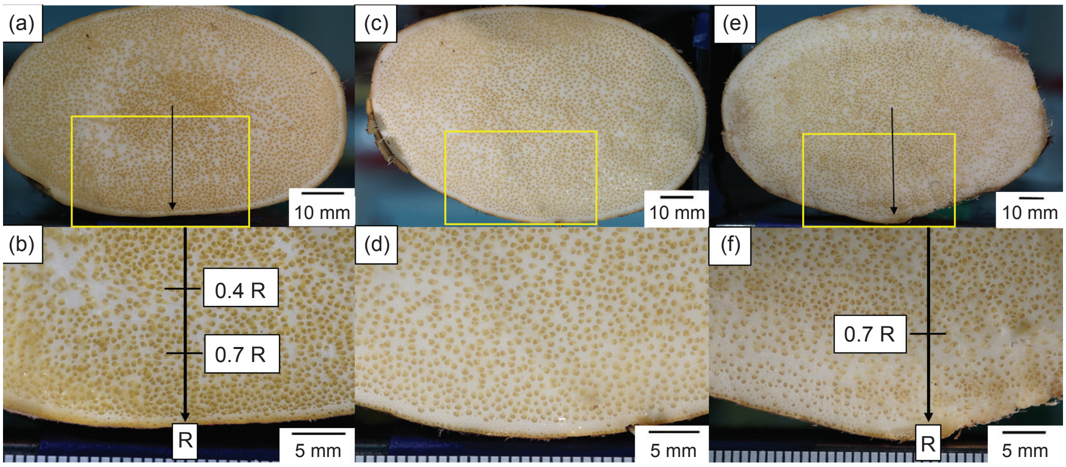

Figure 5. *Cross-sections of stalk F (a) at position No. 1 with x1.7 magnification; (b) at position No. 1 with x10.3 magnification; (c) at position No. 3 with x1.3 magnification; (d) at position No. 3 with x11.4 magnification; (e) at position No. 5 with x1.0 magnification; and (f) at position No. 5 with x11.6 magnification. The yellow box in the upper images highlights the regions that correspond to the bottom images. The black arrows represent an estimation of the radius (R) of the stalk cross-sections.*

are used as variables. Since the PT area ratio is the product of VB density and VB size, an individual term for the PT area ratio is not considered. An equation that weighs the contributions of VB density and VB size towards cutting pressure is postulated as Equation (4):

$$P = R + C_\rho \rho^\alpha + C_A A^\beta \quad (4)$$

where, P is the pressure, C_ρ and C_A is the scaling coefficients, ρ is the VB density, A is the VB size, R is the lump parameters, α and β are the power scaling of VB density and VB size respectively.

From regression analysis, the coefficients are determined to be $R = -0.4637$, $C_\rho = 0.1051$ N, C_A = 74.7465 N.mm^{-1}, $\alpha = 1$ and $\beta = 0.5$. The contribution of VB size to cutting pressure is found to be much smaller compared to VB density. Also, cutting pressure scales by the square root of VB size. The lump parameter, R was found to be close to 0. This indicates that additional systemic contributors to the cutting force are insignificant. These coefficients are used to calculate the cutting pressure required for different VB densities and VB sizes. *Figure 6* shows the comparison between the calculated cutting pressures and their corresponding experimental values and a validation plot using a separate dataset to test the regression equation. The linear regression (R-squared) is 0.7413 with a gradient of close to 1, which shows good agreement between the calculated and experimentally measured cutting pressure.

The validation plot is obtained by processing a separate dataset consisting of 23 sub-section samples using the regression model. The gradient of the validation plot is close to that of the original regression dataset, which is 1.0726 vs. 1.0021 (a difference of 7%), with both datasets intercepting near the origin.

The results in *Figure 6* show that VB density is the primary contributor to cutting pressure. Although, there are no prior reports on the correlation between cutting pressure on the VB density of oil palm FBS, previous studies on OPT have shown a positive correlation between VB density and modulus of rupture (MOR), which is analogous to shear resistance perpendicular to grain (Darwis et al., 2013). Darwis et al. (2013) reported a MoR on OPT samples ranging from 5–20 N.mm^{-2} following a procedure similar to sub-section cutting. This shows that mechanical cutting forces for OPT and FBS are similar. Oil palm VBs, compared with PTs, have a higher amount of α-cellulose – the most stable and polymerised form of cellulose (Abe et al., 2013) which make up the thick-walled, fibrous sclerenchyma cells that surround the VBs. An increased Young's modulus and tensile strength are known to be attributed to increased cellulose content (McLaughlin & Tait, 1980). Thus, the combination of both great cell wall properties and high vascular bundle density would result in high mechanical properties of the overall plant tissue (Gibson, 2012).

VBs of the oil palm act like rebar in concrete by bounding the surrounding parenchyma tissue matrix more firmly, adding to the embedded material's shear resistance and overall mechanical properties (Dickison, 2000). Additionally, fibres going through a material impede the propagation of cracks within the material under stress. The greater the density of fibres the greater the reduction in breaking or cracking in the material/tissue, keeping it intact while the tensile strength of the uncut fibres resists the tissues separation (Moon et al., 2011; Wilson et al., 2013). In addition, the number of VBs that the blade edge comes into contact with at any instance during the cutting process is directly proportional to the VB density and FBS width. Therefore, with increasing VB

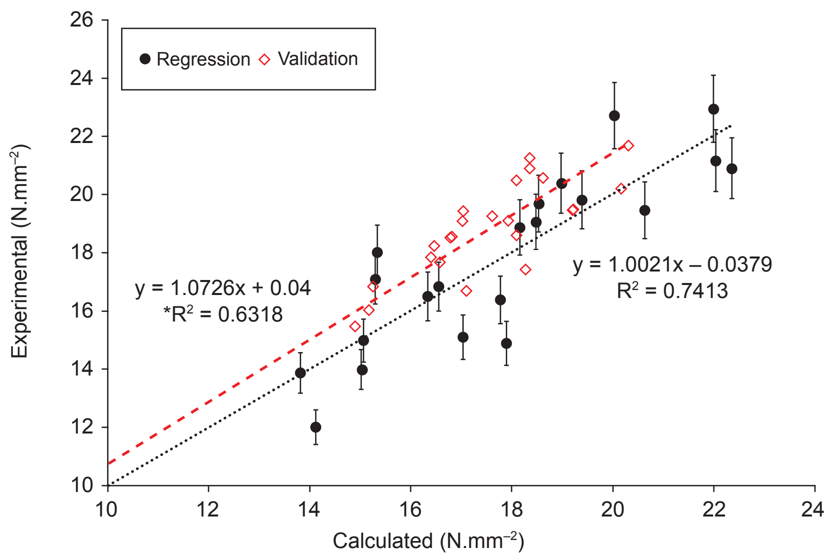

Figure 6. Sub-section cutting regression plot and validation plot.

density, the blade has to cut more VB fibres over the same width, which would increase cutting difficulty.

Complete Fruit Bunch Stalks (FBS) Cutting

Cutting force dynamics through stalk. *Figure 7* shows three different cutting force profiles that were observed when cutting through complete FBS. A smooth cutting process is shown in *Figure 7a*, where the cut force increases at the beginning of the cutting as the blade enters the stalk, and reaches a maximum around the middle section, before decreasing towards the end. The change in measured cut force corresponds to the stalk width profile along the cutting direction.

While a smooth cutting process is desirable, there were instances where in some cutting processes the blade is "caught" by the stalk tissue because of the wedging effect which is a countering force that acts against the separating force exerted by the blade (Libretexts, 2021). Coupled with friction between the stalk cross-section surface and the blade, these effects provide a formidable resistance to cutting which requires additional force to overcome. *Figure 7b* shows a sudden increase in cutting force around 26 mm into the stalk followed by a sudden decrease at 33 mm. This can be attributed to transitions between static and kinetic friction, also referred to as stick-slip motion experienced by the blade against the tissue of the FBS at low cutting speeds and under high friction (Berman et al., 1996; Martins et al., 1990).

In addition, there are also cuttings where the cutting force beyond the mid-section of the FBS remains high and does not return to zero at the end of the cutting process as seen in *Figure 7c*. This can be attributed to the deformation of the blade as it travels through the stalk tissue, which was visually observed. The deformation causes a gap between the blade and the surface of the stem cutter base. This results in the FBS rind remaining intact in the gaps between the blade and the base, while the frame of the blade holder is already pressing on the base.

Based on the analysis of the cutting force dynamics, the stick-slip motion and blade deformation occur after cutting through the centre of the FBS. Therefore, the measurement of cutting force was standardised and made at the instance when the blade is fully embedded (25 mm into the FBS). This coincides with the centre region of the FBS, where the maximum cut force was measured for most of the FBS samples.

Anatomy and pressure results. A total of 11 stalks were analysed for their anatomical variations from proximal to distal positions, while cutting pressure was measured in seven stalks. The total cross-sectional area for all FBS samples increases considerably from the proximal to distal positions. Increasing by 19% from positions No. 1 to No. 2 and a further 15% from positions No. 2 to No. 3. The average PT area ratio increases from proximal to distal positions by 4% across all FBSs. The variation is relatively small due to the simultaneous decrease

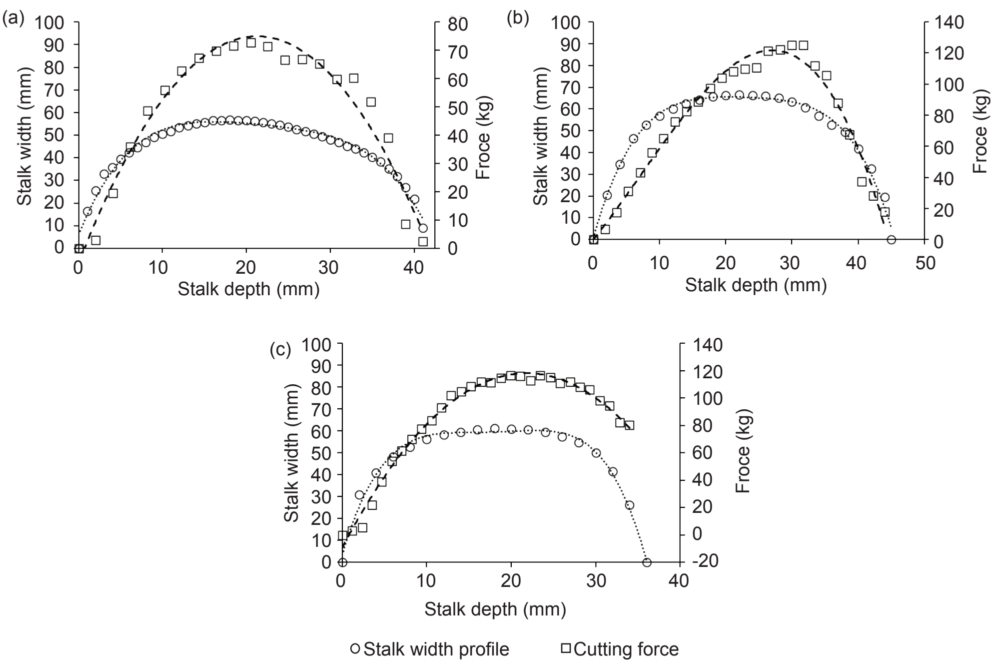

Figure 7. Changes in force at a given cut depth for three force-measured cut samples. (a) A relatively ideal cut with minimal sudden fluctuations in cut force, and a close to zero end cut force, (b) cut force spikes up after approximately 26 mm and cut force drops at 33 mm, and (c) cut force remains high at the end of the stalk.

in VB density and increase in VB size along the FBS. This falls within the measurement uncertainty. Coupled with the fact that the PT area ratio is a product of VB density and VB size, subsequent analysis will focus on the variation of VB density and VB size.

Figure 8a and *8b* shows the VB density and VB size values of each stalk at their corresponding positions as well as their average normalised values for each position. It can be seen that VB density trends downward from proximal to distal, while VB size increases from proximal to distal. For both VB density and VB size, a larger increase (decrease) was observed from positions No. 1 to No. 2 compared to from positions No. 2 to No. 3. On average, VB density decreases by 18% from positions No. 1 to No. 2 and a further 7% from positions No. 2 to No. 3. While VB size increases by 16% from position No. 1 to No. 2 and a further 1% from position No. 2 to No. 3.

The general path of the VBs of monocots along the stem is well understood. VBs spiral upward along the stem, then bend and divide into the leaves, inflorescence, or form bridges with other VBs (Corley & Tinker, 2016; Dickison, 2000). In the current study, the stalk surface is bare in the sense that it lacks leaves or other organs, until it reaches the fruits and spikelets. This limits VB branching at the proximal sections of the FBS to the interconnecting bridges between neighbouring VBs. Thus, up to the fruits, it can be postulated that the number of VBs remains relatively constant, only multiplying upon closer proximity to the fruits of the stalk. The total number of VBs measured across all samples on average increases by 15% from proximal to distal positions. However, VB density still reduces from proximal to distal due to the more significant increase in total cross-sectional area. On the other hand, there are no reports that explain the variation in VB sizes along the same FBS.

Figure 8c shows the measured cutting pressure of each stalk at their corresponding positions as well as their average values for each position. Based on the results, cutting through the complete FBS at the middle section requires the least force. The cutting pressure varies between 39.87 and 63.68 $N.mm^{-2}$, 39.12 and 49.65 $N.mm^{-2}$ and 42.46 and 55.19 $N.mm^{-2}$ for position No. 1, No. 2 and No. 3, respectively and their corresponding average cutting pressure are 54.08, 43.62 and 49.56 $N.mm^{-2}$, respectively.

In summary, results show that cutting through the middle section of the FBS requires about 10% less force compared to cutting close to the fruit bunch rachis. Given that position No. 1 of the FBS is usually covered by the fronds and the fact that the FBS size increases towards the fruit bunch rachis, cutting the stalk at its lowest exposed position will require the least amount of force. Although a 10% reduction in required cut force does not appear substantial, cumulative cutting of a large number of stalks during actual harvesting operations amounts to significant savings in energy expenditure. This will be increasingly essential when the harvesting process becomes further mechanised in the estates,

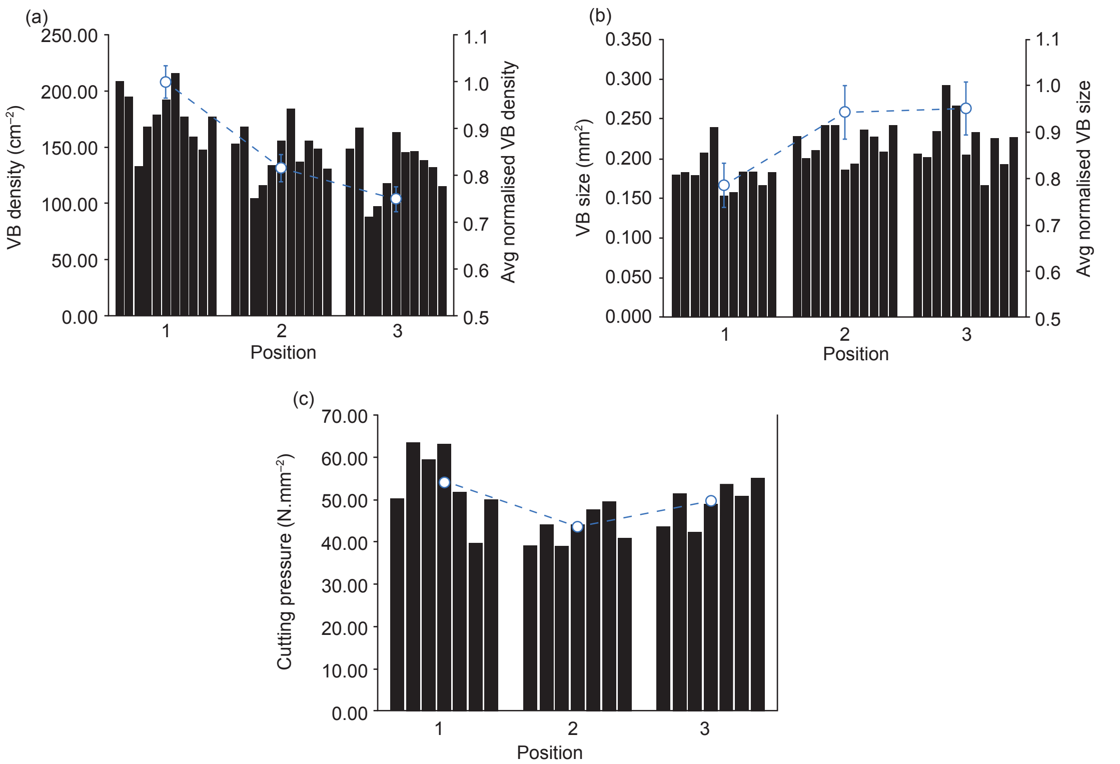

Figure 8. (a) Vascular bundle (VB) density, (b) VB size and (c) cutting pressure for different positions along the fruit bunch stalks (FBS). Each column represents a specific stalk, with the left most column being stalk A ascending to stalk K. The marker plot represents the average values for each position.

where energy consumption has to be strictly managed and optimised.

Note that the cutting pressure required to cut through complete FBS is more than double that for sub-section cutting. This can be attributed to the wedging effect and friction between the stalk cross-section surface and the blade as discussed above. Larger cross-sections introduce a larger friction and greater wedging effect on the blade, while also causing more blade deformation. To verify if the correlation between VB density, VB size and cutting pressure established in sub-section cutting still applies, a comparison between the measured cut pressure and the calculated values is made in the next section.

Comparisons with sub-section cutting. The relationship between cutting pressure and FBS anatomy was analysed using the same equation and coefficients determined from sub-section cutting. *Figure 9* compares the measured cutting pressure with the calculated values. A similar linear trend between experimental and calculated cutting pressure is still observed. In addition, although quantitatively separating the contributions of the material's strength and frictional effects has been difficult (Williams, 1998), the measured cutting pressure for complete FBS cuts is found to be 2.5 times higher than that required for sub-section cutting, with an average measured cutting pressure of 46.87 $N.mm^{-2}$ and the average calculated cutting pressure of 17.85 $N.mm^{-2}$.

Table 1 shows the multivariate regression analysis of both sub-section and complete FBS cutting. The table shows that sub-section cutting produces a better correlation between FBS cross-sectional anatomy and cutting pressure as evident by the better R^2 and significance, F-value. The *P*-values of the coefficients for both sub-sections and complete FBS cutting confirm that VB density is the most significant contributor to cutting pressure since it is the only coefficient with a *P*-value less than 0.05.

CONCLUSION

Cross-sectional anatomical structure analysis was conducted on oil palm FBS and its influence on cutting pressure was investigated. An equation relating VB density, VB size and cutting pressure is established. When cutting sub-sections of the FBS, results show that VB density has the most significant contribution towards cutting pressure. In the analysis of the anatomical structure of complete FBS, VB density was found to decrease from proximal to distal positions by an average of 25%, while VB size and PT area ratio increased from proximal to distal positions of the FBS by 17% and 4% respectively. When cutting through complete FBS, cutting pressure was found to be the least at the middle sections of the FBS, half-way between the stalk ring and fruit bunch rachis. Cutting through the complete FBS introduces additional resistances due to the wedging effect and stalk-blade surface contact friction. For oil palm FBS, specifically this results in 2.5 times increase in required cutting pressure when compared to sub-section cutting.

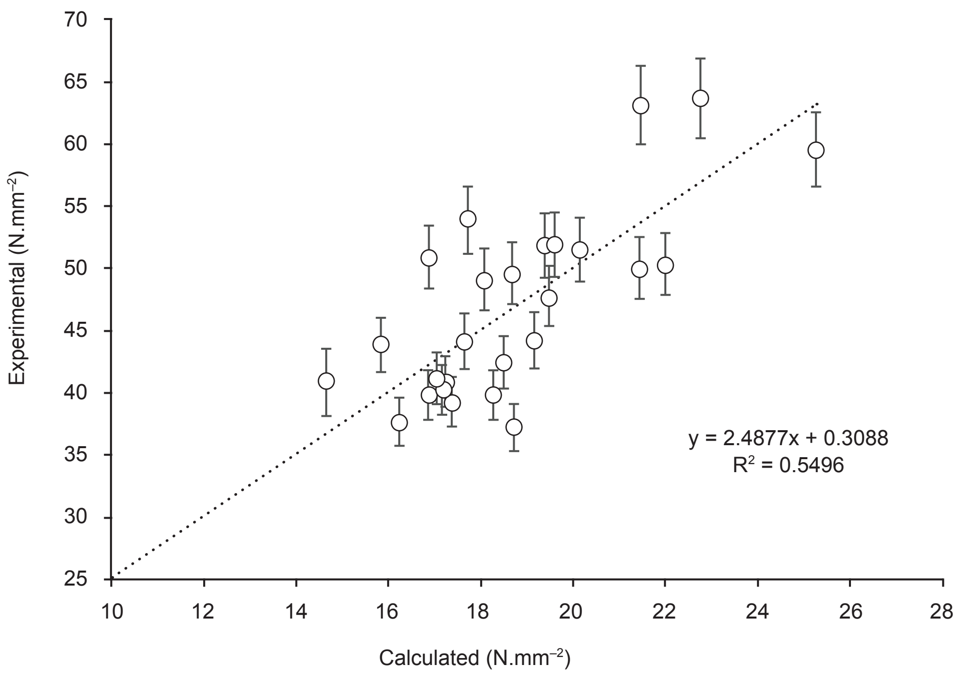

Figure 9. Plot of experiment versus predicted cutting pressure using the equation and coefficients from sub-section cutting.

TABLE 1. STATISTICAL RESULTS FOR MULTIVARIATE REGRESSION ANALYSIS OF SUB-SECTION AND COMPLETE FBS CUTTING

Sub-section cutting						
Item	**Coefficients**	***P*-value**	**R^2**	**Standard error**	**Sample size**	**Significance F**
Intercept	-0.4637	0.9585	0.7413	1.621	21	$5x10^{-6}$
VB density (cm^{-2})	0.1051	0.00011				
VB size (cm^2)	74.7465	0.5594				
Complete FBS cutting						
Item	**Coefficients**	***P*-value**	**R^2**	**Standard error**	**Sample size**	**Significance F**
Intercept	41.01088	0.112391	0.604609	4.950623	25	$4x10^{-5}$
VB density (cm^{-2})	0.19013	0.005156				
VB size (cm^2)	-486.075	0.209861				

Note: VB - vascular bundle; FBS - fruit bunch stalks.

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CHEMICAL PROPERTIES AND BIOCOMPATIBILITY OF MICROCRYSTALLINE CELLULOSE REINFORCED DENTURE BASE RESIN MATERIAL: AN *In Vitro* STUDY

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ABSTRACT

This study aimed to determine the chemical functional groups and cytotoxicity level of denture base resin (DBR) material reinforced with oil palm based microcrystalline cellulose (MCC) at different concentrations. Three MCC-reinforced polymethyl methacrylate (PMMA) specimens were compared with conventional and commercially available high-impact PMMA. The test groups were represented by adding various MCC and acrylic polymer concentrations. Fourier transform infrared spectroscopy (FTIR) was conducted to compare the chemical structure of the specimen groups. Cytotoxicity was evaluated by filter diffusion test. Extracts from study groups were tested using the MTT assay protocol for cell viability and proliferation with normal human oral fibroblast (NHOF). The FTIR analysis showed that good bonding between MCC-OPEFB and PMMA as the functional group strength was reliable in cellulose-treated PMMA. The analysis also confirmed high integrity and successful grafting between the two. Cell viability assays showed that exposure of NHOF to polymer-MCC mixture eluates did not promote cell death or considerable toxic impacts. Suitably processed oil palm-based MCC-reinforced DBR can improve the bonding and integrity of the composite material without compromising its biocompatibility leading to the development of reinforced DBR with enhanced microstructural and chemical properties.

Keywords: cytotoxicity, FTIR, microcrystalline cellulose, oil palm empty fruit bunch, polymethyl methacrylate.

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INTRODUCTION

Polymethyl methacrylate (PMMA) is widely used for removable complete and partial dentures due to its aesthetic qualities, biocompatibility and clinical performance (Im et al., 2017). However, its mechanical properties, specifically its low flexural (Karci et al., 2019) and impact (Mowade et al., 2012) properties, have prompted researchers to explore improving its physical characteristics. One approach is to add natural or synthetic additives, which have been shown to enhance the material's strength and durability (Gad et al., 2017; Soygun et al., 2013).

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Microcrystalline cellulose (MCC), an extracted compound, is deemed one of the richest yielded renewable organic materials (Seddiqi et al., 2021). MCC of the oil palm biomass is utilised to prepare environmentally friendly polymer composites (Soom et al., 2009), with its strengthening function and low density, increases the mechanical properties of the polymers and has appropriate aesthetic values. In addition, MCC has also been reported to enhance polymer composites' thermal stability, barrier properties, and biodegradability (Lang et al., 2022). MCC powder was homogeneously mixed with the PMMA polymer and distributed evenly in the resin dough mixture, despite its insufficient impregnation of fortifying particles like fibres, rods, or flakes within the polymer matrix due to its high polymer/monomer mixture consistency. Studies have reported that PMMA reinforced with MCC oil palm empty fruit bunch (OPEFB) fibre has better flexural strength and modulus compared to conventional and commercially modified PMMA resins (John et al., 2014).

Despite being the material of choice, some denture wearers have reported irritation and allergic reactions induced by the synthetic structure of the acrylic utilised as a denture base when in contact with the oral mucosa, primarily the unreached methyl methacrylate (MMA) residuals (Gautam et al., 2012). The incomplete conversion of MMA monomer to its polymer in the polymeric matrix will affect the properties of the final product. Besides, other toxic agents, such as methacrylic acid and formaldehyde residue, remain within the PMMA denture base after processing (Alqutaibi et al., 2023). One of the measures for biocompatibility is that the material is not toxic to cells (Kohli & Bhatia, 2013). *In vitro* cytotoxicity assays are necessary to screen any newly developed materials before clinical application. The aim of this study was to evaluate PMMA denture base resin biocompatibility when mixed with MCC in different weight percentages and its molecular composition and structure.

MATERIALS AND METHODS

Table 1 shows the commercial details of the conventional, high-impact acrylic and synthesised OPEFB based MCC materials.

Cytotoxicity Testing

Preparation of specimens. A total of five groups of denture base resin (DBR) test specimens (n = 3), measuring 65 x 10 x 3 mm were produced according to International Organization for Standardization (ISO) specifications (ISO, 2013). Samples of groups A and B (*Table 2*) were prepared with the powder-to-liquid ratio recommended by the manufacturer. Both PMMA and MCC powders were weighed according to *Table 2* using a digital weighing device (Sartorius-AX224, Sartorius AG, Germany) at an accuracy of ± 0.01 mg during specimen preparation for groups C, D and E. The specimens were prepared by slicing segments of the previous rectangular test pieces with a diamond cutting disc and made into cylindrical specimens with a measurement of 5.0 ± 0.2 mm diameter and 2.5 ± 0.2 mm thickness.

Cell Culture

Thawing of cells. Cryovials containing frozen normal human oral fibroblast (NHOF) were transported from liquid nitrogen to a water bath at room temperature. During the thawing, the vial was kept on the surface of the water bath by applying an intermittent light pressure. The cells were thawed and processed within a few seconds to ensure viability and relocated to a T-75 culture flask containing 30 mL of growth medium, then incubated at 37°C, 5% CO_2 at a flat position overnight. The medium was repeatedly replaced till the cell monolayers became confluent.

Specimen preparation for MTT assay. A total of 15 acrylic specimens of the five groups (n = 3) were placed in test tubes with Dulbecco's Modified Eagle's Medium (DMEM). An additional test tube was packed with only the medium and cells as a control. All test tubes were placed in a water bath at 37°C for 24 hr, then the acrylic specimens were removed gently from the test tubes and the NHOF extract was filtered using a sterile syringe filter of 0.22 µm. The NHOF were grown with 5% CO_2 at 37°C in the DMEM and 10% fetal bovine serum (FBS) containing penicillin/streptomycin and amphotericin-B. The cells were plated in 24-well tissue culture trays (104 cells/cm) for a 24-hr incubation period. After this period, the

TABLE 1. RELEVANT COMMERCIAL DETAILS OF THE MATERIALS

Material	Product information	Manufacturer
Acron Duo	Heat-cure denture base acrylic	Kemdent Works, United Kingdom
Acron HI	Heat-cure high impact acrylic	Kemdent Works, United Kingdom
MCC of OPEFB	Microcrystalline cellulose from oil palm empty fruit bunch.	Malaysian Palm Oil Board (MPOB), Malaysia

TABLE 2. MASS COMPOSITION OF THE SPECIMENS

	Test material	Powder (g)	Liquid (mL)	MCC (g)
A	Heat-cure denture base acrylic (conventional)	12.00	4.00	Nil
B	Heat-cure high impact acrylic	12.00	4.00	Nil
C	Heat-cure denture base acrylic (conventional) with 2% cellulose (2% of monomer liquid increased)	12.00	4.25	0.24
D	Heat-cure denture base acrylic (conventional) with 2% cellulose (2% acrylic powder reduced)	11.76	4.00	0.24
E	Heat-cure denture base acrylic (conventional) with 5% cellulose (5% acrylic powder reduced)	11.40	4.00	0.60

Note: MCC - microcrystalline cellulose.

cultures in the wells were subjected to cytotoxicity, and assessment was completed by using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test. Cytotoxicity was assessed based on cell viability. A percentage of cell viability greater than 80% is considered non-cytotoxic; 80%–60% weak; 60%–40% moderately and less than 40% strongly cytotoxic (López-García et al., 2014).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

All specimens from Group A, B, C, D and E used for a previous study (Rahaman Ali et al., 2020) were prepared by grinding one cured acrylic specimen from every group using a diamond bur. Before the FTIR testing, each ground specimen was carefully preserved in a dry, sterile and well secured container. The potassium bromide (KBr) method was used for the FTIR analysis. This involved mixing the ground acrylic with KBr in a ratio of 1:200 and compacting the mixture into pellets in a stainless-steel die.

Transmission spectra were sourced from a FTIR spectrometer with a spectral resolution of (SPECTRUM 400, Perkin Elmer, UK) and a wavelength accuracy of 650–4,000 cm^{-1}. Specimens from each group were placed in the window, and analysis was conducted by running the spectrum. The point of substantial transmittance peaks was established with the SPECTRUM™ 10 STD software (Perkin Elmer, USA).

RESULTS AND DISCUSSION

Results showed that all test groups were classified as non-cytotoxic, with no statistically significant difference among all groups; the viability of all groups was more than 90% (*Figure 1*). Among the five groups, the commercially reinforced specimens (Group B) showed the lowest cell viability (91.7%). In contrast, Group E specimens showed the highest cell viability (99.2%).

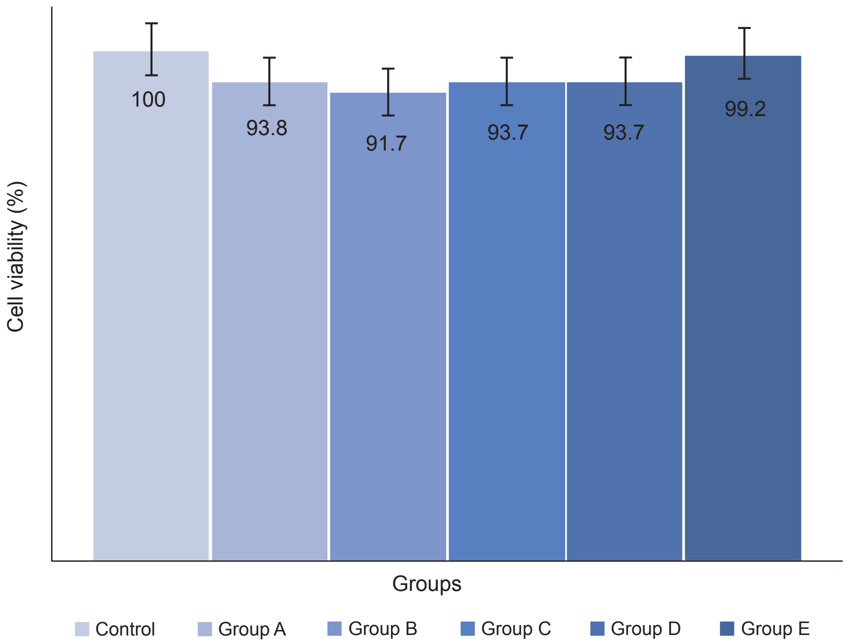

Figure 1. MTT test reporting on the cell viability of the specimens.

The FTIR spectrum for MCC from EFB (*Figure 2*) exhibited two main absorbance regions namely the functional group region (4,000–1,300 cm^{-1}) and the fingerprint region (1,300–400 cm^{-1}). The concentration of the peak at 894.11 cm^{-1}, associated with the β-glycosidic linkages, declined as the crystallinity of the samples intensified. The hydrolysis process affected the cleavage of glycosidic linkages of cellulose to produce MCC. The structural property of MCC was also considered by applying X-ray diffraction (XRD), and the outcome demonstrated that it has a crystallinity index of 72% (Fatiha et al., 2021). With a high crystallinity index, MCC is considered suitable for green bio composite production (Ramli et al., 2015). A high crystallinity index in MCC can significantly enhance the material's mechanical strength, rigidity and thermal stability. The ordered and tightly packed crystalline regions contribute to these improved properties, making the bio composite more durable and resistant to mechanical stress. Additionally, high crystallinity reduces moisture absorption, which is crucial for applications where dimensional stability and resistance to environmental factors are essential. This crystallinity also improves the compatibility between MCC and the polymer matrix, ensuring better dispersion and stronger interfacial bonding within the composite. These attributes make high-crystallinity MCC particularly suitable for producing bio composites used in demanding applications that require strength, stability and durability.

Figure 3 summarises the FTIR spectra of the PMMA specimens with and without MCC reinforced. The prominent bands in the zone ranging from 3,400–3,500 cm^{-1} are associated with OH bending, while the bands between 2,800–2,900 cm^{-1} are responding to –CH aliphatic compounds. The bands at 1,435–1,438 cm^{-1} represent $–CH_2–$ group bending. The penetrability at 1,141–1,158 cm^{-1} is relevant to the C–O–C group. It can be observed that all specimens show similar bands in the 1,000–1,800 cm^{-1} range. However, there are some differences in the intensity of the bands. The specimens modified with MCC show an additional band around 3,300 cm^{-1}, assigned to the hydroxyl (–OH) group of cellulose. This band is more prominent in specimens C, D and E, indicating that adding MCC increases the amount of –OH groups in the composite. In addition, specimens C, D and E show a decrease in the intensity of the bands between 1,720–1,750 cm^{-1}, which are related to the carbonyl (C=O) stretching vibration of PMMA. It suggests that MCC reduces the amount of C=O bonds in the composite. Adding MCC to PMMA modifies the chemical structure of the composite by introducing –OH groups and reducing the amount of C=O bonds. When MCC is introduced into PMMA, the composite undergoes significant chemical and structural changes. MCC is rich in –OH groups, which can interact with the C=O groups of PMMA through hydrogen bonding. This interaction weakens the C=O bonds, decreasing the intensity of the C=O stretching vibrations observed in the FTIR spectra. Additionally, the hydroxyl groups from MCC contribute directly to an increase in the –OH group content in the composite, as reflected by the more prominent –OH stretching bands. There may also be some chemical modification, where the hydroxyl groups from MCC react with the ester groups in PMMA, further reducing the number of carbonyl groups. These interactions and the potential disruption of PMMA's crystalline structure result in a composite material with increased –OH groups and a reduced presence of C=O groups.

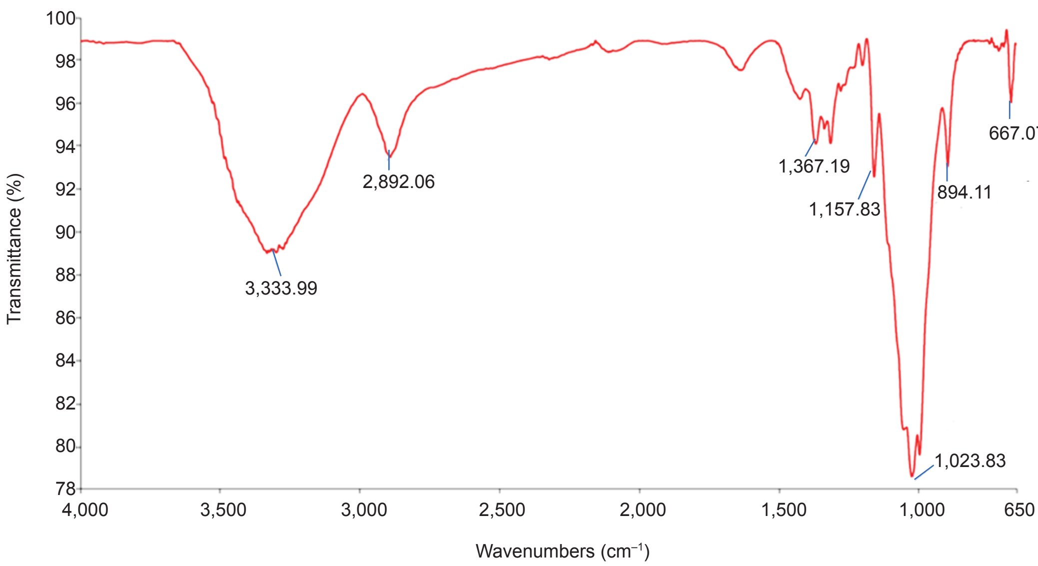

Figure 2. FTIR spectrum of MCC from empty fruit bunch cellulose.

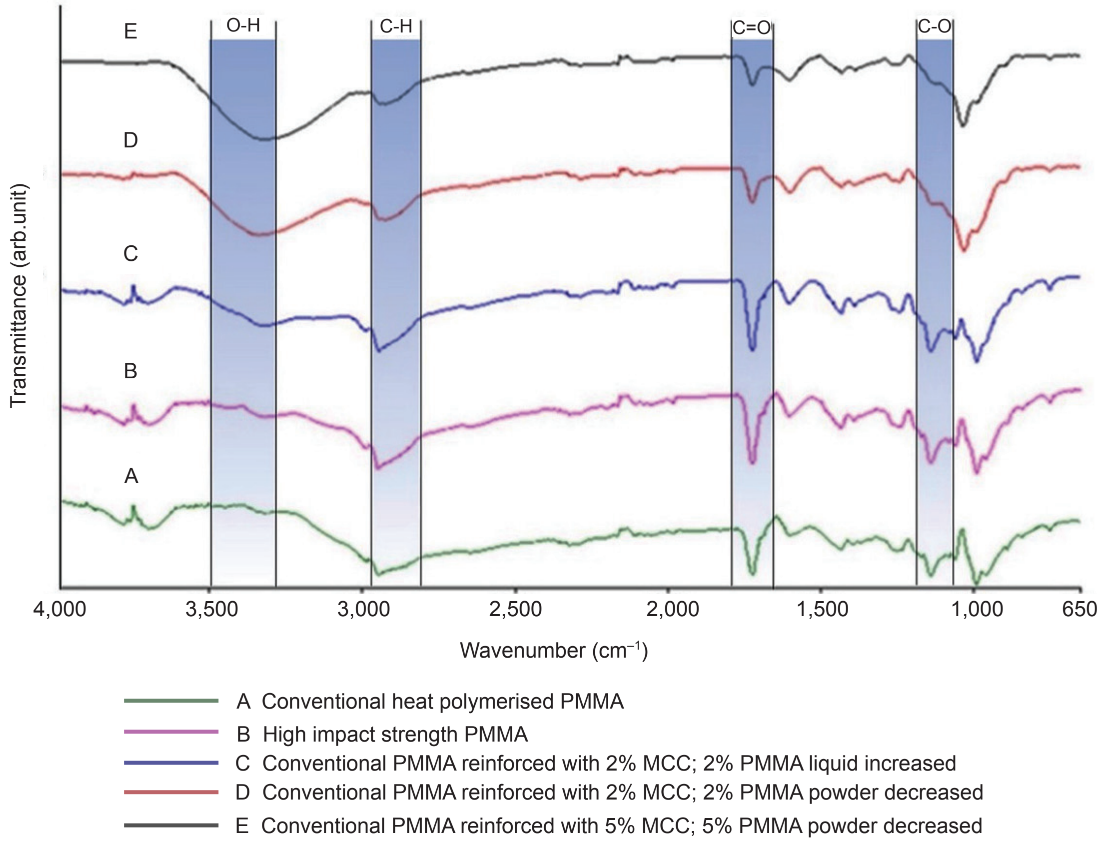

Figure 3. Overlay of the FTIR spectra of the PMMA and MCC specimens.

Developing dental materials with desirable biological and toxicological properties is essential for clinical use (Kulak-Ozkan et al., 2003). A critical step in the testing procedure is the evaluation of *in vitro* cytotoxicity, which helps to determine how cells react when in contact with the material and the process of cell death. The capacity to produce the desired tissue-biomaterial interfacial reaction is critical for the future of biomaterials science (Ratner et al., 2020).

This study used human fibroblast cells as a model for denture bases in direct contact with oral mucosa. The results showed that all test groups were non-cytotoxic, with more than 90% viability. The high cell viability observed in all groups suggests that these materials can be safely used in clinical applications without causing any harmful effects on surrounding tissues or cells. It is vital to consider that although the cytotoxicity of these materials was evaluated *in vitro*, additional studies are necessary to evaluate their biocompatibility *in vivo*. *In vivo* studies are essential to fully understand the biological response to dental materials, as they consider factors such as the immune response, tissue integration and long-term effects.

The main causative factor of denture sore mouth is the hypersensitive response of oral tissue to denture base material. *In vitro* studies have reported that residual monomer can be released from the denture into the oral cavity (Leggat & Kadjarune, 2003; Rashid et al., 2015). The filler in the cured denture resin matrix releases the unreacted residual monomer into the oral cavity causing clinical signs and symptoms of hypersensitivity. This chemo toxic irritant effect can be eliminated by ensuring a complete curing process and storing processed dentures in water for at least one day before use, preferably at 37°C (Bayraktar et al., 2005). In another study (Raszewski et al., 2021), it was reported that bioactive glass fillers were added to PMMA for the release of fluoride ions to inhibit microbial colonisation and the formation of plaque in the oral cavity of the denture wearer. Therefore, it is important to determine the biocompatibility when introducing a new agent into a dental product. The current study confirms that adding MCC-OPEFB to the PMMA acrylic denture resin does not compromise its biocompatibility and cytotoxicity level.

Conversely, the FTIR spectra of PMMA specimens modified with MCC provide valuable insights into the molecular structure and properties of the resulting composites. The spectra reveal distinct bands and variations in their intensities, depending on the concentration of MCC and the type of PMMA used. One of the spectra's most notable observations is the region's changes associated with –OH and C–O–C groups. The presence of added bands at 3,318 and 3,418 cm^{-1} in the MCC-reinforced groups indicates the occurrence of –OH (H-bonded) groups, likely derived from the cellulose component of MCC. Additionally, an extra band at 1,641 cm^{-1} in the MCC groups indicates the presence of –OH groups associated with cellulose. These changes suggest potential improvements in the bond between the PMMA matrix and the filler particles, leading to improved mechanical properties. MCC has a unique character because it can be grafted with other polymers through the hydroxyl group, as reported by Hubbe et al. (2008). The various wavelengths in the FTIR spectra can provide valuable insights into the changes that occur when an additive is introduced into a polymer matrix. For instance, changes in spectral bands' shape or relative intensities indicate that the additive has modified the original compound (Larkin, 2018). Regarding C=O and C–O bands, PMMA reinforced with MCC showed variations in their intensities depending on the concentration of MCC and the type of PMMA used. The greatest concentration of the C=O band was realised in the high-impact acrylic PMMA (Group B), followed by conventional PMMA reinforced with 2% MCC (Group C) and conventional PMMA (Group A). Meanwhile, the C–O band intensity increased with increasing MCC concentration. The cellulose-containing groups showed a stronger intensity of the functional group, which is a positive indication from a chemical standpoint (Tjeerdsma & Militz, 2005).

The changes in the spectral bands observed in the MCC-reinforced groups may have implications on the features of the resulting PMMA composite. The increased presence of –OH groups may enhance the bond between the PMMA matrix and the filler particles, improving mechanical properties. The changes in the C–O–C groups may also have implications for the water sorption and solubility of the composite, which are essential considerations in dental applications. It is worth noting that in Groups C and D, which were reinforced with 2% MCC, there was a lowering of the intensity of the bands at 1,721 and 1,641 cm^{-1}. It may indicate that the adding of MCC has some effect on the crosslinking mechanism of PMMA, which could affect the overall strength and stability of the material. In addition to the abovementioned changes, the spectra of PMMA reinforced with MCC also showed variations in the 1,300–1,000 cm^{-1} region. The band at 1,158 cm^{-1}, which is associated with the C–O–C group, showed an increase in intensity in the specimens with the addition of MCC (Groups C, D and E) compared to the conventional PMMA (Group A) and high-impact PMMA (Group B). This could be ascribed to the presence of cellulose in the MCC, which could enhance the intermolecular interactions between MCC and PMMA.

The FTIR results in this study provide valuable insights into the changes in the molecular structure of PMMA modified with MCC. The spectra obtained from different PMMA specimens reinforced with different concentrations of MCC reveal distinct bands and variations in their intensities. These changes indicate the potential for the composite material to form crosslinks between MCC and PMMA, which can enhance the overall strength and stability of the material, making it a promising option for use in prosthodontics and other dental applications. The changes observed in the FTIR spectra, specifically the increase in –OH groups and the reduction in C=O groups due to the introduction of MCC into PMMA, could have a correlation with the cell viability results. The increase in hydroxyl groups generally enhances the hydrophilicity of the material, which can improve cell attachment and proliferation. This is because cells tend to favor hydrophilic surfaces for adhesion, which can promote better integration and viability. On the other hand, reducing carbonyl groups might reduce the potential cytotoxicity associated with the ester components in PMMA, further supporting a more favorable environment for cell growth. Recent *in vitro* simulation by the authors of this study have demonstrated that incorporating MCC into acrylic resin can significantly enhance the denture flexural strength and modulus, even after thermal cycling (Rahaman Ali et al., 2020). The scanning electron microscope (SEM) result showed that the resin treated with various concentrations of OPEFB-MCC displayed no micro-gaps between the filler particles and the acrylic resin matrix. These results suggest that using MCC as a reinforcing agent can potentially improve denture longevity and prevent denture fractures, which is a significant concern for many denture wearers. Moreover, the ease of adding, blending and handling MCC alongside PMMA resin further supports its possible clinical application. The findings of the current study provide a promising avenue for developing new dental materials with improved mechanical properties, which can significantly impact the oral health and quality of life of denture wearers.

Overall, the use of natural agents that are environmentally friendly, from a renewable OPEFB-MCC to reinforce acrylic denture resin, is a promising method to improve the biological and toxicological functions of the existing commercially available synthetic reinforced PMMA resins. Cytotoxicity tests conducted in this study demonstrated that the natural agent used did not adversely affect human fibroblast cells. However, more *in vivo* studies are necessary to evaluate the clinical performance of these materials. Additionally, adding MCC to conventional PMMA may lead to changes in the composite's molecular structure, affecting its properties and performance in dental materials. These findings suggest that

MCC can be used in other dental applications, and additional studies are necessary to investigate this possibility.

CONCLUSION

The results of this study demonstrated that the addition of MCC to PMMA resin resulted in a copolymer structure, which improved the microstructural and chemical properties of the PMMA resin. The FTIR changes indicating increased –OH and reduced C=O groups likely contribute to improved cell viability, as these modifications result in a more biocompatible surface and conducive to cell viability. The biocompatibility tests confirmed that the resulting composite had an acceptable biological response, making it a promising material for a wider dental application. Furthermore, the outcomes of this study suggest that MCC has the potential to improve the clinical service life of dentures. The research findings presented in this study also have the potential to extend the application of OPEFB-MCC to other dental materials, paving the way for future research into the potential applications of this natural agent in dentistry to promote sustainable use of terrestrial ecosystem which is one of the pillars of the 2030 agenda for the United Nations' Sustainable Developmental Goals.

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EVALUATION OF LAMINATED PANELS FROM SEMANTAN BAMBOO AND OIL PALM TRUNKS FOR SUSTAINABLE COMPOSITE MANUFACTURING

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ABSTRACT

The increasing demand for sustainable materials in the composite industry has driven interest in alternative non-wood resources. Among them, bamboo and oil palm biomass are recognised as potential natural fibres that can be utilised to develop eco-friendly composite materials. This study aimed to evaluate the properties of laminated panels made from Semantan bamboo crush mat and oil palm trunk (OPT) veneer with different layer configurations. Three types of laminated panels are compared in the study: A bamboo mats panel, an OPT veneers panel and a hybrid panel with veneer applied as the core layer while the mats served as surface layers. The composites were fabricated using a hot-pressed technique and urea-formaldehyde (UF) resin as a binder. The panels were tested for physical and mechanical properties and the results showed that layer configuration significantly influenced these properties. The panel consisting entirely of a bamboo mat showed the best performance, while the hybrid panel was comparable in most properties tested. On the other hand, the panel with entirely OPT veneers was inferior, suggesting that the hybrid panel would be a promising alternative to wood-based products.

Keywords: bamboo, laminated composite, layer configuration, oil palm trunk.

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INTRODUCTION

Like laminated panels, engineered wood is constructed by layering thin sheets of wood veneer or lumber to enhance its strength and other properties. Compared to other wood fibre panels, laminated panels display superior performance in both physical and mechanical characteristics due to the strategic use of alternative fibres and optimised layer configurations. Plywood, cross-laminated timber (CLT), veneer, glued laminated timber (glulam) and laminated veneer lumber (LVL) are all prime examples of laminated panels. In the construction industry, laminated panels have diverse applications in roofing, flooring, partitions, furniture and entire building structures. Malaysia plays a significant part in the global timber industry. It is one of the major producers and exporters of a wide range of timber products, including sawn timber, flooring, doors and panels like plywood and medium-density fibreboard (MDF) and this sector contributes substantially to the Malaysian economy (Malaysian Timber Industry Board [MTIB], 2023). However, the focus is shifting toward sustainable alternatives due to concerns about deforestation and resource scarcity (Dieterle & Karsenty, 2020).

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Therefore, bamboo and oil palm trunks (OPT) are recognised as viable alternative materials for use in laminated panel production.

Bamboo is a renewable resource with fast growth and qualities comparable to typical wood resources. Its strength, durability and versatility make it a compelling choice for various applications, including furniture manufacturing and construction (Huang et al., 2019; Siam et al., 2019). As an abundant tropical resource, it encompasses over 80 genera and 1,600 species worldwide, with 70 species grown in Malaysia, including 50 endemics and 13 commercial species (Siam et al., 2019). Despite its many advantages, such as exceptional strength-to-weight ratios and optimum thermal, acoustic and other qualities with high tensile strength, the use of natural bamboo as a building material is limited. This limitation is primarily due to the small diameter of bamboo culms and the wide range of mechanical properties (Wang et al., 2011). To address these restrictions, bamboo composite materials have been developed (Abidin et al., 2023; Yusof et al., 2023; Zhong et al., 2017). Bamboo composite is an engineered material predominantly utilised for structural purposes in the building and construction industries. These composite materials are commonly utilised in various shapes such as beams, boards, lumber and other components. Bamboo composites have been created over the last 40 years, including bamboo scrimber (Rao et al., 2022; Yang et al., 2024), laminated bamboo (He et al., 2024; Manik et al., 2022), ply bamboo (Uyup et al., 2012) and bamboo strand-based composites (Sulastiningsih et al., 2024). Bamboo composites offer excellent dimensional stability, minimal distortion and consistent size. They also possess high wear resistance, stiffness and strength (Mili et al., 2023).

Alternatively, OPT, a biomass of palm oil plantations, offers a way to utilise by-product materials and reduce environmental impact. Oil palm plantations have seen a surge in growth across Southeast Asia, particularly in Malaysia (Parveez et al., 2020). In 2023, the total estimated production of oil palm biomass was 92.37 million tonnes based on a dry weight basis, with OPT contributing 9.83 million tonnes to this total (Malaysian Palm Oil Board [MPOB], 2024). The versatility of palm oil, with applications in food, cosmetics, pharmaceuticals, biofuels and various household products, has solidified its position as a crucial element of the Malaysian economy (Nuryawan et al., 2022; Ropandi et al., 2022; Yusof et al., 2020). In the composite industry, due to its low-grade construction, oil palm biomass has been utilised in various forms, including pellets, adsorbents, briquettes, plywood, particleboard and fibreboard (Badri & Khairul, 2006; Chong et al., 2020; Ibrahim et al., 2013, 2020; Safana et al., 2018). However, laminated panels made solely from OPT veneers exhibit inadequate physical and mechanical characteristics due to the OPT having a lower density ranging from 222–404 kg/m^3 (Nuryawan et al., 2022).

Both bamboo and OPT emerge as promising substitutes for wood in laminated panels due to their abundance, fast growth and similar properties like wood. Thus, the utilisation of bamboo and OPT in hybrid laminated panel production enhances their inherent value proposition. These renewable resources contribute to the aesthetic appearance of the panel and exceptional mechanical properties while also mitigating environmental impact through sustainable sourcing and reduced reliance on traditional timber sources (Suhaily, 2020). The properties of bamboo, specifically Semantan bamboo and OPT are summarised in *Table 1*.

Previous studies have proven that the configuration of layers in laminated panels plays a crucial role because it directly impacts the structural integrity, strength and performance of the final product (Hua et al., 2015; Jantawee et al., 2023; Yahaya et al., 2014). Therefore, this study explores the potential of combining bamboo crush mats and OPT veneers to fabricate laminated panels with varying layer configurations, aiming to investigate their performance and suitability for diverse applications.

MATERIALS AND METHODS

This study utilised crushed bamboo mats (*Figure 1a*) produced from flattened round Semantan bamboo (*Gigantochloa scortechini*) and oil palm (*Elaeis guineensis*) veneer (*Figure 1b*) that were supplied by local suppliers. The bamboo mats received were treated with a borax solution for one week to increase durability and protect against fungi and

TABLE 1. PROPERTIES OF SEMANTAN BAMBOO AND OPT

Properties	Semantan bamboo	OPT
Moisture content	48.6%–90.5% (Hamid et al., 2006)	Up to 500.0% (Bakar et al., 2008)
Density	530–680 kg/m^3 (Hamid et al., 2006; Siam et al., 2019)	240–530 kg/cm^3 (Bakar et al., 2013)
Modulus of rupture (MOR)	125.00 N/mm^2 (Zakikhani et al., 2017)	46.62 N/mm^2 (Bakar et al., 2008)
Modulus of elasticity (MOE)	10,039 N/mm^2 (Zakikhani et al., 2017)	2,843 N/mm^2 (Bakar et al., 2008)

insect infestations. The urea formaldehyde (UF) adhesive was mixed with ammonium chloride (NH_4Cl) hardener in a 100:1.5 ratio to accelerate the curing process of the laminated panels. The UF adhesive was sourced from a local wood-based supplier.

Bamboo mats and OPT veneers were bonded with UF adhesive to fabricate 3-ply laminated panels (300 × 300 mm) using a 250 g/m² glue spread rate. Three panels per configuration were produced, with cores oriented perpendicularly to the outer/inner layers, mimicking plywood. The hybrid panels feature bamboo mats as outer layers and OPT veneer as the core. Additionally, panels entirely of bamboo mats or OPT veneers were fabricated, utilising each material for both outer/inner and core layers (*Figure 2b*). For the bamboo panels, the bamboo mats were placed in the middle layer to enhance shear resistance and energy absorption, which are critical for applications requiring improved toughness and durability. Conversely, in the hybrid panels, the bamboo mats were positioned on the outer layers to maximise flexural stiffness and strength, as the outermost layers primarily resist tensile and compressive stresses during bending. This strategic layering ensures that each panel design meets its targeted mechanical performance criteria. Initial cold pressing was performed at room temperature with 5 kg/cm² pressure for 15 min to enhance ply adhesion and maintain panel shape. Hot pressing was followed at 140°C and 25 kg/cm² for 12–15 min to cure the adhesive and ensure proper bonding, resulting in a final panel thickness of 10 mm. The panels were then conditioned for one week at 25 ± 2°C and 65 ± 2% relative humidity to achieve equilibrium moisture content (EMC). After conditioning, panels were trimmed and cut into test samples for physical and mechanical evaluations. The same procedure was applied to panels composed entirely of bamboo mats and OPT veneers.

Physical and Mechanical Evaluation

Each panel was cut into dimensions of test specimens and evaluated by a series of physical and mechanical tests. A total of five samples were prepared for each test for every panel type, resulting in a total of 45 test specimens in this study.

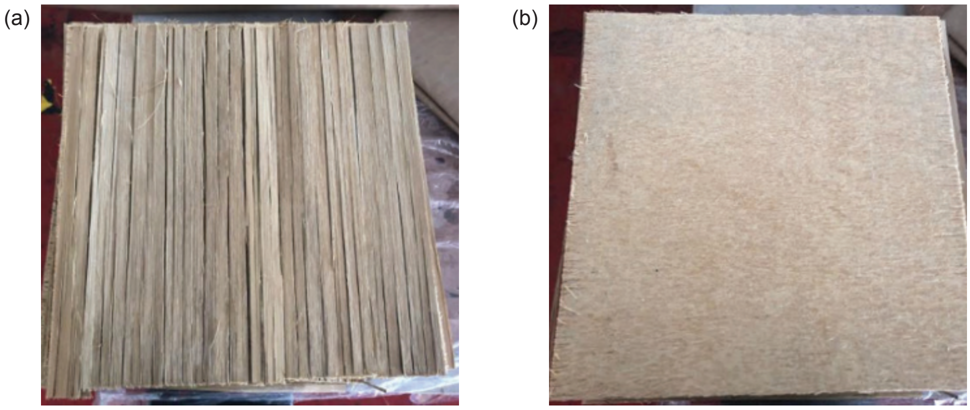

Figure 1. (a) Crushed bamboo mats and (b) oil palm trunk (OPT) veneer.

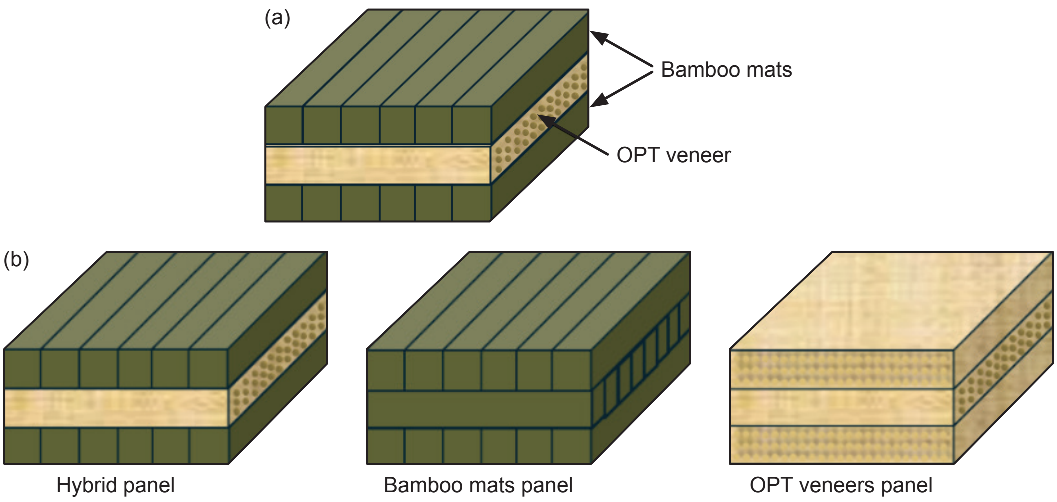

Figure 2. (a) Configuration of the hybrid laminated panel and (b) layup of each panel.

Physical evaluation. The laminated panels were tested to evaluate their physical properties for moisture content (MC) (ASTM D4442-92), density (ASTM D2395-14), water absorption (WA) (ASTM D1037-12) and thickness swelling (TS) (ASTM D1037-12[2020]). MC is expressed in percentage (%) and was estimated by weighing the panels before and after oven drying for 24 hr as per Equation (1):

$$\text{Moisture content } (\%) = \frac{W_f - W_i}{W_i} \times 100 \qquad (1)$$

where, W_i is the initial weight (g) of the test specimen before the oven-drying, and W_f is the weight (g) of the test specimen after the oven-drying. Density is defined as mass per unit volume with the SI unit of g/cm^3 and was calculated using Equation (2):

$$\text{Density } (\text{g/cm}^3) = \frac{M}{V} \qquad (2)$$

where, M is the mass (g), and V is the volume (cm^3) of the test specimen. WA was derived by comparing the original and final weights after soaking in water for 24 hr, which is expressed in %. WA is calculated using Equation (3):

$$\text{Water absorption } (\%) = \frac{W_f - W_i}{W_i} \times 100 \qquad (3)$$

W_i is the initial weight (g) of the test specimen before soaking in water, and W_f is the weight (g) of the test specimen after soaking in water. TS was evaluated by measuring the change in thickness before and after water immersion for 24 hr, expressed in % and was calculated using Equation (4):

$$\text{Thickness swelling } (\%) = \frac{T_f - T_i}{T_i} \times 100 \qquad (4)$$

where, T_i is the initial thickness of the test specimen before soaking in water and T_f is the thickness of the test specimen after soaking in water.

Mechanical evaluation. The mechanical tests incorporated bending (ASTM D7264), compression (ASTM D3501-94), shear (ASTM D2718 [2011]) and wood failure percentage (WFP) (ASTM D5266 [2013]). Bending tests measured the modulus of elasticity (MOE) and modulus of rupture (MOR). MOE is the ratio within the elastic limit of stress corresponding to strain with the SI unit of MPa. The formula of MOE is stated in Equation (5) below:

$$\text{Modulus of elasticity (MPa)} = \frac{PL^3}{4bd^3\,(\Delta d)} \qquad (5)$$

where, P is the load at the limit of the proportion of the force-displacement curve, d is the length of the test specimen, and Δd is the deflection at the mid-length at the limit of the proportion. MOR is the maximum load that the test specimen with the SI unit of MPa can withstand. The formula for MOR is shown in Equation (6):

$$\text{Modulus of rupture (MPa)} = \frac{3P_{max}L}{2bh^2} \qquad (6)$$

where, L is the length of the test specimen, P_{max} is the maximum load that the test specimen can afford, b is the width of the test specimen, and h is the thickness of the test specimen. Compression testing determined the maximum load that the panels could withstand. In the compression test, compressive strength with SI unit of MPa is calculated as per Equation (7):

$$\text{Compressive strength (MPa)} = \frac{P_{max}}{bd} \qquad (7)$$

where, P_{max} is the maximum load that can be applied to the test specimen, b is the width of the test specimen and d is the length of the test specimen. A universal testing machine was used to assess shear strength, and the percentage of broken bonding surfaces was calculated to determine WFP.

Statistical Analysis

The effects of the layer configuration were evaluated using an analysis of variance (ANOVA) at 0.05 levels of significance. Tukey HSD tests were conducted as post-hoc analysis.

RESULTS AND DISCUSSION

Physical Properties

Density and moisture content. *Figure 3* presents the density values for the laminated panels, ranging from 0.64–0.72 g/cm³. The bamboo mats panel had the highest density at 0.72 g/cm³, while the OPT veneers panel had the lowest at 0.64 g/cm³. This is consistent with the known higher density of bamboo compared to OPT, reflecting the material properties. Semantan bamboo densities range from 0.53–0.68 g/cm³ (Norul Hisham et al., 2006; Siam et al., 2019), whereas OPT veneer densities range from 0.15–0.4 g/cm³ (Abdul Khalil et al., 2010; Bakar et al., 2013). Thus, panels made entirely of bamboo mats exhibit higher densities than those made from OPT veneer.

These findings are consistent with previous research. Abdul Khalil et al. (2010), reported a density of 0.63 g/cm³ for OPT plywood using UF adhesive at a 300 g/m² spread rate. Similarly, Sulaiman et al. (2009) reported a density of 0.57 g/cm³ for LVL made from OPT with the same adhesive and spread rate. The density of the bamboo mat panel differed slightly from that reported by Suhaily et al. (2020), likely due to variations in bamboo species. Specifically, *Gigantochloa levis* and *Dendrocalamus asper* bamboo laminated composites were reported to have densities of 0.98 and 1.02 g/cm³, respectively, with a 200 g/m² glue spread rate.

Given that hybrid laminated panels incorporate both crushed bamboo mats and OPT veneers, their density is lower than that of bamboo mat panels but higher than OPT veneer panels. This variation is attributed to the different densities of the materials used. Additionally, the pressing processes during panel fabrication cause volume reduction through cell compression, thereby increasing the density of the laminated panels (Sulaiman et al., 2009). In summary, the density of the manufactured panels falls within Class II (0.6–0.9 g/cm³), according to SNI 03-3527 (1994).

The hybrid panel exhibited the highest MC at 5.18%, followed by the bamboo mats panel at 4.74%, and the OPT veneers panel at 4.38% (*Figure 3*). The lower MC in OPT veneer panels is attributed to more effective drying processes during manufacturing compared to bamboo crush mats (Mokhtar et al., 2011). Differences in density and porosity also affect moisture absorption rates, with OPT veneer potentially having a lower inherent MC due to its natural properties and processing methods (Chai et al., 2011). Consequently, panels with OPT veneers show lower MC than those with bamboo crush mats. The overall MC ranged from 4.38%–5.18% for all panels tested, with a mean of 4.77%, adhering to the plywood standard of a maximum 14.00% MC (JAS 003, 2014) and confirming the suitability of the panels for various applications as noted by Sumardi et al. (2020).

Water absorption (WA) and thickness swelling (TS). WA and TS are critical for assessing the dimensional stability of panels, reflecting their expansion and shrinkage under various environmental conditions. *Figure 4* shows the mean WA and TS values for each laminated panel. The WA values were 39.0% for bamboo mat panels, 41.8% for hybrid panels and 77.8% for OPT veneer panels. The OPT veneers panel demonstrated significantly higher WA, attributed to its greater porosity and void content (Paridah, 2022), which enhances moisture absorption and retention compared to bamboo mat panels (Masseat et al., 2018). Srivaro et al. (2014), also noted that increased porosity correlates with higher WA in wood materials.

The density, porosity and WA values of the panels are interrelated. Higher-density panels typically have lower porosity, resulting in reduced water absorption, and vice versa. In this study, the hybrid laminated panel exhibited a higher WA than the bamboo mat panel, indicating that the OPT veneer has a higher saturation threshold compared to bamboo. This is consistent with Sumardi et al. (2020), who reported that WA values for hybrid panels (34%–43%) were higher than those for strip bamboo panels (29%–35%).

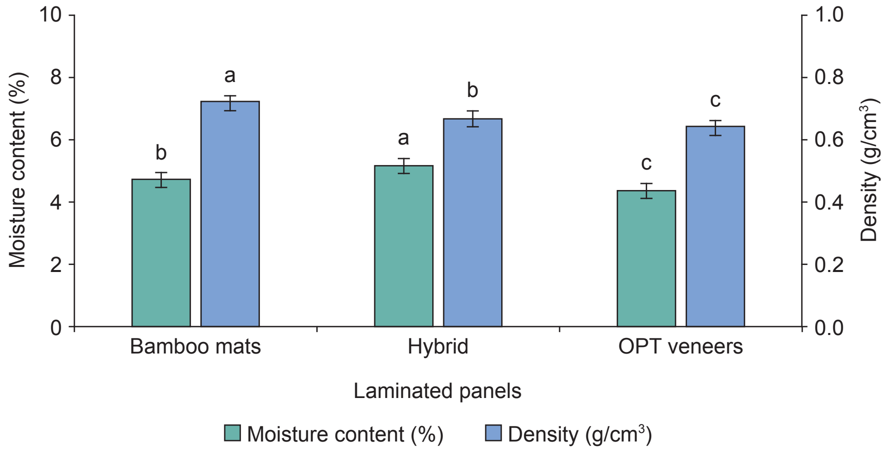

Figure 3. Mean values of density and moisture content for each laminated panel.

The interaction between hydrophilic fibres and adhesives can also affect water absorption. Al-Maharma and Al-Huniti (2019) found that poor adhesion between hydrophilic fibre surfaces and adhesives can create voids, increasing WA. Additionally, the pressing process, sample preparation and other fabrication steps can introduce microcracks in the panels, leading to increased water uptake (Panthapulakkal & Sain, 2007).

The mean TS values were 14.40% for hybrid panels, 19.90% for bamboo mat panels and 18.70% for OPT veneer panels (*Figure 4*). *Figure 5* shows the variation in thickness after 24 hr water soaking. TS values generally follow a trend similar to WA values, reflecting their close relationship. However, the TS value for bamboo mat panels was notably higher than for the other panels. This discrepancy may be due to gaps within the crushed bamboo mats, which become more pronounced during the pressing process and affect swelling (Srivaro et al., 2014).

Consequently, water tends to infiltrate gaps within crushed bamboo mats, causing internal swelling. However, the WA of bamboo mat panels remains low due to bamboo's inherent anatomical structure, despite these gaps. Srivaro et al. (2014) noted that pressing can induce additional swelling through densification in the core layer. In this study, the perpendicular arrangement of laminates was found to enhance dimensional stability by reducing WA and thickness changes. This arrangement effectively balances the panel's stresses, as supported by Lee et al. (2012) and Sumardi et al. (2020).

Mechanical Properties

Modulus of elasticity (MOE) and modulus of rupture (MOR) of bending properties. The mean MOE ranged from 6.6–16.6 GPa, and the MOR ranged from 53.1–108.3 MPa (*Figure 6*). Bamboo mat panels exhibited the highest MOE of 16.6 GPa, followed by hybrid panels at 16.4 GPa, while OPT veneer panels had the lowest MOE of

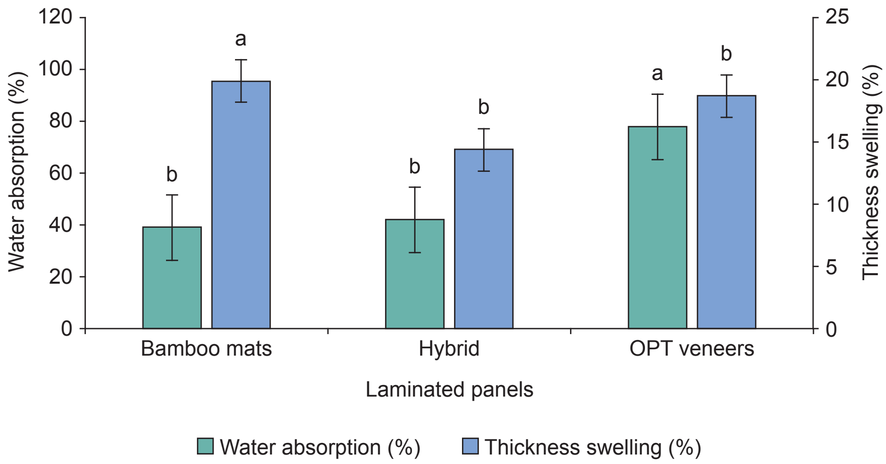

Figure 4. *Mean values of water absorption (WA) and thickness swelling (TS) for laminated panels according to the configuration.*

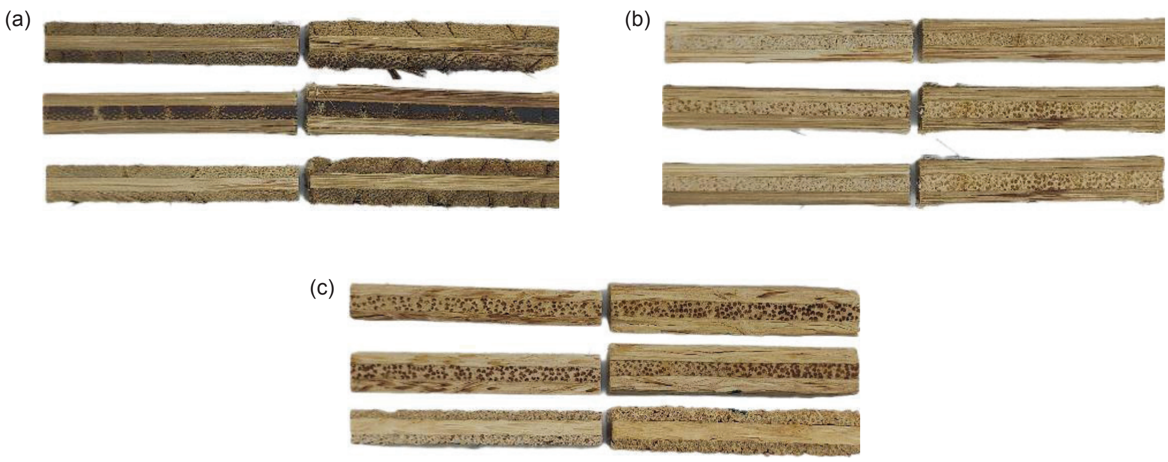

Figure 5. *Changes in the thickness of test specimens after soaking in water for 24 hr. (a) Bamboo mat panels, (b) hybrid panels and (c) OPT veneer panels.*

6.6 GPa. The minimal MOE difference between bamboo mats and hybrid panels contrasts with the larger difference from OPT veneers, indicating superior mechanical properties in bamboo-based and hybrid panels. Statistical analysis confirmed significant differences between the MOE of bamboo mats and hybrid panels compared to OPT veneers.

These results indicate that the MOE of the hybrid panel was nearly as high as that of the bamboo mats panel, despite the hybrid's inclusion of OPT veneer. While OPT veneer has lower strength than bamboo, its role in the hybrid panel only slightly reduces bending strength compared to the bamboo mat panel, suggesting effective compatibility between the crushed bamboo mat and OPT veneer. Trisatya et al. (2021) similarly found that hybrid composite beams of bamboo and damar exhibited superior MOE compared to damar-only composites, demonstrating that hybrid laminates can match or exceed the strength of single-material panels.

The bamboo mats panel achieved the highest MOR at 108.3 MPa, followed by the hybrid panel at 90.9 MPa, with the OPT veneers panel showing the lowest MOR at 53.1 MPa (*Figure 6*). This is consistent with Sumardi et al. (2020), who reported lower MOR for hybrid bamboo panels compared to all-bamboo panels. The OPT core in the hybrid panel was less effective at resisting bending stress due to inherent strength differences, as reflected in the failure modes observed (*Figure 7*). Bamboo's natural strength and stiffness contribute to higher bending strength (Abdullah et al., 2017) in panels made entirely of bamboo mats (Nkeuwa et al., 2022). Conversely, the lower stiffness of the OPT veneer impacts the bending strength of the hybrid panel (Nuryawan et al., 2022). Thus, while the MOE and MOR of the hybrid panel were comparable to or slightly lower than those of the bamboo mats panel, they were higher than those of the OPT veneers panel.

The failure mode of each test specimen from each group is displayed in *Figure 7*. Most of the bamboo mat panel specimens (*Figure 7a*) displayed horizontal shear failure. Bamboo is a highly anisotropic material with excellent strength along the grain (Verma & Chariar, 2012) but lesser strength across the grain. Shear pressures concentrate on the weaker transverse grain direction at the interfaces and within the cores (Bahari & Wan Jaafar, 2011). Because it is perpendicular to the load's direction, the core layer is the most vulnerable to shear stresses, resulting in visible horizontal shear failure. Similarly, hybrid panel specimens (*Figure 7b*) are subjected to significant stress at the interface of the bamboo and OPT layers due to their different mechanical properties. Failure will occur at the interface if the adhesive bond is weaker (Sewar et al., 2024) than the OPT or bamboo layers. The majority of OPT veneer panels exhibit splintering tension (*Figure 7c*). The reason for this is that OPT is not as compressive or tensile as bamboo (Nuryawan et al., 2022), and it fails when the OPT's weaker fibres are subjected to excessive tensile pressure.

The load-displacement graph for the bamboo mat panel (*Figure 8a*) demonstrates a steep elastic area with a drop at failure. This behaviour suggests a high stiffness and brittle failure mechanism. The observed horizontal shear failure correlates with the abrupt load decrease, which occurred because the panel's transverse layers were unable to effectively withstand shear pressures (Yusof et al., 2023).

The hybrid panel's graph (*Figure 8b*) shows intermediate stiffness with a sharp decline at failure. The sturdy bamboo outer layers and the softer OPT core each contribute to this behaviour. The horizontal shear failure in the core corresponds to the graph's sudden load decrease. OPT is more flexible and ductile (Wahab et al., 2008), it can withstand more deformation before failing, as shown by the slow fall following the peak load

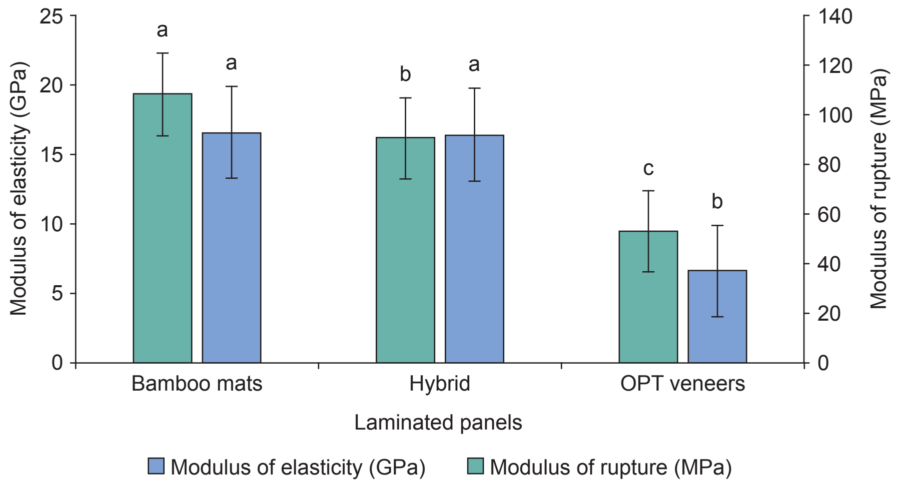

Figure 6. Mean values of modulus of elasticity (MOE) and modulus of rupture (MOR) for laminated panels according to the configurations.

Figure 7. The failure mode of the test specimens after the bending test. (a) Bamboo mats panels, (b) hybrid panels and (c) OPT veneer panels.

(*Figure 8c*), which is indicative of splintering tension failure. This is in line with the bending test's observed failure mode (*Figure 7c*).

Compressive strength. Compressive strength denotes the ability of a material to withstand applied loads that cause fracture or crushing. *Figure 9* shows the compressive strength results, ranging from 17.0–36.4 MPa, with a slight deviation from the bending test trend. The hybrid panel displayed the highest compressive strength (36.4 MPa), followed closely by the bamboo mats panel (34.2 MPa) and the OPT veneers panel again had the lowest value (17.0 MPa).

The compressive strength difference between the hybrid and bamboo mat panels was 2.1 MPa and statistically insignificant. This superior compressive strength in the hybrid panel may result from effective bonding between crushed bamboo and OPT veneer, enhancing overall performance. The load-displacement graph (*Figure 10b*) revealed a sharp slope in the elastic area, indicating that bamboo contributed significantly to stiffness (Rassiah et al., 2018). The slope in the plastic zone is somewhat increased because of the ductile OPT core, allowing the panel to withstand greater deformation before failing. The use of stiff and ductile materials maximises load-bearing capability for compressive strength (Selamat et al., 2019).

Conversely, slightly lower compressive strength in the bamboo mat panel compared to the hybrid panels might be due to gaps filled with brittle resin in the outer layers. In addition, due to the stiffness of bamboo, the slope is comparable to the hybrid panel (*Figure 10a*), but the plastic region is brief due to the absence of considerable ductility (Syaifudin et al., 2022). When the elastic limit is surpassed, the panel collapses due to crushing or buckling. The lower compressive strength observed in the OPT veneer panel could be attributed to the anatomical structure of OPT fibres, which generally have lower compressive strength compared to bamboo due to their higher proportion of vessels and parenchyma cells (Osman et al., 2022). *Figure 10c* shows a short elastic slope as the panel deforms greatly because of OPT's low rigidity. The plastic region will be longer since OPT is more ductile before the fibres in the compressed zone collapse gradually.

Sulastiningsih et al. (2018) reported higher compressive strength for all-bamboo composites compared to hybrid wood plank cores. This difference may be due to better compatibility between bamboo and OPT veneer in this study compared to their bamboo and wood plank study. The use of crushed bamboo mats, which differ in processing from bamboo strips, might also contribute to the observed results. Additionally, a study by Getu et al. (2021) found that bamboo and sisal fibre hybrids exhibited higher compressive strength. *Figure 11* shows the crushing appearance of the test specimens' post-compression. Overall, both hybrid and bamboo mat panels showed similar and superior compressive strength compared to the OPT veneers panel.

Shear strength and wood failure percentage (WFP). *Figure 12* shows the mean values of shear strength and WFP for each panel type, tested in dry conditions following the type of resin used. The shear strength test measures the force required to break the bond between two materials, with the bamboo mats panel exhibiting the highest shear strength at 2.6 MPa, followed by the hybrid panel at 2.3 MPa and the OPT veneers panel at 1.5 MPa. These values indicate significant shear strength, influenced by adhesive penetration and the inherent properties of the materials. The WFP was highest in the hybrid panel (62.7%), followed by the OPT veneers (46.0%) and the bamboo mats panel (30.3%).

The bamboo mats panel exhibited superior performance due to the unique characteristics of bamboo. Bamboo's vascular bundles are rich in cellulose fibres distributed throughout the culm, enhancing resistance to various forces, including shear (Mili et al., 2023). While both bamboo and oil palm materials are less dense than hardwoods, bamboo has a higher density than oil palm.

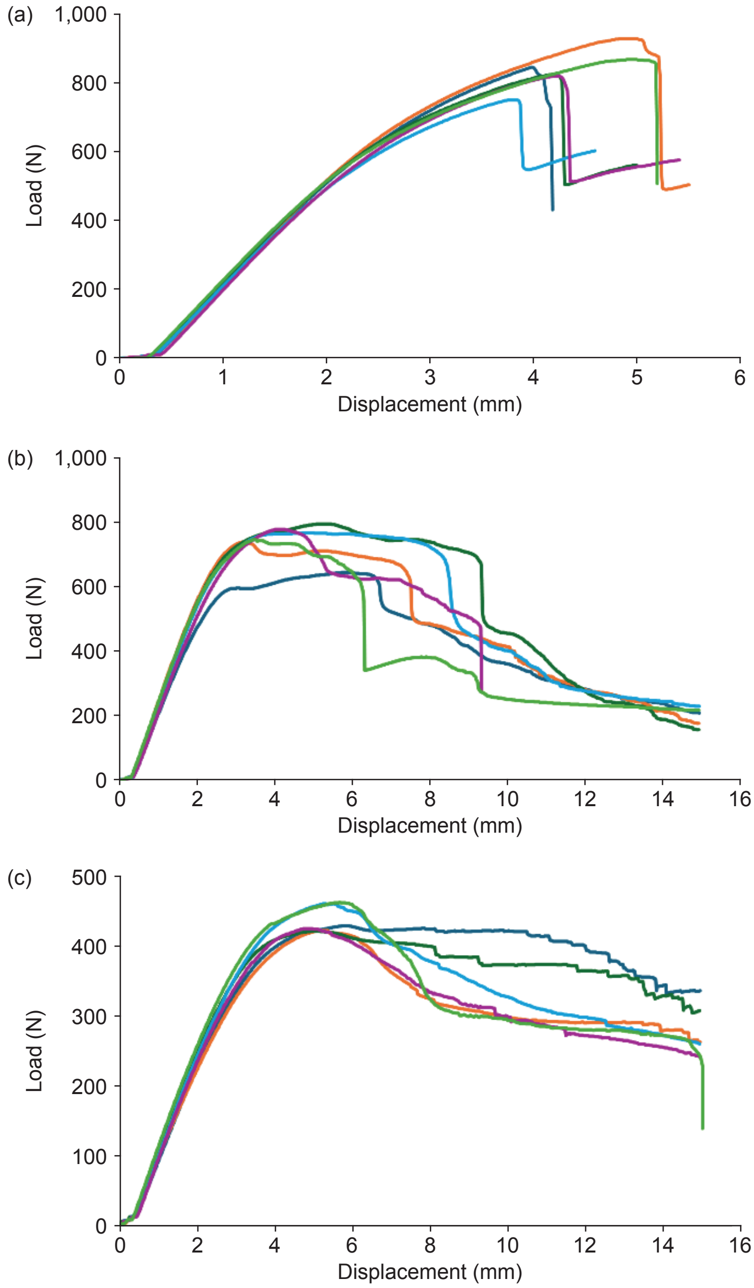

Figure 8. Comparison of the load-displacement values obtained by each panel. (a) Bamboo mat panels, (b) hybrid panels and (c) OPT veneer panels for bending test.

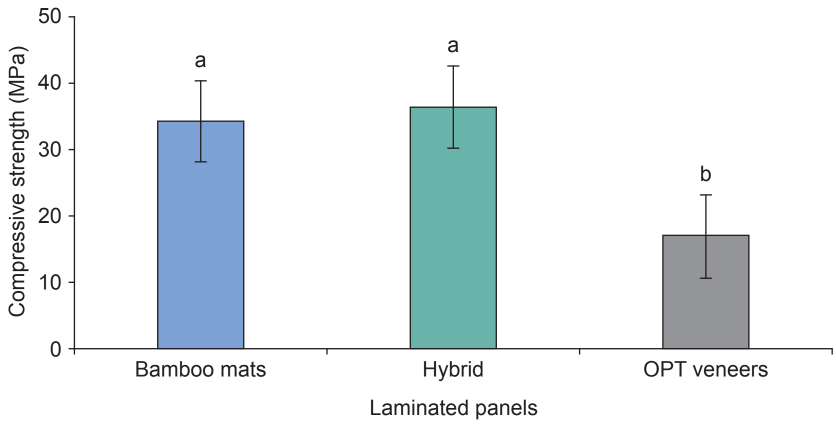

Figure 9. Mean values of compressive strength for laminated panels according to the configurations.

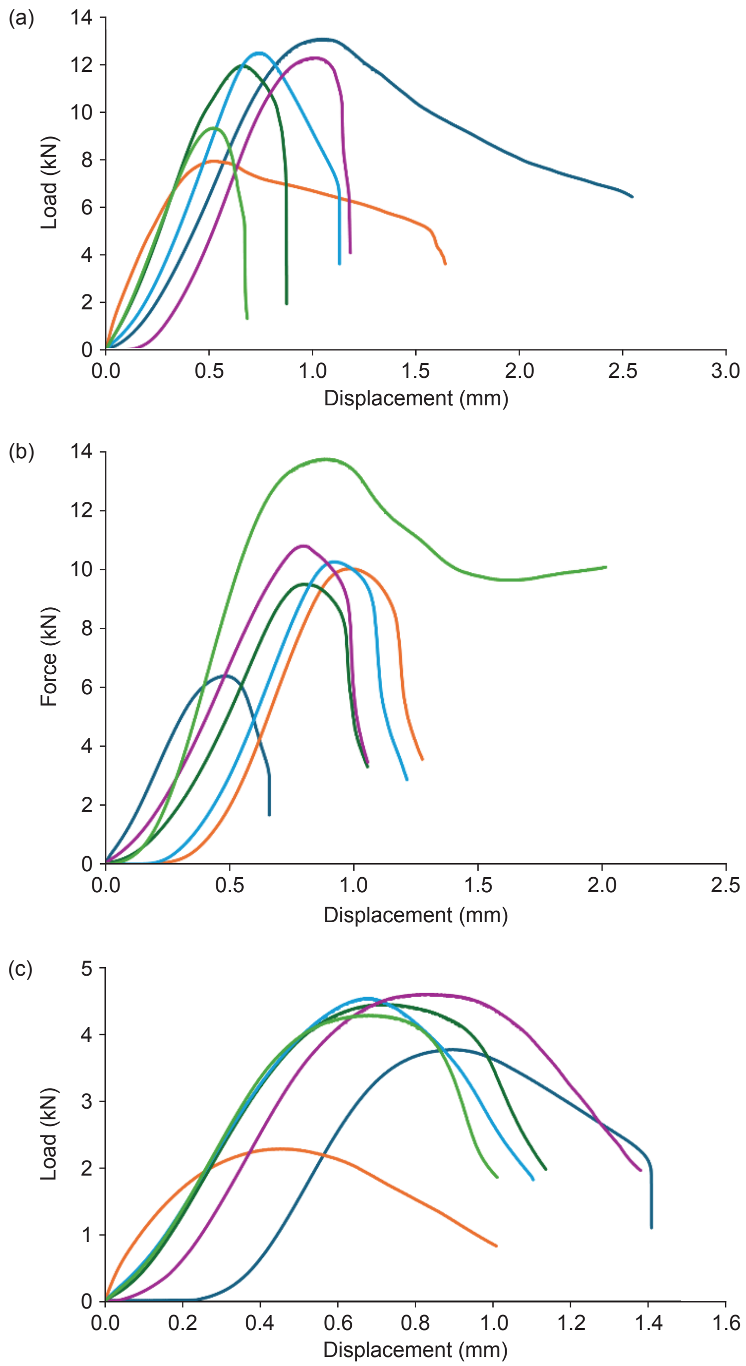

Figure 10. Comparison of the load-displacement values obtained by each panel. (a) Bamboo mat panels, (b) hybrid panels and (c) OPT veneer panels for compression test.

Li et al. (2021) found that bamboo woven panels (BMCP) significantly improved shear strength when incorporated into Hem-fir cross-laminated timber (CLT), thanks to the strategic placement of bamboo fibres in the inner layers to resist in-plane shear stresses. Similarly, Zhang et al. (2023) reported that CLT panels with hybrid bamboo-wood layers exhibited superior interlaminar shear strength compared to those made solely from wood. These findings highlight the potential of bamboo-wood composites to enhance the shear performance of beam structures.

The OPT veneer panel has the lowest shear strength among the other panels. Wahab et al. (2008), reported similar findings, noting that OPT LVL has lower shear strength compared to rubberwood LVL. This is attributed to OPT's lower density, which results in reduced intrinsic strength and stiffness, thereby affecting adhesive bonding efficiency (Wahab et al., 2008). Additionally, the shorter and less oriented fibres in OPT lead to weaker inter-fibre connections and a less cohesive structure, making it more susceptible to shear forces (Nordin et al., 2013). Furthermore, OPT

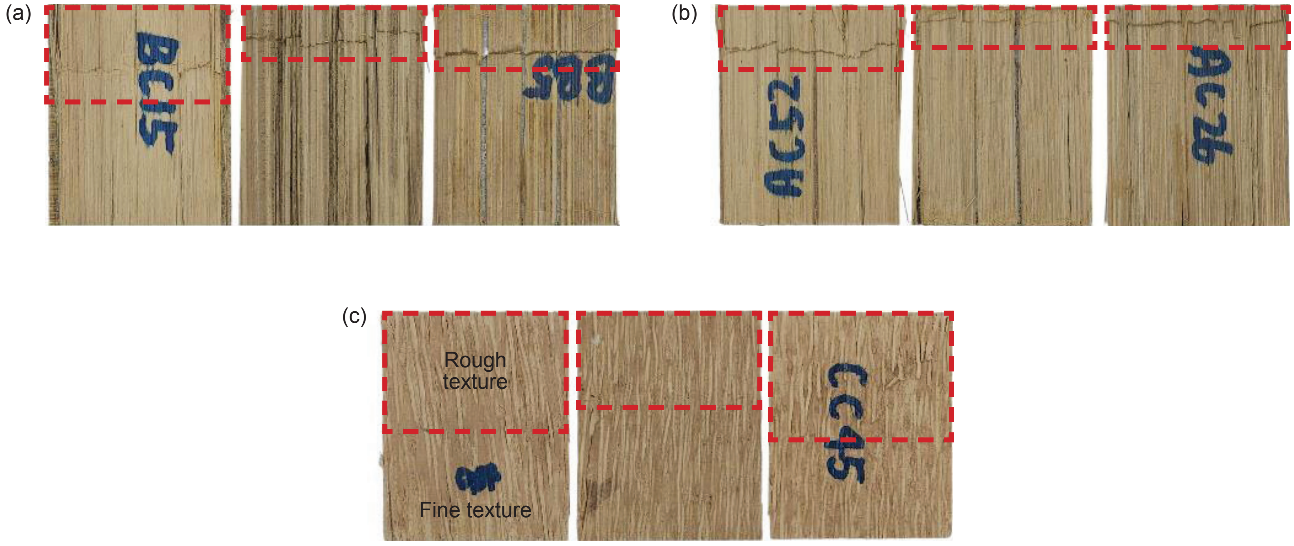

Figure 11. The failure mode of the test specimens after the compression test. (a) Bamboo mat panels, (b) hybrid panels and (c) OPT veneer panels.

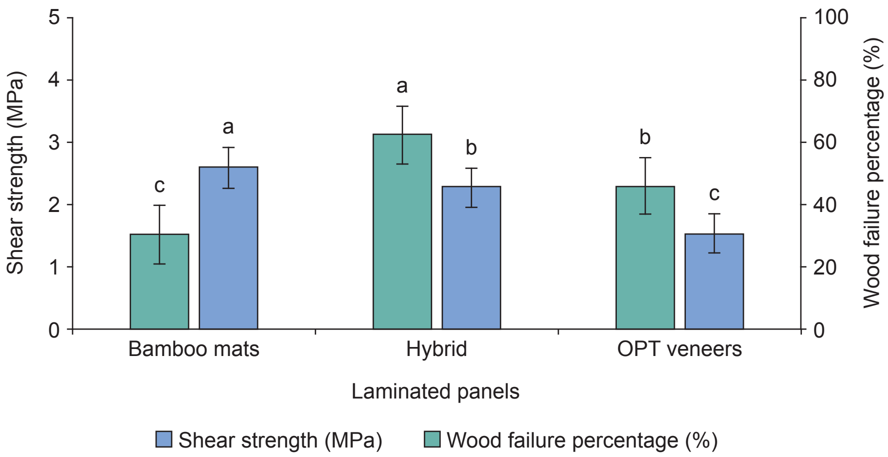

Figure 12. Mean values of shear strength and wood failure percentage (WFP) for laminated panels according to the configurations.

samples contain a larger proportion of parenchyma compared to bamboo, contributing to their lower shear strength.

Each panel initially failed with micro-cracks forming at the centre, which expanded under increasing load, notably in the hybrid and OPT veneer panels. This pattern is consistent with the WFP results, particularly for panels with OPT veneers, as shown in *Figure 13*, where most failures occurred in the parenchyma region. Additionally, higher content of extractives and oils compared to bamboo may disrupt adhesive bonding, leading to weaker joints and reduced shear strength (Prabuningrum et al., 2020).

Differential shrinkage may contribute to the high WFP of the hybrid panel, but this is likely due to the synergistic effect between bamboo and OPT. Bamboo fibres provide inherent strength and good porosity, which benefits adhesive flow (Hoque et al., 2019). The strong bamboo fibres form a solid structural framework, while the pores facilitate adhesive penetration. The pore size in the hybrid material may optimise adhesive distribution while filtering out unwanted particles (Hartono et al., 2023). Despite bamboo's strength, variations in fibre structure and density compared to OPT can lead to mismatched shrinkage during drying, potentially causing internal stresses and cracks (Wei et al., 2019). The interaction between bamboo and OPT might primarily contribute to the lower WFP in bamboo mats and OPT veneer panels. Bamboo with a smooth, waxy surface could impair adhesive bonding compared to the rougher texture of OPT, leading to weaker bonds and increased risk of delamination, which lowers WFP.

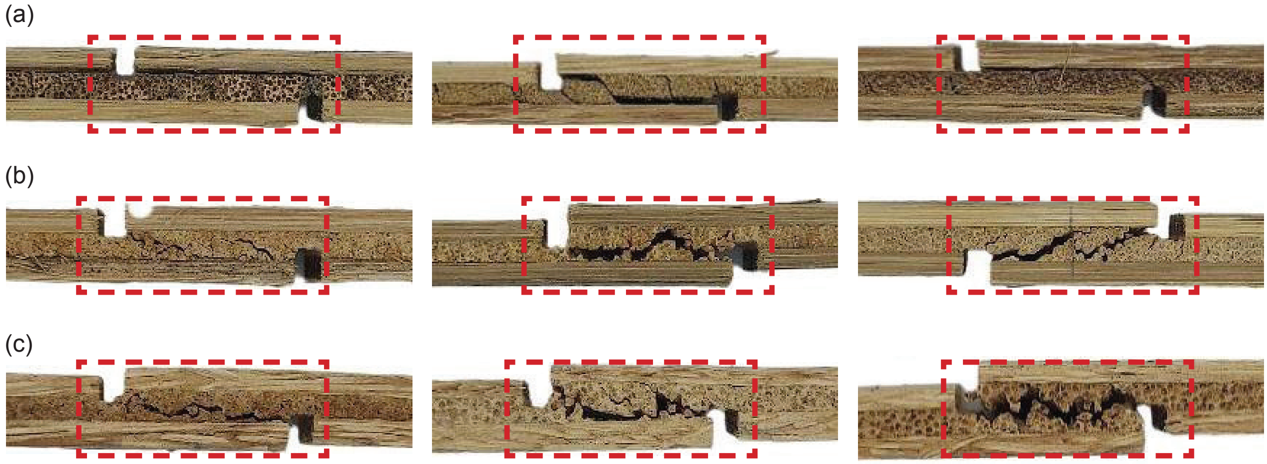

Figure 13. Test specimens after the shear test. (a) Bamboo mat panels, (b) hybrid panels and (c) OPT veneer panels.

Given that the hybrid laminated panel exhibits the highest WFP, it remains suitable for structural applications as its value meets acceptable standards. To further enhance the performance of these hybrid panels, pre-treatment of the bamboo surface through techniques like sanding, corona treatment, or chemical etching is recommended to improve adhesive bonding. Additionally, optimising pressing conditions (temperature, pressure and holding time) is essential to ensure proper adhesive activation and complete curing throughout the panel.

CONCLUSION

In conclusion, the hybrid laminated panel, constructed from crushed bamboo mat and OPT veneer, exhibited favourable physical and mechanical properties. The bamboo mats and hybrid panels demonstrated superior performance compared to the OPT veneer panel in several physical attributes, including density, WA and TS. Specifically, the bamboo mats panel excelled in bending modulus, bending strength and shear strength, whereas the hybrid panel showed enhanced compressive strength and WFP. Statistical analysis revealed no significant difference between the bamboo mats and hybrid panels regarding bending modulus and compressive strength. Conversely, the OPT veneers panel underperformed in both physical and mechanical tests relative to the other panels.

These findings underscore the potential of hybrid laminated panels comprising bamboo crushed mat and OPT veneers as viable alternatives to solid wood for interior furniture applications, offering comparable advantages over bamboo mat panels. Combining bamboo with OPT veneers makes production more economical, especially when larger quantities are required, as it reduces dependency on bamboo alone. In addition, it addresses the underutilised potential of OPT, promoting sustainable practices.

The capacity of hybrid panels to resist fire or flame for structural purposes, as well as other layer combinations and designs, should be investigated in future studies. Furthermore, the hybrid panels enable property fine-tuning; therefore, by varying the bamboo to OPT veneers ratio, a particular strength, stiffness and cost balance may be optimal for furniture application.

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PHOTOCATALYTIC DEGRADATION OF ANAEROBIC TREATED PALM OIL MILL EFFLUENT BY MODIFIED ZnO WITH LEMONGRASS AND KINETIC STUDIES

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ABSTRACT

The incorporation of green synthesis nanoparticles has a high photocatalytic degradation capability for treating anaerobic treated palm oil mill effluent (AnT-POME). Nevertheless, studies using zinc oxide-lemongrass nanoparticles (ZnO-L NPs) in photocatalytic processes for the treatment of AnT-POME are still in the early phases. Therefore, the goal of this study was to assess the photocatalytic degradation of AnT-POME in terms of colour, turbidity and chemical oxygen demand (COD) removal upon introducing modified ZnO-L NPs. The outcomes of this study demonstrate that the ideal conditions for photocatalytic degradation of AnT-POME have been established in the presence of ZnO-L NPs loading 0.1 g/L and pH 8, which results in a higher percentage of removal for colour (50.88%), turbidity (85.62%) and COD (97.02%). The photocatalytic process in the presence of ZnO-L NPs proved an improvement in colour, turbidity and COD removal when compared to without the addition of ZnO-L NPs. According to the experimental results, ZnO-L 1:3 NPs exhibit superior photodegradation of AnT-POME via pseudo first-order kinetics because of higher R^2 (0.9169) and K value (0.0167). Thus, the current study will gain a lot of attention because of the advantages of ZnO-L NPs' environmental performance in photocatalytic systems.

Keywords: anaerobic treated palm oil mill effluent, degradation, nanoparticles, photocatalytic.

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INTRODUCTION

The oil palm industry serves as one of the world's rapidly growing tropical sectors. Malaysia is presently regarded as the second-biggest exporter of palm oil after Indonesia because of its tropical environment and abundant natural resources. The problems associated with pollution brought on by palm oil mill effluent (POME) are getting worse as the palm oil business grows. POME contains a substantial number of organic compounds, that may negatively impact water quality and endanger both the environment and human health adversely (Sidik et al., 2020). The secondary biological treatment of POME results in the production of anaerobic treated POME (AnT-POME). The breakdown of

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lignocellulosic substances is responsible for AnT-POME's brownish colouration and elevated colour intensity (1,500 pt/Co). Additionally, AnT-POME has substantial concentrations of chemical oxygen demand (COD) (1,200 mg/L), turbidity (2,300 NTU), and total suspended solids (1,800 mg/L), all of which exceeded the Malaysian government's standard discharge guideline (Mohamad et al., 2021; Muhamad et al., 2022). Therefore, improper AnT-POME treatment could have a negative impact on water bodies and the environment.

Palm oil industries are currently utilising biological treatment, which has various problems, including the production of secondary pollutants (AnT-POME), which may have a severe environmental impact. Over 85.00% of milling companies in Malaysia generally use an open ponding method for POME treatment (Sayuti & Azoddein, 2015**)** and the Department of Environment (DOE) will enforce the requirements in accordance with the Environmental Quality Act of 1974 and palm oil mills are regulated under Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations 1977. The safe limit for POME waste being released, referring to the DOE Malaysia, is established at 100 ppm of biological oxygen demand (BOD), and no regulation for COD concentration for palm oil mills (Muhamad et al., 2022; Razali et al., 2021). However, the current limit is generally complied by the mills using ponding system. Only those mills with BOD 20 ppm struggling to achieve consistently. In recent years, numerous technologies, and polishing techniques for AnT-POME have been established to enhance the performance of treated AnT-POME in accordance with the requirements of the DOE prior release to a water body.

In recent years there has been a significant rise in interest in alternative technologies for POME treatment. The foremost examples are the photocatalytic (Tan et al., 2023), hybrid plasma and acoustic (Chan et al., 2023), integrated photovoltaic-electrocoagulation (Mohamad et al., 2022), coagulation-flocculation process (Lim et al., 2022) and membrane technology (Som & Yahya, 2021). The drawbacks of the traditional open ponding approach are successfully addressed by the aforementioned solutions, which are efficient and practicable. Even though there are numerous cutting-edge technologies accessible from previously conducted studies, there are still several obstacles that prevent them from being used for POME treatments in industry. These obstacles include those related to cost, feasibility, performance and environmental impact. Even after numerous treatments, the POME's characteristics still do not meet the DOE's standards for discharged materials since the levels of COD, BOD and colour intensity are still excessive and considered dangerous to discharge.

The photocatalytic process, which uses the notion of advanced oxidation processes (AOPs), that is associated with the transfer of electrons in the degradation of organic matter, has become another viable advanced wastewater treatment for POME (Moksin et al., 2021; Puasa et al., 2021). Theoretically, the mechanism of the photocatalytic degradation process begins with a photoexcitation stage that occurs when electrons (e) in the valence band (VB) are excited to the conduction band (CB), producing positively charged holes (h^+). Then, as h^+ and e are formed in the system, the hydroxyl radical (•OH) is created. This radical (•OH) is essential for the breakdown of organic matter pollutants into simple molecules like methane (CH_4), carbon dioxide (CO_2), water (H_2O) and other compounds (Chin et al., 2022). Generally, photocatalysts are crucial for the efficiency of the photocatalytic process in producing good-quality treated wastewater (Puasa et al., 2021). Additionally, Ren et al. (2023) asserted that photocatalytic degradation is one of the promising techniques since it can totally mineralise organic pollutants under simple circumstances like ambient temperature and pressure. Aside from that, according to Chin et al. (2022) the advantages of the photocatalysts used in the photocatalytic system include their ability to be recycled or reused and yet still maintain the degrading efficiency. The photocatalytic degradation process is therefore the most pertinent method to treat AnT-POME out of all those mentioned previously. Consequently, this study suggests photocatalytic degradation as one of the promising strategies.

The development of highly efficient semiconductors, such as titanium dioxide (TiO_2), zinc oxide (ZnO), cadmium sulfide (CdS), tin dioxide (SnO_2), Copper (II) oxide (CuO), tungsten trioxide (WO_3), iron (III) oxide (Fe_2O_3), strontium titanate ($SrTiO_3$), cerium dioxide (CeO_2) and calcium oxide (CaO), has been the subject of numerous studies to date (Chin et al., 2022; Ren et al., 2023). ZnO has been recognised as a notable photocatalytic material due to its environmental compatibility, cost-effectiveness and excellent photocatalytic efficiency (Ng et al., 2019; Shkir et al., 2024d). Furthermore, ZnO has also received a lot of attention for its photocatalytic potential due to its advantageous properties, which include strong excitation binding energy, a substantial surface area, high reactivity, biodegradable, photosensitivity and higher stability (Chin et al., 2022; Ng et al., 2019; Shkir et al., 2024c).

The surface morphology of ZnO has been improved by several methods including doping with suitable transition elements enables tuning its properties, making it suitable for many applications (Alagarasan et al., 2024; Jansi et al., 2024; Khan et al., 2024; Shkir et al., 2024d). The particle size and morphology of nanoparticles can also be enhanced to promote efficient photocatalytic application by adding capping agents such as polyvinylpyrrolidone (PVP) (Hairom et al., 2015), polyethylene-glycol (PEG) (Desa et al., 2020) and plant-based extracts (Shkir et al., 2022a) during the formation of ZnO nanoparticles. An additional surfactant called a capping agent is utilised to prevent specific nanoparticles from aggregating and to hinder their growth (Agarwal et al., 2017). Importantly, the capping agent can affect the structural properties of nanoparticles in surface engineering, including their size and shape. Lemongrass leaves are useful as a capping agent since they are simple to get, affordable and environmentally-friendly. Previous studies on the biosynthesis of ZnO photocatalyst demonstrated ZnO's adaptability as a photocatalyst that can degrade a significant number of organic materials for various types of wastewaters (Rao et al., 2022; Sayadi et al., 2022).

Sidik et al. (2020) have demonstrated the efficacy of a hybrid photocatalytic membrane reactor system in treating palm oil mill secondary effluent (POMSE). Nonetheless, the biggest obstacle to the application of membranes in wastewater treatment is their frequent replacement requirement, which might increase maintenance and operational costs. In addition, the usage of ZnO-L NPs in a photocatalytic system alone is still regarded as new. As a result, the current work intends to investigate the efficacy of ZnO-L NPs in AnT-POME treatment utilising the photocatalytic system to fill the gap of membrane fouling concerns employing a membrane system.

MATERIALS AND METHODS

Materials

The AnT-POME sample was collected in an airtight container from a neighbouring palm oil plantation's open pond in Pagoh, Johor, Malaysia. APHA 2012 standard techniques were used to analyse the original AnT-POME characteristics such as pH, COD, turbidity and colour. The AnT-POME samples were stored at 4°C in a chiller before being used. R&M Marketing, UK, provided commercial ZnO (Sigma-Aldrich). In the meantime, BT Scientific, Malaysia supplied the hydrochloric acid (HCl) and sodium hydroxide (NaOH) that were used to modify the pH of the AnT-POME solution.

Green Synthesis of Zinc Oxide-Lemongrass Nanoparticles (ZnO-L NPs)

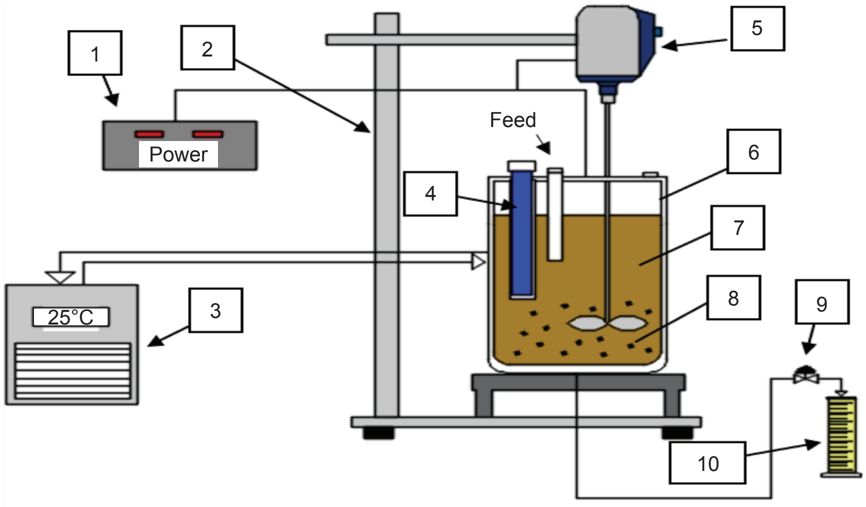
Ten g of powdered form of dried lemongrass leaves were heated with 100 mL of deionised water for 2 hr at 80°C. The lemongrass solution was subsequently filtered using filter paper (Brand: Whatmann No. 1). The filtrate was used to synthesise ZnO-L NPs in accordance with the previous protocol of Sidik et al. (2020). The precipitation method involved the addition of 0.10 M zinc acetate, 0.15 M oxalic acid and lemongrass leaves extract based on various ratios of ZnO solution to lemongrass leaves extract (1:3 and 1:9). A magnetic stirrer was used to vigorously stir the mixture for 24 hr. The precipitated material was then filtered from the mixture and dried in an oven at 100°C for 1 hr. Upon synthesising, the extracted material was calcined in a furnace for 2 hr at 500°C. Fourier transform infrared spectrophotometer (FTIR Model; Agilent Tech Cary, USA), field-emission scanning electron microscopy (FESEM Model; JEOL-JSM 7600F) and energy dispersive X-ray analysis (EDS) were used to characterise the ZnO-L NPs.

Photocatalytic Anaerobic Treated Palm Oil Mill Effluent (AnT-POME) by Modified Zinc Oxide-Lemongrass Nanoparticles (ZnO-L NPs)

Various parameters influencing the photocatalytic activity of the ZnO-L NPs were examined during the photocatalytic degradation process of AnT-POME. First and foremost, the photocatalytic procedure was carried out utilising a technique established by Puasa et al. (2021) using different ZnO-L NPs (1:3 and 1:9), commercial ZnO and without ZnO under pH 8, AnT-POME initial concentration of 50% and photocatalyst loading of 0.1 g/L. *Figure 1* depicts the schematic diagram of the photocatalytic process at the laboratory scale that was used in this study. The photocatalytic process of AnT-POME was carried out in a 2.0 L reactor with an ultraviolet (UV) lamp and ZnO-L NPs. The light source was a UV lamp that emitted mostly at 253.7 nm and had a defined power of 18 W. Before beginning the photocatalysis procedure, the mixture was rigorously stirred using an impeller (PL111) with an overhead stirrer (WireStir HS-30D) at 150 rpm in the dark for 30 min to achieve photocatalyst adsorption-desorption equilibrium. A water chiller was used to circulate cooling water throughout the process, keeping the temperature at ambient (25°C) levels. At intervals of 10 min, approximately 5.0 mL of the sample was taken out of the reactor for COD analysis. The whole process was repeated by using a different photocatalyst loading (0.1, 0.3 and 0.5 g/L) and pH (4, 6 and 8) to determine the best operating condition for the photocatalytic process. To prevent contamination, the photocatalytic treated effluent was collected and kept at 4°C before the performance study.

Analytical Method

The treated AnT-POME samples were separated by using a centrifuge for 20 min at 8,000 rpm under



Note: 1 - Power source; 2 - Retort stand; 3 - Water chiller, 4 - UV lamp; 5 - Overhead stirrer, 6 - Photocatalytic reactor; 7 - AnT-POME; 8 - ZnO-L NPs; 9 - Valve; 10 - Measuring cylinder.

Figure 1. Schematic diagram of the photocatalytic reactor.

4°C. The treated AnT-POME samples were analysed in terms of colour intensity, COD, turbidity and pH. According to the ADMI standard procedure (97 Color ADMI 1 inch), the colour intensity was measured using a DR6000 UV-Vis Laboratory Spectrophotometer (Hach, Germany). The treated AnT-POME (2 mL) was added into COD digestion reagent vials then was inserted into the preheat DRB200 (Hach, Germany) reactor and heated at 150°C for 2 hr. The sample was then cooled to room temperature before further analysis using the DR6000 Spectrophotometer. The turbidity was determined via a portable turbidity meter (Model: Cole Parmer Oakton, Cole-Parmer, USA) and pH was measured using a portable milwaukee pH meter (Milwaukee Instrument, USA). The percentage of removal was calculated according to Equation (1) (Sidik et al., 2020).

$$\text{Removal } (\%) = \frac{(C_0 - C_t)}{(C_0 \times 100)} \tag{1}$$

where, C_0 and C_t denote the colour intensity, COD and turbidity at time zero and at time (t), respectively.

Kinetic Study of Photocatalytic Anaerobic Treated Palm Oil Mill Effluent (AnT-POME)

The photocatalytic activity of AnT-POME in the presence of ZnO-L NPs was established via a pseudo-first-order kinetic model according to the Langmuir-Hinshelwood (L-H) rate law [Equation (2)].

$$-\frac{dC_A}{dt} = (-r_A) = \frac{kC_A}{1 + K_A C_A} \tag{2}$$

It is possible to further simplify the expression for the denominator to $(1 >> K_A\, C_A) \approx 1$ at low reactant concentrations, reflected in this investigation by the organic content of the AnT-POME. As a result, a single Power Law model may easily explain the AnT-POME decomposition behaviour. The batch photocatalytic kinetics that adheres to the pseudo-first-order reaction kinetics is as shown in Equation (3).

$$-\frac{dC_A}{dt} = kC_A \tag{3}$$

The expression shown in Equation (4) is obtained by integrating Equation (3):

$$\text{Ln}\left(\frac{C}{C_O}\right) = kt \tag{4}$$

where, C_O is the starting COD concentration (*mg/L*), C is the COD concentration at time (mg/L), t is the time (min), and *k* is the apparent specific response time (min^{-1}). The particular reaction time, *k*, for each experiment, as well as the R^2 values, will be established by plotting a graph of Ln $\left(\frac{C}{C_O}\right)$ vs. time (Schnabel et al., 2022).

RESULTS AND DISCUSSION

Characterisation of Zinc Oxide-Lemongrass Nanoparticles (ZnO-L NPs)

As shown in *Figure 2a*, FTIR spectroscopy was used to demonstrate the functional groups composition presence in ZnO-L 1:3 NPs within the 400–6,000 cm^{-1} infrared adsorption band. According to *Figure 2a*, the structures of the O–H groups that define the polysaccharides from lemongrass leaves had a vibration frequency of 3,390.199 cm^{-1}. This phenomenon might be brought on by some biomolecules that were isolated from lemongrass leaves, like phenolic compounds. In accordance with Ajayi and Afolayan (2017) and Devi et al. (2016), the protein's aromatic stretching of –C–N, which serves as both a capping and a reducing agent, is the cause of the extremely strong absorption peak at 1,444.402 cm^{-1}. Based on Sidhu et al. (2022), the amide bond and aromatic ring both played a role in the production of the metal nanoparticles and could serve as their capping agents. The function of amide bonds and aromatic rings as capping agents is attributed to their distinctive chemical structures. Amide bonds possess strong conjugative effects and hydrogen bonding capabilities, which significantly enhance the stability of materials. Meanwhile, the π-π stacking interactions inherent in aromatic rings facilitate robust intermolecular interactions at interfaces, thereby augmenting the material's physicochemical properties (Cao et al., 2024; Shi et al., 2020a, 2020c).

The tiny particles were seen to be well-shaped. The FESEM image (*Figure 2b*) revealed aggregates of particles, and the aggregation may have been caused by the ZnO-L NPs' high surface energy. According to the TEM images (*Figure 2c*), the ZnO-L NPs 1:3 observed in this investigation are agglomerates with a hexagonal and spherical structure, having an average particle size between 20 and 60 nm. The results of the elemental analysis using EDX spectroscopy are shown in *Figure 2d*. A qualitative and quantitative status of the elements that might have been involved in the creation of ZnO-L NPs is provided by EDX analysis. Carbon, oxygen and zinc are the three elements that make up the synthesised ZnO-L NPs. Strong signals for zinc and oxygen in the ZnO-L NPs show that ZnO NPs are formed.

Effect on Different Types of Zinc Oxide (ZnO) in Photocatalytic

In order to improve the photocatalytic performance, it is critical to investigate the ideal amount of photocatalyst used in the process. From an economic perspective, this is also a crucial criterion. The inclusion of ZnO-L NPs in the photocatalytic process allows the

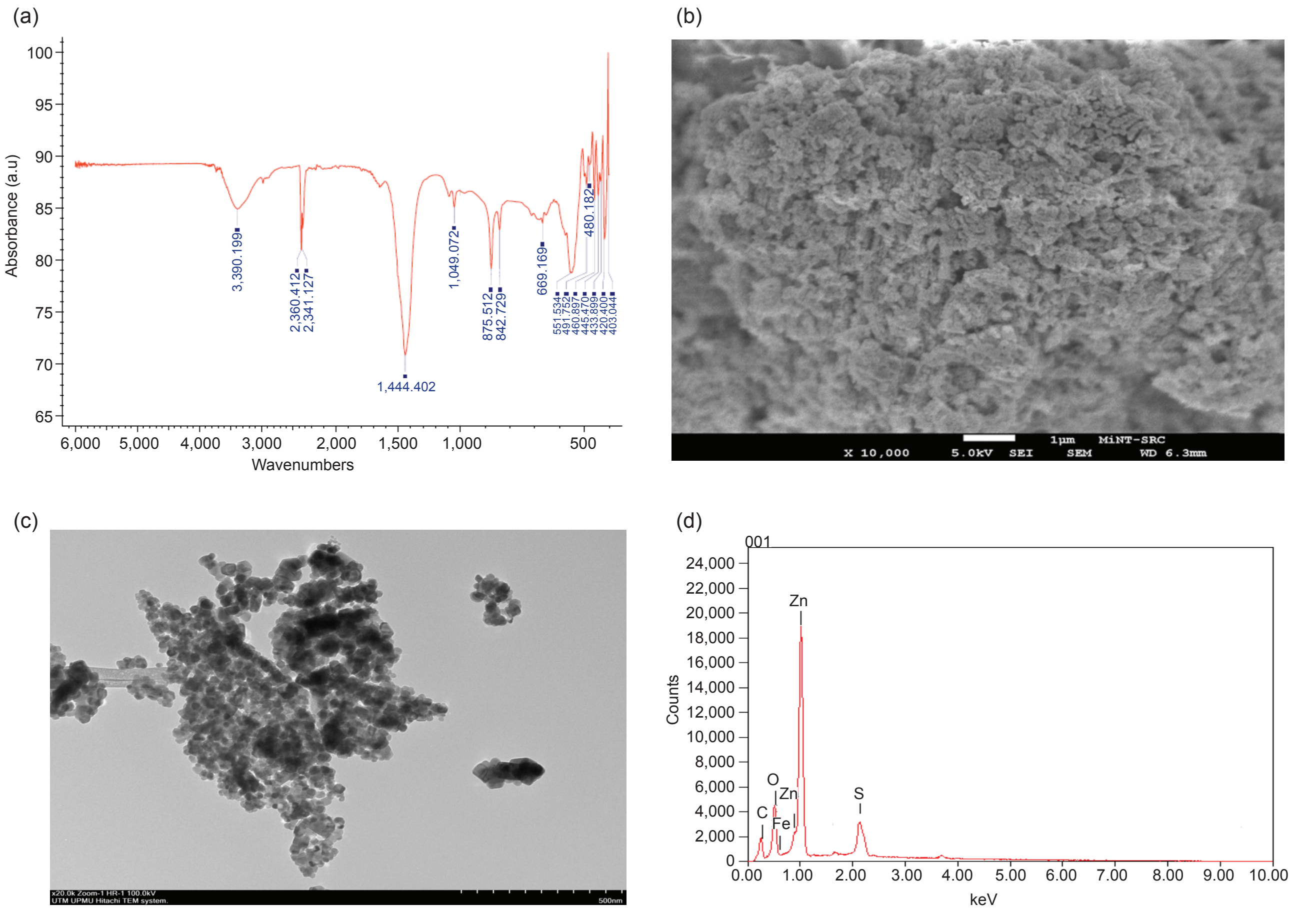


Figure 2. Characterisation of ZnO-L 1:3 NPs utilising (a) FTIR spectrum, (b) FESEM image, (c) TEM image and (d) EDX profile.

pigment particles in AnT-POME to be degraded, resulting in lower colour intensity, turbidity and COD.

Photocatalytic activity may vary depending on the catalyst's surface or characteristics, which impact pollutant adsorption and electron-hole pair rate. Thus, it is critical to understand the impacts of various photocatalysts, such as ZnO-L 1:3 NPs, ZnO-L 1:9 NPs, without ZnO and commercial ZnO, on the decolourisation and degradation of AnT, as demonstrated in this study. In comparison to ZnO-L 1:9 NPs, without ZnO and commercial ZnO, *Table 1* demonstrates that treated AnT-POME in the occurrence of ZnO-L 1:3 NPs has the best performance in terms of colour, turbidity and COD value. The colour intensity of AnT-POME that was treated using ZnO-L 1:3 NPs was 573.6 ADMI after undergoing photocatalytic treatment for 60 min. The value still surpasses the usual DOE criterion, which is that it must be less than 200.0 ADMI (Bello & Raman, 2017). Previous studies utilising various types of photocatalysts, such as ZnO-Clay (Awang et al., 2024), TiO_2 (Nawaz et al., 2020), and ZnO-PEG (Zainuri et al., 2018), have similarly encountered issues with the colour removal efficiency of AnT-POME. In order to diminish the colour till it reaches the specified value, AnT-POME must still undergo tertiary treatment.

Furthermore, the COD value obtained in the presence of ZnO-L 1:3 NPs is 20.3 mg/L lower than the minimum requirements set by DOE (50.0 mg/L) (Muhamad et al., 2022). Turbidity data for ZnO-L 1:3 NPs show the lowest value when compared to ZnO-L 1:9 NPs, commercial ZnO, and no ZnO are present. The fact that COD values were greater when no catalyst was introduced indicates that only a small quantity of the pollutant was broken down, demonstrating that the pollutant's molecular structure was stable and challenging to eliminate (Shi et al., 2020d). According to these findings, ZnO-L 1:3 NPs are thought to be the most efficient photocatalyst for treating AnT-POME through the photocatalytic treatment technique. This result can be explained by the tiny well-shaped ZnO-L 1:3 NP particles that were discussed in the previous section.

Figure 3a demonstrates that following photocatalytic treatment, the greatest COD elimination percentage is 97.02% at 0.1 g/L loading of ZnO-L 1:3 NPs. The proportion of COD removed from the treated AnT-POME without ZnO and commercial ZnO is 72.62% and 70.62%, respectively. This high COD removal from ZnO-L NPs may be attributed to the green synthesis capping agent's increased ability to stabilise nanoparticles and break down organic compounds in AnT-POME, which in turn improves photocatalytic performance. It is evident that the presence of ZnO-L 1:3 NPs resulted in a higher percentage of colour and turbidity removal compared to ZnO-L 1:9 NPs, commercial ZnO and without ZnO. As a result, the AnT-POME was treated with green ZnO-L NPs, which improved AnT-POME performance.

Photocatalysis is a surface-oriented process involving the adsorption of organic contaminants in the form of COD on the surface of ZnO NPs. *Figure 3b* shows the outcomes of an experiment in which the contact time for various types of ZnO was varied in order to determine the effect of contact time on COD removal AnT-POME. The graph clearly shows that COD elimination percentages improved significantly when the contact duration was increased from 10–90 min, reaching a maximum of 97.02%. The COD removal (%) capacity was sluggish from 30–90 min. It is beneficial to use modified green ZnO-L 1:3 NPs to increase the adsorption of AnT-POME contaminants on the photocatalyst surface, resulting in greater interaction between the pollutants and the catalyst. As a result, more pollutants can be degraded, increasing COD removal after 30 min.

Effect on Different Loading of Zinc Oxide-Lemongrass Nanoparticles (ZnO-L NPs) in Photocatalytic

The performance of the photocatalytic process is significantly influenced by the impact of various loadings of photocatalyst. The total photo-degradation process is greatly influenced by the number of ZnO-L NPs required to initiate a photocatalytic reaction, and the concentration of ZnO-L NPs is also crucial for developing a genuinely heterogeneous photocatalytic system. Following photocatalytic treatment, the treated AnT-POME from various ZnO-L NPs loadings (0.1, 0.3 and 0.5 g/L) are shown in *Table 2*. After 60 min

TABLE 1. CHARACTERISTIC OF UNTREATED AND TREATED AnT-POME UNDER DIFFERENT TYPES OF ZnO

Parameter	Unit	Untreated AnT-POME	Type of ZnO NPs			
			Without ZnO	Commercial ZnO	ZnO-L NPs 1:3	ZnO-L NPs 1:9
Colour	ADMI	1,168.00	605.33 ± 0.47	607.33 ± 0.47	573.67 ± 0.47	612.67 ± 0.47
Turbidity	NTU	29.20	10.56 ± 0.01	14.65 ± 0.13	4.20 ± 0.10	9.32 ± 0.08
COD	mg/L	683.00	211.00 ± 0.00	200.67 ± 0.47	20.33 ± 0.47	192.67 ± 0.47

Note: AnT-POME - anaerobic treated palm oil mill effluent; ZnO - zinc oxide; ZnO NPs - zinc oxide nanoparticles; ZnO-L NPs - zinc oxide-lemongrass nanoparticles; COD - chemical oxygen demand.

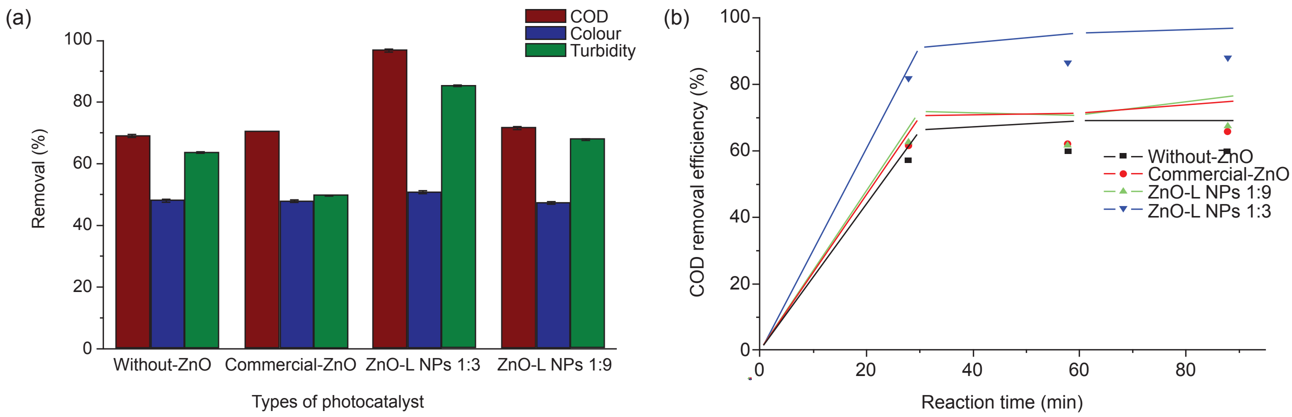


Figure 3. (a) Removal percentage of anaerobic treated palm oil mill effluent (AnT-POME) and (b) chemical oxygen demand (COD) removal efficiency over reaction time using different types of photocatalyst.

TABLE 2. CHARACTERISTIC OF TREATED AnT-POME UNDER DIFFERENT LOADING OF ZnO-L (1:3) NPS

Parameter	Unit	Untreated AnT-POME	Different loading of ZnO-L NPs		
			0.1	0.3	0.5
Colour	ADMI	1,168.00	573.67 ± 0.58	602.00 ± 0.00	600.33 ± 0.58
Turbidity	NTU	29.20	4.20 ± 0.12	7.88 ± 0.13	5.27 ± 0.02
COD	mg/L	683.00	20.33 ± 0.58	217.33 ± 0.58	250.00 ± 0.00

Note: AnT-POME - anaerobic treated palm oil mill effluent; ZnO-L NPs - zinc oxide-lemongrass nanoparticles; COD - chemical oxygen demand.

of treatment, it was discovered that ZnO-L NPs with a loading of 0.1 g/L produced the best results, 573.6 ADMI for colour, 4.2 NTU for turbidity and 20.3 mg/L for COD were reported.

The proportion of COD, colour and turbidity removal from treated AnT-POME is shown in *Figure 4*. After the photocatalytic process, the small variation in AnT-POME percentage for colour removal is negligible. Increased photocatalyst loading boosts photocatalytic activity, however once a saturation phase is reached, it causes cloudiness in the AnT-POME. Additionally, as shown in *Figure 4*, the percentage turbidity removal trend and colour removal trend are similar. The maximum rate of turbidity reduction, 85.62%, is demonstrated by ZnO-L NPs with a loading of 0.1 g/L. This may be due to the fact that a small concentration of ZnO-L NPs had an impact on the percentage of turbidity removal. While ZnO-L NPs loading of 0.3 and 0.5 g/L results in turbidity removal percentages of 73.03% and 81.96%, respectively.

The COD removal percentage trend indicates that the maximum COD reduction of 97.08% for AnT-POME was achieved at 0.1 g/L of ZnO-L NPs. Meanwhile, the COD percentage removal of the treated AnT-POME decreased significantly as the loading increased. One possibility is that the smaller size of ZnO-L NPs' results in higher surface areas for UV light absorption. Thus, for light to enter and degrade the organic molecules in AnT-POME, a lower catalyst concentration is preferred, leading to a higher COD removal.

Effect on Different pH

In the heterogeneous photocatalytic process, the impact of pH has a major impact on the efficiency of photocatalytic degradation. The AnT-POME sample was tested at different pH levels of 4, 6 and 8. Following a 60 min photocatalytic process, *Table 3* shows the results of treated AnT-POME at various pH levels, while *Figure 5* depicts the pattern of colour, turbidity and COD removal, respectively.

According to the data, the photocatalytic process of AnT-POME at pH 6 has the lowest reading of the colour value, which is 551 ADMI, and demonstrates the largest proportion of colour degradation, which is 52.83%, in comparison to pH 4 and pH 8, where colour degradation is 51.28% and 50.88% respectively. The photocatalytic process with pH 6 also has the best turbidity reduction (90.11%) compared to pH 4 and pH 8, which had 88.32% and 85.62% reductions, respectively. The increased trend of removal at pH 6 may be caused by more ZnO-L NPs surface area available for UV light absorption, which will accelerate the photocatalytic process's degradation.

The tendency of pollutants in AnT-POME to aggregate under acidic conditions reduces the amount of active photocatalyst surface sites available for UV light to start the adsorption process, which may be the reason that acidic conditions cause less degradation. As a result, this barrier might slow down photocatalytic

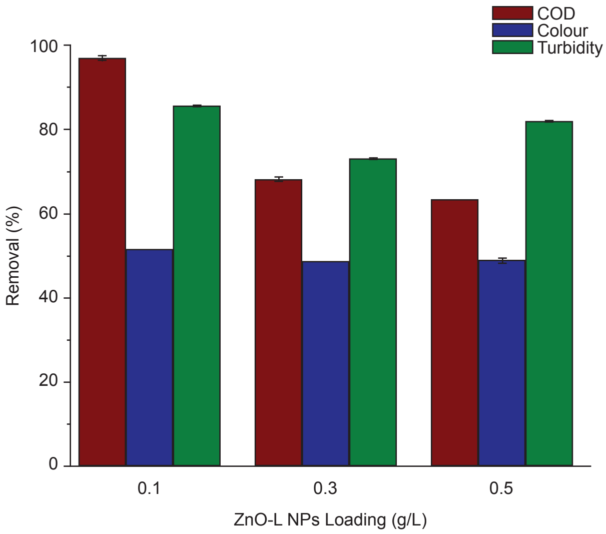


Figure 4. Percentage of anaerobic treated palm oil mill effluent (AnT-POME) from different loading of ZnO-L NPs.

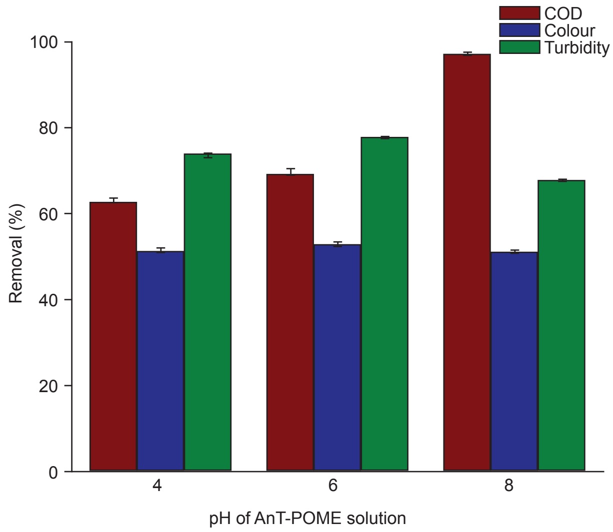


Figure 5. Removal percentage of anaerobic treated palm oil mill effluent (AnT-POME) under different pH.

TABLE 3. CHARACTERISTIC OF TREATED AnT-POME UNDER DIFFERENT pH CONDITIONS

Parameter	Unit	Untreated AnT-POME	Different pH conditions		
			4	6	8
Colour	ADMI	1,168.00	568.67 ± 0.58	550.67 ± 0.58	573.67 ± 0.58
Turbidity	NTU	29.20	3.41 ± 0.20	2.89 ± 0.08	4.20 ± 0.12
COD	mg/L	683.00	255.00 ± 1.00	209.67 ± 1.15	20.33 ± 0.58

Note: AnT-POME - anaerobic treated palm oil mill effluent; COD - chemical oxygen demand.

degradation and boost AnT-POME's colour intensity. The maximum COD elimination (97.02%) at pH 8 may be attributed to the more hydroxyl groups present in the basic state, which are able to breakdown more organic pollutants in AnT-POME. However, the presence of hydroxyl ions in base conditions above a particular limit may reduce colour removal. This behaviour might be caused by the competing molecule interfering with the photocatalytic breakdown of AnT-POME pigment.

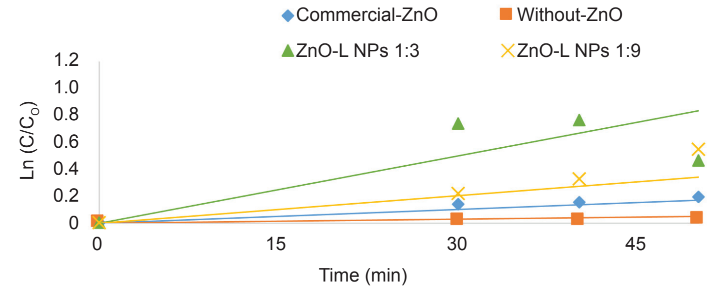
Kinetic Study of Photocatalytic Anaerobic Treated Palm Oil Mill Effluent (AnT-POME)

Photocatalysis is the term used to describe the photoreaction-based pollutant degradation process that occurs on the ZnO-L photocatalyst surface. The process of degradation may differ depending on the type of pollutants and the organic materials they include. AnT-POME degradation kinetic models were evaluated for various photocatalyst types, ZnO-L NPs loading (0.1, 0.3 and 0.5 g/L), and pH (4, 6 and 8). A 60 min contact time was used throughout the entire experiment. As shown in *Figure 6*, the graph between Ln (C/C_O) and time exhibits a linear pattern with pseudo-first-order kinetics for different conditions in photocatalytic degradation of AnT-POME. The correlation coefficient (R^2) and rate of constant value (K) were calculated and presented in *Figure 6*.

The experimental findings with R^2 greater than 0.90 for each condition significantly suited the Langmuir-Hinshelwood model. The R^2 of commercial ZnO and ZnO-L 1:3 NPs were greater than 0.90, indicating that the photodegradation of AnT-POME followed the pseudo-first-order kinetics better than ZnO-L NPs 1:9 and without ZnO. However, the higher K, for various ZnO applications reveals that ZnO-L NPs 1:3 (0.0167) degrade at a faster rate than commercial ZnO (0.0034) conditions. As evidenced by the results, ZnO-L NPs exhibit greater photocatalytic activity.

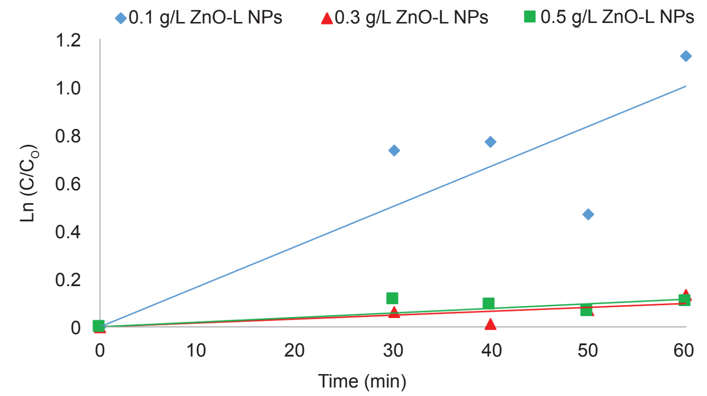
The R^2 (0.9172) and K (0.0167) values are maximum at 0.1 g/L loading. Meanwhile, at 0.3 and 0.5 g/L loading of ZnO-L, the R^2 and K values decline. This result is supported by the higher removal of COD, colour and turbidity for 0.1 g/L ZnO-L loading as mentioned in the previous section. The shortage of a binding area found in ZnO-L photocatalyst causes the AnT-POME degradation efficiency to decrease with increasing loading. As a result, the optimal loading of ZnO-L NPs of 0.1 g/L was obtained in order to assess the future experimental process in large-scale applications to treat AnT-POME.

(a)



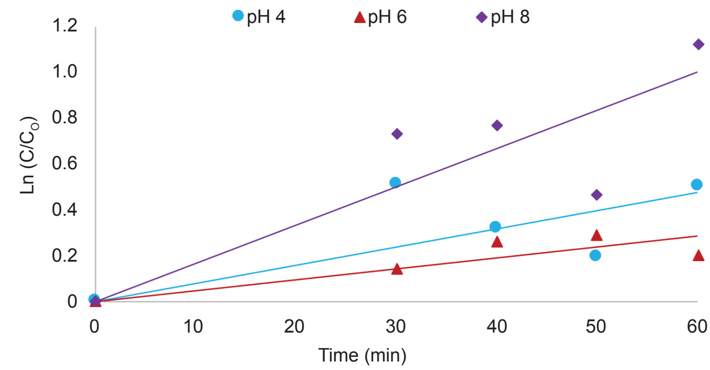
Condition	Value	
	R^2	K
Commercial ZnO	0.9621	0.0034
Without ZnO	0.7603	0.0010
ZnO-L NPs 1:3	0.9169	0.0167
ZnO-L NPs 1:9	0.8116	0.0069

(b)



Condition	Value	
	R^2	K
0.1 g/L ZnO-L NPs	0.9172	0.0167
0.3 g/L ZnO-L NPs	0.8376	0.0016
0.5 g/L ZnO-L NPs	0.8795	0.0019

(c)



Condition	Value	
	R^2	K
pH 4	0.8254	0.0080
pH 6	0.9297	0.0048
pH 8	0.9169	0.0167

Note: The Langmuir-Hinshelwood model evaluation along with the rate constant values (K) and the correlation coefficient (R^2) for the degradation of AnT-POME.

Figure 6. Kinetic study for the degradation of AnT-POME at the different (a) types of ZnO, (b) ZnO-L loading and (c) pH of AnT-POME.

It is evident that as the pH rises from 6–8, the photocatalytic degradation increases. In fact, the maximum K value of 0.0167 has been achieved at pH 8, which also has the highest COD removal effectiveness (97.02%). More hydroxyl radicals are produced under basic conditions, which leads to an increase in high photocatalytic activity at higher pH. According to Chin et al. (2022), hydroxyl radicals are crucial in the breakdown of POME into simple compounds.

Numerous researchers are working on photocatalytic treatment. *Table 4* summarises previous studies on photocatalytic efficiency for pollutant degradation using various published photocatalysts. The removal efficiency and rate constant for the degradation of pollutants have been influenced by the photocatalyst's size. The higher particle size during the photocatalytic process may result in a lower adsorption rate, which could explain the lower degradation efficiency and first order kinetic rate (Ramesh et al., 2021; Shkir et al., 2024a). Most studies in *Table 4* demonstrate the impact of modified photocatalysts, which drastically reduce their size while increasing their specific surface area and photocatalytic performance. This is beneficial for UV light absorption and helps break down more contaminant molecules (Chandekar et al., 2020; Shi et al., 2020b; Shkir et al., 2024b). Additionally, the outcomes of this investigation demonstrate that the morphological change of biosynthetic ZnO-L NPs 1:3 is attributed to the improvement of the photocatalytic performance of AnT-POME COD removal. Therefore, the specific role of biomolecule compounds in biosynthetic nanoparticles might be investigated further to show the precise mechanisms or contributions of these compounds to the material that improves photocatalyst morphology.

CONCLUSION

In conclusion, the addition of ZnO-L NPs in the photocatalytic process for treating AnT-POME improved the performance in terms of colour, turbidity and COD elimination compared to without ZnO and commercial ZnO. The findings of this investigation confirmed that AnT-POME treatment via photocatalytic process in the presence of green ZnO-L NPs 1:3 significantly improved the treated AnT-POME quality owing to the reduced size of nanoparticles (20-60 nm). The optimal photocatalyst loading for AnT-POME treatment via the photocatalytic degradation process was 0.1 g/L due to the high rate of AnT-POME particle degradation. Furthermore, water quality analysis revealed that the treated AnT-POME under the ideal photocatalyst loading (0.1 g/L) and pH (6) approaches the effluent standard as per the Environmental Quality (Prescribed Premises) (Crude Palm Oil) Regulations 1977, under the Environmental Quality Act 1974. It was discovered that the treated AnT-POME had percentages of removal for turbidity (85.62%), COD (97.02%) and colour (50.88%). As a result, it is thought that this study has a great deal of potential for use in the palm oil mill business. This study's weakness is that it has yet to be carried out on a pilot scale due to the high cost and lack of resources. Additionally, more study on the behaviour of ZnO-L NPs across

TABLE 4. COMPARISON OF PHOTOCATALYTIC EFFICIENCY OVER VARIOUS REPORTED PHOTOCATALYSTS FROM PREVIOUS RESEARCH

Photocatalyst	Pollutant	Removal/ degradation efficiency (%)	Rate constant, k (min^{-1})	Size photocatalyst (nm)	Reference
LaCa	POME	54.0	0.0036	~85.0	Ghazali et al. (2019)
Cu @ ZnO	Methylene green	75.0–80.0	~0.0400	26.4–28.9	Chandekar et al. (2020)
Cu_3P-ZSO-CN p-n–n	Antibiotic	55.0–98.0	0.0543	NA	Guo et al. (2021)
$BiPO_4/CuBi_2O_4$	Tetracycline	92.0	0.0241	150.0–200.0	Lu et al. (2022)
TiO_2/RGO	Bisphenol-A	98.5	0.0009	23.0–30.0	Ramesh et al. (2021)
CO_5/H-CN	Tetracycline	86.0	~0.0130	NA	Shi et al. (2020b)
$AgPO_4/CO_3(PO_4)_2$/g-C_3N_4	Tetracycline	88.0	0.0159	40.0–100.0	Shi et al. (2020d)
Tb@ZnO	Methylene green	~90.0	~0.0200	NA	Shkir et al. (2020b)
Sr:ZnO	Methylene blue	~70.0	~0.0300	16.0–24.0	Shkir et al. (2020a)
Ni:ZnO	Methylene blue	94.0	0.0133	20.0–100.0	Shkir et al. (2022b)
$BaFe_2O_4$/BiOCl	POME	61.0	0.0045	NA	Tan et al. (2023)
MgO/Ag:MO	Rh-B dye	84.0–92.0	0.0131–0.0199	21.0–28.0	Shkir et al. (2024a)
Ag:NiO	Methylene blue/Rh-B dye	88.0–99.0	0.0153–0.0309	15.0–40.0	Shkir et al. (2024b)
ZnO-L	AnT-POME	50.0–97.0	0.0167	20.0–60.0	This study

a range of hues is advised to determine their adaptability and wider usefulness in a variety of real-world situations. Therefore, the results of this study will serve as the foundation for further investigation into the photocatalytic treatment of AnT-POME on a pilot scale.

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SMALL SCALE FIELD EVALUATION OF A NOVEL PALM-BASED INSECTICIDE FORMULATION USING COLD AND THERMAL FOGGING AGAINST *Aedes aegypti*

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ABSTRACT

A new insecticide formulation with deltamethrin as an active ingredient (F1) has been developed in order to suppress the worldwide spread of mosquito-borne diseases. The formulation is naphtha-free and utilises locally sourced palm-based materials to obtain a cost-effective product. The objective of this study is to assess the performance of the formulation, in terms of droplet size and efficacy, in comparison to a commercial naphtha-based insecticide (F2). Open-field bioassay of the formulations against adult Aedes aegypti *was conducted using cold and thermal fogging methods and measured at distance points 25, 50, 75 and 100 m. Overall, droplet size and efficacy significantly decreased with increasing distance from the spray path. No significant differences in volume median diameter (VMD) were observed between fogging methods and formulations. In general, both formulations showed significantly higher efficacy as cold fogs than thermal fogs. Significant differences in knockdown effect were observed between methods, time and formulations. Nevertheless, the definite effect of insecticidal formulations, i.e. mortality, was not significantly different between F1 and F2. In conclusion, our results show that the palm-based formulation was able to achieve comparable performance with the commercial formulation without the use of naphtha.*

Keywords: deltamethrin, droplet size, efficacy, space spray, vector control.

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INTRODUCTION

Dengue, an arboviral disease, has spread rapidly across the globe and is becoming endemic in more than 100 countries (Selvarajoo et al., 2020). Regular dengue outbreaks have occurred in Malaysia since the 1960s, and the case numbers continue to increase alongside Malaysia's rapid population and infrastructure growth. Malaysia recorded the biggest spike in dengue cases in four years in 2019 (AbuBakar et al., 2022). Dengue vector control activities in Malaysia rely heavily on human resources (Yazan et al., 2021). Thus, lockdowns or movement control order (MCO) to contain the spread of Coronavirus disease 2019 (COVID-19) have significantly curtailed regular dengue control operations (Rahim et al., 2021). At present, there are no effective treatments available for dengue. Furthermore, the current vaccine exhibits moderate efficacy and fails to provide balanced protection against all four dengue serotypes (Stanaway et al., 2016). Hence, control of the dengue vector population remains the mainstay strategy for containing the spread of the disease.

Space spraying using thermal and cold foggers is an often-used intervention strategy for controlling mosquito-borne diseases (Pryce et al., 2018). However, space sprays are only effective when the droplets remain airborne. Thus, multiple applications are necessary for effective control of the dengue vector population (World Health Organization [WHO], 2009). With the ever-increasing fuel cost and concerns for adverse environmental impacts from frequent space spray interventions, the use of water-dilutable water-based formulation is turning into an appealing and trendy alternative to oil-

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based formulation. Questions have been raised about the efficacy of water-based formulations as the vapour pressure of water causes droplet evaporation and consequently size reduction, creating insecticide droplets that are prone to drift (Farajollahi & Williams, 2013). Nevertheless, efficacy studies using water-based formulations have demonstrated that they are equally or more effective than formulations using diesel fuel or mineral oil as a diluent (Farajollahi & Williams, 2013; Harburguer et al., 2012a).

In recent years, bio-based formulations incorporating plant and microbial bioactives have been explored to address environmental impacts and insecticide resistance in mosquito control. Bioformulations of plant extracts from *Carica papaya* and *Parthenium hysterophorous* have shown promising larvicidal activity against *Aedes aegypti* (Jayaraman et al., 2023). Entomopathogenic fungi, *Beauveria bassiana* JN5R1W1, when formulated as inverted emulsions with soybean oil demonstrate high mortality rates against adult mosquitoes through both direct and indirect contact methods (Yong-Lee et al., 2023). Under the field-simulated trial, *Ocimum kilimandscharicum* oil formulation achieved 98% larvicidal mortality against *Anopheles gambiae* larvae after 24 hr, as opposed to 54% mortality achieved by *Bacillus thuringiensis* subsp. *israelensis* granules (Ochola et al., 2022). While these bioformulations comprising bioactives show promise, they often lack the robustness and field applicability of synthetic chemicals. Thus, while bioactives provide potential supplemental strategies, synthetic chemicals remain the cornerstone of effective vector control in the field.

In response to these challenges, a novel water-based insecticidal formulation (F1) comprising palm-based solvents and surfactants has been developed to restrain dengue outbreaks and minimise the environmental impact of vector control (Mohsin et al., 2019). The formulation contains deltamethrin, a larvicide and adulticide that causes an instant knockdown by paralysing the nervous system of insects (Kumar et al., 2011). F1 is free from highly volatile and flammable naphtha solvent and incorporates palm-based methyl esters (PME) as a substitute. PME is less volatile and possesses higher flash point compared to petroleum-based solvents (Mohsin et al., 2017). Additionally, F1 comprises non-toxic and readily biodegradable palm-based surfactants which are instrumental in improving the stability of pesticide emulsions (Ghazali & Ahmad, 2004; Ismail et al., 2014; Shaari et al., 2022; Siti Afida et al., 2016). Malaysia's export revenue of palm-based oleochemicals in 2023 declined by 30.1% from the previous year despite the marginal improvement in export volume (4.8%) due to lower imports by the major buyers of Malaysian palm oil (Parveez et al., 2024). Given the recent decline, leveraging these locally sourced bio-based components offers both economic and strategic benefits by reducing dependency on imported hydrocarbons.

This study addresses the novel application of F1 as an environmentally friendly and cost-effective solution for dengue vector control. By comparing F1 to a commercially available deltamethrin formulation (F2) in an open-field setting, we aim to evaluate their respective efficacies in terms of droplet size distribution, knockdown and mortality of the primary dengue virus vector *Ae. aegypti* using cold and thermal fogging methods. This investigation is critical in determining whether a palm-based water-dilutable insecticide can meet or exceed the performance of conventional formulations, providing a new, sustainable avenue for managing dengue outbreaks in Malaysia and other endemic regions.

MATERIALS AND METHODS

Biological Material

Sucrose-fed adult *Ae. aegypti* females (aged 2–5 days) from the Vector Control Research Unit, Universiti Sains Malaysia (USM), Penang, Malaysia, were used for the adulticidal assessments.

Chemicals

PME comprising a mixture of methyl caproate, methyl octanoate, methyl decanoate and methyl laurate at 3.2 wt%, 50.8 wt%, 44.0 wt% and 2.0 wt%, respectively, was provided by Emery Oleochemicals Sdn. Bhd., Malaysia. Deltamethrin (98.0%) was donated by Hextar Chemicals Sdn. Bhd. Malaysia. Xanthan gum derived from *Xanthomonas campestris* was purchased from Sigma-Aldrich Sdn. Bhd., Malaysia. Bio-based surfactants, C12–C14 fatty alcohol ethoxylate and polyoxyethylene (20) sorbitan monooleate, are used in the study. The former was obtained from Huntsman Singapore Pte. Ltd., Singapore, while the latter was purchased from Chemweb Sdn. Bhd., Malaysia.

Insecticide Formulations

F1 was prepared using the method in a previous study by Mohsin et al., (2019). In brief, the formulation was developed using 2.0 wt% deltamethrin, 5.0 wt% mixture of the previously mentioned bio-based surfactants and 13.0 wt% PME as the oil phase and distilled water and 0.4 wt% xanthan gum as the aqueous phase. The hydrophilic-lipophilic balance of the surfactant was kept constant at 10. The oil phase was prepared by stirring for 10 min at 55°C, while the aqueous phase was prepared by dispersing xanthan gum in distilled water at 55°C. Thereon, the oil phase was gradually incorporated into the aqueous phase using a Kinematica Polytron® PT3100 homogeniser (Kinematica AG, Switzerland) operated at 7,000 rpm for 10 min. The commercial insecticide (F2) is an oil-in-water (o/w) emulsion formulation containing 2.0 wt% deltamethrin and petroleum-based naphtha from Bayer Environmental Science, Spain. The blank formulation (F3) was similarly produced according to the previously mentioned process without deltamethrin to examine the possible insecticidal activity of the emulsion system.

Fogging Method

All insecticide formulations were water-diluted at a dilution ratio of 1:100 v/v. Then, thermal fogging was applied at 5,000 mL ha^{-1} using a hand carried Agrofog Hand Held Thermal Fogger (Agro Technic Pte. Ltd., Singapore). On the other hand, cold fogging was performed at 500 mL ha^{-1} using a truck mounted LECO ULV Fog Generator Model 1800 (Clarke, USA) with its sprayer head nozzle angled 30° upwards to the horizontal plane. The truck then travelled 6–9 km hr^{-1} at a distance of 200 m perpendicular to the spray angle.

Droplet Size Measurement and Efficacy Assessment

Field tests were performed in accordance with the WHO guidelines WHO (2009) in an open field in USM, with no tall grasses that should hinder fog movement. The study was conducted during fair weather. The temperature during treatment was 27°C–30°C, the relative humidity was 70%–80% and the wind speed was 1.5–3.0 m s^{-1}. A total of 20 mosquitoes were held in nylon mesh-screened cylindrical cages (1.2 mm mesh size) with wire frame support (10 cm diameter × 15 cm height × 10 cm tapping cover). The cages were suspended at 1.5 m from the ground on poles distanced at 25, 50, 75 and 100 m from the spray nozzle. Knockdown was recorded after 15 min, and the mosquitoes were transferred into mesh covered cups using battery operated aspirator and returned to the laboratory. The cups were provided with a cotton pad soaked with 10% sucrose solution. Then, knockdown was recorded again at 30 min and 60 min post-treatment while adult mortality was recorded at 24 hr post-treatment. Three replicates were conducted for each formulation (including water control i.e., water without insecticide). Results from the water control treatment were excluded from reporting as knockdown and mortality activities were not observed. In each test, one set of adult cages was kept in the laboratory as a control. If mortality was above 20% in the concurrent controls, the whole test would be rejected. Results from the treated samples will be corrected using Abbott's formula if mortality in the controls was above 5% (Abbott, 1925). Abbott's formula for calculating corrected mortality is as Equation (1):

$$\frac{\begin{array}{c}\text{\% Mortality in treated} - \\ \text{\% Mortality in control}\end{array}}{100\% - \text{\% Mortality in control}} \times 100 \qquad (1)$$

To record fog droplet sizes at varied pole distances, magnesium oxide (MgO)-coated slides were placed approximately 1.5 m above the ground on a slide rotator at each pole. The droplets were examined under a microscope and their volume medium diameter (VMD) in mm was calculated using Microsoft Excel developed by Clarke Engineering Technologies, USA.

Statistical Analysis

Data were analysed using IBM SPSS Statistics 25 (IBM, USA). Two-way analysis of variance (ANOVA) followed by Tukey's Honestly-Significant-Difference (Tukey HSD) post-hoc test was carried out to determine significant differences in fog droplet sizes between formulations (F1, F2 and F3) and distance (25, 50, 75 and 100 m). Similarly, two-way ANOVA and Tukey HSD were carried out to determine significant differences in the efficacy results (knockdown and mortality) between distance and knockdown time (15, 30 and 60 min). Differences between means were considered statistically significant at $p<0.05$. In addition, t-tests were carried out to compare the means of droplet sizes between fogging methods and means of efficacy results between formulations (F1 and F2) and fogging methods.

RESULTS AND DISCUSSION

Droplet Size

Fog droplet size is an important factor influencing droplet deposition and the efficacy of insecticides. During space spraying, large droplets may fail to stay airborne or penetrate obstacles, while very small droplets may fail to land on target insects due to aerodynamics and convection currents (Farajollahi & Williams, 2013). VMD is a commonly used parameter to describe the droplet diameter, i.e., where 50% of the spray liquid volume consists of droplets smaller than this value (He et al., 2022). Accordingly, the optimum droplet size for efficacious ground adulticiding was determined to be between 12 and 20 μm VMD (Bonds, 2012). The VMD of droplets produced in this study are within said range until up to 50 m but decline below the optimal size at further distances (*Table 1*).

Two-way ANOVA analysis showed no significant difference in VMD between formulations (*Table 2*), indicating that droplets produced using palm-based formulations F1 and F3 were comparable to the commercial product, F2. The water-based formulations exhibited significant droplet size reduction with increasing distance. The finding is in line with previous studies by Bengoa et al. (2014) and Farajollahi and Williams (2013), which attributed the droplet size reduction to water evaporation in the outdoor environment as they travelled further away from the spray nozzle. Additionally, t-test analysis revealed that there is no significant difference in the droplet sizes produced by cold and thermal foggers (*Table 2*), which is in agreement with the findings obtained by Harburguer et al. (2012b) and Megat Nabil Mohsin et al. (2023). There was a statistically significant interaction between method and distance. In contrast, the interaction between formulation and distance and the interaction between formulation and method were non-significant (*Table 2*).

Knockdown of Adult Mosquitoes

Quick knockdown from exposure to insecticidal formulation ensures a reduction in feeding and insect reproduction and diminishes the chances for insects to escape (Georgia et al., 2018). In the absence of deltamethrin, F3 showed negligible knockdown with the maximum being only 3.33% at all checkpoints via cold fogger and 1.67% at the 25 m checkpoint via thermal fogger after 60 min (*Figure 1*). The results showed that the palm-based emulsion system alone had no knockdown activity and thus is excluded from statistical analysis.

A significant decrease in knockdown was observed as the distance increased, similar to the trend reported for droplet size. However, unlike droplet size, knockdown was significantly different between methods and between formulations F1 and F2 (*Table 3*). With cold fogging, knockdown by F1 was slightly lower than the commercial insecticide at the 15 min interval (*Figure 1a*). However, the opposite result was observed with thermal fogging (*Figure 1b*). In general, it was observed that knockdown significantly increased with time. At the 30 and 60 min intervals, cold fogging using F1 and F2 had achieved 100% knockdown at all checkpoints intervals, indicative of a low recovery rate for the knocked-down mosquitoes. Nevertheless, lower knockdown percentages were observed with thermal fogging compared to cold fogging as both F1 and F2 had failed to achieve complete knockdown even at 60 min (*Figure 1b*).

Two-way ANOVA revealed significant interactions between method and distance, method and formulation, method and time, time and distance, as well as time and formulation. However, there was no significant difference in the interaction between formulation and distance (*Table 3*). A note of caution is due here since the knockdown effect, i.e., the paralysis of insects due to insecticides

TABLE 1. DROPLET MEASUREMENT AT SPECIFIED DISTANCES USING DIFFERENT FOGGING METHODS

Fogging method	Formulation	VMD (μm) (mean ± S.E.)			
		25 m	50 m	75 m	100 m
Cold	F1	13.40 ± 0.12	12.60 ± 0.10	11.57 ± 0.06	10.20 ± 0.06
	F2	13.40 ± 0.06	12.73 ± 0.03	11.50 ± 0.06	10.23 ± 0.09
	F3	13.33 ± 0.12	12.63 ± 0.03	11.97 ± 0.18	10.10 ± 0.00
Thermal	F1	12.83 ± 0.12	12.30 ± 0.12	11.87 ± 0.20	10.30 ± 0.06
	F2	12.70 ± 0.10	12.27 ± 0.03	12.07 ± 0.09	10.30 ± 0.12
	F3	12.87 ± 0.07	12.23 ± 0.07	11.90 ± 0.06	10.30 ± 0.06

Note: F1 - novel water-based insecticidal formulation; F2 - commercial insecticide; F3 - blank formulation; VMD - volume median diameter; S.E. - standard error.

TABLE 2. THE EFFECTS OF FOGGING METHOD, FORMULATION AND DISTANCE ON FOG DROPLET SIZE

Variable	df	F-value	*P*-value
Fogging method	70	4.417	0.580 (2-tailed)
Formulation	2	0.219	0.804
Distance	3	885.136	<0.001
Fogging method × distance	3	24.004	<0.001
Fogging method × formulation	2	0.237	0.790
Formulation × distance	6	0.972	0.453

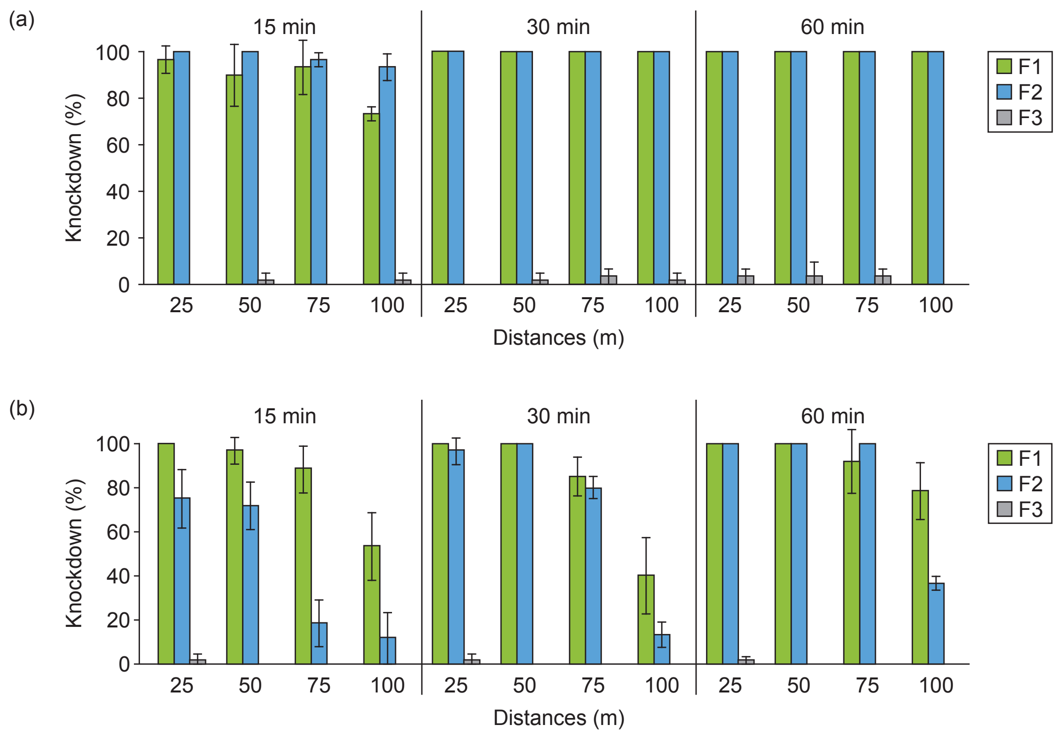
Note: df - degree of freedom.

is potentially reversible (Tsaganou et al., 2021). Therefore, we must also look into the delayed but definite effect of insecticidal formulations, i.e., mortality.

Adult Mortality

The objective of chemical space spraying is to minimise disease transmission by reducing the target infectious adult mosquitoes and their populations (Zuharah et al., 2019). As shown in *Figure 2*, negligible adult mortality was observed with F3, ranging from 0%–3% for cold fogging and 0%–5% for thermal fogging. This result further reinforces that the palm-based emulsion system used in the formulation provided no insecticidal activity.

The statistical analysis demonstrated no significant differences in adult mortality between F1 and F2 (*Table 4*). Despite the absence of highly volatile and flammable naphtha solvents, the palm-based formulation was able to provide comparable adulticidal efficacy with the commercial formulation. In this study, mortality



Note: F1 - novel water-based insecticidal formulation; F2 - commercial insecticide; F3 - blank formulation.

Figure 1. Percentage knockdown (mean ± S.E.) of adult Ae. aegypti *after exposure to fogging formulations using (a) cold and (b) thermal fogger.*

TABLE 3. THE EFFECTS OF FOGGING METHOD, FORMULATION, KNOCKDOWN TIME AND DISTANCE ON PERCENTAGE KNOCKDOWN

Variable	df	F-value	*P*-value
Fogging method	142	97.787	<0.001 (2-tailed)
Formulation	142	16.819	0.049 (2-tailed)
Knockdown time	2	24.530	<0.001
Distance	3	60.943	<0.001
Fogging method × distance	3	44.297	<0.001
Fogging method × formulation	1	36.872	<0.001
Fogging method × time	2	7.390	0.001
Knockdown time × distance	6	2.378	0.033
Knockdown time × formulation	2	4.279	0.016
Distance × formulation	3	2.500	0.063

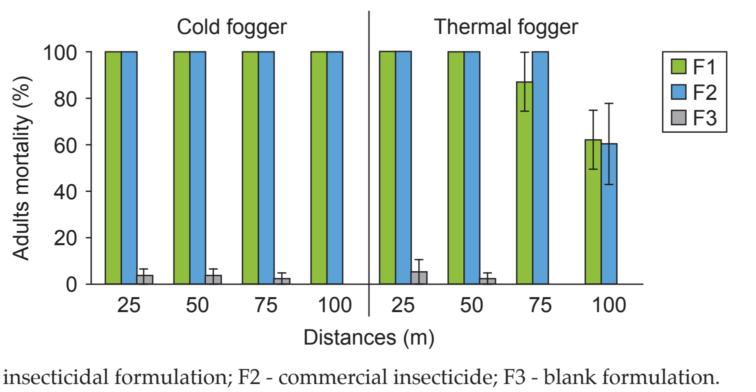
Note: df - degree of freedom.

was seen to decline significantly with increasing distance, a similar pattern was observed with knockdown. This may be attributed to droplet size reduction with increasing travel distance. Larger droplets are able to accomplish higher efficacies compared to smaller droplets because they are harder to transport by air currents because of their larger inertial force (Sugiura et al., 2011). In contrast to knockdown, mortality was significantly different between fogging methods (*Table 4*). It is worth highlighting that the cold fogging method was more effective in controlling adult *Ae. aegypti*, achieving 100% mortalities at all distance points (*Figure 2*). This pattern is in accord with previous studies, which attributed the poorer performance of their water-based products via thermal fogging to excessive heat by the applicator, resulting in reduced efficacy (Fulcher et al., 2016; Megat Nabil Mohsin et al., 2023). Nevertheless, additional studies are required to assess the efficacies of this palm-based formulation against immature *Ae. aegypti* stages, pupae and larvae.

Two-way ANOVA revealed that similarly with knockdown, a significant interaction was observed between method and distance, whereas no significant interaction was observed between formulation and distance. Nonetheless, contrary to the trends observed with knockdown, a significant difference was not observed in the interaction between method and formulation (*Table 4*).

CONCLUSION

In conclusion, this study successfully demonstrated that the novel palm-based insecticide formulation (F1) offers comparable efficacy to the commercial naphtha-based formulation (F2) in controlling *Ae. aegypti* mosquitoes, without the environmental and safety concerns associated with petroleum-derived solvents. The field trials using both cold and thermal fogging methods showed no significant differences in droplet sizes between formulations, while the performance of cold fogging was generally superior in terms of knockdown and mortality. Importantly, this research highlights the potential of utilising palm-based ingredients, particularly PME and surfactants, which are biodegradable and locally abundant, reducing dependence on non-renewable resources. The use of F1 also provides a cost-effective and sustainable alternative for dengue vector control, aligning with global trends toward greener, more environmentally responsible pest control solutions. This study is unique in its focus on replacing volatile, flammable naphtha with a bio-based formulation, which is particularly relevant in regions like Malaysia, where palm oil is a major agricultural product. The findings suggest that palm-based insecticidal formulations could play a significant role in future vector control strategies, particularly in tropical and subtropical regions where mosquito-borne diseases are prevalent.



Note: F1 - novel water-based insecticidal formulation; F2 - commercial insecticide; F3 - blank formulation.

Figure 2. Percentage mortality (mean ± S.E.) of adult Ae. aegypti *after exposure to fogging formulations using cold and thermal foggers.*

TABLE 4. THE EFFECTS OF FOGGING METHOD, FORMULATION AND DISTANCE ON ADULT MORTALITY

Variable	df	F-value	*P*-value
Fogging method	46	57.982	0.004 (2-tailed)
Formulation	46	0.239	0.725 (2-tailed)
Distance	3	27.445	<0.001
Fogging method × distance	3	27.445	<0.001
Fogging method × formulation	1	0.665	0.420
Distance × formulation	3	0.955	0.425

Note: df - degree of freedom.

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PROCESSING OF UNDER AND OVERRIPE OIL PALM FRUITS AND ITS EFFECTS ON YIELD AND OIL QUALITY

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ABSTRACT

Fresh fruit bunches (FFB) harvested between 19 and 24 weeks after anthesis (WAA) were processed in a laboratory to extract crude palm oil (CPO). The CPO was mechanically extracted from oil palm fruits at various stages of maturity and analysed for its physico-chemical properties, including deterioration of bleachability index (DOBI), carotene content, total chloride content (TCC) and chlorophyll (CHL) pigments. Underripe fruits at 19 WAA had a higher moisture content (MC) (72.69%) compared to ripe fruits at 23 WAA (26.84%). As the fruits fully matured, the oil content (OC) increased, exceeding 40.00% of the total fruit mass. The quality of CPO extracted from underripe fruits was characterised by lower carotene content and DOBI values (175.00 mg kg^{-1} and 2.19, respectively), but higher levels of CHL pigments (5.38 mg kg^{-1}) and TCC (7.00 mg kg^{-1}). In contrast, the CPO from fully developed fruits exhibited higher carotene content and DOBI values (762.00 mg kg^{-1} and 3.45, respectively), while CHL pigments and TCC decreased. Good quality CPO is identified by a higher DOBI value and lower CHL pigments and TCC. A higher DOBI value indicates easier bleaching and refining, while lower CHL pigment levels contribute to better oxidative stability.

Keywords: anthesis, chlorophyll, total chloride content, underripe fruits.

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INTRODUCTION

Fresh fruit bunches (FFB) are harvested when they reach optimal ripeness. The degree of FFB ripeness can be categorised into unripe, underripe, ripe and overripe. The optimal ripeness of FFB is reported to be between 20–22 weeks after anthesis (WAA), based on oil content (OC) in the fruit (Prada et al., 2011; Sambanthamurthi et al., 2000). At palm oil mills, FFB maturity assessment is carried out by graders based on the number of loose fruit sockets on the bunch. The FFB is considered overripe when there are more than five loose fruit sockets on the bunch, while ripe FFB exhibits two to five loose sockets. The FFB are categorised as unripe and underripe when no loose fruit sockets are observed on the bunch (Malaysian Palm Oil Board [MPOB], 2015). To differentiate between unripe and underripe FFB, the colour of the fruits plays an important role, with unripe fruits usually being yellow, while underripe fruits are yellowish-orange in colour. The processing of unripe FFB has been associated with low OC, resulting in a lower oil extraction rate (OER) at palm oil mills (Basiron, 2007; Parveez et al., 2023). In addition to low OER, other pertinent issues related to unripe fruit processing include inferior crude palm oil (CPO) quality (Tan et al., 2023) and low kernel extraction rate (KER) (Amir et al., 2022).

Chlorophyll (CHL) and carotene content in CPO are some of the quality issues reported to be associated with the degree of ripeness of oil palm fruit (Siew, 2001; Tan et al., 2023). CPO extracted from fruits at 17 WAA maturity showed higher CHL content but lower carotene content compared to CPO extracted from fruits at 23 WAA maturity (Tan et al., 2023). At 19 WAA, FFB produced CPO with as high as 12 mg kg^{-1} CHL content (Tan et al., 1997). Conversely, CHL was undetectable in CPO extracted from 22 WAA fruits. It was also highlighted that CPO extracted from underripe fruits exhibited low deterioration of bleachability index (DOBI), which is associated with lower

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carotene content (Siew, 2001; Tan et al., 1997). Oils with significant CHL content could lead to inferior quality due to its adverse effects on bleachability, hydrogenation and oxidative degradation (Tan et al., 2023). Higher CHL content in crude canola oil resulted in dull, dark brown refined oil and required large amounts of bleaching earth for refining (Diosady, 2005).

The presence of chlorinated compounds in CPO has been comprehensively examined by Nagy et al. (2011), while Tiong et al. (2018) identified different types of organochlorine compounds in CPO derived from oil palm fruits at different stages of maturity. The chlorinated compounds can be categorised as organic and inorganic. The most abundant organochlorine in CPO is primarily phytosphingosines (Nagy et al., 2011), which have been found to be the most reactive chlorine precursor in the formation of 3-monochloropropane-1, 2-diol esters (3-MCPDE) (Tiong et al., 2018). The occurrence of sphingolipid organochlorine compounds is dependent on the OC in the mesocarp, which correlates with the stages of maturity and fruit ripeness (Tiong et al., 2018). On the other hand, the inorganic chlorines are mostly found in the form of iron (II), iron (III), magnesium and calcium chloride.

Studying the processing of under and overripe fruit is important to address the potential adverse effects on oil quality and to explore ways to mitigate the formation of contaminants throughout the supply chain. The reactive chlorinated compounds, which are associated with the formation of 3-MCPDE, are closely linked to the ripeness of the fruits. Colour darkening or colour reversion of refined oil may be caused by the CHL content in CPO, which originates from underripe fruit processing.

MATERIALS AND METHODS

Materials

A total of 18 commercial oil palm trees of *tenera* (DxP) variety, 15 years old, were identified at MPOB's Kluang Research Station, Johor, Malaysia as the source of FFB (*Figure 1*). The oil palm trees were grown on Rengam and Jerangau soils, with the Rengam series providing well-drained, loamy conditions and the Jerangau series contributing heavier, clayey soil with more water retention. These soil types were selected to evaluate their influence on oil palm fruit development at various maturity stages. The selection of oil palm trees was based on the availability of female flowers during the field trials. The flowers were marked as Week-0 immediately after anthesis. At the end of each maturity stage, FFB from 19–24 WAA FFB were harvested and delivered to the laboratory for further processing. The FFB from each maturity stage were collected in triplicate. All chemicals used were of analytical and GC grades.

Methods

Extraction of CPO. The FFB was first chopped to obtain the spikelets before undergoing sterilisation in an autoclave at 120ºC for 20 min. The mesocarp and kernel of the fruits were then separated using a knife. The mesocarp was mechanically pressed using a hydraulic press to obtain crude oil, which was later centrifuged at 10,000 rpm using a Thermo Fisher Scientific Floor Model Centrifuge (Sorvall RC 5C+, USA) to separate water and sludge. Finally, the CPO was filtered using a Whatman No. 1 filter paper and stored in a freezer at –20ºC prior to subsequent analyses.

Moisture content (MC) and OC. The fruits were sampled from the FFB and the mesocarp was sliced into small pieces. The MC of the fruits was determined by heating the mesocarp samples at 105ºC until constant weight was achieved. The mesocarp samples were kept in an oven for 24 hr and the MC was recorded as an average from 12 measurements.

The determination of OC was carried out using two units of the Gerhardt Soxtherm 6-place apparatus (Model SOX416, Gerhardt GmbH, Germany). About 10 g of dried mesocarp sample was placed into 12 extraction thimbles. The oil from the dried mesocarp was extracted using petroleum ether at 150ºC for 8 hr. The weight of the oils was recorded as an average from 12 measurements.

Analyses of oil samples. The DOBI, carotene and CHL contents were analysed according to the International Organization for Standardization (ISO) Method (ISO 17932:2011) (ISO, 2018) and American Oil Chemists' Society (AOCS) Official Method (Cc 13i-96) (AOCS, 2015), respectively. Analysis of total chloride content (TCC) was carried out using American Society for Testing and Materials (ASTM) D 4929-04, Method B for crude oil (ASTM, 2004), with slight modifications (Hammid et al., 2022).

Statistical analyses. Analyses were conducted in triplicate and reported as means ± standard deviation (SD) of independent analyses. Data obtained from the analyses were subjected to one-way analysis of variance (One-way ANOVA) with Fisher's Multiple Comparison to determine significant differences among the samples, defined at a 95% confidence interval ($p < 0.05$). The statistical analyses were performed using a Minitab software Version 16 (Minitab Inc., USA).

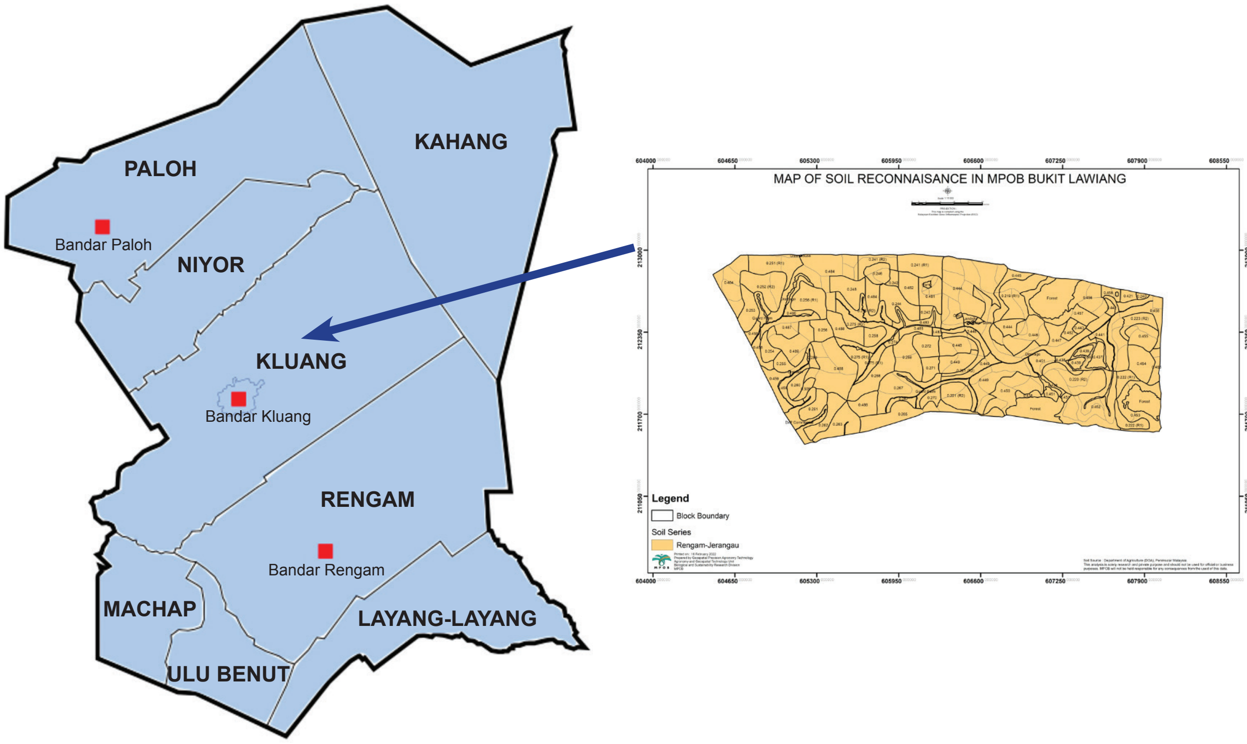

Figure 1. Location of the sampling of fresh fruit bunches (FFB).

RESULTS AND DISCUSSION

Biosynthesis of oil in oil palm mesocarp begins from 15–16 WAA and increases over the next 6–7 weeks (Tan et al., 2023). *Figure 2* illustrates the OC and MC in the fruits at different degrees of maturity. At the early stages of fruit maturity between 19–21 WAA, biosynthesis of the oil was almost stagnant and the OC was not significantly different ($p > 0.05$), hovering between 14.7%–17.8%. However, as the fruit ripened, the OC increased significantly ($p<0.05$), with the highest OC of 43.9% reached at 23 WAA, slightly reducing a week later at 24 WAA. On the other hand, MC in the mesocarp peaked at the early stages of fruit maturity. The maximum MC in the fruit was observed between 19–21 WAA before rapidly decreasing between 22–24 WAA. Hence, it could be suggested that the optimal harvesting time is 23 WAA, when the OC is highest while the MC shows the opposite trend. At 24 WAA, the fruits had entered an overripe period where the OC had slightly reduced.

It was reported that the accumulation of oil peaked at 22 WAA and slightly decreased at 24 WAA (Prada et al., 2011). The reduction in OC at 24 WAA was in agreement with the over-ripening of fruit and bunches. Bafor and Osagie (1986) and Oo et al. (1986) reported that the highest OC in mesocarp occurred at 22 WAA. However, Sambanthamurthi et al. (2000) found that the highest OC was achieved at 20 WAA. Variations in OC could be attributed to differences in the age of the oil palm, cultivar and environmental conditions (Bafor & Osagie, 1986).

Figure 3 demonstrates the changes in carotene content and DOBI values during the ripening of the fruits. Carotene content and DOBI values significantly increased ($p < 0.05$) as the fruits developed to maturity. As shown in *Figure 3*, the carotene content and DOBI were lowest during the early stages of fruit development. As the fruit ripened, carotene were continuously produced, concurrently with lipid synthesis. At the optimum harvesting time of 23 WAA, the carotene content and DOBI were found to be 535 mg kg^{-1} and 2.85, respectively, which are within the range of typical values for CPO quality. Unlike other CPO qualities, carotene content and DOBI kept increasing a week after the optimum harvesting time (24 WAA). This similar trend was also reported by Tzuan et al. (2022). Ruswanto et al. (2020) highlighted that fruit quality is one of the most important factors affecting the DOBI values of the extracted oil. Low DOBI values are observed in CPO extracted from unripe or underripe fruits, particularly those that are still blackish in colour. Black bunches may have CPO with a DOBI below 1.50, whereas CPO extracted from fruits at optimum ripeness could exhibit a DOBI above 3.00. The free fatty acid (FFA) content was also higher in overripe fruits (Soetrisno et al., 2024).

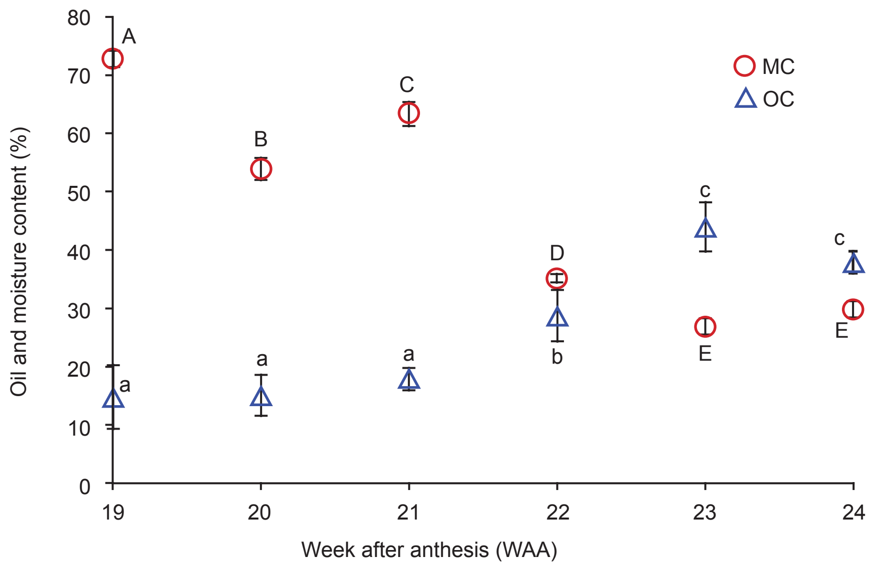

Figure 2. Transient of moisture content (MC) and oil content (OC) of FFB harvested at different degrees of maturity. Values with different letters are significantly different ($p < 0.05$).

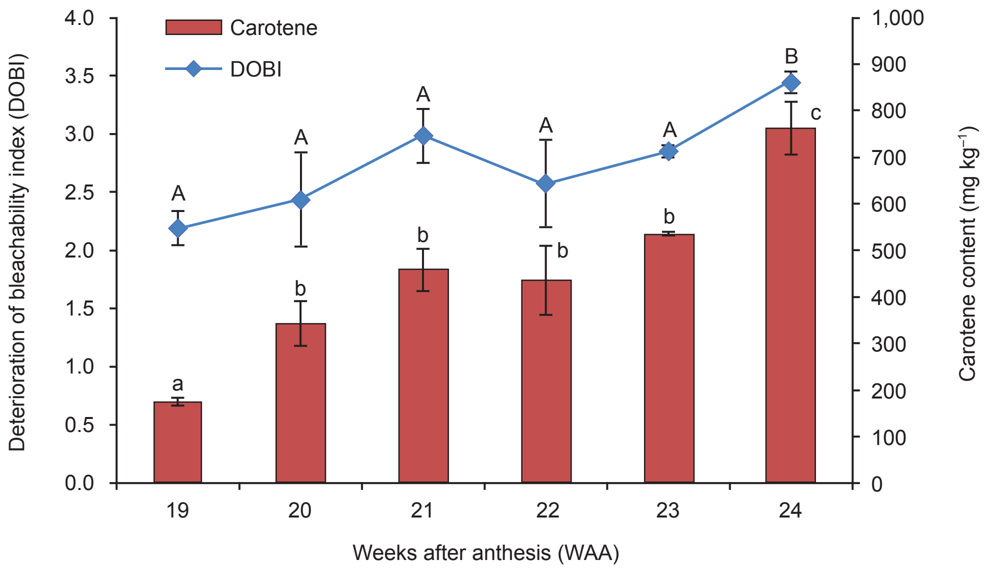

Figure 3. Changes in carotene content and DOBI during the ripening of fruits. Values with different letters are significantly different ($p < 0.05$).

Chlorophylls (CHL) are another group of pigments in CPO besides carotenoids. CHL are fat-soluble pigments and are undesirable because of their adverse effects on oxidative deterioration and bleachability (Li et al., 2019; Tan et al., 2023). CHL contents in CPO extracted from underripe fruits were significantly higher ($p < 0.05$) compared to mature fruits (*Figure 4*). During the early stages of maturity (19–21 WAA), the CHL content was highest and began to decrease as the fruits ripened to 22–24 WAA. The results of this study support the earlier findings by Tzuan et al. (2022), where CHL was found to be higher in immature fruits. As the fruit fully developed (23–24 WAA), the CHL content was almost undetectable.

The surface of oil palm fruits is usually green to black in colour during the unripe stage due to the high CHL content. During the ripening stage, the fruits turn orange-red in colour due to the accumulation of carotene and the degradation of CHL. At the early stages of fruit development, the CHL on the FFB surface is actively involved in photosynthesis, producing energy essential for the growth and fruit development. As the fruit matures, the CHL becomes less crucial, and the fruit begins to accumulate phytonutrients and other compounds, such as carotene, which give it its final orange-red colour (Tan et al., 2023).

TCC in CPO extracted from fruits at different degrees of ripeness is illustrated in *Figure 5*. The TCC in CPO extracted from underripe fruits between 19–21 WAA was significantly higher (p < 0.05) compared to the TCC in CPO extracted from ripe and overripe fruits of 22–24 WAA. The TCC trend corresponded to the OC and MC in the fruits (*Figure 2*). It was noted that fruits with maturity between 19–21 WAA had higher MC as well as significantly higher TCC in the extracted CPO. When the fruits ripened to 22–24 WAA, a significant reduction in MC and TCC was observed. According to Tiong et al. (2021), CPO extracted from unripe fruits (18–19 WAA) contained higher amounts of organochlorines, namely fatty acids, diacylglycerols and other unknown organochlorines. As the fruit fully developed, the reduction of organochlorine content was significant (p < 0.05).

Chloride is considered a beneficial macronutrient for plants. It increases fresh and dry biomasses, promotes greater leaf expansion, enhances the elongation of leaf and root cells, improves water relations, increases mesophyll diffusion of carbon dioxide (CO_2) and boosts water- and nitrogen-use efficiency (Colmenero-Flores et al., 2019). In the case of oil palm, chloride may have been metabolised for the development of oil palm fruits, which could lead to reduced TCC in the ripened fruits (Viégas et al., 2020).

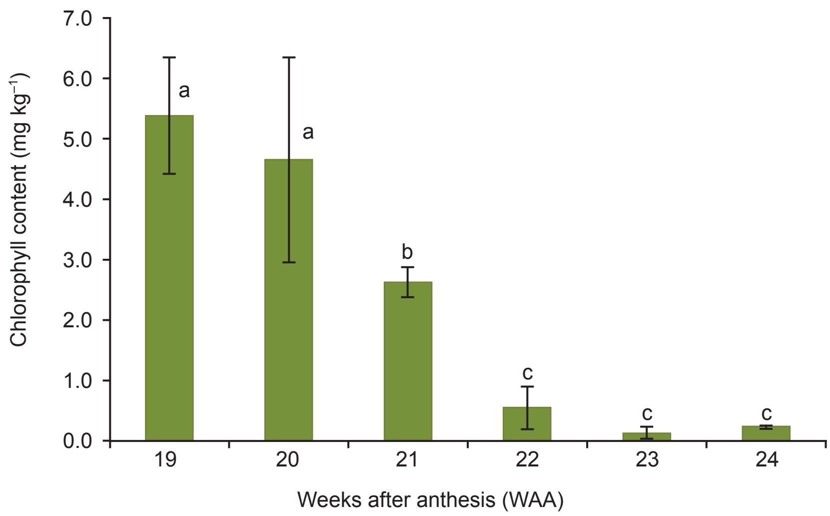

Figure 4. Changes in chlorophyll (CHL) content during the ripening of fruits. Values with different letters are significantly different (p < 0.05).

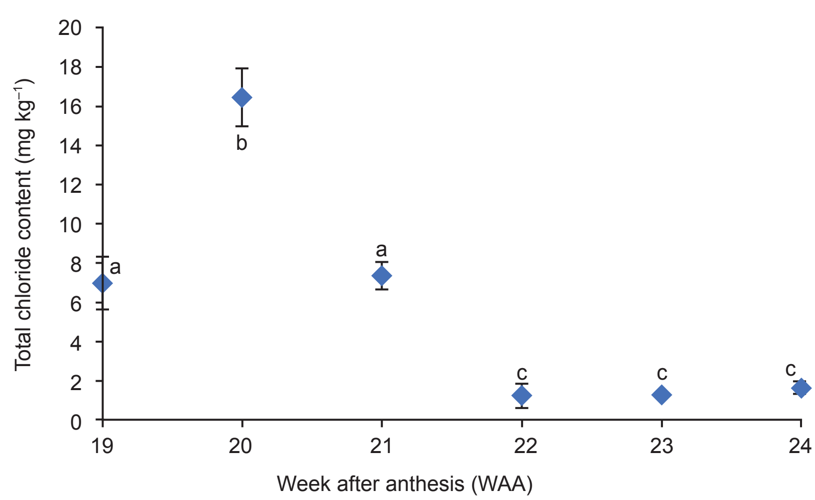

Figure 5. Total chloride content (TCC) in crude palm oil (CPO) extracted from fruits at different degrees of maturity. Values with different letters are significantly different (p < 0.05).

CONCLUSION

Underripe fruit processing presents several challenges that require remedial action. The underripe fruits contained higher MC than oil. CPO extracted from underripe fruits exhibited low carotene content and DOBI values but higher levels of CHL pigments and TCC. As the fruit development progressed, the carotene content and DOBI values of the extracted CPO increased, while the CHL pigments and TCC were depleted in the oil extracted from ripe fruits. The presence of CHL pigments may affect colour while TCC contributes to the formation of 3-MCPDE in refined oil. Therefore, processing of underripe fruits should be avoided to safeguard the quality of CPO.

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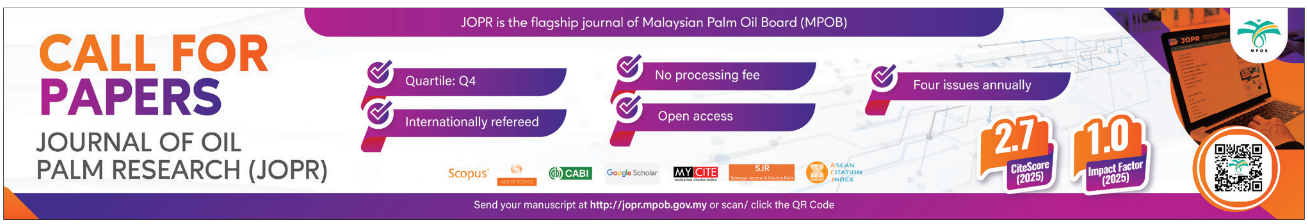

Contents of the Coming Issue

Journal of Oil Palm Research

Vol. 38 (3) September 2026*

- Sustainable Innovations in Palm Oil Extraction: A Comprehensive Overview of Green Technologies
  Nicky Rahmana Putra; Yustisia Yustisia; R Bambang Heryanto; Ni Wayan Trisnawati; Damasus Riyanto; Nyoman Ngurah Arya and Bramantyo Airlangga

- Biogenic Silica from Oil Palm Biomass: A Review on the Potential, Extraction Methods and Properties
  Hoerudin Hoerudin; Tatang Hidayat; Aton Yulianto; Siti Agustina; Fajriyan; Karnadi; Abdul Majid; Ahmad Suhendra; Eddy Sapto Hartanto and Achmad Kamil

- Prediction of Oil Palm Bunches Production Using Artificial Neural Network
  Emanuelly Canabrava Magalhães; Carlos Alberto Araújo Júnior; Helio Garcia Leite; Gianmarco Goycochea Casas; Juliana Machado Lisboa; Carlos Henrique Garcia and Rodrigo Rabe Sales Bicalho

- Cytotoxic and Anti-acne Activity of Sugar Fatty Acid Esters (SFAE) from CPO and Palm Oil Hydrolysate
  Tuti Wukirsari; Ade Andriani; Obie Farobie; Sri Handayani and Sumi Hudiyono

- Genetic Performance of 3-Way (MPOB-Nigeria × Deli) *Dura* × *Pisifera* Oil Palm Crosses
  Nor Azwani, A B; Zulkifli Yaakub; Noraziyah Abd Aziz Shamsudin; Marhalil Marjuni and Fadila Ahmad Malike

- Biosynthesis of Silver Nanoparticles Using Palm Oil Mill Effluent (POME) Extract
  Abdulrahman Sani Aliero; Nurliyana Ahmad Zawawi; Nik Ahmad Nizam Nik Malek; Muhammad Hariz Asraf; Iqbal Jalaluldin; Mohd Firdaus Abdul Wahab and Mustapha Isah

- Spray Coverage and Effectiveness of Unmanned Aerial Vehicle Point-to-Point Spraying Technology for the Control of *Oryctes rhinoceros* in Immature Oil Palm
  Meor Badli Shah Ahmad Rafie and Samsudin Ami

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