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REVIEW ARTICLES

- Climate Variability and Water Stress Effects on Oil Palm (*Elaeis guineensis* Jacq.) Productivity in Malaysia
- Precision Agriculture Implementation to Improve Sustainability and Productivity of Oil Palm Plantations in Indonesia: A Review

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CLIMATE VARIABILITY AND WATER STRESS EFFECTS ON OIL PALM (*Elaeis guineensis* Jacq.) PRODUCTIVITY IN MALAYSIA

SANI IDRIS[1,2]; CHRISTOPHER TEH BOON SUNG[1]*; SYAHARUDIN ZAIBON[1] and SIM CHOON CHEAK[3]

ABSTRACT

Oil palm is a key pillar of Malaysia's socio-economic development, contributing to the nation's economic stability and is also a major driver of the global oil industry. However, climate variability has progressively reduced the productivity of oil palm by subjecting it to water stress through inadequate and irregular rainfall, prolonged dry spells and elevated temperatures. This article reviews past literature and provides useful insights into the effects of climate elements and the physiological and agronomic effects of water stress on oil palm. Water stress impairs the physiological and metabolic functions of oil palm, particularly stomatal conductance, leaf water potential, proline synthesis, sex differentiation and water use efficiency. These combined effects diminish the biomass and yield of oil palm. This review also highlights the temporal variability of climate and identifies the role of various soil properties related to water stress. It presents climate projections threatening oil palm sustainability and presents possible solutions. Additionally, the specific fraction of plant-available water necessary for triggering water stress remains under-researched. The relationship between various physiological and genetic mechanisms that control stomatal response during water stress is unclear. The efficiencies of various irrigation approaches and water conservation measures must also be re-evaluated based on climate predictions.

Keywords: climate change, dry spell, global warming, irrigation, water deficit.

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INTRODUCTION

Oil palm has the highest oil yield, and it is harvested regularly throughout the year (DoCampo et al., 2021; Yawson, 2015). Globally, its production expands steadily (Dislich et al., 2017; Kawamura et al., 2014; Paterson & Lima, 2017) and typically reaches its maximum yield at 9–10 years after field planting, with an average fresh fruit bunch (FFB) yield of 16.6–25.6 t ha^{-1} in Malaysia (Kushairi et al., 2019; MPOB, 2022). Although oil palm originated from Africa, Indonesia, Malaysia, China and India are now the major producers and importers (Carr, 2011), thus dominating the global oil palm industry (Abubakar & Ishak, 2022; Voora et al., 2019; World Bank, 2021). Oil palm greatly contributes to Malaysian socioeconomic performance (Norizan et al., 2021).

Oil palm production in Malaysia is limited because of water stress resulting from water deficits (Mutert et al., 1999; Norizan et al., 2021), which is identified as the primary constraint to optimum yields (Woittiez et al., 2017). Water for oil palm production comes primarily from precipitation (Miller & Donahue, 1992; Shevade & Loboda,

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2019), which is often insufficient or not distributed uniformly throughout the year, leading to water stress and a decline in yield.

Global warming due to climate change (CC) causes water shortages (Jagtap, 2007; Teh, 2017). Mueller (2009) and Okon et al. (2021) reported that CC is a phenomenon that affects different regions of the world in various negative ways, particularly the tropics (Idowu et al., 2011; Williams et al., 2018). The impact of CC through water stress inducement places more pressure on crop production globally than in any other sector (Intergovernmental Panel on Climate Change [IPCC], 2014). This suggests that more study is required on the impact of CC on crop production (Koh et al., 2011; Okon et al., 2021). The adverse effects of CC on crop production are both direct and indirect, as well as short- and long-term (Fitton et al., 2019; Obioha, 2008). For instance, CC-induced water stress causes oil palm seedlings to wilt, experience stunted growth and suffer from impaired root development (Okon et al., 2021). Over the past decades, efforts to combat CC have been costly, placing a strain on countries both economically and technically (Abubakar et al., 2021; IPCC, 2021).

The effects of CC on the oil palm industry are evident. Intense solar radiation and temperature are twin CC elements that cause oil palm yield reductions (Hoffmann et al., 2015) through their effects on critical physiological processes (photosynthesis, respiration, and transpiration) (Cheah et al., 2020; Teh & Cheah, 2023). Henson and Harun (2005) linked the impact of CC to seasonal fluctuations in the heat energy balance, which affects the oil palm FFB yields at many sites in Malaysia. Henson and Harun (2005) acknowledged that the effects of CC in some parts of Kedah, Malaysia, were so severe that rainfall during April, May, and June was significantly reduced, with average rainfall amounting to only one-third of the reference evapotranspiration (ETo). For instance, February recorded a mere 0.4 mm ETo. Hoffmann et al. (2015) observed a wide range of FFB reduction under various water stress scenarios. Ministry of Plantation Industries and Commodities (MPIC, 2018) emphasised the need for accurate and real-time data on oil palm response to water stress and its causal factors for biotechnology and breeding projects aimed at developing climate-resilient oil palm planting materials.

Against this backdrop, this review aims to navigate through previous studies to harmonise various submissions on oil palm behaviour when exposed to water stress under varying climate conditions. The goal of this study was to identify research gaps and suggest possible ways forward. We utilised search engines like Google and Edge, as well as academic databases such as ScienceDirect and Google Scholar, to conduct a comprehensive literature search.

IMPORTANCE OF OIL PALM PRODUCTION

Oil palm is central to the global oil market (DoCampo et al., 2021). Although it has the least cultivated area for oil crops (Oil World, 2022), its oil output greatly surpasses (20 times) those of *Glycine max* L. (soybean), *Arachis hypogaea* L. (groundnut), *Brassica napus* L. (canola) and *Helianthus annus* L. (sunflower) (Chang et al., 2014; Low, 2019; Moraidi et al., 2012; Woittiez et al., 2017). Corley and Tinker (2016) and Murphy (2021) stated that worldwide, palm oil accounts for approximately 39% of the total vegetable oil consumed (Murphy, 2021) and is being used for the production of critical additives, reagents, polymers, and organo-minerals (Adileksana et al., 2020; Bognár et al., 2020; Cheah & Hoi, 1999; Chin et al., 2020; Gao et al., 2020; Jamshaid et al., 2022; Liew et al., 2021; Masharuddin et al., 2021; Otsuka et al., 2006; Uke et al., 2021).

Oil palm production is crucial for Malaysian socioeconomic development (Malaysian Palm Oil Council [MPOC], 2019; Pacheco et al., 2017); thus, guides central decisions and policy-making (Department of Statistics Malaysia [DOSM], 2020; Malaysian Palm Oil Board [MPOB], 2022; Singh et al., 2021). It also provides gainful employment to a large population (Abdul Rahman, 2018; AsianAgri, 2023; Parveez et al., 2023), including 70% of job-seeking foreigners (Hamid et al., 2013; Hanafiah et al., 2022; Nkongho et al., 2014; Parveez et al., 2022; Pirker et al., 2016; Qaim et al., 2020). All these describe oil palm as a multi-utility crop worth sustainable production for enhanced global industrialisation and national growth.

OIL PALM GROWTH, YIELD AND PHYSIOLOGICAL RESPONSE TO WATER STRESS

Yield Gap

United States Department of Agriculture (USDA, 2023) data reveals a significant yield gap in Malaysian oil palm production. On average, the country achieves its expected maximum yield in only one out of 12 months (November). Bakoumé et al. (2013), Chalvantharan et al. (2023), and USDA (2023) confirmed that optimal yields in Malaysia are achieved in only 4–5 months yr^{-1}. Woittiez et al. (2017) reported that actual oil palm yields remain less than 50% of their potential (3.3 vs. 8.0 $\mathrm{t\ ha^{-1}\ yr^{-1}}$). Rhebergen et al. (2018) suggested this gap could be even wider in subsistence production systems. Consequently, addressing this substantial yield gap could significantly impact global oil production. Woittiez et al. (2017) estimated that closing this gap could improve world oil production by 17.5 t yr^{-1}. In a study of an oil palm plantation in Central Kalimantan, Indonesia, Hoffmann et al. (2017) estimated that closing the yield gap through management practices alone could increase its FFB production by 1.2 t ha^{-1} yr^{-1}. These projected increases underscore the significant potential of addressing yield gaps, highlighting how such improvements could substantially enhance food security. The persistent yield gap is closely linked to climate variability (Abdul Rahman, 2018; Bakoumé et al., 2013). These climatic challenges limit the expression of full yield potential in improved oil palm germplasm (Ariffin et al., 2002; Rhebergen et al., 2020).

Climate Variability and Its Effects on Oil Palm

Climate change or climate variability (University Corporation for Atmospheric Research [UCAR], 2022) is said to occur when changes in climate variables are observed in an extended manner (IPCC, 2013). This means that sudden weather anomalies that occur for one to three years, then disappear would not be considered as climate variability. Climate variability is typically caused by natural and anthropogenic interplay (Idris & Yahaya, 2022), consisting of orbital revolution of the earth, volcanic eruption, movements of the crust (MetOffice, 2023), burning of fossil fuels, and increased concentrations of greenhouse gases (GHGs) from cultivation and deforestation (Green Peace, 2023; Ogle et al., 2014). Therefore, the history of oil palm production has been a critical anthropogenic source by which GHGs are added to the earth system (Butler & Laurance, 2009; Fitzherbert et al., 2008).

Saifan et al. (2021) revealed that 25% of farmers in Malaysia are vulnerable to declining yields due to climate variability-related problems. Abubakar and Ishak (2022) confirmed a link between climate variability and consistent yield downward trend (7.5%) in various regions in Malaysia. Changes brought about by climate variability intensify the water deficit, thereby restricting growers to drought-tolerant oil palm cultivars that are not high-yielding (Masud et al., 2017), thus reducing FFB output (Shobande, 2021). Increased ETo losses from weather variability events (Wang et al., 2014) negatively impact productivity, as well as reducing the efficient distribution and utilisation of fertilisers and herbicides (Gustafson et al., 2015). Variations in climate conditions often shorten the length of the oil palm production cycle (Abubakar et al., 2021; Morton et al., 2017; Saifan et al., 2021) and increase the prevalence of crop diseases and new pests, resulting in low productivity (Melillo et al., 2014; Saifan et al., 2021).

Climate-related limitations affect perennial crops, such as oil palm, as their performance is influenced not only by current weather conditions but also by the lingering effects of past climate events (Carr, 2011). In 2018, oil palm production was negatively affected by climate factors, likely exacerbated by the cumulative effects of previous years' climate variability (Kushairi et al., 2017). However, an adequate understanding of the legacy or residual effects of CC either at the spatial or temporal scale is still scarce.

Although climate variability in Malaysia is less pronounced than in African countries like Nigeria (NiMet, 2022), it still significantly leads to unexpected yield variations across different locations and seasons (Nelson et al., 2006; Sarkar et al., 2020; Tang, 2019). Based on Kushairi et al. (2019) and MPOB (2018), between 2015–2018, yield variance stood at 17% ha^{-1} basis (from 15.91 t ha^{-1} in 2016 to 19.92 t ha^{-1} in 2017). They further confirmed that the observed yield variance was linked to changing climate patterns, as the water deficit in 2017 was far less than in 2016.

Economically, Zainal et al. (2012) estimated that a 1°C increase in temperature due to climate variability would result in losses of USD10.63 ha^{-1} in Peninsular Malaysia, USD10.89 ha^{-1} in Sabah and USD9.01 ha^{-1} in Sarawak. Furthermore, over the next 6, 36 and 76 years from 2023, oil palm net revenue is projected to decline by an average of USD81.52, USD30.44 and USD12.37 ha^{-1}, respectively, due to the continued effects of climate variability (Zainal et al., 2012). Malaysia faces potential economic insecurity if erratic climate patterns lead to decreased oil palm yields and reduced palm oil prices (Swaray et al., 2021).

Lim et al. (2022) pointed out that the occurrence of abrupt global hydrological phenomena such as *El Niño*, which amplifies the water stress problem, is another form of climate variability. *EI Niño* is an abnormality that occurs when less rainfall occurs in the Western Pacific but more is experienced in the eastern part, resulting in drought and flooding, respectively (National Geographic, 2023). Although its occurrence was only five times between 1980 and 2000, it resulted in severe consequences of production reductions (Goh et al., 2011). Oil palm production was severely affected by *EI Niño* in 1997–1998 (Lim et al., 2008) and in 2016 (Parveez et al., 2022). Findings from a long-term study (spanning 33 years) indicated that *EI Niño* increased potential ET by up to 95% (Tui & Arifin, 2013). Kamil and Omar (2017) attributed a significant loss of yield to *El Niño* in Malaysia and other tropical countries.

Globally, Hekstra (1986) maintained that climate variability, particularly through changes in precipitation and temperature, has led to low productivity by 5%–20% and decreased crop production quality (Alam et al., 2017).

In summary, insufficient rainfall, partly driven by *El Niño* and climate fluctuations, leads to water stress conditions that severely threaten palm oil production.

Temperature Variability and Its Effects on Oil Palm

Pour et al. (2014), Wang et al. (2014) and The Star (2022) stated that the escalation of global and regional temperatures is a product of long-term climate variability. IPCC (2013) and Shahid et al. (2017) confirmed that the rising number of hot days and sustained temperature increases in various regions of Malaysia are clear indicators of climate variability, which directly influences oil palm productivity (Corley & Tinker, 2016). Tang (2019) reported that over three decades, temperature trends varied across different zones in Malaysia, with increase of 7.55% in Kota Kinabalu, 7.27% in Kuching and 10.20% in Malacca, Kuantan and Subang Jaya. Ministry of Natural Resources and Environment (NRE, 2015) reported that the 10 year temperature increase in the Peninsular was 56.40% higher than that experienced in Sarawak. Abdul Rahman (2018) corroborated that although an increase in temperature was recorded in all regions, the Peninsular was 33.30% warmer than the eastern part of Malaysia based on approximately five decades of data.

Sammathuria and Ling (2009) reported that the climate variability experienced in Malaysia included unusually high temperatures that occurred five times between 1972 and 1998. Al-Amin et al. (2015) and Murad et al. (2010) pointed out that increased heat, which was observed around 1983-1987, originated from anomalous temperature rise. In Malaysia, day-to-day temperature variation is more obvious and anomalous than the average year-to-year temperature variation (Murad et al., 2010).

However, the relationship between ambient and soil temperatures and their simultaneous effects on oil palm growth and development has not yet been identified. Although Nuruddin and Tokiman (2005) sought to establish ambient temperature as the best explanatory variable for soil temperature, finding a high regression coefficient ($R^2 = 0.96$), the 1 cm depth at which they measured soil temperature was not sufficiently reliable to represent the active rhizosphere of oil palm roots.

Temperature has diverse effects on various biochemical and anatomical processes in oil palm, particularly by reducing activation energy, which influences metabolic rates and enzymatic activities (Kim, 2010; Kirkham, 2005; Mazlan et al., 2021). Generally, mature oil palm requires a temperature of 24°C–28°C for optimal growth and development (Lim et al., 2008), above which the dry matter yield is reduced up to 16% due to excessive ET rates (Okon et al., 2021). Lim et al. (2021) reported that prolonged periods of temperatures below 21°C led to a high rate of flower abortion, which in turn resulted in a 3.2% reduction in FFB yield. The growth of young seedlings was impeded at temperatures below 15°C but stimulated at above 20°C (Lim et al., 2008). In a separate study, Ferwerda and Ehrencron (1977) found that exposing oil palm to 22°C and 8°C day and night temperatures, respectively, for 120 days resulted in a complete cessation of growth in oil palm. However, when temperatures increased to between 12°C and 27°C, frond leaf production increased in a quadratic pattern. Okon et al. (2021) suggested that temperature sensitivity varies among oil palm cultivars, with some cultivars demonstrating a better ability to withstand prolonged exposure to high temperatures compared to others. However, a key question remains: How long will the effects of high temperatures continue to impact oil palm growth and yield after the initial exposure? In addition, little is known about the lower and upper critical limits for optimum oil palm performance.

Rainfall Variability and Its Effects on Oil Palm

Gleick (1989) found that the rate of rainfall reduction due to climate variability across the globe was 100%–200% compared to pre-industrial times. In Malaysia, Tangang et al. (2012, 2018) reported that changes in the pattern and intensity of rainfall have been observed since the early 2000s. Observations by Abdul Rahman (2018) revealed that Sabah and some parts of the Peninsular (e.g., Pahang and Kelantan) received 5% less rainfall on average due to climate change. Noor et al. (2018) analysed rainfall patterns in Malaysia using intensity-duration-frequency (IDF) curves, which describe the probability of rainfall intensity occurrence over given time periods. These curves, generated from hourly rainfall data from 1971 to 2005, revealed high rainfall intensity with wide variability across the Peninsular. However, the state of Kedah showed a consistent decreasing trend in rainfall intensity, particularly within the first ten years. Both observed and simulated hourly rainfall records confirmed highly variable trends throughout the studied areas.

Carr (2011), Finucane and Keener (2015) and University of Nottingham Malaysia (2022) stated that rainfall variability can be observed through fluctuations in the groundwater hydrology, especially in areas close to the shoreline, such as parts of Sabah, Penang, and Sarawak (Mayowa et al., 2015). This becomes clearer during the southwest monsoon season (Tang, 2019).

Rain is the major source of water for the agricultural production of all crop types (Benešova et al., 2012; Fischer et al., 2007; Ibrahim et al., 2020; Najihah et al., 2022; Norizan et al., 2021; Sadiq et al., 2022a), making it the most limiting factor in oil palm production (Goh, 2000; Jazayeri, et al., 2015). Goh et al. (2011) added that the effect of rainfall on oil palm production has been underscored since 1965 (Tui & Arifin, 2013), with most results showing a linear rainfall-yield relationship (r = 0.89). Similarly, DoCampo et al. (2021) corroborated that the harvesting peak for oil palm always coincided with months of high rainfall. Furthermore, Hermantoro et al. (2018) found that in Lampung and Palembang, where rainfall supplied 100 mm less water month^{-1}, the yield decreased by 9.0% and 3.5% in the first and subsequent years, respectively. This is expected because all metabolic processes governing oil palm growth and development are water-dependent, either as major constituents or catalysts (Karananidi et al., 2020; Weil & Brady, 2017). In addition, the role of rainfall as a principal water source extends to nutrient dissolution, availability, and subsequent uptake (Norizan et al., 2021), resulting in the formation of high-quality and high-quantity FFB (Woittiez, 2019). Therefore, even a narrow difference in the rainfall among locations can lead to a wide difference in yield outcomes (Donough et al., 2011; Paterson & Lima, 2017).

However, Corley and Tinker (2016) noticed that the relationship between the total rainfall and FFB yield has been inconsistent. This may be due to the contribution of other rainfall attributes, namely, distribution and intensity (Jadhav, 2019; Oettli et al., 2018; Sarkar et al., 2020). According to Najihah et al. (2022), Usman et al. (2013) and Woittiez (2019), the frequency of rainfall occurrence reasonably dictates the oil palm yield by determining the sex ratio, number of spikelets, percentage of fruit set, and weight per fruit bunch. A similar effect of precipitation variability was reported to constrain oil palm production in neighbouring countries of India and Bangladesh (International Rice Research Institute [IRRI], 2007).

Additionally, the interplay of other factors, notably soil hydraulic properties, the presence of surface cover, and the crop's rooting system significantly influences the effectiveness of rainfall received per unit area. The question is, can we have studies dedicated to experimenting the effect of the amount, distribution, and intensity of rainfall over a complete production cycle of oil palm under various weather conditions in Malaysia?

Climate Events Variability and Its Effect on Oil Palm

Climate variability can also be examined through the occurrence of extreme conditions such as flooding and drought (IPCC, 2013, 2014). Al-Amin and Leal Filho (2014) observed that historical climate variability in Malaysia has led to an increased frequency of floods in some areas, while exacerbating water scarcity in many others (The Star, 2022). Flood occurrence, which has been steadily increasing since 1980, showed a sharp increase between 2000 and 2005 and peaked around 2010 (Laudicina & Peterson, 2015). Nashwan et al. (2019) and Tam et al. (2021) believed that climate variability that triggered heavy rainfall during the northeast monsoon was the leading cause of the unprecedented flood that the Kelantan River Basin experienced towards the end of 2014. Data presented by Tam et al. (2021) indicated that floods occurred consecutively every year from 2001 to 2014, except in 2002, along the Kelantan River Basin area. However, its magnitude varied widely (0.2 m in 2011 to 6.8 m in 2014). Similarly, the floods that occurred in 1998 and 2007 suggest climate variability (Al-Amin et al., 2011). Murad et al. (2010) noted that the reoccurrence of these extreme meteorological hazards could potentially turn productive oil palm land into marginal or permanently unsuitable. Similarly, Lamade et al. (1998) and Henson et al. (2008) reported that long-term flooding deleteriously affects photosynthesis and transpiration and leads to premature death of young palms, stunted growth and productivity loss (Carr, 2011).

Drought variability over a temporal scale is not only peculiar to Malaysia but also to other Asian countries where both intensity and frequency of droughts are increasing (Manikandan & Tamilmani, 2015; Tabari et al., 2013). According to Hasan et al. (2021), results from 40 years data revealed that approximately 50% of the basin areas in Malaysia have been experiencing drought at different time scales. Hasan et al. (2021) and Huang et al. (2023) showed that the highest drought intensities were observed from 1997 to 1999 and 2016 to 2018, and were more prevalent in several areas in Peninsular with a frequency of 39.25%. Specifically, the 1998 drought was very severe and as such, increased water scarcity. The historic variability trend of drought (1985–2019) indicated that critical droughts around the Muda River occurred for 12 years (1991–2016), with the highest frequency occurring from 2003 to 2007 (Luhaim et al., 2021). Moreover, Sukarman et al. (2022) concluded that all 18 of the studied oil palm plantations showed evidence of drought spells equivalent to a 450 mm water deficit annually between 2000 and 2004, which were more frequent between January and May.

Drought is characterised as a short-term cessation of rainfall (>5 days) or an annual precipitation below 1200 mm yr^{-1} (Hartley, 1988). This phenomenon can lead to soil moisture depletion beyond the crop's tolerance threshold, resulting in severe bunch failure, abortion, and decreased yield due to water deficit (Chi & Qi, 2021; Kirkham, 2005; Sukarman et al., 2022; National Geographic, 2023).

The oil palm tree possesses unique morphological features, including a highly lignified cuticle and hypodermis, which can mask overt physical symptoms of drought-induced water stress (Rees, 1961). However, prolonged drought periods of three to six months can significantly reduce yield, biomass accumulation, and leaf area index (Carr, 2011; Corley & Tinker, 2016; Goh, 2000; Grossiord et al., 2020). Moreover, drought-facilitated water stress has been associated with a consequential reduction in the quantity of extractable oil in the subsequent year (Muhamad Rizal & Tsan, 2008; Neto et al., 2021).

In the context of Southeast Asian oil palm cultivation, particularly in Malaysia and Indonesia, climate variability-induced droughts lasting one to three consecutive months can have significant impacts (Abubakar & Ishak, 2022). Such drought events can potentially cause yield losses of up to 10 t of FFB ha^{-1} yr^{-1} (Olivin, 1986).

Mohd Arif (2005) revealed that most edaphic production constraints in unsuitable areas of Malaysia, such as soil acidity, nutrient imbalances, sandiness, and hardness, are linked to drought. These conditions exacerbate water limitations by reducing groundwater recharge and soil moisture reserves (African Centre of Meteorological Applications for Development [ACMAD], 2022), which are essential for continuous water uptake by oil palm. However, according to Carr (2010) and Corley and Hong (1982), less frequent drought spells caused minimal reductions to oil palm yields as well as insignificant impacts on leaf budding and initiation rates.

In summary, the recurrence of both dry spells (droughts) and floodings reduces oil palm production capacity in Africa, Asia and Latin America (Fischer et al., 2007; Lee & Ong, 2006; Malay Mail, 2015; Marengo et al., 2009; Paeth et al., 2009). As such, ACMAD (2022) emphasised that any serious scientific community must be motivated to act swiftly to mitigate their consequences since they most often occur unnoticed (NiMet, 2022).

Effects of Solar Irradiance on Oil Palm

Oil palm generally requires approximately 5 hr day^{-1} of sunshine hours for optimal photosynthesis (Lim et al., 2008). Several studies have reported a strong positive linear relationship between sunshine hours and FFB yield, with sunshine potentially enhancing yield by up to 80% (Cheah & Hoi, 1999; Lamade & Setiyo, 2002; Lim et al., 2008).

Reduced solar irradiance due to self-shading (when upper fronds shade lower fronds of the same palm) has been reported to reduce photosynthetic efficiency by 57%, drastically reducing yield. Hoffmann et al. (2014) explained that the lower fronds, likely because they were blocked from direct sunlight, contributed very little to FFB formation (Hartley, 1988; Henson, 2002).

A yield simulation using an oil palm potential growth model named PALMSIM estimated that FFB could reduce to 10 t ha^{-1} when constrained by irradiation (Hoffmann et al., 2014). Caliman and Southworth (1998) observed reduced yield from solar radiation obstruction due to open burning. They established that shortened sunshine hours decreased FFB by 1.3–4.7 t ha^{-1} yr^{-1}. Haze conditions tend to reduce solar radiation reaching the oil palm trees via reflection or absorbance. Aziz et al. (2018) revealed that haze decreased solar radiation by 22.0%–45.0%, which resulted in declined photosynthesis by 12.9%–53.2%.

In the Peninsular, the average sunshine hours recorded by Tui and Arifin (2013) was 5.57 hr day^{-1} from 1979 to 2011, representing the lower limit of the optimum amount reported by Carr (2011). Therefore, optimisation of solar radiation reception through research could help enhance assimilate production (Corley & Tinker, 2016; Lim et al., 2021). Proper planting density and planting orientation that avoids self-shading and leaf overlap are important for maximum radiation interception.

Effects of Relative Humidity and Vapour Pressure Deficit on Oil Palm

Oil palm thrives in humid environments (DoCampo et al., 2021). This means that relative humidity (the amount of water vapour in a particular air volume) (Van der Pol et al., 2015) is a critical parameter for oil palm productivity (Maikasuwa, 2013; Obioha, 2008; Rhebergen et al., 2019). Van Ierland et al. (2006) stated that relative humidity (RH) significantly affects key internal mechanisms and the surrounding oil palm system, influencing the final yield. Lim et al. (2022) reported a relationship between stomatal aperture and ambient RH, where lower RH reduces stomatal apecture, leading to reduced photosynthetic efficiency and a decrease in net biomass weight. Oil palm seeds perform poorly during the germination stage when RH is lower than 75% and higher than 90% (Lubis, 1992).

Kirkham (2005) states that vapour pressure deficit (VPD) and RH are interrelated in function because RH is the ratio of the actual vapour pressure to the saturated air vapour pressure (Miller & Donahue, 1992; Van der Pol et al., 2015). Jacquemard (1998) asserted that a high RH is required to offset the effect of high temperatures so that the VPD is maintained at the optimum state. Jacquemard (1998) inferred that VPD above 1.8 kPa and RH = 58% triggered stomatal closure at 30°C, causing significant yield reduction.

Based on the regression output, a high VPD would reduce stomatal conductance, which in turn, reduce photosynthesis (Henson, 1995; Van Ierland et al., 2006). Furthermore, Henson (2009) confirmed that considerably high VPD reduces total dry matter production, and its severity increases if VPD is coupled with soil moisture deficit (Lim et al., 2008). In a separate study, Henson and Harun (2005) and Price and Black (1990) noted that VPD, together with temperature, explained 20%-31% of the carbon dioxide (CO_2) flux. This directly influenced the rate at which oil palm generated photosynthates for FFB production. Suboptimal CO_2 levels adversely affect the oil palm internal hydraulic system, causing an appreciable (13.2%) yield loss (Grossiord et al., 2020). Fieldwork evidence affirmed that at a very high VPD, oil palm metabolic activities are hampered or ceased completely, affecting the reproduction phase (Setyo et al., 1996; Tani et al., 2003; Villalobos et al., 1993). The negative effect of high VPD on oil palm yield and growth attainment was significant at $p = 0.05$, and these negative impacts could not be adequately reversed by re-watering efforts (Goh et al.,, 2011). The specific VPD threshold beyond which recovery becomes impossible still remains unclear.

Climate Prediction

Paterson et al. (2017) remarked that the climate over the next 70 years will be particularly challenging for oil palm production, thus requiring a paradigm shift in cultivation methods (Rival, 2017). Fleiss et al. (2017) and Paterson et al. (2015) stated that, like other crops, oil palm is highly dependent on climate. Therefore, the projected temperature increase of more than two-fold (IPCC, 2007) could potentially reduce its productivity by exacerbating water stress and weakening its defense mechanisms (Fleiss et al., 2017). Teh and Cheah (2018) reported that CORDEX SEA (https://cordex.org) projected the air temperature in Malaysia may rise by up to 3.2°C and the country may experience lower rainfalls by 20% by the end of the 21st century. Loh et al. (2016) further estimated that the projected temperature rise across all the climate change scenarios ranged from 2.3°C to 3.7°C.

Leta et al. (2018) and Siderius et al. (2018) stated that the rate of drought occurrence and its intensity were projected to increase by 1%-30% within the 21st century. Based on National Water Research Institute of Malaysia (NAHRIM) prediction, from 2025 until 2030, Terengganu will be vulnerable to intermittent drought experiences (Malay Mail, 2020; Reuters, 2023). The rainfall forecast showed a highly variable status across seasons, but dry spells will persist longer by 30% in the driest months (December-May), while wet spells will increase by the same percentage around mid-year (Loh et al., 2016).

Shanmuganathan et al. (2014) observed that the triangular benefits of oil palm production-high quantity, quality, and net profit-are likely to decline if climate conditions become unfavorable, as projected by Al-Wabel et al. (2020) and Teh and Cheah (2018). Extreme temperatures, irregular rainfall, and prolonged dry spells contribute to harsh weather conditions (Tang & Al-Qahtani, 2020). Therefore, closing all potential water loss gaps through enhanced conservation measures is essential for ensuring sustainable oil palm production.

EFFECTS OF WATER STRESS ON OIL PALM

The response of oil palm to water stress involves multiple physiological and genetic mechanisms (Hanafiah et al., 2022; Jaleel et al., 2009; Shao et al., 2008), making it challenging to fully understand and predict its behaviour under such conditions. In addition, the time taken for oil palm to reach maturity and reproduction stage complicates the understanding of the water stress-yield connection (Carr, 2010; Corley & Tinker, 2016).

Lim et al. (2008) and Suharyanti et al. (2020) found that water stress triggers various physiological responses as adaptive strategies, such as midday stomatal closure, shading of older leaves, extension of root system, and conversion of stored trunk starch to support bunch and inflorescence development (Carr, 2011). As a result, this process inhibits growth of new leaves and delays shoot development (Méndez et al., 2012).

Under persistent water stress conditions, canopy thinning, dropping of developing bunches, and eventual tree death can occur (Goh et al., 2011). Jazayeri et al. (2015) observed that young oil palm seedlings in nurseries often failed to recover even after water was resupplied following periods of extreme water stress. This vulnerability is likely due to the absence of well-established root system and insufficient starch reserves in the trunk, which are characteristics typically found in mature palms (Lim et al., 2008). The underdeveloped state of these young plants makes them particularly susceptible to drought-induced physiological damage, from which they struggle to recover even when water becomes available again. This signifies that water stress is associated with poor yield and less vegetative vigour and could lead to permanent wilting and death at higher severity. Lim et al. (2008, 2021) posited flower abortion is a more immediate and severe consequence of water stress in oil palm compared to changes in sex differentiation.

Henson and Harun (2005) stated that during the 4–5 dry months in the northern part of Kedah, oil palm initially maintained high ETo rates in response to water stress, driven by high sensible and latent heat. However, as the stress persisted, ETo decreased, and only returned to normal levels when sufficient moisture (5 mm day^{-1}) was restored in June. However, under mild water stress, the ETo rate showed no significant difference compared to that of fully watered crops (Ibrahim et al., 2020).

Jazayeri et al. (2015) experimented with the *tenera* hybrid of oil palm tolerance to water stress levels. They observed that high and severe water stress levels (50.0% and 25.0% of ETo, respectively) caused a drastic decline in leaf water potential by 66.7%, photosynthetic rate by 28.6% and water use efficiency (WUE) by 26.87%. The rate of photosynthesis in week 4 and 8 was reduced by 23.0% and 53.0% for IRHO7010 germplasm, respectively. For the IRHO1001 germplasm, the rate was reduced by 46.0% and 74.0%, respectively. Similar reduction in photosynthesis have been linked to chlorophyll and carotenoid degradation and reduce ATP synthesis in other crops (Boughalleb & Hajlaoui, 2011; Cha-um et al., 2013; Pan et al., 2020), which in turn stunted shoot growth (Farooq et al., 2009). This suggests that different oil palm cultivars may respond differently to water stress, though more empirical evidence are needed to confirm this assertion.

High concentrations of proline, malondialdehyde, abscisic acid, and relative electrolyte leakage have been observed, all of which play a central role in moderating the stomatal responses to mitigate the effects of water deficit (Cha-um et al., 2013; Henson et al., 1992; Najihah et al., 2022). Proline is the most prominent chemical indicator of water stress (Cao et al., 2011), and it is a biochemical solute produced through glutamate intermediates and oxidation by P5CR (Sun et al., 2011).

The flux of CO_2 peaked during the morning hours but gradually declined as water stress intensified by midday, reflecting maximum assimilation in the morning and minimum assimilation at midday, as observed in oil palm (Henson & Harun, 2005) and similarly reported for groundnut (Reddy et al., 2003). This was more marked in the driest month of February when CO_2 was as low as 1 g m^{-2} hr^{-1} (Reddy et al., 2003). Henson and Harun (2005) and Price and Black (1990) reported that physiological responses to water stress, as measured through nighttime gaseous exchange, were difficult to interpret due to large hourly variability, and that this variability might be attributed to reduced VPD and the absence of solar irradiation during nighttime.

Oil palm primarily responds to water stress through two initial mechanisms: Stomatal closure and cell membrane depolarisation, as reported for oil palm (Jazayeri et al., 2015) and similarly observed in other crops (Hopper et al., 2014; Jaleel et al., 2009; Reddy et al., 2003). Depolarisation refers to a change in the electric charge distribution across the cell membrane. This process is initiated by the activation of anion channels in the stomatal guard cells (Brault et al., 2004). As a result, the interior of the cell becomes less negatively charged relative to the exterior (Nuhkat et al., 2021). This change in membrane potential plays a crucial role in facilitating communication between cells and coordinating various physiological responses within the plant (Nuhkat et al., 2021).

Zhou and Yarra (2022) identified several genetic transcription factors, including bZIP, EgbZIPs, and specifically 11 EgbZIPs, that are activated when oil palm is exposed to water stress. This discovery of genetic indicators has significant implications. Parveez et al. (2023) suggested that such discovery could broaden the scope for developing genetically modified oil palm varieties and enhance advanced conservation practices. However, despite these advancements, a key question remains unanswered: Among the various genetic, enzymatic, and hormonal responses to water stress, it remains unclear which occurs first to trigger stomatal closure. This gap in our understanding highlights the complex nature of plant responses to water stress and indicates areas for future research.

WATER STRESS IN RELATION TO SOIL PROPERTIES

The limited availability of water to plant roots, which is primarily determined by soil properties, is a critical factor that can lead to poor performance and, in severe cases, complete crop failure (Lindh et al., 2022; Miranda et al., 2021). This phenomenon explains the observations made by Safitri et al. (2019), Sukarman et al. (2022), and USDA (2017) regarding oil palm performance under different soil conditions. These studies found that oil palms receiving equal amounts of water exhibited varying degrees of water stress severity depending on soil texture. This variation in water stress, influenced by soil properties, ultimately contributes to the observed yield gap in oil palm cultivation (Hoffmann et al., 2015; Nasution et al., 2017; Woittiez et al., 2015). The prominent role of soil texture in water availability underscores the argument made by Norizan et al. (2021) against the practice of applying uniform irrigation across different soil types. As Kirkham (2005) explains, this approach is unjustifiable due to the varying hydro-physical attributes of different soil textures. These differences significantly influence how water is retained and made available to plants.

Paramananthan (2003), Kushairi et al. (2019) and Idris (2020) maintained that soil texture considerably influences the water productivity and yield of oil palm and other arable crops. Kirkham (2005) and Gunawan et al. (2020) highlighted that soil texture strongly determines water accessibility to crops, mostly in relation to drainage, infiltration, and hydraulic conductivity. Carr (2011) and Goh (2000) identified soil texture variation as a key factor influencing productivity differences among oil palm cultivated lands in Asia. Soil texture plays a crucial role in determining how much water the soil can retain and release at different stages of moisture content, such as when the soil is fully saturated (saturation), at its optimal water-holding capacity (field capacity), or when plants can no longer extract water (permanent wilting point) (Saxton et al., 1986; Teh & Iba, 2010). These distinct moisture levels in soil water retention significantly impact oil palm productivity (Mutert et al., 1999; Woittiez et al., 2017).

Oil palm's susceptibility to water deficit stress is closely linked to soil texture, particularly the soil's capacity to retain plant-available water (PAWC) (Carr, 2011; Hoffmann et al., 2014). This relationship becomes evident when comparing different soil types. For instance, Hoffmann et al. (2015) observed that sandy loam and clay loam soils possess significantly higher PAWC compared to sandy clay soils. The variation in PAWC among soil types has important implications for oil palm cultivation. Soils with intrinsically low PAWC make oil palms more vulnerable to water stress, potentially affecting crop productivity. This vulnerability is likely due to variations in the critical water depletion limit specific to each soil type.

In a separate study, Jourdan et al. (2000) examined oil palm root expansion and distribution, especially within the active root zone. Sandy soils retain less water and drain faster than clay soils (Kasno & Subardja, 2010; Teh, 2016). While oil palms can grow in various soil types due to its tolerance for different soil conditions, achieving maximum yield requires soils that retain water well yet make it easily accessible to the trees (Norizan et al., 2021).

Coarse sandy soils and fine clayey soils (vertisols) significantly reduce oil palm yields (Paramananthan et al., 2000). Heavy clay soils have very low infiltration rates, potentially causing waterlogging that can lead to sudden mortality in immature palms or yield reductions of up to 25% (Abram et al., 2014; Lee & Ong, 2006). These findings indicate that neither coarse- nor fine-textured soils meet the optimal water requirements for oil palms. The varying clay and sand content in soils directly affects water availability.

Additionally, soil bulk density (BD) is strongly linked to water stress (Michael & Dunn, 2000). BD increases in deeper soil layers, implying that roots may increasingly struggle to penetrate the entire soil profile, limiting their ability to access water throughout the soil solum (Nasrul et al., 2002). Wiratmoko et al. (2015) advised that good tillage operations can be undertaken to improve BD and soil tilth for root development. BD also affects root anchorage and soil attachment. Gray et al. (2015) observed that poor anchoring due to low BD can render soil less suitable for sustainable oil palm production due to lodging and uprooting tendencies. Othman et al. (2011) reported that lodging exposes oil palm roots to excessive dehydration and breakage, reducing their water uptake potential (Lim et al., 2008). Kirkham (2005) and Venturas et al. (2017) maintained that when crop roots are injured, the xylem tissue responsible for conducting water also becomes affected; hence, water transport system becomes impaired. This indicates that exposed and broken root systems can increase water stress severity. Dolmat et al. (1993) and Tie (2004) recommended mechanical compaction (Mutert et al., 1999) as a strategy to curtail root lodging and uprooting in low BD soils.

Poor soil cohesion leads to weak root attachment, potentially reducing water uptake (Paramananthan, 2013). Other soil morphological and chemical properties also influence water availability through various mechanisms.

Coarseness in the plough layer hinders hydraulic permeability, limiting water availability to roots (Nasrul et al., 2002). Similarly, studies by Afandi et al. (2022) and Woittiez et al. (2017) indicated that soil shallowness restricts vertical root growth and reduces root density. Root density, particularly within the upper 30 cm of soil, is crucial as it directly influences the amount of water available for plant uptake (Afandi et al., 2022). In Malaysia, soil shallowness is a significant issue, exacerbating water stress and limiting optimal yields (Fairhurst & McLaughlin, 2009). In contrast, deep soils promote extensive root systems that enable better water exploration by oil palm (Dufrêne et al., 1992; Rey et al., 1998).

However, soil salinity also plays a critical role in controlling water transmission and uptake, as noted by Mutert et al. (1999). Furthermore, water stress is aggravated in soils with moderate to extreme acidity, which negatively impacts oil palm performance (Mutert, 1999; Paramananthan, 2013). Adam et al. (2011) found that when oil palm is exposed to both water stress and soil acidity, the plant produces more male inflorescences and fewer female flowers, which directly lowers its productivity.

Olivin (1986) categorised soils based on their oil palm yield productivity under water deficit conditions, assuming all other production factors remained constant. Soils yielding 25–27 t ha^{-1} yr^{-1} under 0 mm water deficit and 16–18 t ha^{-1} yr^{-1} under 200 mm water deficit were deemed suitable. In contrast, soils yielding 22–16 t ha^{-1} yr^{-1} with no water deficit and 9–13 t ha^{-1} yr^{-1} under a 200 mm water deficit were considered the least suitable for oil palm cultivation.

MITIGATION EFFORTS IN OIL PALM PRODUCTION

Irrigation

When rainfall fails to meet oil palm ETo demands, irrigation is applied to meet the shortfall. A fully irrigated oil palm has a crop coefficient (Kc) between 0.9 and 1.0, indicating minimal water stress (Carr, 2011; Teh, 2016). Early study by Ochs and Daniels (1976) revealed a significant effect of irrigation on yield, with results showing threefold increase. Further studies by Tui and Arifin (2013) reported a 57% higher mean annual yield under irrigation conditions than under rainfed conditions over approximately 33 years. Henson (2009), using OPRODSIM, found a 25% yield increase with irrigation, with further improvements when comparing the two irrigation schedules. Teh (2017) used an oil palm growth model to estimate that even a daily supplementation of 1 mm could boost yields by 1.5 t ha^{-1}, particularly in water-deficient areas where yields commonly increase by 50% (Lee & Izwanizam, 2013). Woittiez et al. (2017) found a direct link between irrigation water volume and FFB yield, and Nasir et al. (2014) showed that oil palm irrigation increased bunch weight, number, FFB and average fruit weight by 10.0%, 44.0%, 27.0% and -22.3%, respectively.

Rao et al. (2008) discovered that irrigating oil palm progeny hastened fruit production by 20% and increased yield by 55%. Chalvantharan et al. (2023) reported an 18.2% yield increase with sprinkler irrigation, although Palat et al. (2008, 2012) observed no significant yield variation across different irrigation methods. Prioux et al. (1992) reported that irrigation doubled the mass of tertiary roots and increased their spread by 20–100 cm, and Lee et al. (2005) noted a 5% increase in bunch number and oil-bunch ratio over three years.

Despite the high initial costs, the long-term yield benefit makes irrigation a viable option (Norizan et al., 2021; Sadiq et al., 2022b). However, these costs pose a challenge to smallholder farmers (Teh and Cheah, 2018). Sadiq et al. (2022a, 2022b) and Tui and Arifin (2013) found that irrigation investment is profitable in tomato cultivation, and Tui and Arifin (2013) similarly reported profitability, although economic benefits can vary, as seen in the negative ROI findings for smart irrigation by Chalvantharan et al. (2023) and the modest yield increases reported by Corley and Hong (1982). Variability in returns can be attributed to differences in water costs, production scale, and other factors (Miller and Donahue, 1992). The lack of technical expertise among Malaysian growers presents a significant barrier to effective irrigation (Chalvantharan et al., 2023; Khan et al., 2018; Norizan et al., 2021) and Mason et al. (2019) recommended tailored irrigation strategies based on climate forecasts.

Surface Mulching

Water conservation efforts have focused on optimising the productivity of increasingly scarce freshwater due to climate variability for higher yield and sustainability (Abdullah & Sulaiman, 2013; Donough et al., 2011; Fereres & Soriano, 2007). Mulching with oil palm residues, such as shredded trunks and fronds, mitigates excessive evaporation and runoff, thereby enhancing water conservation (Khalid et al., 2000; Moraidi et al., 2013; Morgan, 2005). DoCampo et al. (2021) noted that such mulching reduces water stress impact, which is prevalent in dry months. Khalid et al. (2000) found that mulching with shredded residues increased soil moisture by 28.0%, compared to 25.2% in other treatments. Mulching with empty fruit bunches (EFB) led to a 39.0% increase in yield by improving water storage and mitigating heat effects (Rudolf et al., 2021), with similar benefits observed using other mulching materials under maize (Li et al., 2018). Moreover, Donglin et al. (2019) reported 5.7%–19.8% and 7.1%–20.9% increases in energy and water productivity, respectively, owing to mulching.

Economic analysis shows a maximum marginal rate of return of 5.75 ha^{-1} yr^{-1} from mulching (Wairegi & Van Asten, 2010) and a 27.0%–53.8% increase in net income compared to unmulched systems (Donglin et al., 2019; Jianguo et al., 2014). For every 1 m^3 of water used, mulching achieved a 5.0% higher value than that of the control (Adetoroa et al., 2020). Nwokocha et al. (2017) concluded that a mix of 12.00 t ha^{-1} EFB and 4.00 t ha^{-1} palm bunch ash provided the best net return. Abubakar et al. (2021), Khalid et al. (2000) and Moraidi et al. (2012) documented significant improvements in the hydrophysical properties following EFB mulching.

Despite the benefits, the use of oil palm biomass as mulch or incorporating it into the soil as an economical practice remains debated. Moreover, addressing water deficits through irrigation on large oil palm farms is both challenging and costly (Abdullah & Sulaiman, 2013; Miller & Donahue, 1992; Teh, 2016). While mulching with 30 t ha^{-1} of EFB can reduce surface evaporation (Lim et al., 2008). Carr (2011) argued that such high application rates are impractical. Rudolf et al. (2021) suggested that motivating farmers to adopt mulching is difficult due to the large quantities required for significant benefits. The costs associated with transportation and application can increase total expenses by as much as 73.3% (Rudolf et al., 2021; Wairegi & Van Asten, 2010).

Sari et al. (2022) highlighted farmers' reluctance toward mulching because of the cost-yield trade-off. Furthermore, effective weed suppression requires a thick mulch layer, which can result in impractically large volumes (Nwokocha et al., 2017; Wairegi & Van Asten, 2010).

Furthermore, mulching can lead to pest infestations, affecting soil temperature and potentially inhibiting germination owing to allelopathy (Benoit, 2022; Cabona et al., 2021; Donglin et al., 2019; Iqbal et al., 2020; Korkança & Sahin, 2021; Ni et al., 2016). It also increases the risk of fire hazards and wild animal-human conflict (Ni et al., 2016; Rongbin et al., 2020). Thus, the choice of mulch material must be tailored to the specific climate conditions to mitigate these risks.

Silt-pit and Cover Crops

Mechanical techniques for water harvesting, such as silt-pit and bund terraces, significantly increase resilience to water stress risks by reducing runoff and enhancing soil water reserves (Gabrielle et al., 2018; Murtilaksono et al., 2011). For example, the silt-pit technique reduced water run-off by 89% and increased soil water reserves by up to 183 mm (Bohluli et al., 2012; Murtilaksono et al., 2011; Yuswar et al., 2020). Moreover, different silt pit sizes affect soil water content, with larger dimensions showing varying outcomes (Bohluli et al., 2012; Masnang et al., 2022; Ping et al., 2012). However, this technique has limitations, including its suitability only for highlands and areas with contrasting slopes and potential soil structure disruption (Bohluli et al., 2015; DoCampo et al., 2021).

Cover cropping also enhances water stress tolerance. Planting of leguminous crops, such as *Brachiaria* and *Pueraria javanica*, improves water infiltration and retention, reduces dry days, and enhances water use efficiency (WUE) (Agusta et al., 2020; Ariyanti et al., 2017; Nouy et al., 1999; Zhang et al., 2023). The integration of diverse cover crops can help mitigate runoff water loss (Gabrielle et al., 2018; Morton et al., 2017; Nabara & Norsida, 2018).

Nonetheless, challenges include interference with mechanisation operations, inter-crop competition, and increased risk of pest and disease transfer, which may complicate the oil palm-legume cropping pattern (Chalmers, 2017; Dowling et al., 2020; Echarte et al., 2011).

Application of Synthetic Polymer

Synthetic soil conditioners, notably referred to as super absorbent polymers (SAPs), are an alternative for water deficit management (Lentz & Sojka, 2009; Zhang et al., 2021). SAPs are networks of flexible porous polymers (Zhang et al., 2021) that are tridimensionally cross-linked (Kiatkamjornwong, 2007). They can retain a large quantity of water (Lucero et al., 2010; Zhang et al., 2019; Zhao et al., 2021), and make water more accessible to roots (Azman, 2013; Rafiei et al., 2013). SAPs application effectively minimises water stress in many field crops (Han et al., 2010; Ibrahim et al., 2020; Zohuriaan-Mehr et al., 2010). Yang et al. (2022) found after a decade of research that conditioning the soil with 45 kg ha^{-1} SAP significantly enhanced soil macroaggregates (>0.25 mm) by 16.5%–36.33%, WUE by 16.0%, and rate of photosynthesis by up to 18.5% (Yang et al., 2020). The application of SAPs reduced runoff by up to 103.0%, and improved the soil moisture by 29.0% (Yuanbo et al., 2017). Additionally, meta-analysis by Zheng et al. (2023) indicated a 15.0% yield increment from SAPs treatment.

However, Idris and Yahaya (2022) and Nasereldin et al. (2023) noted the unavailability of SAPs in local markets and asserted their high purchasing cost as the core constraints limiting their accessibility. Furthermore, SAPs require a specialised storage environment that is relatively dark, dry, and cool to maintain their shelf life (Mechtcherine et al., 2021; Snoeck & De Belie, 2019). Unfortunately, most farmers do not have such required storage facilities.

To date, there has been no published study on the effectiveness of SAPs on water stress reduction in oil palm in Malaysia or other Asian countries. This is a knowledge gap that needs to be addressed.

CONCLUSION

Over time, Malaysia's climate has become increasingly variable, marked by a rise in temperature (7.5%), reduced rainfall (-5.0%) and more frequent extreme weather events. This variability has exacerbated water deficits, constraining sustainable oil palm production. Future climate projections suggest higher temperatures, decreased rainfall, and consequently, more severe water stress.

While the use of oil palm biomass and cover crops has been tested and shown to mitigate water deficits, further research is needed to understand the short-, medium- and long-term effects of these practices. Similarly, treatment methods such as biochar, mulching and use of silt pits require deeper investigation. Additionally, the potential of synthetic polymers as a solution for managing water stress in oil palm plantations remains to be explored.

Table 1 provides a summary of water stress effects for various plantation crops, and *Figure 1* shows the water stress levels experienced by oil palms across the 12 months.

TABLE 1. SUMMARY OF YIELD, GROWTH AND PHYSIOLOGICAL EFFECTS OF WATER STRESS FOR DIFFERENT CROP CATEGORIES

S/N	Mode/level of water stress inducement	Crop	Resultant effect	Source
01	100 mm less deficit	Oil palm	10%–15% FFB yield reduction	Caliman and Southworth (1998)
02	Intermittent water stress from rainfall cessation and inadequacy	Oil palm	The number and yield of FFB downsized by >91.00% and 88.46%, respectively	Gawankar et al. (2003)
03	Natural water deficit stress from rainfall cessation was monitored	Oil palm	Declination of leaf water content, WUE and photosynthetic rate and increased chlorophyll content were observed	Noor (2006)
04	Experimental sites were under yearly water deficit of 150, 250 and 400 mm	Oil palm	Higher water stress got the least number of bunches (-82%), least bunch weight (-79%), and least FFB (-88%)	Dwarko et al. (2008)
05	Water balance and fraction transpirable soil water approaches were adopted to predispose the test crop to equivalent water stress	Oil palm	Yield is more sensitively affected by water stress at 2 ½ years prior to bunch maturity	Legros et al. (2009)
06	Consistent non-watering for 24 days after full establishment (30 days) in the glasshouse	Oil palm	All gas exchange variables declined drastically, and at 24 days of severe water stress, photosynthesis stopped while 200% leaf water potential became 2 times higher	Suresh et al. (2010)
07	Natural water stress equivalent to an annual shortfall of 450 mm due to an unimodal rainfall pattern	Oil palm	Stages most badly affected were fruit filling, sex differentiation and state central arrow	Renny et al. (2011)
08	Superimposition of stress by supplying 0.5 of full FC water volume	Oil palm	The root/shoot ratio improved by 23.0% Number of leaves decreased by 11.4%	Sun et al. (2011)
09	Water deficit was induced by maintaining moisture at -0.042, -0.500, -1.000, and -2.000 MPa tensions	Oil palm	Assimilation of CO_2 completely ceased, and bulb diameter decreased by 48% at -2 MPa; stomatal conductance was not affected by genotype-water potential interaction	Méndez et al. (2012)
10	Water stress imposed for 12 and 16 days equivalated to 13% and 6% SWC, respectively	Oil palm	Chlorophyll was disrupted by 59.0% and 95.9%, while photosynthetic rate diminished by 71.7% and 91.1% respectively	Cha-um et al. (2013)
11	4 and 8 weeks without watering after water deficit at -1.50 MPa pressure was attained	Oil palm	Photosynthesis declined by 23% and 53% for the respective duration, and WUE steadily went down	Jazayeri et al. (2015)
12	Crop exposed to moderate water deficit of -0.5 MPa	Oil palm	Significant reduction in the stomatal conductance, rates of photosynthesis and transpiration, as well as vegetative development were recorded	Rivera-Méndes et al. (2016)
13	Only half and a quarter of FC water content were given as deficits for 60 days after 30 establishment days	Oil palm	Except root-to-shoot ratio, all growth variables reduced drastically, but proline status increased greatly	Duangpang et al. (2018)
14	Water stress was induced under bio-silica-treated soils	Oil palm	Water-stressed crop with no bio-silica showed higher proline status by up to 90%, and nitrate reductase activity (NRA) decreased by 93%	Amanah et al. (2019)
15	Studied the impact of dry season water stress abiotic factor	Oil palm	32.5% decrease in photosynthesis, a significant drop in gas exchange, but WUE and leaf sugar content improved by up to 27% and 1%, 14%, respectively	Bayona-Rodriguez and Romero (2019)
16	No-irrigation + fertiliser and no-irrigation without fertiliser were studied against full irrigation	Oil palm	The final harvested FFB was 17% lower due to the water deficit	Rhebergen et al. (2019)

TABLE 1. SUMMARY OF YIELD, GROWTH AND PHYSIOLOGICAL EFFECTS OF WATER STRESS FOR DIFFERENT CROP CATEGORIES (continued)

S/N	Mode/level of water stress inducement	Crop	Resultant effect	Source
17	Studied the damaging effect of water stress on leaf attributes only in areas of moderate to severe water stress due to sub-optimal rainfall	Oil palm	Multicollinearity results indicated that the number of green broken leaves (NGBL), number of folded leaves (NFL) and number of trees with central leaf cabbage toppled (NLCT) were more strongly correlated with water stress than unopened leaves (NUL) and number of base leaves dry out (NBLD) parameters which showed moderate correlation status	Yehouessi et al. (2019)
18	100 mm less water at sex determination and floral abortion phases	Oil palm	Yield loss occurred at both phases by 6% and 7%, respectively	Suharyanti et al. (2020)
19	Molecular study based on 2-week induced-water stress on seedling	Oil palm	Identified more than 1293 genes associated with water stress response across biosynthetic and metabolic, transportation and homeostatic processes	Wang et al. (2020)
20	Irrigated and non-irrigated parental stocks	Oil palm	Water deficit badly affected male and female inflorescence ratio and lowered bunch number significantly. The water deficit negatively impacted FFB and oil yield by 20%	Abdul Wahid (2021)
21	Monitored water stress induced by extreme *El-Niño* Southern Oscillation (ENSO)-facilitated drought	Oil palm	Reduction of the total fruit produced by 31%	Mauro et al. (2021)
22	Dry season water stress	Oil palm	Inflorescence and fruit formation stage was staggered	Mendoza-Hernández et al. (2021)
23	Water stress induced to young seedlings at "bifd" saplings developmental stage. By water deprivation for 14 consecutive days. At the end of this period, the substrate water potential, as measured, equivalent to -13.61 ± 1.79 MPa	Oil palm	Distortion of starch, sucrose, glyoxylate and dicarboxylate metabolism pathways occurred. Also, alanine, aspartate, glutamate, arginine and proline synthesis were positively affected	Neto et al. (2021)
24	Studies the agronomic impact of the 2015 *El Nino*-facilitated water stress at 4-12 months and 24-30 after the occurrence	Oil palm	Reduction in harvestable FFB stood at 23%–30%, but the older palms recorded the maximum values. The decline of the oil extraction ratio was found to be explained by the water stress effect	Sidhu et al. (2021)
25	Observational study due to normal climate dryness in low-rainfall receiving areas	Oil palm	Naturally tolerant cultivars had 44% and 38% greater FFB than susceptible ones in the first and second trials, respectively. Exactly 56 Single nucleotide polymorphisms (SNPs) were observed in the genetic information of water stress-tolerant lines. *MRL1*, *At1g35710*, *RNP1*, and *BDA1* genes were found to be closely associated with the SNPs detected	Yono et al. (2021)
26	14 consecutive days of water deprivation	Oil palm	Water stress-associated miRNAs and genes specific to oil palm, namely *egu-miR28ds* and *egu-miR29ds* and MYBs, HOXs and NF-Ys, were identified	Salgado et al. (2022)
27	Irrigation was withheld for 20 consecutive days for different young progenies	Oil palm	Biomass of all progenies tested decreased by 34%; proline content increased by up to 300%; stomatal conductance reduced significantly, and nine moisture stress-responsive miRNA were identified	Ithnin et al. (2022)
28	1.0, 1.5 and 2.0 L per polybag per day were supplied as deficits	Oil palm	Plant height went down in 1.0 and 1.5 L by 23.0% and 20.0% and the former reduced shoot dry weight by 12.0%	Kautsar et al. (2022)

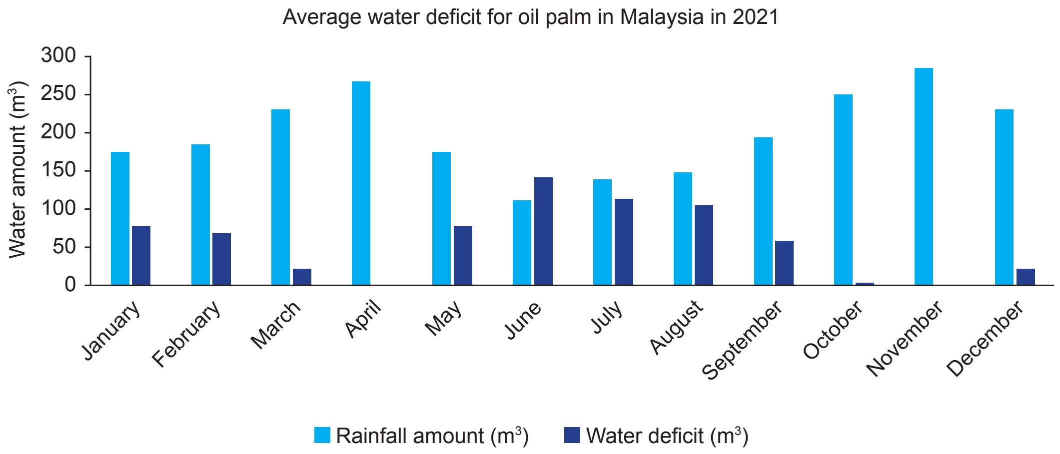
TABLE 1. SUMMARY OF YIELD, GROWTH AND PHYSIOLOGICAL EFFECTS OF WATER STRESS FOR DIFFERENT CROP CATEGORIES (continued)

S/N	Mode/level of water stress inducement	Crop	Resultant effect	Source
29	Used Fraction of Transpirable Soil Water approach to consider PWP = FTSW 0 and 15 FC = FTSW 0.15 as deficit	Oil palm	Seedling weight was reduced by 9.7% and 8.3% at PWP and 15% FC, respectively Generally, growth by height was reduced by one-third	Pangaribuan and Akoeb (2022)
30	Oil palm was predisposed to water deficit on Histosols, Entisols and Spodosols	Oil palm	Water stress was more severe in Entisols and Spodosols up to 22% than in Histosols (max. 19%)	Sukarman et al. (2022)
31	Chemically induced water stress at 0% to 30% (m/v) of polyethylene glycol (PEG 8000)	Date palm	Progressive growth reduction was obviously observed, and increased manufacturing of proline (inducer of stomatal closure) was noticed	Al-Khayri and Al-Bahrany (2004)
32	Irrigation was only given after 50, 100, 150 and 200 mm soil water had been depleted by evaporation	Date palm	Fruit diameter and fruit weight significantly lowered but were more chronic at 200 mm depletion	Alihouri and Torahi (2013)
33	Zero water supply was considered a deficit due to scanty rainfall	Date palm	Fruit size went down by 53%–76%, pulp content of date fruit reduced drastically by 57%–75% across three development stages examined, while cell wall lignification reduced insignificantly by 1.9% only in Stage 1	Gribaa et al. (2012)
34	Under the growth chamber environment, water was supplied fully at one-day intervals for two weeks to get the date palm acclimatised. The water supply was then stopped to induce water stress	Date palm	Water-stressed germplasms showed increased accumulation of fucose and glucose compounds, signifying an adaptive switch to carbohydrate metabolism	Safronov et al. (2017)
35	Involved application of 60% and 80% ETc	Date palm	Seedling establishment percentage was not affected by the 80% ETc but was significantly affected by 60%. However, the 60% competed well for the trunk perimeter index	Moheb (2019)
36	Only half of ETc applied using reclaimed wastewater and well-water	Date palm	A reduction of 86 kg of fruit output per palm was recorded. Total sugar and non-reducing sugar content enrichment occurred	Mattar et al. (2021)
37	Triggered water stress by administering 10%, 20%, 30%, 40% and 50% of actual water demand	Date palm	Fruit yield compressed by 4.5%–12.3%	Isaid et al. (2021)
38	Bubbler irrigation system was used to supply 0.25 and 0.5 of full ETc targeting flowering, hababouk, and Rutub and Tamr (third fruit development phase when moisture is lost and sucrose is converted to sugar) stages	Date palm	Decreased final yield by 28.39% compared to full ETc	Al-Mansor et al. (2021)
39	50% and 25% less than full ETc was delivered using drip and sub-surface irrigation systems	Date palm	10.8% and 6.65% reduction for 50% and 25% deficit were obtained for chlorophyll. However, while a declination of 1.7% for the 50% deficit was observed for photosynthesis, the 25% deficit numerically got higher by 0.15% relative to the full ETc	Mohammed et al. (2021)
40	Tested only one deficit level (25% FC)	Date palm	Photosynthetic rate, relative moisture content of leaf, chlorophyll enrichment, stomatal conductance and transpiration became statistically lowered. Over half of the EST (water stress-responsive mRNAs) detected were associated with photosynthesis, metabolism and gaseous exchange.	Alhajhoj et al. (2022)

TABLE 1. SUMMARY OF YIELD, GROWTH AND PHYSIOLOGICAL EFFECTS OF WATER STRESS FOR DIFFERENT CROP CATEGORIES (continued)

S/N	Mode/level of water stress inducement	Crop	Resultant effect	Source
41	This involved water stress interval viz: 3, 5 and 7-day intervals at 100%, 50% and 25% FC	Cocoa	A perfect linear trend was observed between the deficit levels and physiological and morphological attributes measured. 75% water deficit at 7-day intervals had the poorest performance e.g. for plant height, it was 26.7% below the average	Ayegboyin and Akinrinde (2016)
42	Enforcement of strong (10%–15% of vol. WC) and moderate (16%–22%) water stress level	Cocoa	29%–62% seedling mortality occurred, retarded growth, proline content increased by 937%	Niether et al. (2020)
43	Zero irrigation under harmattan season (dry season accompanied by harsh, dry, cloudy air)	Cocoa	The leaf area index decreased by 33%	Sala et al. (2021)
44	Subjected to artificial water stress until the leaf water potentials were at -3.0 and -3.5 MPa	Cocoa	Photosynthesis dropped by 98% and energy metabolism was completely distorted	Zambrano et al. (2021)
45	80%, 60% and 40% fraction of full water required administered	Cocoa	A highly significant reduction in pollen grain production was recorded	García-Cruzatty et al. (2023)
46	Observation of anomalous dry spell effect	Rubber	Yield reduced by 50%	Thomas et al. (2011)
47	Water stress from seasonal drought	Rubber	Partial stomatal conductance, impaired transpiration and loss of vigor were observed	Kunjet et al. (2013)
48	26, 33 and 40 days of drought stress after two months of proper establishment	Rubber	Stuntedness: Nig 801 and RRIM628 cultivars increased in height only by 1.0% and 6.0%	Korieocha et al. (2015)
49	7 days withholding of water during the summer period	Rubber	Maximum quantum yield, leaf wax content and photosynthetic rate indices became negatively affected	Thomas et al. (2015)
50	Water deficit consisted of: FTSW > 0.75 (control); 0.1 < FTSW < 0.20 (severe), and FTSW > 0.75 after rewatering (recovery)	Rubber	Many biochemical and enzymatic complexes, including superoxide dismutase, peroxidase, and hydrogen peroxide content, were adversely affected	Cahyo et al. (2022)

Note: FC - field capacity; WC - water content; ETc - crop evapotranspiration; FSTW - fraction of transpirable soil water; PWP - permanent wilting point; SWC - soil water content; WUE - water use efficiency.



Source: Chalvantharan et al. (2023).

Figure 1. Water deficit based on 251 m^3 month^{-1} ha^{-1} oil palm requirement from average monthly rainfall in Malaysia in 2021.

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PRECISION AGRICULTURE IMPLEMENTATION TO IMPROVE SUSTAINABILITY AND PRODUCTIVITY OF OIL PALM PLANTATIONS IN INDONESIA: A REVIEW

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ABSTRACT

Oil palm plantations have emerged as a strategic catalyst for Indonesian economic growth, with planted areas expanding by 71% from 2010 to 2020. The expansion of cultivated regions contributes significantly to state revenue and attracts domestic and international criticism. Two major issues are associated with oil palm plantations in Indonesia: (1) The adverse consequences of land conversion and (2) the limited productivity of these plantations. The conversion of land, mainly deforestation and peatland transformation, is widely believed to be responsible for biodiversity depletion and a substantial surge in greenhouse gas emissions. However, many oil palm plantations in Indonesia suffer from poor productivity, making such sustainability ventures questionable. Over the years, the application of Precision Agriculture (PA) techniques has demonstrated their effectiveness in minimising agricultural inputs, optimising yields and mitigating environmental impacts. This article explores how PA practices can elevate oil palm plantation productivity and ensure sustainability through dependable field monitoring systems, adept database management, transparency and traceability, site-specific agriculture and a robust decision support system. The article will also delve into the challenges faced by smallholder farmers who require support to adopt PA due to high initial investment, socio-cultural resistance and limited capacity for embracing new technology.

Keywords: ISPO, oil palm plantations, precision agriculture, RSPO, sustainability.

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INTRODUCTION

Indonesia, one of the most significant crude palm oil (CPO) producers, produced 44.76 million tonnes in 2020. About 61.7% of the production was exported worldwide, generating state revenue of 18.69 billion dollars. This massive production came from plantation areas of 14.59 million hectares spread throughout the country (Badan Pusat Statistik [BPS] Indonesia, 2021), suggesting land productivity is around 3.1 t of CPO ha^{-1}. Malaysia had a land productivity of 3.2 t of CPO ha^{-1} in the same year after producing 19.14 million tonnes of CPO from planted areas of 5.87 million hectares (Parveez et al., 2021). The most updated data cannot be interpreted further since the COVID-19 pandemic of 2020-2022. The pandemic has disrupted the supply and demand of CPO and made the prices uncertain. The CPO price volatility continued the following year after the pandemic (Gandhy et al., 2022). However, in the previous study based on the data released by BPS Indonesia (2017) and Malaysian Palm Oil Board (MPOB, 2017), Indonesian palm oil (productivity per hactare) falls constantly below Malaysia's

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productivity level. Malaysia reached an average productivity of 4.28 t CPO ha^{-1}, while Indonesia reached 3.41 t CPO ha^{-1} from 2000 to 2015. Malaysia has already achieved more than 4 t ha^{-1}, while Indonesia only achieved the best productivity of 3.68 in 2015 (Hudori, 2017). Increasing productivity and sustainability is a challenge for the Indonesian oil palm industry. *Vice versa*, in the late 1990s, Malaysia committed to preserving a minimum of 50% forest cover, consequently leading the Malaysian oil palm industry to prioritise intensification within their territory. Indeed, Malaysian investors have continued to enlarge their oil palm cultivation areas within Indonesian land.

Besides the land productivity issue, the environmental issue of oil palm plantations in Indonesia has long been in the spotlight. The heightened environmental concerns have come from the European Union (EU), one of the biggest importers of CPO. Despite efforts made by palm oil-producing countries to ensure the sustainability of their palm oil, the EU insists that it is hard to establish sustainability in the oil palm industry. The EU has even committed to reducing palm oil use and will stop using it for biofuel in 2030. One of the criticisms of the palm oil industry is that oil palm plantation is an incredibly destructive and malicious form of agriculture due to massive expansion. The expansion, especially in forests and peatlands, causes more CO_2 emissions and biodiversity losses. Sustainable palm oil management can be achieved by intensifying, rather than expanding, land tenure security with vital forest conservation and better support for smallholders for a fair negotiation (Idriyadi, 2022). Based on data from Statistic Indonesia, the planted area has increased by 71% over 10 years, from 8.55 million hectares in 2010 to 14.59 million hectares in 2020 (BPS Indonesia, 2017, 2021). The transformation of forests and peatlands into oil palm plantations has been identified as a significant contributor to greenhouse gas (GHG) emissions, accounting for 15%–25% of Indonesia's total carbon emissions. The previous research concluded that the land cover changes to commercial oil palm plantations are responsible for the increase in total carbon emissions in Indonesia, especially on the islands of Sumatra and Kalimantan, leading to Indonesia being placed among the ten biggest carbon emitters (Shahputra & Zen, 2018).

Productivity and sustainability should not be seen as conflicting goals for oil palm plantations. In agroecological practices, productivity can be an indicator of agroecosystem sustainability. Reducing input consumption (e.g., water, pesticides, fertiliser) can ease the environmental burden. Hence, it enhances efficiency, boosts productivity, and reinforces sustainability (Wezel et al., 2014). The article discourse revolves around this concept. This article considers sustainability based on Indonesia Sustainable Palm Oil (ISPO) and Roundtable Sustainable Palm Oil (RSPO) in the discussion since these two certifications have been recognised and applied in Indonesia. The Indonesian Government has implemented ISPO to promote sustainability. However, a perspective emerges that the ISPO standard holds complexities, aiming to reassert national governance authority and accommodate the domestic palm oil sector, all the while assimilating norms advocated by international private governance. Conversely, some contend that ISPO serves as a counterbalance to RSPO, alleviating foreign pressures on the oil palm industry by positioning it as a domestic enterprise. Although there is a gap between ISPO and RSPO, both have the understanding to minimise the adverse effects with different emphases on their side (Choiruzzad et al., 2021).

The focus of the discussion is the possibility of adopting Precision Agriculture (PA) practices in Indonesian oil palm plantations to achieve better productivity and sustainability through increasing compliance with ISPO and RSPO principles. PA is a management strategy that gathers, processes and analyses temporal, spatial and individual plant and animal data and combines it with other information to support management decisions according to estimated variability for improved resource use efficiency, productivity, quality, profitability and sustainability of agricultural production (International Society of Precision Agriculture [ISPA], 2024). PA has characteristics as an integrated and production-oriented farming system aimed at improving long-term, site-specific and whole farming production, typically in productivity, efficiency, and profitability, while simultaneously minimising unwanted impacts on the environment and wildlife (Joint Research Centre, 2014). PA is practised in the oil palm industry, particularly in crop, water, and soil management (Tan et al., 2022). However, implementing all existing technologies must follow the conditions under which they are applied. So, this article also discusses the challenges that exist in Indonesia in implementing PA. The discussion in the article takes a technocentric and ecocentric perspective, especially regarding how to improve land productivity and sustainability because the oil palm industry has many intersecting and broadsides. The principles of RSPO and ISPO discussed in this article represent an ecocentric perspective which is the core of sustainability. Technocentric focuses more on technology and science as a way to repair the damage done to the environment rather than changing the ethical perspectives on environmental issues (Salman, 2019). This discussion aims to provide new insights into PA applications in oil palm plantations in Indonesia.

MATERIALS AND METHODS

The scope of this article varies based on the differences in the oil palm plantations in Indonesia, which comprise large companies, smallholders, or state plantations. This review article is focused to draw the formulation around two focal themes: Low productivity and low adherence to sustainability principles in managing oil palm plantations in Indonesia. The discussion in this article started with the understanding that increasing productivity results from maintaining sustainability. Sustainability standard in Indonesia refers to ISPO and RSPO, so the discussion starts with how ISPO and RSPO are positioned in Indonesia's oil palm industry. By understanding the gap between the implementation of ISPO and RSPO conducted in Indonesia and the standards that must be achieved, it is hoped that there will be an overview of areas that can be improved through applying PA to increase compliance with sustainability principles.

The authors reviewed several journal articles, proceedings and conferences to determine the implementation of ISPO and RSPO in Indonesia, the challenges to increasing adherence to sustainability principles and the possibility of implementing PA in Indonesian oil palm plantations. Utilisation of search engines like Scopus, PubMed, IEEE Explore and Google Scholar are used to explore previous publications related to the topic discussion. The primary statistical data used in this article are taken from annual reports issued by Statistics Indonesia. Work report from related Indonesian ministries and leading non-governmental organisations (NGOs) are also used as a reference for the latest data on the development of Indonesian oil palm plantations. The authors also reviewed some previous research about the application of PA and discussed the possibility of implementing PA by considering the existing conditions in Indonesia.

RESULTS AND DISCUSSION

Sustainability Through RSPO and ISPO

Defining the gap and similarity between RSPO and ISPO is a base to identify the essential steps to improve adherence to sustainability principles, and in the end, can improve productivity. Sustainability and productivity are linked in one direction. Sustainability practices such as enhancing agroecosystem balance through agroecological practices are proven to generate high palm oil yields (Bessou et al., 2017). On a broader understanding, sustainability increases fresh fruit bunches (FFB) production and gives social, economic, and environmental benefits (Nurliza et al., 2022). ISPO is a certification launched by the Indonesian Ministry of Agriculture that is mandatory for oil palm plantation companies, plantations with no mills, and mills without plantations (Hutabarat, 2018).

TABLE 1. DIFFERENCES BETWEEN ISPO AND RSPO

Item	ISPO	RSPO
Source of regulations	Regulation of the Minister of Agriculture of the Republic of Indonesia regulation No. 38 of 2020.	Implemented based on RSPO principles and criteria 2018.
Regulatory entanglement	Mandatory in 2025.	Voluntary.
Principles	• Compliance with laws and regulations. • Implementation of good agriculture practices (GAP). • Good management of the environment, natural resources and biodiversity. • Implementation of sustainable business transparency.	• Legality of respect for land rights and community welfare. • Optimise productivity, positive impacts and resilience. • Protect, conserve and enhance ecosystems and the environment. • Respect for human rights, including labour rights and working conditions.
The land used	Oil palm land development must be legally sound and its location refers to the spatial planning determined by the Indonesian government.	No new plantings in areas of high conservation value in forests with high carbon stock, in areas with steep slopes of more than 22°, in peat areas and in the regions that do not have free, prior and informed consent (FPIC) from indigenous peoples, local communities, or other users.
Focus and recognition	ISPO focuses more on business legality and compliance with Indonesian laws and regulations. Its certificates are valid nationally.	More concerned with realising a sustainable oil palm plantation business and its certification applies internationally.
First audit	ISPO certification bodies must conduct a first-phase audit within three months of signing the ISPO certification agreement.	The first audit is conducted after becoming an RSPO member and implementing the RSPO principles and criteria and has prepared for the first audit.
Conservation area	At least 30% of the area is designated as conservation area.	Requires 75% protection for areas classified as high conservation value (HCV) and a minimum of 35% designated as conservation areas.

In contrast, RSPO is a non-governmental certification programme founded by multi-stakeholder groups, including representatives from the private sector, NGOs and investors, that promote sustainable oil palm production (Apriani et al., 2020). The party supporting RSPO argues that ISPO is the way for the government to protect its industry by adopting environmental values as a trading tool. The other party that rejected RSPO insists that RSPO is the tool from the West to regulate domestic and strive to transcend nation sovereignty (Choiruzzad et al., 2021). This divergent viewpoint has frequently been attributed to the perception that sustainability principles in the oil palm industry can be disputed and disobeyed by the stakeholders, even though both standards fundamentally aim to uphold environmental and social responsibility.

The differences between ISPO and RSPO are primarily in the criteria for high conservation values (HCV), free prior and informed consent (FPIC), peatland and new planting procedures (NPP). RSPO is more stringent in tying commitment, and demands more transparency and more detailed mandatory steps, especially concerning environmental and social impact. For example, in using peat as a planted area, RSPO encourages making a voluntary commitment to avoid peatlands and demands the implementation of best management practices (BMPs) for peatland management. However, the previous ISPO regulations still permit peatland cultivation under specific conditions, demanding the prevention of negative repercussions and preserving water levels within defined parameters. The specified conditions that would enable peat to be planted are peat areas constituting less than 70% of the concession area, with depths of less than 3 m (EFECA, 2018). RSPO has more comprehensive details and requirements than ISPO, particularly on indicators of labelling, trust, fair treatment for smallholders, smallholder credit, farmer market access and conflict resolution (Wulandari & Nasution, 2021). There is even an assumption that RSPO emphasises sustainable oil palm plantation practices to reduce deforestation and protect biodiversity. In contrast, ISPO is the state initiative to raise global market competitiveness by paying attention to environmental challenges and commitment to GHG emissions (Sylvia et al., 2022).

RSPO and ISPO impel the stakeholders to maintain sustainability. The principles of ISPO overlap with those of RSPO, encompassing aspects like legal compliance, environmental stewardship, social accountability and sound business ethics (Hutabarat, 2018). Both certifications aim to encourage palm oil production by considering economic, environmental and social pillars. Also, both demand compliance with laws and regulations, good management practices for sustainable plantations, environmental responsibility, sensitivity to the needs of workers and communities and continuous improvement (Widiati et al., 2020). The journey toward ISPO and RSPO implementation is also always dynamic, primarily to accommodate smallholder farmers. The RSPO sets a standard acceptable to oil palm smallholders in Indonesia. The RSPO implementation flow should be as simple as possible to relieve barriers to smallholders. An example is the relaxation to address the GHG effects, social impact assessment, and the burden caused by the administrative system to assist smallholders in complying with the principles and criteria of RSPO (Sylvia et al., 2022). Simultaneously, the revised ISPO has integrated the principle of transparency as an evaluative factor, though concerns persist regarding its effective execution. ISPO also implements less stringent conditions for smallholders by excluding principle requirements of responsibilities towards workers' rights, social responsibilities, and community economic empowerment (Barahamin et al., 2022).

Indonesia is committed to strengthening the implementation of ISPO since the compliance rate is still low. According to the Head of Plantation Service for Riau Province, only 30.00% of the oil palm companies have fulfilled ISPO certification in Riau until 2022 (Wulandari & Nasution, 2021). Moreover, only 37.00% of the oil palm industries in the country received ISPO certification in 2019. The most frequent obstacles to increasing ISPO compliances are legality issues of land use rights and lack of global market stimulus. The legality issues are prevalent among smallholder oil palm holdings. Many of these holdings are being established in areas not included in the spatial plan (Sylvia et al., 2022). The obligation to get ISPO certification was strengthened by Indonesian Presidential Regulation No. 44 of 2020, which aimed to improve ISPO compliance (JDIH BPK, 2020). The obligation encourages all oil palm industry players to obtain ISPO certification no later than five years after the issuance of this Presidential Regulation (Badan Standardisasi Nasional [BSN] Indonesia, 2020). Another challenge after issuing Presidential Regulation No. 44 of 2020 is to ensure transparency. It will force the palm oil mills to refuse FFB from smallholders or plasma plantations that are not ISPO-certified because only 0.21% of plasma plantations were certified in 2019. The plasma plantations face a great challenge in fulfilling the requirement of land legality and origin of seeds certification as a prerequisite of transparency. The application of certified seeds by smallholder farmers is still low. The use of forests illegally due to weak oversight makes it difficult to clarify land legality. This condition will be more challenging since many smallholders or plasma plantations need to understand the principles of environmental management that meet ISPO standards (Purwanto, 2020). The plantations owned by big corporations also have not yet fully implemented ISPO principles, especially the third principle. Based on assessing five oil palm plantation companies in East Kalimantan Province, implementing the environmental management and monitoring the 3rd principle could only reach the average achievement level of 54.69% (Anwar et al., 2016).

In order to fulfil the Paris Agreement, the implementation of ISPO and RSPO will be necessary in Indonesia. One of the objectives of this agreement is to reduce GHG emissions with a reduction target of 29% and up to 41% compared to business as usual in 2030 (Ministry of Environment and Forestry Republic of Indonesia, 2022). The commitment to reduce GHG obliged the Indonesian Government to evaluate the policies to prioritise conservation rather than expansion, significantly reducing peatland cultivation (Maskun et al., 2021). Indonesia's commitment can be seen in the latest ISPO changes published in 2020. There is a more robust requirements indicator pointing to a ban on peatland use. The new ISPO does not permit new planting on peatland, regardless of depth, after 15 November 2018, in existing plantation areas and new development areas (Kehati et al., 2021). The strong commitment from the central Government should drive regional regulators and plantation management to improve agriculture practices. Some practices in the field can support the GHG reduction commitment like reducing land clearing in areas with high carbon content, conducting soil and water conservation, utilising no over-fertilising, promoting the use of empty fruit bunches (EFB) for fertilisation, controlling the use of pesticides and practising good water level management for peat areas (Sylvia et al., 2022).

Increasing Productivity by Maintaining Sustainability

The critical step to maintaining sustainability and reducing GHG emissions is to slow down the expansion of land use conversion, particularly on the conversion of forests and peats and Indonesia has taken policies such as a permanent extension of the forest and peatland moratoriums based on Presidential Instruction No. 5 of 2019 (United Nations Framework Convention on Climate Change [UNFCC] , 2021). This new policy direction demanded that the plantation be more able to intensify and improve land productivity. Smallholdings or plasma plantations with financial, knowledge and supply chain barriers face low productivity problems. Insufficient availability of certified seeds and adequate fertiliser hinders smallholders aiming to enhance productivity (Herdiansyah et al., 2020). Efforts to maintain the balance of nature by paying attention to sustainability principles can have a strong impact on the productivity of oil palm plantations in the long term as shown in *Figure 1*.

Smallholdings face greater vulnerability to low productivity issues. Expanding the cultivated area yields more detrimental outcomes than benefits. Larger planted areas necessitate increased

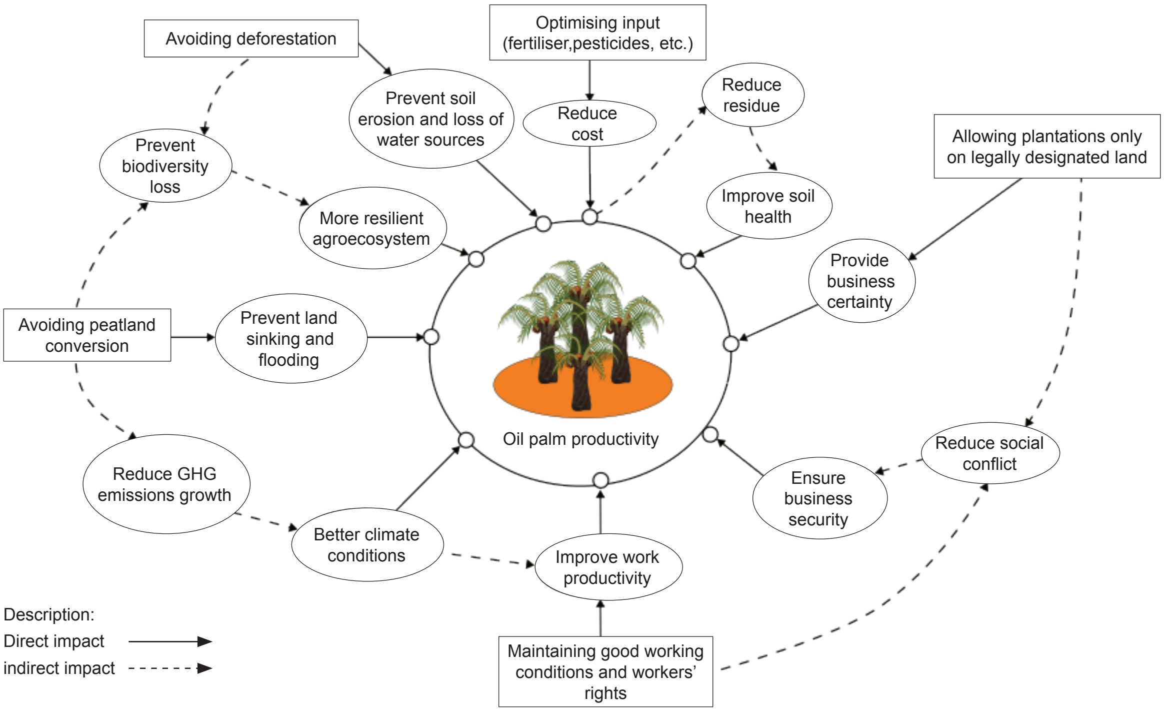

Figure 1. Illustration of operations that consider sustainability can affect palm oil productivity.

quantities of oil palm trees, ultimately demanding higher fertiliser and labour inputs, despite yielding below-expectation due to these adverse effects. Since deforestation triggers ecosystem imbalance, it needs more resources to handle the impacts such as the spread of plant pests and diseases, land degradation and loss of water resources. Based on the research conducted by Sari et al. (2021), ecosystem imbalance causes the effort to control pests to be higher than fertilisation and reduces the yield of the oil palm tree. Hence, all the significant factors in palm oil production, such as land maintenance, fertiliser and pesticides, must be maintained more efficiently (Yanita & Suandi, 2021).

Trade-offs and mutualism always exist among ecosystem services in an agroecosystem. Good agriculture management practices can reduce losses among ecosystem services and maximise the provisioning among ecosystem services (Power, 2010). The conversion of land use and land cover is an appropriate example of the dynamics of trade-offs and mutualism among ecosystem services. The relationship of the ecosystems is complex, but at least, based on ecological footprint examination, two opposing processes can be determined. The first process is the biological capacity of the agroecosystem to produce needs and absorb the by-products, and the second is the demand for agroecosystem services by a group population. Deeper comprehension can be explored through an integrated model considering economic, geographic and ecological issues to capture multiple drivers of changing land use and cover (United Nations Department of Economic and Social Affairs [UN-DESA], 2012). High demand for palm oil products was reported as the main driver in Indonesia and Malaysia's conversion of land use and land cover. To attain sustainability, opting for best management practices offers a more effective route to increasing oil palm yield than converting new land for cultivation (Wicke et al., 2011). The best management practice in oil palm plantations covers many areas, including agronomy, soil-water management, financial management, harvesting and transportation, people management, and innovation (Pardamean, 2017). Best management practices could reduce pressure to convert up to 1.6 million hectares of land to new plantations by 2050. Crop recovery, canopy management and soil, moisture and nutrient management are the backbone of best practice management implementation (Paoli et al., 2014).

The productivity of FFB and CPO is also contingent upon land suitability and the implementation of technical cultivation practices. Land suitability is classified based on soil types, rainfall and soil content. The technical cultivation standard adheres to the principles of good agricultural practices. Enforcing these technical cultivation practices entails employing well-suited land, quality seeding, appropriate fertilisation, meticulous maintenance and effective harvesting techniques (Anwar et al., 2014). Furthermore, pests and disease control must be considered. The technical cultivation standard also encompasses specific targets to accomplish, including the quantity of prenursery seeds that experience delayed transfer to the main nursery, the count of unripe FFB harvested and the frequency of harvesting rounds (Suryani et al., 2019). The study on production enhancement carried out by Hidayati et al. (2014) has definitively established a hierarchy of priority factors for boosting productivity, listed from the highest to the lowest level: Plant health, reseeding, fertiliser type, fertiliser dosage, suitability of tools and working materials, soil type, frequency of fertiliser application, work procedures, weed control, pest management, fertiliser technology and seed fertilisation. The oil palm tree population and uniformity are significant factors in improving productivity. Good plant health, especially in nursery and planting, will minimise reseeding and ultimately, suppress heterogeneity. The reseeding is replanting activities due to plant seed failure to grow and is done to ensure a fixed number of trees and low variation of different planting years per planting area. The uniformity of tree crops creates a better set up to apply the same treatment in nutrient application fertilisation, weed, and pest control. The nutrient application is also necessary to improve palm oil production per hectare. Fertiliser type, amount and application method have significant effects on productivity.

Precision Farming for Maintaining Sustainability and Improving Productivity

The Indonesian Government has shown commitment to sustainability and has proven it by implementing new ISPO to increase palm oil sustainability and productivity. The Government understands that low crop productivity rates, high production cost, low practical awareness of sustainability, land legality and suitability are challenges to the improvement of oil palm plantations, be more competitive and hence sustainable for the industry in Indonesia (Ministry of Industry of the Republic of Indonesia, 2021). However, the implementation of the Government's commitment is still doubtful. Furthermore, the problems that become critical and significant challenges are weak supervision and poor law enforcement against illegal practices, so many oil palm plantations are being established inside unspecified areas. This situation makes it challenging to enforce land legality as required by ISPO and RSPO (Santoso & Saputra, 2020). Based on the shortcomings described above, the barriers faced in upholding the sustainability principles in the oil palm industry can be determined by several factors. These factors are weak supervision, both for illegal land use changes or wrong plantation operations that damage the environment; low continuous improvement system; lack of transparency and traceability implementation to ensure sustainability; the difficulty of applying best management practices and good agricultural practices; and inaccurate policy making directions, both from regional government or strategy policy from the plantation management. These barriers need to be overcome, and PA is an option to overcome them.

PA can be defined as improving crop yields and assisting management decisions using high-technology sensors and analytical tools (Singh et al., 2020). PA can assist good agricultural practices or best management practices in the oil palm cultivation business (Tan et al., 2022). PA tools and techniques include but are not limited to, variable rate technology, soil mapping, remote sensing technology, unmanned aerial vehicles (UAV), tractor guidance systems and monitoring and mapping yield (Mcconnell, 2019). Based on the five barrier sectors mentioned above that challenge the enhancement of sustainability and productivity of oil palm plantations in Indonesia, five parameters of PA applications can be proposed. The proposed five parameters of PA applications appear in *Table 2*, consisting of reliable field monitoring systems, database management systems, transparency and traceability systems, site-specific agriculture and a decision support system.

Reliable Field Monitoring System

The authority in Indonesia has implemented coordination, control and surveillance to prevent deforestation. However, it should be strengthened because oil palm canopy cover remains in the middle of a protected forest (Nughara, 2019). Although the Central Government has already imposed a moratorium on new land clearing from virgin forest and peatland to oil palm plantations, deforestation and peatland fires are caused by many factors, including poor supervision and control (Drost et al., 2021). Immediate and accurate monitoring in the field is vital to control and supervise land use. Reliable methods are necessary to recognise, detect, monitor, analyse and predict. The adoption of technologies such as GPS, GIS, UAVs and satellites for remote sensing has already been set up to monitor land cover changes. Even aerial photographs have been used to map land cover and land use since the 1940s (Wavan et al., 2006). An example is satellite imagery used for the estimation of leaf nitrogen content based on Sentinel 1-A imagery. The estimation accuracy is quite high using a random forest regression model. The estimation results can be used for planning, monitoring and providing fertiliser recommendations (Munir et al., 2023). PA implementation can help monitor soil conditions, land suitability and feedback from communities living around the plantation in real-time as shown in *Figure 2*.

A reliable field monitoring system is not only used to monitor and protect from illegal land use changes but can also help monitor the

TABLE 2. SUMMARY OF THE PRACTICAL APPLICATION OF PA IN OIL PALM PLANTATION

Parameters of PA applications	Practical application in oil palm plantation
Reliable field monitoring system	Innovative development in oil palm seedling nursery (Lahuri et al., 2021); Drone technology in oil palm plantations (Khuzaimah et al., 2022); Monitoring oil palm plantation blocks (Yuniasih et al., 2019); GIS data collection for oil palm with smartphone-based (Abdullah & Muhadi, 2015); Prediction of palm oil yields using machine learning (Khan et al., 2022); Modelling oil palm phenology based on remote sensing (Hernawati et al., 2022); Oil palm detection using satellite imagery (Nurmasari & Wijayanto, 2021); Multisensor approach to monitoring oil palm plantation (Pohl et al., 2015).
Database management system	The framework of oil palm PA (Fairhurst et al., 2003); Agronomic management information system (Tropical Crop Consultants [TCCL], 2010); Oil palm gene database (Sanusi et al., 2018); Information system for oil palm breeding (Fauzi et al., 2020).
Transparency and traceability system	Standard methods to trace agriculture products based on data centres (Cheng et al., 2013); Tracing and tracking agricultural batch products (Ruiz-Garcia et al., 2010); GPS-based trace and track and sustainable global supply chains (Kandel et al., 2011); RFID application strategy in agri-food supply chains (Zhang & Li, 2012); Enabling technology for food supply chain transparency (Astill et al., 2019); Chemical and biological sensors for food monitoring and intelligent packaging (Mustafa & Andreescu, 2018); Food traceability and transparency using blockchain (Yiannas, 2018).
Site-specific agriculture	Variable rate sprayer for oil palm plantation (Ishak et al., 2011); Diagnostic tools for optimising fertiliser (Dubos et al., 2019); Oil palm water balance tool (Safitri et al., 2017); Variable rate application in palm oil (Ishola et al., 2012).
Decision support system	The decision support system in agriculture (Zhai et al., 2020); Data life cycle management supported by information and communication technology for decision-making (Demestichas & Daskalakis, 2020); Expert system in oil palm PA (Tan et al., 2022).

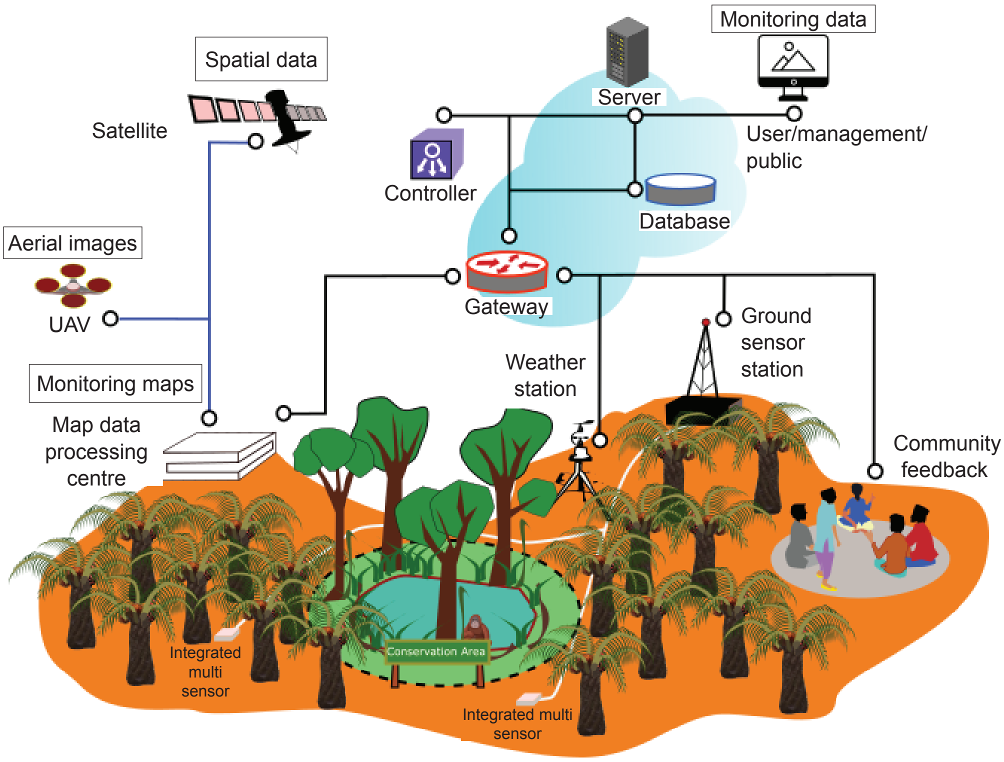

Figure 2. An alternative concept of PA for monitoring oil palm plantations.

comprehensive condition of the oil palm plantation more effectively. For example, the use of UAVs or drones for monitoring, crop yield assessment, spraying, health assessment, weed mapping, irrigation management and disease detection since UAVs can produce a high-resolution aerial photograph with more economical operational cost, rapid and more accurate than the manual method (Norasma et al., 2019). These technologies can also be used inexpensively for surveillance in marking planted areas' boundaries, thereby avoiding land legality problems, especially for smallholders. The capability of drones or UAVs to do 3D mapping makes it possible to do geological surveying, topographic mapping, volumetric calculation and generate site images in 3D format. The UAV application is also decisive for observation, tree counting and layout preparation for replanting (Khuzaimah et al., 2022).

GIS can handle spatial data containing geo-references and time-references. GIS has lower operating costs than UAV since the map data can be downloaded from satellite operators; some are paid, but some are free. The Sentinel-2 and Landsat-8 optical satellite imagery can be used in Indonesia (Nurmasari & Wijayanto, 2021). Some developers have just developed a smartphone-based mobile app to monitor *Ganoderma* disease based on GIS data (Abdullah & Muhadi, 2015). GIS and GPS can be used for data modelling and predicting or building an automated system by incorporating multiple sensors. The data from weather stations and soil data from sensors with time references and geo-references can be used for predicting oil palm yields. The data are compiled and modelled using machine learning to predict the future trend (Khan et al., 2022). The most interesting is that the data interpretation result has high accuracy. Multisensory data from optical and radar remote sensing satellites can improve tree classification accuracy by up to 96%. It means that the data provide a more complete perception of the object and more complete information (Pohl et al., 2015). The most recent example was reported by Lahuri et al. (2021), who described the management of multiple tools and sensors to develop an intelligent farming system for oil palm seedlings in a wide area. The micro-sprinkler powered by the solar system was controlled using the Internet of Things (IoT) application and operated automatically based on soil humidity, temperature and pH. There is a GIS system that ensures the location of the tree. Every tree gets the appropriate water, fertiliser, and herbicide in accordance with its needs.

Database Management System

Many oil palm industry stakeholders in Indonesia believe that digital transformation can escalate production performance and reduce costs.

The digital transformation will streamline field data collection and statistical tasks, enabling real-time execution (Haryanti et al., 2021). The database has an essential role in making a decision. The user records all required information in regular structured information, which can be used as a basis for decision-making (Çelikyürek et al., 2019). Growing awareness of managing big data from the plantation for continuous improvement is happening in Indonesia. Several domestic companies have developed technology to collect and manage data in oil palm plantations by using information system technology. A reliable database management system (DBMS) is needed because the palm oil industry has an extended production chain (Yuwono, 2020). Reliable DBMS should have many methods of keeping records, keeping data for a long time, reusing data when needed, accessing data quickly, filtering records according to specific features and sharing data among users efficiently (Costa et al., 2022).

A DBMS is a tool to store and analyse intricate datasets. The field monitoring system generates numerous datasets encompassing seedlings, planting, harvesting and replanting stages. DBMS in agriculture can store information such as soil maps, farm maps, landscape maps, annual field maps, crop records, environmental conditions, weather conditions, pest records, fertiliser applications, chemical records and flood maps (Sarmah et al., 2018). The datasets collected in oil palm plantations should have geo-references and time references. The data usually consist of soil and land mapping, FFB production, fertilisation dose, environmental sampling, pests and diseases, palm census and climate (Fairhurst et al., 2003). Accurate and reliable DBMS could record and manage datasets for many years. The output of DBMS should be reliable and ready to be used by management for evaluating, planning and forecasting. The meaning of reliability includes the ability to recover in the event of system failure, having suitable security mechanisms and can be integrated with the existing systems (Gunjal & Koganurmath, 2003). An example of DBMS implementation in oil palm plantations is the agronomic management information system (AIMS). AIMS is integrated software that stores and processes data in oil palm plantations using SQL and GIS-compatible software. This system has been used widely by oil palm companies and has been proven to assist the decision-making process by top management (TCCL, 2010).

Transparency and Traceability System

Trade barriers have become an external challenge that haunts Indonesian palm oil exports. The most apparent issue is transparency and traceability since the importers, mainly from the EU, demand apparent food safety and sustainability assurance from the CPO exporters (Gunawan et al., 2021). Moreover, transparency and traceability have been listed in the RSPO as tools to ensure reliability and sustainability by knowing exactly how palm oil flows through the supply chain, including which mills and plantations process the palm fruit and kernels (Voora & Andrade, 2016). The new ISPO also implies transparency and traceability in sustainability implementation practices, though not as clear as the RSPO. Although there is a risk that this change will make it even more difficult for smallholders to implement the new ISPO, this policy is still a mandatory regulation (Purwanto, 2020). Following the fact that the commitment to transparency and traceability principle in Indonesia is still low since the percentage of plantations that have successfully implemented ISPO is still under 50% (Hasnah et al., 2021).

Transparency is how all parties can monitor and perceive how the industry operates by applying the principle of sustainability. Transparency and traceability are required in modern agriculture to ensure food safety and improve supply chain transparency (Cheng et al., 2013). Traceability is the ability to trace an entity's history, application, or location through recorded identifications. It means the customer can trace the origin of the palm oil they consume. The agricultural process data service with transparency and traceability capability usually consists of data acquisition, data transfer, data storing and analysis, data transfer interface and data user utilisation (Ruiz-Garcia et al., 2010).

Data acquisition with manual tracking still exists in the current food supply chain. A prominent example is oil palm surveying to monitor the soil and palm trees' health, ecosystem balance, existing pests and water source conditions, primarily conducted using manual field inspection methods. However, automated data acquisition will be preferred in the future using the newest technologies, including various sensors, vocalisation analysis systems and imaging technologies. The most recent example is the utilisation of GPS and RFID in the supply chain, which have been widely implemented to retrieve the time and location of the product. This technology gives assurance for real-time visibility, from the raw material to the end product, which may reduce food contamination and spoilage (Kandel et al., 2011). Besides that, IoT's utilisation for data transfer to data storage and big data analytics that can provide deeper comprehensive decision options with minimal errors has already been implemented in agricultural supply chain management (Astill et al., 2019). These examples show that the technology that supports the transparency and traceability requirements has been proven and is ready to be implemented in oil palm plantations.

Site-specific Agriculture

The use of production inputs such as fertiliser doses, pesticide doses, organic matter and the number of workers can affect the yield of FFB. There is often inefficiency in using these inputs, especially among smallholder farmers in Indonesia (Maulida et al., 2022). Site-specific management in PA aims to optimise water, fertiliser, herbicide and other inputs into the field. Furthermore, excessive inputs in agriculture can pollute the environment. Three mandatory things to do in site-specific management are knowing the position, gathering live information at the location and conducting the variable rate application. GPS is a standard system for knowing the location. Gathering information like yield monitoring, soil electrical conductivity (EC) condition, remote imagery, soil compaction sensing, or soil pH condition is conducted using sensors at sequential times and with geo-references. Variable-rate controllers can be applied for any required inputs. The inputs are spread with varying doses as needed at each point in a specific location that has been gathered previously (Franzen, 2018). There are some methods for collecting and interpreting data in the PA implementation. Dubos et al. (2019) used long-term fertilisation data to diagnose the sustainability of nitrogen (N) and potassium (K) in the soil since both are important for oil palm trees. This data was linked to past yield data and mature period data to detect the depletion of soil nutrient reserves. This method can predict soil nutrient conditions before measuring the actual soil nutrient condition in the field to avoid the risk of excessive fertilisation without hindering FFB production.

Another example of site-specific agriculture is using a water balance model to predict water content distribution in the root zone. The water balance tool is developed by inputting the data on climate, soil properties, crop stage, root density and root zone layer. Then, this data is modelled to conceive water behaviour in the root zone. This method can be a tool for knowing the actual water needs in the oil palm root zone (Safitri et al., 2017). An example of variable rate application implementation in oil palm plantations was shown by Ishak et al. (2011) , who developed a variable rate sprayer for herbicide application based on colour detection from camera vision. The camera vision is used to distinguish between weeds and palm trees and calculate the percentage of weeds in a particular location. Thus, the computer controller decided the amount of herbicide application. This application was expected to make a site-specific application for reducing the use of herbicides. This application can also be implemented in other liquid inputs such as fertiliser or insecticides.

Decision Support System

PA implementation needs massive and integrated infrastructure to take data from the field, send and store it in the database, be accessed and tracked by concerned parties and apply site-specific application inputs for optimisation. When all these different parameters of PA are already running, this is then the appropriate time to implement a decision support system. An agricultural decision support system (DSS) is a human-computer system that utilises data from various sources to provide farmers with many optional recommendations for supporting their decision-making under different circumstances. Although the system gives recommendations, the farmer still chooses the decision. The agricultural DSS has become more critical since changes in the climate, soil nutrient balance, water balance, pest attack and other conditions have affected the sustainability and productivity of oil palm plantations (Ren et al., 2022).

The general framework of agricultural DSS can involve many aspects of the dataset and can utilise different types of machine learning to enhance capabilities, as shown in *Figure 3*. The DSS can cover field data models, such as crop and irrigation models, and can be influenced by economic and human intervention factors (Zhai et al., 2020). The recommendation options can be generated based on the dataset stored in a database that fits the data lifecycle model. The data lifecycle model may consist of data collection and the IoT, data analysis and artificial intelligence, and data storage and distribution. The quality of the data life cycle model will influence the quality of the DSS output (Demestichas & Daskalakis, 2020). Agricultural DSS can also give the farmer recommendation options based on big data on planting seeds, managing transportation in the field, managing irrigation, applying inputs (fertiliser, herbicides, insecticides), or predicting labour needs (Tan et al., 2022).

The Challenges in Indonesia

PA practices have been implemented in Indonesia, especially in plantations managed by big corporations. However, smallholders still have limitations in implementing PA in their plantations (Ginting & Wiratmoko, 2021). The number of plantations managed by smallholders and plasma is large. Based on data from BPS Indonesia, (2021), almost 42.0% of palm oil plantations in Indonesia are smallholder holdings with limited ability to implement ISPO. Though the new ISPO is mandatory for every plantation in Indonesia, only 33.0% of the total oil palm area obtains ISPO certificates, and those are mostly from plantations managed by big corporations. The oil palm

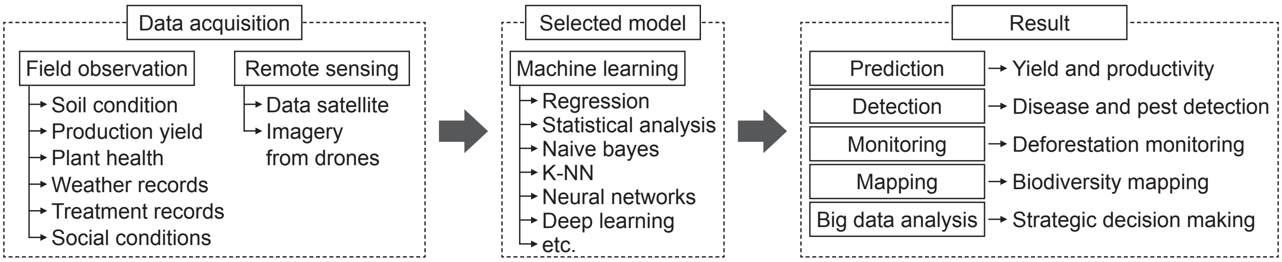

Figure 3. The instance of data flow for palm oil decision support system.

plantations, primarily owned by smallholders and plasma farmers, still need assistance implementing ISPO (Hasnah et al., 2021). The smallholder's readiness to conduct environmental management, monitoring, and continuous improvement as part of ISPO implementation is still low. Only 60.9% of ISPO indicators had been applied by smallholders using gap analysis. The lowest percentage of implementation is environmental management and monitoring principles, with only 43.0% that can be fulfilled (Soebirin et al., 2021).

It is undeniable that PA implementation requires expensive investment costs. Some tools of PA and cost estimation can be seen in *Table 3*. The utilisation of UAV technologies has various technical issues that must be considered: The need for expert pilots and data processing teams, the high-speed ultra-low scenario, data downloading tasks in a real-time application, size and payload to prevent obstructions and software for automatic analysis. In addition, it requires an expensive initial investment (Khuzaimah et al., 2022). Similar difficulties will be encountered when building field monitoring systems integrated with the database system to ensure transparency and traceability. A reliable transparency and traceability system needs a suitable arrangement of the successive links between batches and logistic units throughout the supply chain. Data standardisation among stakeholders along the supply chain is required and the consequence is that they should have infrastructure and process standards that make it integrated (Ruiz-Garcia et al., 2010).

The economic sustainability of the oil palm industry can be affected due to the activation of a transparency and traceability system associated with new costs and risks. Oil palm plantations have a long supply chain involving various sectors hence making it difficult to standardise the requirements among the stakeholders except in large companies with integrated infrastructure from upstream to downstream. Moreover, there are some barriers to ensuring effortless flow of data streams and smoothly from the downstream to upstream and, ultimately, to the customer due to the lack of IoT connectivity, the complexity of the supply chain, and economic sustainability (Astill et al., 2019). It relates to Indonesia's conditions since more than 40% of oil palm plantation areas in Indonesia are blank spots or have no internet connection, particularly in the hinterland (Redaksi Sawit Indonesia, 2022).

Human resources readiness is another challenge because plantation workers' rejection, social and cultural rejection, and low advanced technology adaptability are still common in oil palm plantations. The education level of plantation workers is varied, forcing plantation managers to expend more effort to implement PA. Even more, there is an assumption that PA can reduce employment, causing workers' rejection (Ginting & Wiratmoko, 2021). There is a need for more research to produce new technologies that fit with the socio-cultural character and nature of oil palm smallholders in Indonesia. High expectancy comes from the young farmers being more adaptable to new technology since they respond better to PA implementation. However, the number of young generations in Indonesia interested in agriculture is becoming less and less (Sondakh et al., 2021).

Palm oil data often differ due to methodological differences and data processing criteria, and the Ministry of Agriculture has been synchronising data from the palm oil stakeholders (Republika, 2019). Asynchronous data among stakeholders can also be an obstacle in decision-making and the implementation of PA. Considering the high expectations of PA applications to support sustainability and productivity, the Government should be bridging and setting up the infrastructure and standards, especially for the smallholders, so that PA can be applied nationally. If the infrastructure for implementing PA cannot be built simultaneously, it should start by developing a pilot project in a small area first. It would be better if that pilot project is integrated with the development strategy of the Indonesia palm oil industrial cluster (POIC).

The idea of POIC is to cluster the palm oil industry to become more competitive and organised. Clustering is applied to the palm oil industry in Indonesia to improve competitiveness and

TABLE 3. SOME TOOLS OF PA IMPLEMENTATION AND COST ESTIMATION

Tools	Function	Cost
Drone	It can be used for monitoring vegetation levels, monitoring vegetation stage, oil palm tree detection, estimating chlorophyll density, crop health monitoring, drone spraying, crop growth monitoring and harvest prediction (Khuzaimah et al., 2022).	Based on the latest research, the average price of drones worldwide in 2023 will be around USD530 (Laricchia, 2023). However, the prices can vary depending on the type of drone. A high-end drone, like the Dji Matrice 350 RTK, can cost more than USD17,000.
Satellite data	Retrieve data from satellites like Landsat, Modis, UK-DMC 2 Imagery Data, Worldview -2 multispectral data, LiDar, Palsar-1, Palsar-2, etc. The satellite data can be used for remote sensing to observe, analyse and assess the landscape conditions of oil palm plantations (Tan et al., 2022).	The cost of satellite data can vary widely depending on several factors, such as resolution, area of interest, sensor type, date of acquisition, licensing and usage. Landsat data with multispectral imagery can be obtained for free with limited resolution. However, more spectral and higher-resolution imagery can be obtained at a cost, like Worldview-3 with 8-band multispectral imagery for USD48 per km^2 (Land Info, 2024).
GPS (Global Positioning System) and GNSS (Global Navigation Satellite System)	GPS or GNSS are key tools to develop specific location data for PA. Data received from GPS or GNSS becomes mandatory for implementing GIS or carrying out site-specific treatment.	The price of GPS can vary depending on its accuracy. GPS with an accuracy of 50 m can range from around USD100–USD500. But for devices that have an accuracy of one to two metres, they can reach USD25,000 (FieldBee, 2022)
Sensors	Sensors are usually coupled with an IoT system for real-time field monitoring. The types of sensors used can vary. They can be soil sensors, environmental sensors, or multispectral camera sensors that take pictures of oil palms to monitor environmental conditions and oil palm plantation crops (Tan et al., 2022).	The cost of sensor technology may vary, but the trend is declining. The accelerometer sensor for plant monitoring can cost USD87–USD430. Infrared sensors can cost USD24–USD188 (Lahuri et al., 2021). Soil sensors may vary from 10 USD for basic models and can be over thousands of dollars for high-end sensors with data logging systems (NiuBol, 2023).
RFID (Radio Frequency Identification)	RFID in oil palm plantations is very useful to improve traceability, efficiency and data management. It can be used for plant tracking and inventory management, harvesting and fruit tracking, supply chain management, worker identification and safety.	The cost of building a system with RFID may vary depending on the scale, type of RFID and method of integration in the information system. The cost of passive RFID ranges from USD0.1–USD1.5 per tag, while the cost of active RFID ranges from over USD10 per tag. However, there are other costs besides the price of tags, such as the reader system, which for passive RFID can reach USD3,000, installation costs, software, and licences (Halstead, 2023).
Decision support system	A system that collates various data from sensors, remote sensing, field observations, and other data into meaningful information. DSS is able to optimise solutions, identify situations, analyse alternative strategies and find data correlations (Rinaldi & He, 2014)	The main costs incurred in applying a decision support system are infrastructure and labour costs. The amount of cost depends on the framework used, the scale of the project, the level of complexity, the cost of data acquisition and expert wages in a particular country.

increase the value of palm oil production (Yahya & Gunawan, 2020). Malaysia and Indonesia have POIC, which is now continually developed. POIC in Indonesia has a larger plantation area than in Malaysia, indicating a larger potential production capacity. However, the infrastructure and logistic network of POIC in Indonesia is more underdeveloped than in Malaysia (Raharja et al., 2021). As a priority development, the POIC plan in Indonesia can align with the functions and objectives of developing PA by developing supporting infrastructure, creating global connectivity, increasing transparency, improving infrastructure and increasing product innovation (Raharja et al., 2021). Implementing PA in the new POIC will be more easily integrated with all lines of business processes than building connectivity on long-established plantations.

CONCLUSION

PA implementation has the potential to be a breakthrough for maintaining the sustainability and productivity of oil palm plantations in Indonesia. The PA performance can encourage the readiness of the oil palm industry to implement ISPO and RSPO because of its ability to carry out monitoring and surveillance in the field, connect with many users in real-time and retrieve and process data for evaluation and decision-making. Five parameters of PA implementation are proposed in the oil palm plantations: A reliable field monitoring system, a database management system, transparency and traceability, site-specific agriculture, and a decision support system. Smallholders and plasma plantations have the biggest challenge in leveraging precision farming practices. The barriers to implementing PA in Indonesia include more investment costs, an inadequate number of experts, lack of infrastructure, socio-cultural rejection, refusal by farmers and workers and the need for business process synchronisation among the stakeholders for running an integrated PA. If it cannot be implemented simultaneously, the best suggestion is to start with a small pilot project that can be integrated into the POIC strategy, which will develop new plantations with an industrial cluster system.

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GENETIC DIVERSITY OF MPOB-ZAIRE OIL PALM (*Elaeis guineensis* Jacq.) GERMPLASM POPULATION BY MULTIVARIATE ANALYSIS

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ABSTRACT

Information on the genetic diversity of the oil palm germplasms is important for the establishment of an ex-situ *core collection. In this study, the assessment of genetic diversity in the MPOB-Zaire oil palm germplasm collection may prove to be valuable in developing appropriate sampling strategies for conservation. Data for 18 phenotypic variables for 55 populations were analysed for principal component analysis (PCA) and cluster analysis (CA). Five paramount principal components with eigenvalues >1.0 accounted for 82.7% of the total variability. PC1 revealed the highest contribution and predominantly attributed to average bunch weight, leaf area index, and bunch weight variables. PC2 was highly associated with oil to bunch, mesocarp to fruit and oil to dry mesocarp. The evaluated populations were grouped into two major clusters, each comprising a few sub-clusters based on phenotypic variables. The study revealed that the MPOB-Zaire germplasm has potential, where selected populations e.g. ZER21, which show dwarf (28.12 cm yr^{-1}) and high kernel (8.03%) characteristics can be used in introgression programmes to further improve advanced breeding lines and develop new breeding material. These results may assist in the selection strategies of populations for regeneration purposes and secure a greater range of diversity compared to sampling at random. Molecular-based diversity patterns could be integrated in future to effectively conserve and exploit.*

Keywords: cluster analysis, core collection, oil palm, principal component.

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INTRODUCTION

The oil palm (*Elaeis guineensis* Jacq.) was introduced to Southeast Asia as an ornamental plant by the British in the early 1870s (Kushairi & Parveez, 2017). In 1917, oil palm was planted commercially and is now the foundation of Malaysia's economy (Kushairi & Parveez, 2017). Malaysia was the world's largest producer of palm oil before being overtaken by Indonesia in 2006, both have tropical climates that are conducive to oil palm plantation. The oil palm plantation grew rapidly from 54,638 ha in 1960 to 5.74 million hectares in 2021 (Parveez et al., 2022). Palm oil is known as a valuable commodity, used in the production of diverse sectors from food to cosmetics and personal care products, as well as in animal feed and biofuel. By the end of the century, the global demand for vegetable oil is expected to reach 240 million tonnes (Corley, 2009). The high productivity and low production costs of oil palm enable it to meet rising oil demand with the least amount of negative impact on the environment (John-Martin et al., 2022).

Good planting materials are heavily reliant on gene pools that can offer diversity, which is the backbone of plant breeding (Acquaah, 2012). Since 1973, the Malaysian Palm Oil Board (MPOB) has expanded the genetic resources of oil palm breeding

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materials by extensively collecting wild germplasm from oil palm origin areas, which includes the 11 African countries: Nigeria, Cameroon, Zaire, Tanzania, Madagascar, Angola, Senegal, Gambia, Sierra Leone, Guinea and Ghana (Rajanaidu et al., 2017). *Ex-situ* living collections of oil palm germplasm are exclusively maintained in the fields at the MPOB Kluang Research Station in Malaysia. These collections provide an accessible resource for the evaluation, characterisation, and utilisation to improve the current planting materials. However, field gene bank upkeep is costly in terms of labour, field management and large land requirement. Maintaining germplasms through this approach also involves the risk of attacks by pests and diseases that could potentially lead to the loss of these valuable genetic resources. Conservation of oil palm genetic resources is critical to establishing a reservoir of genes for the creation of novel traits such as high yield, dwarf palm, long stalk, large kernel, oil quality, disease resistance, drought and flood tolerance. Exploiting the genetic diversity of the MPOB-Zaire oil palm germplasm is important to understand its characteristics and population structures. The establishment of a core collection would lower the cost of maintaining the oil palm germplasm by conserving the maximum genetic resource with minimal land space.

Zaire, now known as the Democratic Republic of Congo (DRC), is geographically the second-largest country in Africa. Distribution of grove palms in Central Africa focuses mostly in Zaire and Angola (Corley & Tinker, 2016). The bulk is located between 3° N and 7° S, but there are a few scattered palms as far south as 15° S. Semi-wild palms exist on the Congo-Uganda border, but the weather in most of eastern Africa is not suitable for growing commercial oil palms; it is either too arid, too high in altitude, or both. In the early 1900s, most of the African oil palm plantation activity was in the Congo, where local farmers were encouraged to plant oil palms (Berger & Martin, 2000). Later, in the 1930s, the major scientific discovery of the relationships between *tenera*, *pisifera* and *dura* was found in the Belgian Congo at the L'Institut National pour l' Étude Agronomique du Congo Belge (INEAC), Yagambi (Susan, 2003), which drove plantation development in the country and later, in the global oil palm industry. In 1984, MPOB, in collaboration with Unilever, conducted the first systematic prospection of the oil palm germplasm in Congo (Rajanaidu et al., 2017).

Evaluation of genetic diversity based on morphological traits to ease the utilisation and conservation of germplasm materials is important for breeding programmes. Multivariate analysis is a group of methods that are often used to analyse the traits of breeding materials. It is important to classify germplasm, organise variability among accessions, and analyse genetic relationships among traits (Zafar et al., 2008). Principal component analysis (PCA) is one of the multivariate statistical techniques used to reduce the dimensionality of large data sets. It accomplishes this by computing little explaining variables that accurately describe the original data set. Hierarchical cluster analysis (HCA) however categorises similar observations based on the observed values of variables for each individual in the dataset. PCA and HCA have been successfully applied in crop germplasm evaluation for many years including pigeon pea (Hemavathy et al., 2017), rice (Mvuyekure et al., 2018), coffee (Ferraz et al., 2019) and cotton (Ullah et al., 2022). Both methods have been used to depict oil palm germplasm collected from Nigeria (Li-Hammed et al., 2016), Sierra Leone (Suzana et al., 2016), Ghana (Sapey et al., 2017), India (Balakrishna et al., 2017), Senegal (Myint et al., 2019) and Tanzania (Suzana et al., 2020). In this study, principal component (PC) and cluster analyses were used to classify and group MPOB-Zaire oil palm *dura* germplasm samples based on morphological traits and to also evaluate their genetic diversity.

MATERIALS AND METHODS

The prospection for oil palms in Zaire covered more than half of the country's area. Zaire has a dense tropical rainforest and experiences a tropical climate with two distinct seasons, dry and wet. During the expedition, 369 open-pollinated bunches (283 *dura* and 86 *tenera*) were collected, including 40 samples assembled by the Institute for the Environment and Agricultural Research Stations (INERA) in Bas-Zaire. Five to 10 palms were randomly sampled at 56 sites (each defined as a population) (Rajanaidu et al., 2017) (*Figure 1*). However, one population (ZRE45) did not survive due to unsuccessful germination. Seedlings from 55 Zaire germplasm populations were planted in four experimental trials, designated as trials 0.220 (17 populations), 0.221 (15 populations), 0.222 (12 populations) and 0.223 (11 populations), incorporating two standard cross progenies, MS3516 and MS3554. The planting design involved two replicates with 10 seedlings per replication at a density of 148 palms ha^{-1} on inland soil, covering nearly 60 ha at Bukit Lawiang, Kluang, Johor, Malaysia. These trials were planted in 1986. Breeding data covering yield recording, bunch analysis and vegetative measurements of 4467 *dura* palms from 55 populations were analysed for PCA and HCA analyses. The bunch analysis (BA) method, developed by Blaak et al. (1963) and improved by Rao et al. (1983), was used to estimate the bunch quality traits. Vegetative measurements (VM) and calculations were conducted according to

Note: 1 - Binga 1; 2 - S. Binga II; 3 - S. Bingga III; 4 - Libonas; 5 - Gwenzale; 6 - Gwaka; 7 - Lisala I; 8 - Ndeke; 9 - Mongana; 10 - Bumba; 11 - Lisala II; 12 - Lisala III; 13 - Lisala IV; 14 - Ebonda I; 15 - Ebonda II; 16 - Basoko; 17 - Ligase; 18 - Yahuma; 19 - Lukutu Islands; 20 - Aruwimi; 21 - Kisangani; 22 - Isangi; 23 - Yangambi Station; 24 - Boteka I; 25 - Boteka II; 26 - Boteka III; 27 - Boteka IV; 28 - Boteka V; 29 - Mbanza Ngungu; 30 - Bukayu; 31 - Uvira; 32 - Lusanga I; 33 - Lusanga II; 34 - Tango; 35 - Imbongo; 36 - Kikongo; 37 - Mapangu I; 38 - Mapangu II; 39 - Mapangu III; 40 - Mapangu IV; 41 - Bongimba I; 42 - Bongimba II; 43 - Bongimba III; 44 - Bongimba IV; 45 - Bongimba V; 46 - Matadi; 47 - Tshela I; 48 - Tshela II; 49 - Tshela III; 50 - Lukula; 51 - Kondo I; 52 - Maternie; 53 - Kisantu; 54 - Kondo II; 55 - Gimbe; 56 - Luki.

Figure 1. Collection sites of Zaire oil palm germplasm. Site numbers (in red) show the locations of the corresponding germplasm.

Corley et al. (1971) and Breure and Powell (1988). Yield record (YR) and BA data were recorded from 1990 to 1995. VM were recorded earlier, from 1988 to 1995.

Eighteen phenotypic traits were extracted from an internal MPOB breeding database known as MPOB-Breeding Information System (MPOB-BIS^{TM}). These traits include two yield component traits, mean bunch number (MBNO) and, mean bunch weight (MABW); 10 traits of bunch quality components, bunch weight (BWT), mean fruit weight (MFW), mean nut weight (MNW), mesocarp to fruit (MTF), kernel to fruit (KTF), shell to fruit (STF), oil to dry mesocarp (OTDM), fruit to bunch (FTB), oil to bunch (OTB), kernel to bunch (KTB); and six traits of VM, frond production (FP), petiole cross-section (PCS), rachis length (RL), height increment (HI), bunch index (BI) and leaf area index (LAI). The raw data for these 18 traits were analysed using a box plot in the ggplot2 R package to visualise the data distribution of the germplasm. The variables were then standardised to mean 0 and standard deviation 1 before executing the PCA and Ward's HCA using FactoMineR and Factoextra R packages. All analyses were conducted in R ver. 4.1.3 and R studio ver. 2021.09.1.

RESULTS AND DISCUSSION

Performance of Yield, Bunch Quality and Vegetative Traits

Performances of Zaire *dura* germplasm for yield, bunch quality components and vegetative traits were evaluated based on individual palm (*Figure 2*). The box plots provide a clear visual representation of the data distribution together with its outliers, mean, median and variance in a single concise diagram. ZRE46 produced the highest MBNO, demonstrating the highest number of bunches produced, with a median value of above 15 bunches palm^{-1} yr^{-1} (*Figure 2a*). However, the high MBNO in ZRE46 was not associated with high fresh fruit bunches (FFB) due to its low MABW (*Figure 2b*). Arolu et al. (2017) and Myint et al. (2019) suggested that an increase in the number of bunches with moderate bunch weight may boost FFB yields. Among the Zaire germplasm, ZRE03 and ZRE48 produced the highest and lowest MABW, respectively.

Commercial oil palm planting materials typically produce bunches weighing from 10 to 25 kg with 500 to 4,000 individual fruits, depending on the bunch size (Corley & Tinker, 2016). The Zaire germplasm materials demonstrated a moderate bunch weight (BWT) ranging from 5 to 30 kg (*Figure 2c*). The formation of fruit bunches is influenced by numerous factors including nutrition, water, the supply of carbohydrates, and pollination, such as pollen supply and pollinator activity. Tanya et al. (2013) reported that BWT was strongly associated with oil yield. The fruit weight (MFW) involves the weight of three components, i.e. the mesocarp, shell and kernel. The MFW of the Zaire populations ranges from 5 to 25 g, with the individual palms of ZRE27 and ZRE40 producing the heaviest fruits (*Figure 2d*). The MNW of this germplasm ranged from 5 to 8 g, with a particular ZRE40 palm possessing the highest nut weight (>15 g).

ZRE17 exhibited the highest mesocarp to fruit ratio, with a mean MTF of above 50% (*Figure 2f*). The best individual palm for this trait, however, was found in the ZRE30 population (78%). The mesocarp component contains 95% of palm oil (Corley & Tinker, 2003) and therefore, to cope with the economic trends towards high oil yield, it is important to develop large mesocarp planting materials. Fruits with small kernel and shell sizes generally exhibit an increase in mesocarp content and this will thus enhance oil productivity (Okoye et al., 2009; Shi et al., 2019; Tanya et al., 2013). In line with this, ZRE17 also produced the smallest KTF and KTB, which are components for the kernel trait. The highest KTF (13%) was observed in ZRE15, while the other populations exhibited a KTF median of above 10%, which complies with the KTF criteria for the minimum requirements of *dura* parent selection (5%) in the current Malaysian Standard MS 157:2017 (Department of Standards Malaysia [DOSM], 2017). The individual palm in ZRE40 mentioned earlier that produced the highest MNW and MFW, was also among the highest in KTF. The ZRE51 population produced the highest KTB, and the highest KTF.

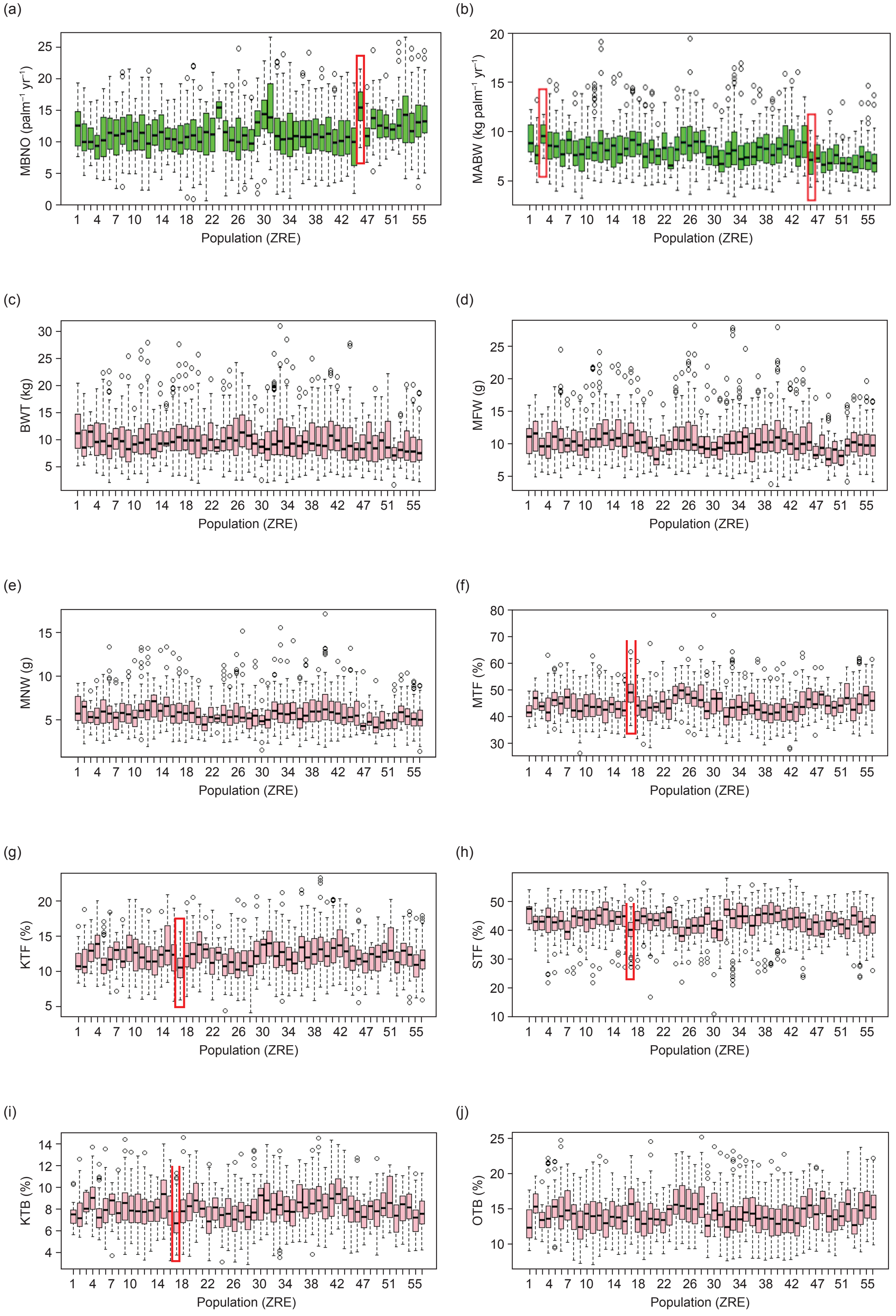
Most of ZRE populations produced oil to dry mesocarp of >75%, with ZRE48 population producing the highest. The highest fruit to bunch ratio was recorded in ZRE35. For both traits, the best individual palm was found in the ZRE40 population (>80%), supported by optimum performances in MFW, MNW, and KTF traits as well. The highest OTB was exhibited by ZRE25 though ZRE28 had an outstanding palm producing 25% oil content. ZRE17 was among the highest in OTB ratios, which are above 15% and this is also associated with the production of high MTF and OTB. A population with good quality features is ideal for further selection for breeding programmes, to incorporate traits related to high oil extraction, thus increasing oil yields.

Besides yield and bunch quality components, VM traits also influence the production of palm oil. Populations with desirable vegetative traits are suitable for introgression with advanced breeding materials. FP has a significant impact on the quantity of bunches as the fruit stalks are joined to each of the axil fronds (Noh et al., 2012). The ZRE populations produced 20 to 28 fronds yr^{-1}, with the highest production observed in ZRE41. In an oil palm breeding program, smaller petiole, shorter rachis length and low HI are desirable vegetative traits (Myint et al., 2019) for the development of compact and dwarf planting materials for higher planting density ha^{-1} (Nor-Azwani et al., 2020). Low HI is a notable factor in oil palm breeding programmes as dwarf palms facilitate easier bunch harvesting and extend the economic lifespan of the palms. ZRE21 exhibited the lowest HI and shortest petiole cross-section (PCS) which is thus ideal for breeding programmes. Meanwhile, ZRE23 exhibited the shortest rachis length which can also be considered a source for the development of compact planting material.

According to the box plot, the kernel and dwarf traits performed well for this germplasm. The trial means of KTF and KTB in the MPOB-Zaire germplasm were 12.21% and 8.01%, respectively. This was higher than the average value of 6.51% and 4.18% in the DxD palm (Trial 0.332), which was the mother palm used in the development of the DxP palm in Malaysia (MPOB, Unpublished Data). While the dwarf population, ZRE21, with the HI of 28.12 cm yr^{-1}, was in range with the dwarf oil palm planting materials (26.0 to 32.5 cm yr^{-1}), which was 57.33% shorter than commercial oil palm planting materials (Arolu et al., 2017).

Principal Component Analysis

Principal Component Analysis (PCA), a reduction variables technique results in sturdy patterns of a dataset, which assists plant breeders in exploring and discovering intriguing traits for future breeding programmes. In PCA, eigenvalues of >1.0 is recommended as a general guideline to retain the important components (Iezzoni & Pritts, 1991). The proportion of variance accounted for in individual principal components (PC) can also be adopted as a standard for targeted PC (Ahmadizadeh & Felenji, 2011). Five paramount PC (PC1-PC5) with eigenvalues >1.0 computed 82.69% of the total variability in the 55 MPOB-Zaire oil palm germplasm populations (*Table 1*). The variances for the PC imply that PC1, PC2 and PC3 correspond to approximately 13 individual variables. Therefore, the variables from PC1 to PC3 need to be prioritised for further Zaire germplasm conservation and breeding programmes. As expected, PC4 and PC5 have smaller eigenvalues of 1.70 to 1.18 with variabilities of 9.44% and 6.55% respectively, equivalent to a single variable, and are only relevant if it has any biological significance.


(a)
MBNO (palm^{-1} yr^{-1})
0
5
10
15
20
25
(b)
MABW (kg palm^{-1} yr^{-1})
5
10
15
20
(c)
BWT (kg)
5
10
15
20
25
30
(d)
MFW (g)
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(g)
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(h)
STF (%)
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(i)
KTB (%)
4
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(j)
OTB (%)
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Population (ZRE)

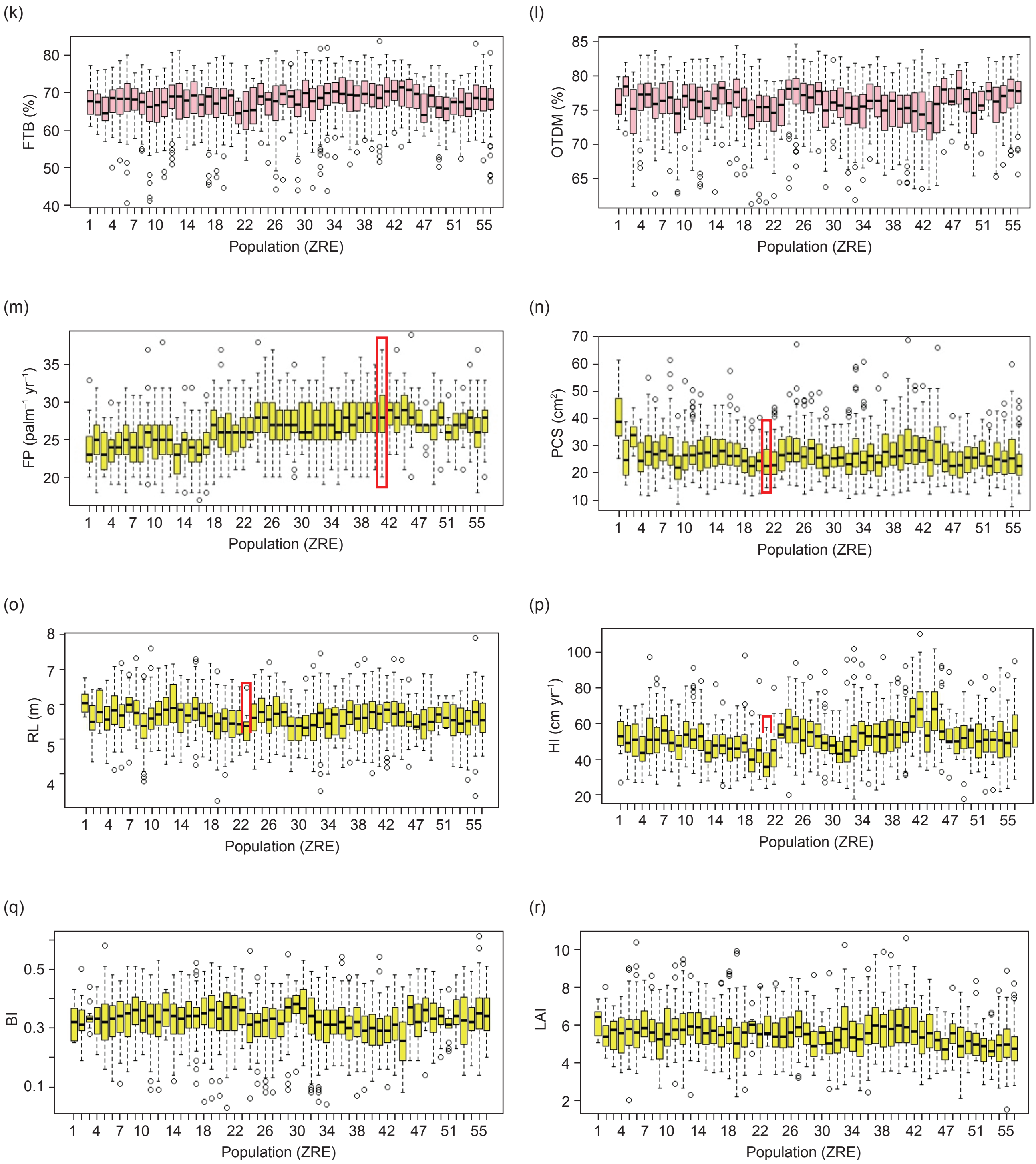

Figure 2. Box plots of the 55 Zaire oil palm populations for each of the 18 traits. (a) Mean bunch number (MBNO), (b) mean bunch weight (MABW), (c) bunch weight (BWT), (d) mean fruit weight (MFW), (e) mean nut weight (MNW) ,(f) mesocarp to fruit (MTF), (g) kernel to fruit (KTF), (h) shell to fruit (STF), (i) oil to dry mesocarp (OTDM), (j) fruit to bunch (FTB), (k) oil to bunch (OTB), (l) kernel to bunch (KTB), (m) frond production (FP), (n) petiole cross-section (PCS), (o) rachis length (RL), (p) height increment (HI), (q) bunch index (BI), and (r) leaf area index (LAI).

TABLE 1. PRINCIPAL COMPONENTS WITH EIGENVALUES >1.0

Principal components	PC1	PC2	PC3	PC4	PC5
Eigenvalue	5.45	4.14	2.41	1.70	1.18
Variance (%)	30.30	23.03	13.37	9.44	6.55
Cumulative variance (%)	30.30	53.33	66.70	76.14	82.69

PC1 is contributed mainly by MABW (0.812), LAI (0.779), BWT (0.767), PCS (0.723), MNW (0.701), MBNO (-0.695) and RL (0.690) (*Figure 3*). PC2 is influenced by bunch yield components such as OTB (0.883), MTF (0.873), OTDM (0.802), KTF (-0.749), KTB (-0.685) and STF (-0.677). The positive or negative coefficient values in the PC explained the interpretation of variability in oil palm breeding germplasm (Ahmad et al., 2014).

The ordination of the overlapping pattern showed strong intra-variable correlations, such as that between MBNO and BI (*Figure 4a*). The right and left quadrants of the plot may also contain the factors responsible for the majority of the variations. Positive correlations exist between variables plotted on the same PC and which are located relatively close together, suggesting that an increase in one causes an increase in the other. An increase in one variable is associated with a decrease in another when the variables are plotted at opposite ends of the plot, indicating negative correlations. MTF, OTDM and OTB traits were negatively correlated with KTF, KTB and STF traits. This suggests that mesocarp content was influenced by the kernel and shell sizes, which was reported by (Shi et al., 2019), and was subsequently reflected in the oil yield production. *Figure 4b* represents the score plot in a map of the distribution of 55 Zaire populations. The ZRE populations that are located close to each other on the bi-plot have similar traits and performance profiles, whereas those far from each other are dissimilar. ZRE17, ZRE24, ZRE25, ZRE26 and ZRE28 are located close together in the Q1 quadrant, suggesting similarities in the performance of MTF, OTB and OTDM traits. These five ZRE populations were also located around Boteka, Zaire, where this village is noted for its abandoned oil palm plantations (Jennifer, 2020). ZRE10 is located close to the centre (origin) of the plot, suggesting its performance is average for all the traits studied.

The bi-plot allows visualisation of the combination of both loading vectors (variables) and the PC scores (Zaire populations) in a single plot (*Figure 4c*). The angles between vectors of different variables demonstrate the correlation in the plot. Firincioglu et al. (2009) reported that this diagram can be used to verify populations with interesting trait combinations that might be valuable in a breeding programme. From the bi-plot, ZRE21 was located at the opposite angle to the HI trait, indicating a high negative correlation, which suggests that this population possesses dwarf feature that could be used to develop a shorter oil palm. One of the main goals in oil palm breeding is

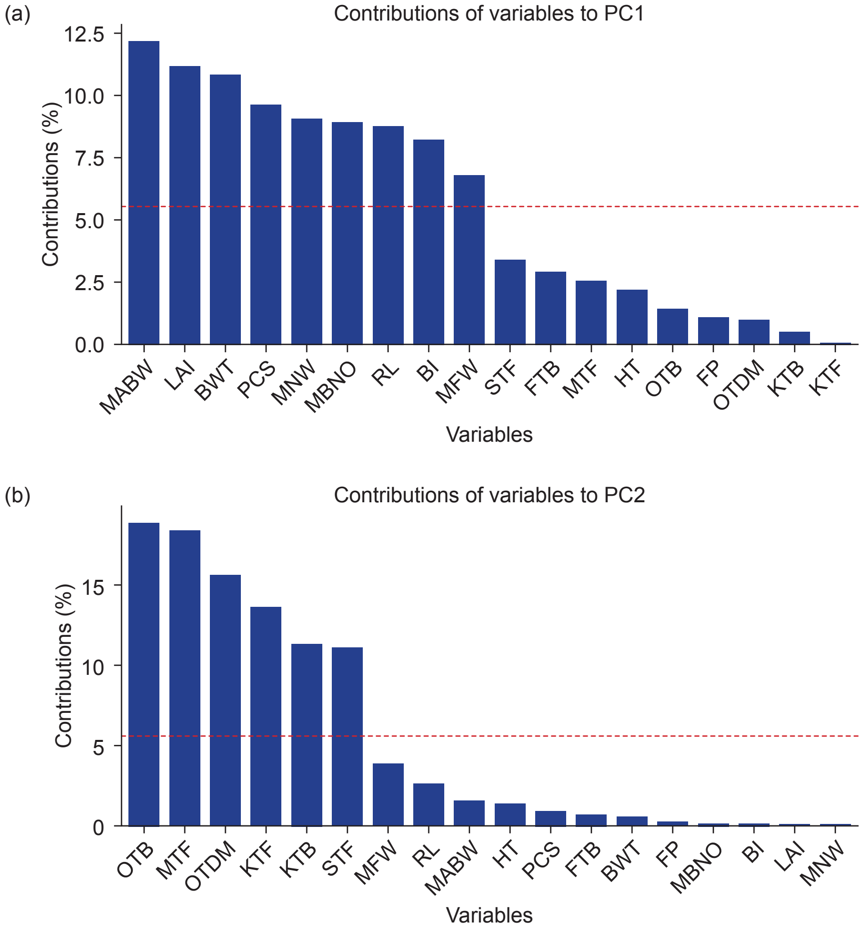

Figure 3. Contribution of variables to (a) PC1, and (b) PC2.

to reduce the HI to 30 cm yr^{-1} compared to 40 to 70 cm yr^{-1} in current planting materials (Kushairi et al., 2011; Rajanaidu et al., 2000). One of the approaches to reach this goal is to introgress the height trait from germplasm samples into commercial planting materials (Ong et al., 2018). Aside from that, this ZRE21 population shows good performance in KTF and KTB traits, indicating a favourable association to develop novel breeding material. Those traits are promising for breeding oil palms with high kernel content which could be important sources of lauric acids for the oleochemical industry (Jalani et al., 2003). Population ZRE01 appears to be an outlier and has the strongest positive association with variables in PC1, such as BWT, LAI and MNW. However, the fact that this population was plotted at an angle greater than 90° between the OTB, MTF and OTDM implies that there is no association with these economic features. On the other hand, ZRE17 showed the strongest positive association with the traits in PC2, suggesting that this population could be a source of breeding material for yield improvement of the Zaire germplasm.

Cluster Analysis

Clustering methods are used as a complementary analytical task to PCA. The ZRE populations were classified according to the variables associated with each cluster using Ward's clustering method based on Euclidean distance. A dendrogram is formed by the levels of similarity at which the observations are merged, which gives a visual summary of the clustering process and depicts an image of the clusters and their proximity with a sharp reduction in the dimensionality of the original data (Shrestha & Kazama, 2007). Two major clusters were observed for the 55 ZRE populations with a coefficient of 15 (*Figure 5*). Cluster 1, which is the larger cluster, comprised 34 populations, which was divided into two sub-clusters. Cluster 2 comprised 21 populations. This clustering is in accordance with the PCA bi-plot, whereby Cluster 1 is made up of the combination of the variables in quadrants Q1 and Q4 and is positively associated with PC1. As ZRE01 is an outlier in the bi-plot, it was placed as a singleton in the subcluster.

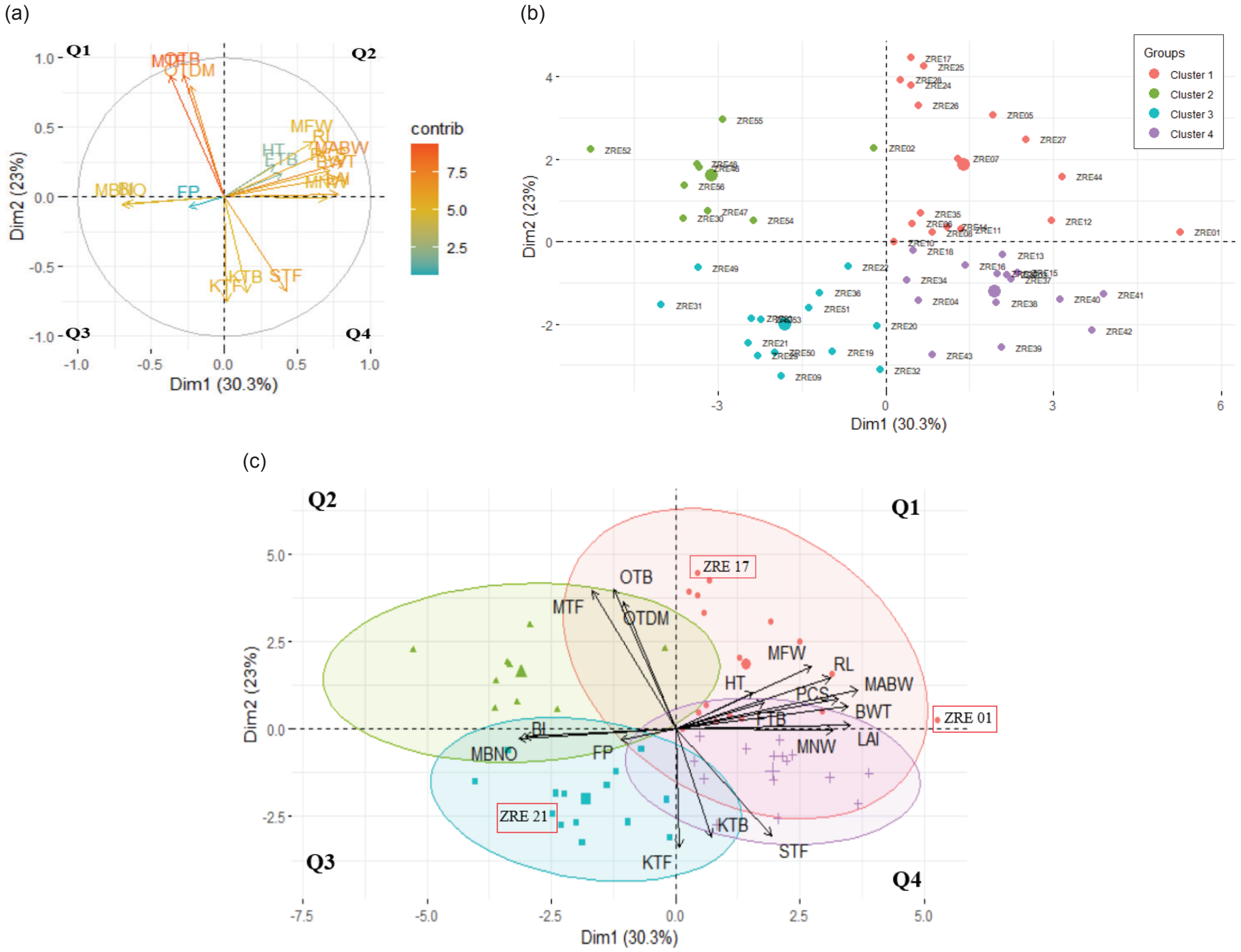

Figure 4. (a) Variables loaded into PC1 and PC2, (b) distribution of the Zaire populations on a score plot of the first two PCs, indicative of the relationships between the populations and (c) PCA bi-plot showing both the loadings of the 18 trait variables and the PC scores of 55 populations. Vectors with parallel variables to the PC axis have a higher direct contribution to the PC, with small angles indicating a high positive correlation.

Cluster 2 however, is associated favourably with PC2 and is built by the combination of variables from quadrants Q2 and Q3.

For the development of core collections, populations in the same cluster can be minimised in a regeneration scheme, thus the repetitive genetic materials could be reduced. By providing a working collection representing the entire range of variability, the core collection can play a significant role in the design of new cultivars (Singh, 2009). The fact that unique genes do not necessarily need to be a part of the core collection is well known. Thus, if it is present by chance, the gene ought to be directly added to the sample core set (Mahajan et al., 1996). The ability to identify desirable traits for oil palm improvement could be achieved by providing a core collection with no redundancy, and accurate information for each accession (Frankel, 1984). Thus, the amount of land required for planting and field maintenance costs for an *ex-situ* core collection can be reduced. This study also revealed the ability of morphological traits to assess the genetic diversity of crop populations. This genetic divergence analysis is vital for selecting highly diverse genotypes for a breeding program. As ZRE01 has a greater genetic distance to ZRE46, a cross between both populations will assist in obtaining the maximum genetic divergence of this germplasm.

Both clusters appeared to be comparable based on the performance of several phenotypic traits (*Table 2*). Some economic features, such as KTF (12%), KTB (8%) and OTDM (76%) appear to be promising for introgression with advanced breeding material. The KTF and KTB values for Zaire germplasm were equivalent to the Nigerian (*dura*) germplasm, but higher than Angola (*dura*) which features 11% KTF and 7% KTB (Rajanaidu et al., 2017). Upon the introduction of the pollinating weevil *Elaeidobius kamerunicus* to Malaysia, the KTB rose from 5% to 7% (Rajanaidu et al., 1996). As a result, the net gain of palm oil is directly impacted by the rise in KTB. Therefore, as proposed by Myint et al. (2019), the development of breeding material with high KTB yields will boost large economic returns. In addition, the average HI for both clusters were 39 and 37 cm yr^{-1}, which demonstrated dwarfism prospects. This is about 63% shorter than the 45-75 cm yr^{-1} in current oil palm planting materials (Arolu et al., 2017). This value is also lower than several other *dura* germplasms such as Angola (42.65 cm), Senegal (46.76 cm), Gambia (43.74 cm) and Sierra Leone (42.30 cm) (Rajanaidu et al., 2017).

Clustering of the majority of the populations appeared to be influenced by their geographical locations. However, several populations from Cluster 2 did not appear associated with geographical factors possibly as a consequence of intensive human activity originating in the Congo Basin, along the Zaire River (Choong et al., 1996). Human intervention in natural habitats can lead to an increase in the level of genetic diversity. Furthermore, as populations used in this study covered the entire geographic range from the rainy to the dry areas, considerable genetic diversity across oil palm populations may be related mainly to geographical differences. ZRE30 and ZRE31 germplasms exhibit low genetic variation due to geographic and climatic factors (Choong et al., 1996). Those populations were located at the edge of the palm belt, and it is assumed that diversity of a species decreases outward from its centre of diversity. The high genetic variability in Zaire germplasm suggested that this germplasm can potentially be a genetic source for oil palm improvement (Zulkifli et al., 2012). Based on multivariate analysis, in conjunction with more advanced molecular tools, the use of the MPOB-Zaire germplasm in breeding and regeneration programmes could be properly strategised.

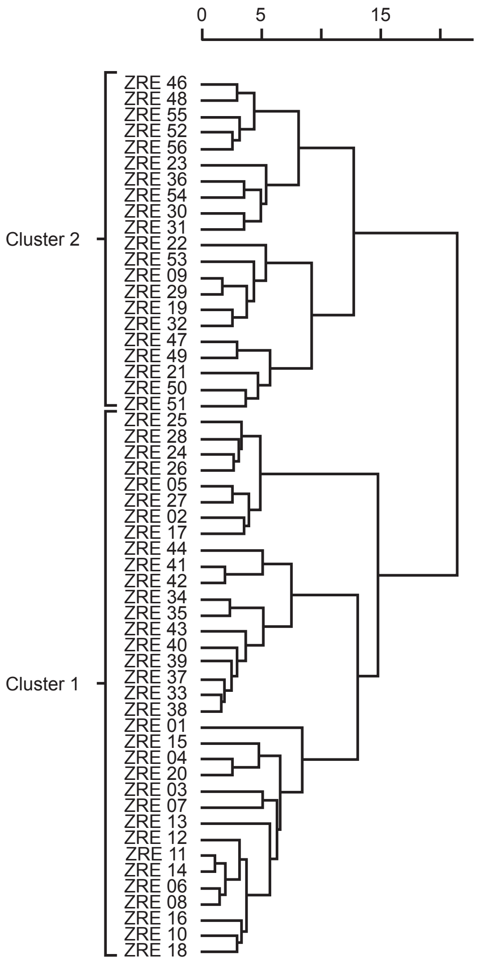

Figure 5. An agglomerative hierarchical cluster dendrogram constructed using the 55 populations, ZRE01 to ZRE55. Populations for the corresponding sample clusters have their initial pairings. Progression through the hierarchy places paired populations into appropriate sample clusters.

TABLE 2. CLUSTERS AND MEAN VALUES OF PHENOTYPIC FEATURES

Cluster	MBNO	MABW	BWT	MFW	MNW	MTF	KTF	STF	OTDM
1	9.87	10.64	10.28	10.67	5.91	44.57	12.16	43.26	75.90
2	11.98	9.19	9.02	9.53	5.26	44.95	12.41	42.64	75.79
Cluster	**FTB**	**OTB**	**KTB**	**FP**	**PCS**	**RL**	**HI**	**BI**	**LAI**
1	68.11	14.30	8.04	25.91	27.66	5.69	39.48	0.31	5.71
2	66.95	14.23	8.01	26.94	24.54	5.47	37.21	0.34	5.21

Note: MBNO - mean bunch number (bunch palm^{-1} yr^{-1}); MABW - mean average bunch weight (kg palm^{-1} yr^{-1}); BWT - bunch weight (kg); MFW - mean fruit weight (g); MNW - mean nut weight (g); MTF - mesocarp to fruit (%); KTF - kernel to fruit (%); STF - shell to fruit (%); OTDM - oil to dry mesocarp (%); FTB - fruit to bunch (%); OTB - oil to bunch (%); KTB - kernel to bunch (%); FP - frond production; PCS - petiole cross section (cm^2); RL - rachis length (m); HI - height increment (cm); BI - bunch index; LAI - leaf area index.

CONCLUSION

This study successfully characterised 55 populations of the Zaire oil palm germplasm with 18 traits for yield components, bunch quality components and VM using multivariate analysis. PCA revealed possible economic traits in this germplasm, i.e. MABW, BWT, OTB and MTF, that can be used as material for breeding programmes. ZRE21 germplasm can potentially be used in the development of planting materials with dwarf palm and high kernel components. The findings of the PCA are in accordance with the CA constructed. The Zaire populations grouped into two major clusters which appear to be strongly associated with their geographical origins. The information from this study can greatly assist oil palm breeders by allowing systematic management to be implemented in breeding and conservation strategies. Optimum efficiency in core collection of germplasm could be obtained by integrating these results with molecular genotyping information.

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ANTIFUNGAL ACTIVITY OF ETHANOLIC EXTRACT FROM INDIGENOUS BACTERIUM *Bacillus subtilis* STRAIN MN704394.1 CULTURE CONTAINING EICOSANE AND ETHYL STEARATE AGAINST *Ganoderma boninense*

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ABSTRACT

Ganoderma boninense *is a fungal pathogen responsible for causing basal stem rot disease in oil palm. This study focuses on exploring the biocontrol potential of indigenous bacterium by detecting several bioactive compounds capable of inhibiting the growth of* G. boninense*. The 16S rRNA sequence analysis proved that the bacterium was* Bacillus subtilis *strain MN704394.1. The ethanolic extracts of bacterium culture supernatant, spanning 8 to 24 hr, were collected and tested on their inhibitory effects against* G. boninense *using simultaneous, preventive, and curative methods. The one obtained at the 8th hour using the preventive method demonstrated the highest inhibition percentage at 96.44%. By using GC-MS analysis, three compounds were consistently detected at all time points, which are eicosane, ethyl stearate and methyl palmitate. Eicosane and ethyl stearate were identified as bioactive compounds. Specifically, at the 8th hr, eicosane constituted 68.7467% of the total area, while ethyl stearate accounted for 10.3018%. Eicosane, a straight-chain alkane consisting of 20 carbon atoms, exhibited antifungal activity. Ethyl stearate, belonging to the group of fatty acid esters, demonstrates significant inhibitory activity against* G. boninense*. The potential of these two compounds is substantial, with prospects for further development, particularly in controlling* G. boninense*.*

Keywords: *Bacillus subtilis*, eicosane, ethyl stearate, *Ganoderma boninense*, oil palm.

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INTRODUCTION

Oil palm (*Elaeis guineensis* Jacq.) is a vital commodity for Indonesia's economy due to its substantial contribution as a primary source of vegetable oil (Latifah & Kadir, 2021). However, the prevalence of basal stem rot disease caused by *Ganoderma boninense* presents a significant challenge to Southeast Asia's oil palm cultivation, accounting for 86% of global palm oil production, particularly in Indonesia (Wong et al., 2022). The disease leads to reduced productivity, with affected trees experiencing a decline in both quantity and quality of fruit bunches, subsequently diminishing oil yield (Evizal & Prasmatiwi, 2022). In certain regions of Sumatra Island, Indonesia, disease incidence rates have been reported spanning from 37% to 52%, intensifying concerns about production losses (Paterson, 2019). Notably, *G. boninense*-infected oil palm trees yield as low as 4 t of crude palm oil ha^{-1} yr^{-1}, considerably lower than the potential 19 t ha^{-1} yr^{-1} under healthy conditions (Rebitanim et al., 2020).

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To address this challenge, biological control using environmentally friendly strategies, particularly through biofungicides, has gained prominence in the oil palm industry. *Bacillus subtilis* emerges as a compelling bioagent due to its effective antimicrobial activity against various pathogens in agricultural contexts (Chandrasekaran & Chun, 2016; Zhang et al., 2022). Its colonisation of plant roots inhibits *G. boninense* growth by producing antimicrobial substances and engaging in competitive interactions (Puspita et al., 2019).

Exploration of *B. subtilis* metabolites reveals the presence of eicosane compounds in strain BS-01 and fatty acid ester compounds in strain SVUNM4 (Awan et al., 2023; Sreenivasulu et al., 2017). While eicosane's potential as an antifungal against *G. boninense* remains understudied, its high flash point poses challenges for storage. Similarly, ethyl stearate, identified among the fatty acid esters, has exhibited inhibitory activity against *Candida albicans* (Huang et al., 2010). The main mechanism of fatty acids (ethyl stearate) as antifungals is through the insertion of fatty acids into the fungal lipid bilayer membrane which damages membrane integrity, causes uncontrolled release of electrolytes and intracellular proteins, and ultimately causes disintegration of the fungal cell cytoplasm. The mechanism of the compound as an antifungal can be that the compound spreads through the fungal membrane and interferes with the synthesis of important components such as ergosterol, glucan, chitin, protein and glucosamine (Tay & Chong, 2016). Interestingly, higher concentrations of these metabolites are found in oil palm plants displaying resistance to *G. boninense*, hinting at their potential as effective antimicrobial agents (Said et al., 2015).

This study explores the biocontrol potential of *B. subtilis* strain MN704394.1 against *G. boninense*, shedding light on bioactive compounds inhibiting its growth. The investigation is significant considering the adverse impact of *G. boninense* on oil palm productivity and introduces potential antifungal agents, aiming to address their limited research and storage challenges.

MATERIALS AND METHODS

Time and Location

The research was conducted from June 2022 to April 2023 at the Biotechnology and Chemistry Laboratory, National Research and Innovation Agency, South Tangerang City, Banten Province, Indonesia.

Biological Materials and Molecular Identification

The bacterium was isolated from healthy oil palm tissue in susceptible areas to serve as stock isolates. The bacterium's stock culture was routinely maintained in the Laboratory for Biotechnology, National Research and Innovation Agency, South Tangerang City, Banten Province, Indonesia. The *G. boninense* strain SSU008, a pathogenic fungus used in this study, was obtained from the collection at Indonesian Oil Palm Research Institute (IOPRI), Marihat, Simalungun Regency, North Sumatra Province, Indonesia. To rejuvenate the stock cultures of indigenous microbes, specific solid media were employed. Nutrient agar (NA) medium was utilised for the bacterium, while potato dextrose agar (PDA) medium was used for *G. boninense*. To identify the antagonistic bacterium strain, DNA extraction was performed using Instagene™ Matrix, followed by sample amplification using 16S rRNA primers (5′-AGA GTT TGA TCC TGG CTC AG-3′) and (5′-GGA TAC CTT GTT ACG ACT T-3′) (Ibrahim et al., 2016) with a base length of 1,500 base pairs. The amplified sample was then sequenced by 1st Base and further analysed with ClustalW and MEGA6 programme to construct a phylogenetic tree (Tamura et al., 2013).

Bacterial Growth Curve

Bacterial growth measurement commenced with a pre-culture step, involving the inoculation of 1-2 colonies of *B. subtilis* MN704394.1 into 50 mL of nutrient broth (NB) media. From the pre-culture, 1 mL was reinoculated into 150 mL of NB media and incubated on a shaker at 150 rpm and 27°C. The total plate count (TPC) method was employed to measure bacterial growth, with measurements taken every 4 hr over a 28 hr period.

To perform the measurement, 1 mL of the bacterial culture was mixed with 9 mL of a 0.85% NaCl physiological saline solution in a test tube. Serial dilutions were prepared from 10^{-1} to 10^{-8} in duplicates. Duplicates from the 10^{-5} to 10^{-8} dilutions, at 0.1 mL each were spread onto nutrient agar (NA) plates. The plates were incubated at 27°C for 24 hr (Irma et al., 2018).

Subsequently, microbial colonies grown on each sample plate were counted using a colony counter. Data analysis involved describing the TPC results for each sample, which were presented using the standards plate counts (SPC) in a table for clarity. SPC is a method used to determine the microbial count within the range of 30–300 colony forming units (CFUs) from dilutions 10^{-5} to 10^{-8}. This approach was employed to minimise potential errors in the analysis process, particularly statistical errors (Yunita et al., 2015).

Extraction of Bioactive Compounds

Bacillus subtilis strain MN704394.1 was cultivated in NB medium. One or two colonies of the bacteria were inoculated into 100 mL of NB medium to create a starter culture. Subsequently, 10 mL was further inoculated into 2 L of NB medium. The bacterial culture was harvested at specific time points, during both the exponential and stationary growth phases, totalling 100 mL of culture. Bacterial cells were separated from the supernatant by centrifugation at 10 000 rpm for 10 min. Furthermore, 100 mL of the supernatant was mixed with 100 mL of ethanol (1:1 v/v) as solvent. To extract metabolites with a reasonably wide polarity spectrum, ethanol solvent is used, as presented by Chan and Chong (2020) and Liu et al. (2007). The resulting mixture was added to a separating funnel and shaken for 15 min, forming two distinct layers. The upper layer consisted of the organic solvent, while the lower layer contained the medium and cell biomass (pellet). The organic solvent layer was concentrated using a rotary vacuum evaporator at approximately 40°C. The obtained extract was then dried at room temperature to remove any remaining solvent (eluent) (Irma et al., 2018).

Activity Test of Crude Extracts Against *G. boninense*

The activity testing of the bacterial extract aimed to identify an extract that effectively inhibited the pathogenic fungus. This involved employing the agar well diffusion method by creating wells on PDA media using a cork borer. The tests were conducted simultaneously, both preventively (pre-infection) and curatively (post-infection) (Syed-Ab-Rahman et al., 2019).

Prior to subjecting the bacterial extract to *G. boninense*, it was dissolved in methanol at a concentration of 10,000 ppm (10 mg 100 mL^{-1}). The supernatant extract from the bacterial culture was introduced into the created wells. Subsequently, *G. boninense* fungus, measuring approximately 1 cm in size, was placed on the PDA media adjacent to the well containing the extract. For comparative purposes in the extract testing, both positive and negative controls were utilised. Benzoic acid (Merck), at the same concentration as the extract, served as the positive control and was dissolved in methanol at a concentration of 10,000 ppm (10 mg 100 mL^{-1}). The negative control involved the application of methanol without the addition of the extract. The inhibition percentage (IP) of the bacterial extract was quantified following the method described by Irma et al. (2018) using the formula: $IP = (R_1 - R_2)/R_1 \times 100\%$, where R_1 signifies pathogen growth in the control and R_2 indicates pathogen growth in the dual culture treatment.

Profiling Bioactive Compounds Using GC-MS

The ethanol extract of *B. subtilis* strain MN704394.1 underwent compound identification through GC-MS analysis at the Chemistry Laboratory, National Research and Innovation Agency, South Tangerang City, Banten Province. GC-MS analysis of the bacterial extract was performed using an Agilent 7890 B gas chromatograph coupled in tandem with MSD 5977 A mass spectrometer. A total of 1 µL volume of the extract solution was injected into a capillary column measuring 30 m × 250 µm × 0.25 µm (Agilent, Type 19091S-433: 93.92873 DB-5MS UI, 5% phenyl methyl silox). The initial oven temperature was set to 40°C with a 1 min hold time, then gradually ramped up at a rate of 10°C min^{-1} until reaching 300°C, where it was maintained for 4 min. Helium served as the carrier gas at a flow rate of 1 mL min^{-1}. The injection mode used was splitless, with an injector temperature of 250°C. The detector employed was a mass-spectrometry (MS) system with ion source and interface temperatures set at 230°C and 250°C, respectively. For the analysis process, electron impact (EI) ionisation was utilised with an ionisation energy of 70 eV. Data processing was performed using the GC-MS Postrun Analysis software. The peak constituents were cross-referenced with the data available in the NIST-17 (National Institute of Standards and Technology) mass spectral library.

RESULTS AND DISCUSSION

Analysis via BLAST of all consensus sequences revealed a high degree of similarity and substantial query coverage between the bacterial strain and the 16S rRNA sequence in the NCBI database. Numerous similar sequences were retrieved from the BLAST analysis and employed for both the reconstruction of a phylogenetic tree and the analysis of genetic distances. Phylogenetic analysis using the neighbour-joining method conclusively identified the bacterial isolate as *B. subtilis* strain MN704394.1, demonstrating 100% sequence similarity (*Figure 1*). According to established taxonomic criteria, species are delineated when the similarity exceeds 97%, species identification is considered a "match" when the similarity surpasses 99% (Drancourt et al., 2000; Janda & Abbott, 2007), while any similarity below 97% suggests the possibility of a novel species (Stackebrandt & Goebel, 1994).

Growth assessment of *B. subtilis* strain MN704394.1 was conducted at 4 hr intervals during a 24 hr period, utilising the TPC calculation method and NB media for bacterial incubation. *Figure 2* shows the growth curve indicating that *B. subtilis*

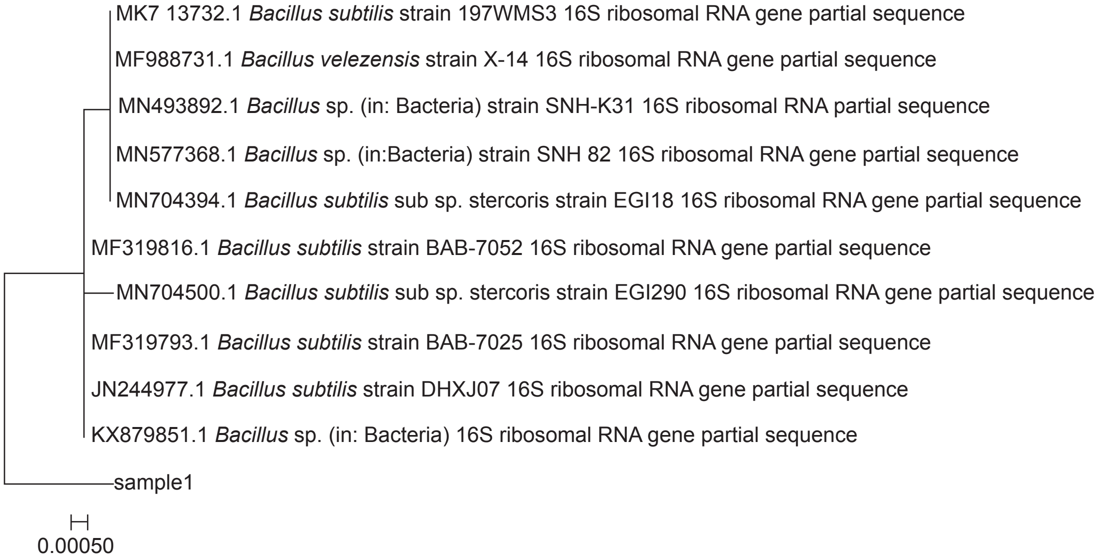


Figure 1. Phylogenetic tree depicting the bacterial isolate based on the 16S rRNA gene.

strain MN704394.1 displayed a brief lag phase, facilitating its rapid entry into the logarithmic (exponential) phase, which was notably extended. This pattern was discernible from hr 0 to hr 12, during which bacterial cell counts increased from 6.550 × 10^7 to 2.595 × 10^8 CFU mL^{-1}. Following the attainment of the logarithmic phase, a stationary phase ensued and persisted until the 20th hr, with bacterial cell counts at the 20th hr (2.515 × 10^8 CFU mL^{-1}) showing no significant deviation from those at the 12th and 16th hr. Subsequently, a death phase commenced, continuing until the 28th hr, characterised by a decline in bacterial cell numbers. *Bacillus subtilis* strain MN704394.1 exhibited decreasing cell counts until the 28th hr, reaching 1.205 × 10^8 CFU mL^{-1}. These bacterial growth phases served as reference points for determining the optimal time for bacterial cell harvest in the extraction process. In this treatment, bacterial cell harvesting was performed between the 8th and 24th hr.

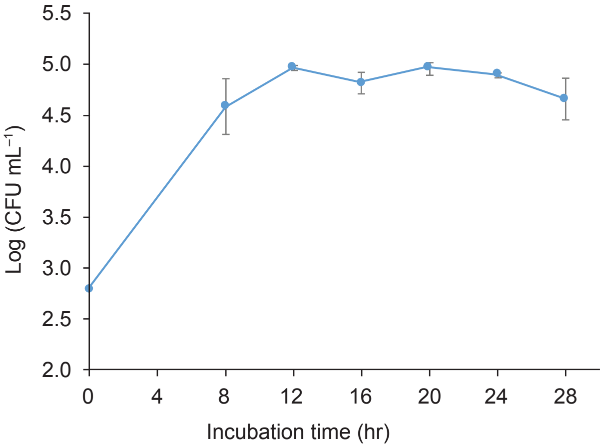


Figure 2. Growth curve of B. subtilis *strain MN704394.1 on NB media (Each data point represents the average of two values and error bars depict the standard deviation).*

The measurement of growth curves aimed to ascertain the optimal time for metabolite production during bacterial proliferation. The bacterial growth curve elucidates the distinct phases in bacterial proliferation, encompassing the lag phase (adaptation phase), the logarithmic phase (exponential phase), the stationary phase and the decline phase (death phase) (Wang et al., 2015). The growth curve analysis revealed that the *B. subtilis* strain MN704394.1 exhibited a brief lag phase. This phenomenon arose due to the pre-culture preparation conducted before growth curve assessment, expediting bacterial growth and propelling it into the logarithmic phase.

The lag phase or adaptation phase in bacterial growth is influenced by new environmental conditions and the initial inoculum size (Schultz & Kishony, 2013). When testing the bacterial growth curve under the same growth environment, the bacteria do not require much time to adapt to their surroundings, thus accelerating their entry into the logarithmic phase (Irma et al., 2018). The logarithmic phase in *Figure 2* observed in *B. subtilis* strain MN704394.1 indicates an increase in the number of bacterial cells until the 12th hr. According to Sulistiana et al. (2021), the mass and volume of cells increased during the logarithmic phase. Andriani et al. (2017) also stated that during the logarithmic phase, there is regular cell division where bacteria double their cell numbers at a constant rate, maintain constant metabolic activity and experience balanced growth conditions.

The logarithmic growth phase yields beneficial metabolites crucial for bacterial growth. Primary metabolites, the products of metabolic pathways, play a pivotal role in microbial growth. Ethyl stearate qualifies as a primary metabolite as it emerges during the logarithmic phase of *B. subtilis* strain MN704394.1, rendering it indispensable for this bacterium's growth. The ethyl stearate quantity increases throughout the logarithmic phase (0th to 12th hr) and stabilises during the stationary phase (12th to 20th hr). This observation aligns with Rezvani et al. (2017) findings, which highlighted the production of essential primary metabolites by *Lactobacillus* sp. during the logarithmic phase (6th to 30th hr).

According to Azizah et al. (2015), *B. amyloliquefaciens* SAHA 12.07 underwent the logarithmic phase until the 6th hr, during which it produced enzymes capable of lysing fungal cell walls. *Bacillus subtilis* entered the stationary phase from the 12th hr until the 20th hr, followed by the death phase. During the stationary phase, the number of dividing cells equalled those succumbing to nutrient depletion (Wang et al., 2015). Secondary metabolites, such as antibiotics or other bioactive compounds toxic to microorganisms, were produced during this phase and acted as a defence mechanism against unfavourable conditions (Irma et al., 2018).

Li et al. (2016) reported that *B. amyloliquefaciens* produced antifungal metabolites during both the logarithmic and stationary phases. These metabolites served as a defence mechanism against extreme environmental conditions, leading to the subsequent death phase. During this phase, there was a decline in cell numbers due to the accumulation of excess toxins and nutrient depletion, resulting in a higher proportion of bacterial cells dying (Irma et al., 2018). Wang et al. (2015) also noted that during the death phase, bacteria lost their ability to divide, with the number of dead cells surpassing the live ones.

The crude extract was derived from bacterial culture supernatant via ethanol extraction, encompassing phases from logarithmic growth to the death phase. Ethanol extracts of *B. subtilis* strain MN704394.1, obtained at 8th, 12th, 16th, 20th and 24th hr, and dissolved in methanol at a concentration of 10,000 ppm, were assessed for their efficacy against *G. boninense*.

Results revealed that the crude extract from bacterial culture spanning 8 to 24 hr, utilising simultaneous, preventive, and curative methods, displayed inhibitory effects on *G. boninense*. Among these approaches, the simultaneous method exhibited the highest inhibition of *G. boninense* growth at the 8th hr in comparison to other time points (*Figure 3*). The inhibition percentage of the ethanol extract at the 8th hr in the simultaneous method was 79.47% (*Table 1*). In the preventive method, the most pronounced inhibition of *G. boninense* growth was also observed at the 8th hr, with an inhibition percentage of 96.44% (*Table 1*). This 8th hr inhibition in the preventive method represented the highest inhibition value among the three methods (*Figure 4*). Meanwhile, in the curative method, the highest inhibition of *G. boninense* growth was noted at the 20th hr when compared to other time points (*Figure 5*). The inhibition percentage of the ethanol extract at the 20th hr in the curative method was 50.76% (*Table 1*). Positive and negative controls were employed as reference points during the extract evaluation. The positive control, utilising benzoic acid alone, demonstrated inhibition solely in the curative method, with an inhibition percentage of 9.85%, whereas the negative control, employing methanol, exhibited no inhibitory activity.

To assess the influence of *B. subtilis* strain MN704394.1 bacterial culture extract on *G. boninense*, three methods were employed: Preventive, curative, and simultaneous (Syed-Ab-Rahman et al., 2019). The preventive method aims to avert fungal infections in plants by applying the bacterial culture extract before planting *G. boninense*. Conversely, the curative method provides substances or compounds at the site of fungal infection after planting *G. boninense* to halt its further development. The simultaneous method involves applying substances or compounds simultaneously with the planting of the pathogenic fungus (Doble & Kumar, 2005).

TABLE 1. INHIBITION PERCENTAGE OF ETHANOL EXTRACT FROM *B. subtilis* STRAIN MN704394.1 CULTURE AGAINST *G. boninense* GROWTH

No.	Extract	Inhibition percentage (%)		
		Simultaneous	Preventive	Curative
1	Negative control (without extract)	0.00 ± 0.00	0.00 ± 0.00	9.85 ± 1.07
2	Positive control (benzoic acid)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
3	Extract at 8 hr after incubation	79.47 ± 3.54	96.44 ± 5.04	50.00 ± 0.00
4	Extract at 12 hr after incubation	68.94 ± 2.79	94.02 ± 1.61	48.48 ± 4.29
5	Extract at 16 hr after incubation	74.24 ± 1.29	91.52 ± 8.14	44.70 ± 5.36
6	Extract at 20 hr after incubation	73.26 ± 4.61	90.45 ± 2.36	50.76 ± 5.36
7	Extract at 24 hr after incubation	70.83 ± 5.89	87.80 ± 6.54	49.24 ± 7.50

Note: ( ) - no inhibition observed; values are presented as the average of duplicate data standard ± deviation.

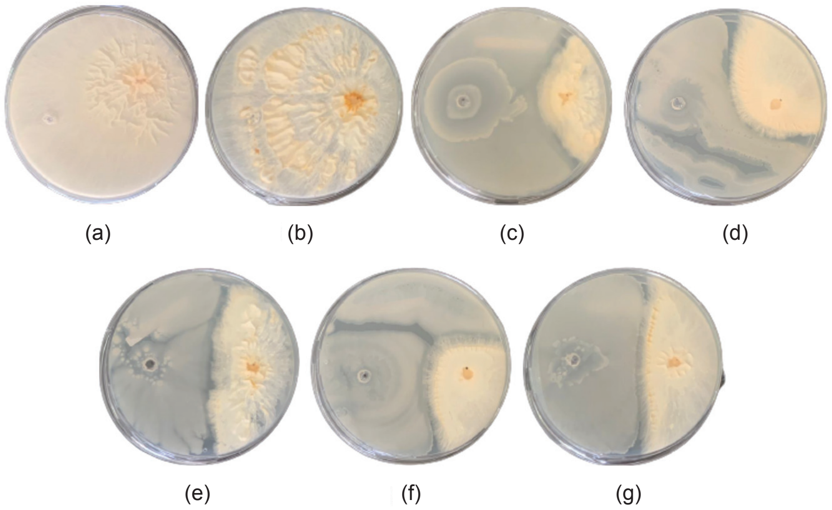


Figure 3. Antifungal activity of ethanol extract on simultaneous growth of G. boninense. *(a) Negative control, (b) positive control, ethanol extract at (c) 8th, (d) 12th, (e) 16th, (f) 20th and (g) 24th hr.*

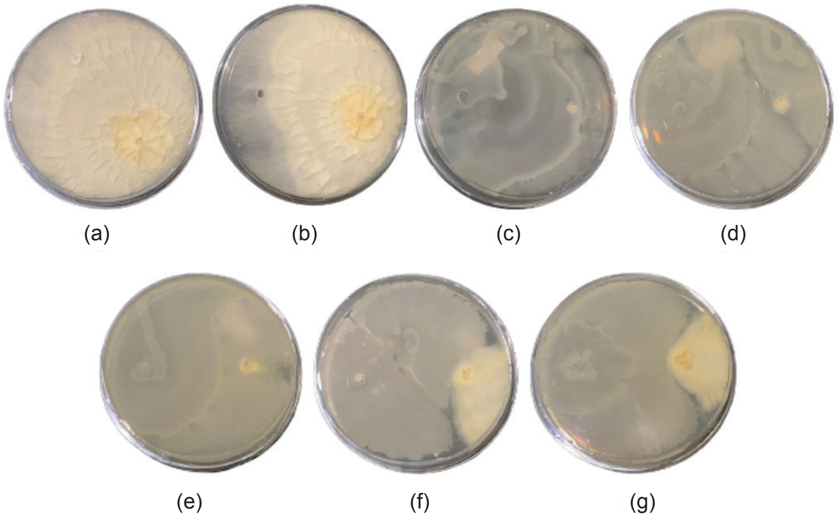


Figure 4. Antifungal activity of ethanol extract on preventive growth of G. boninense. *(a) Negative control, (b) positive control, ethanol extract at (c) 8th, (d) 12th, (e) 16th, (f) 20th and (g) 24th hr.*

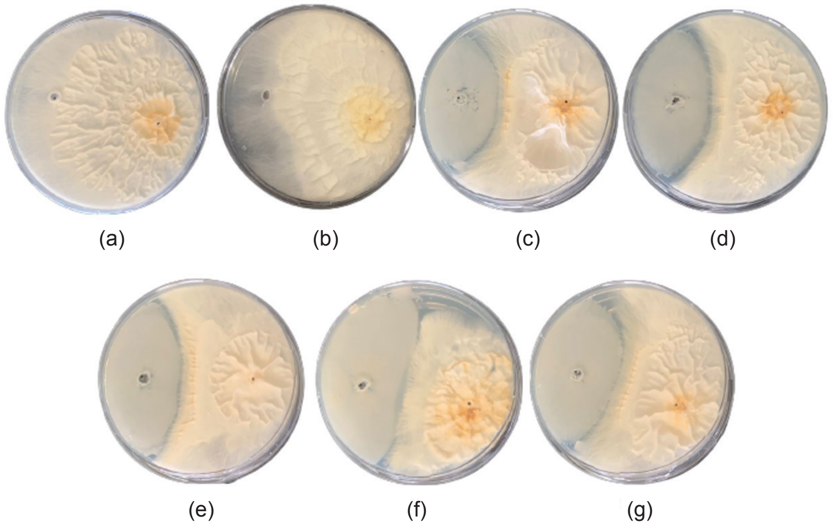


Figure 5. Antifungal activity of ethanol extract on curative growth of G. boninense. *(a) negative control, (b) positive control, ethanol extract at (c) 8th, (d) 12th, (e) 16th, (f) 20th and (g) 24th hr.*

Ethanol serves as the solvent for extracting the *B. subtilis* strain MN704394.1 culture. Chen et al. (2015) indicated that lower ethanol concentrations enhance the solubility of polar antifungal compounds, while higher ethanol concentrations were suitable for extracting semi-polar or non-polar compounds. This range of solvent polarities effectively extracted the antifungal compounds present in the bacterial culture supernatant. The concentrated extract resulting from this process was evaporated to prevent any potential interference during testing of the bacterial extract's activity. Additionally, methanol was employed in the extract testing due to its efficient dissolution of chemical compounds in the bacterial extract, ensuring complete dissolution of any residue adhering to the tube walls (Irma et al., 2018).

The extraction of supernatant from *B. subtilis* strain MN704394.1 cultures using the preventive method exhibited substantial inhibition, particularly at the 8th hr, achieving a remarkable 96.44% inhibition of *G. boninense*. Further application of the simultaneous method at the 8th hr resulted in 79.47% inhibition, followed by the curative method at the 20th hr, displaying a 50.76% inhibition. Bacterial extracts of pronounced efficacy yield more abundant metabolite outcomes, which can be efficiently extracted using ethanol, thus producing significant inhibitory effects.

The produced metabolites exhibit antifungal properties, evident from their inhibitory activity against fungal growth during extraction testing. Factors affecting bacterial growth include the nutrients in the media and environmental conditions, such as temperature, agitation, and pH (Irma et al., 2018). These factors influence bacterial metabolism in synthesising specific metabolite products. The culture supernatant extract of *B. subtilis* strain MN704394.1 at 8th hr was obtained from bacterial cultures in the logarithmic growth phase. Generally, primary metabolites such as enzymes and fatty acids can be produced during this phase (Awan et al., 2023; Azizah et al., 2015). However, secondary metabolite products, usually formed in the stationary phase, can also be produced in the middle of the logarithmic phase (Zvanych et al., 2014). The coarseness of the supernatant from *B. subtilis* strain MN704394.1 culture imparts antifungal properties against *G. boninense* growth by disrupting cellular activities, leading to inhibited fungal growth. The metabolite compounds produced by the bacteria elicit inhibitory responses on the growth of the pathogenic fungus (Irma et al., 2018). These findings demonstrated the effectiveness of compounds generated by *B. subtilis* in hindering *G. boninense* growth. As a positive control, benzoic acid was employed. Benzoic acid, widely used for its antifungal properties and preservation capabilities in various human consumption products, was chosen as a positive control (Loya-Rodriguez et al., 2023). In contrast, the negative control using methanol yielded different results from both the positive control and the treatments, as it did not exhibit any toxic effects on *G. boninense* mycelium growth.

The ethanol extract from *B. subtilis* strain MN704394.1 underwent GC-MS analysis to identify its bioactive compounds. Three compounds were consistently detected at all time points (8th, 12th, 16th, 20th and 24th hr): Eicosane, ethyl stearate, and methyl palmitate. Eicosane and ethyl stearate were identified as the predominant bioactive compounds across all time points and were present at elevated concentrations. Specifically, at the 8th hr, eicosane constituted 68.75% of the total area (at a retention time of 20.32), while ethyl stearate accounted for 10.30% (at a retention time of 22.12). The GC-MS analysis of the ethanol extract from *B. subtilis* strain MN704394.1 at 8th, 12th, 16th, 20th and 24th hr consistently revealed that ethyl stearate had the second-largest area value at each time point. At the 8th hr, ethyl stearate was identified with an area value of 10.30% at a retention time of 22.12 (*Table 2*). At the 12th hr, in the bacterial ethanol extract, ethyl stearate was found to have an area value of 9.02% at a retention time of 22.12 (*Figure 6*). Meanwhile, the GC-MS analysis of the ethanol extract from *B. subtilis* strain MN704394.1 at the 16th, 20th and 24th hr consistently showed that ethyl stearate maintained the same area value of 11.11% at a retention time of 22.12 (*Figure 6*).

Analysis of the crude extract from *B. subtilis* strain MN704394.1 using GC-MS revealed the consistent presence of three compounds across all monitored time points (8th, 12th, 16th, 20th and 24th hr). These compounds are eicosane, ethyl stearate, and methyl palmitate.

Prior investigations have substantiated the bioactivity of these aforementioned compounds. In a study conducted by Awan et al. (2023), compounds extracted from *B. subtilis* BS-01 and identified through GC-MS analysis yielded eicosane, palmitic acid and stearic acid compounds, displaying substantial potential as antifungal agents against *Alternaria solani*. Most fatty acids, including fatty acid ethyl ester (ethyl stearate) and fatty acid methyl ester (methyl palmitate), exhibit antifungal properties (Astiti & Ramona, 2021).

According to Tay and Chong et al. (2016), fatty acid esters identified via GC-MS analysis in papaya leaf extracts demonstrate potential inhibitory activity against *G. boninense*. One such compound within this group is ethyl stearate, an ethyl ester derivative with a 20-carbon chain. Additionally, fatty acid ethyl esters have exhibited inhibitory and antifungal properties against *Candida albicans*

TABLE 2. THE PROFILING OF BIOACTIVE COMPOUNDS IN THE ETHANOL EXTRACT AT THE 8TH HR USING GC-MS

No.	Retention time	Area (%)	Compounds	Formula	MW (g/mol)	Bioactivity
1	6.27	1.77	Cyclopentane, 1,2,3,4,5-pentamethyl-	$C_{10}H_{18}$	138.3	-
2	17.18	0.54	Tetracosane, 1-iodo-	$C_{24}H_{49}I$	464.6	-
3	17.90	6.65	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahydro-	$C_7H_4N_2O_2$	154.2	Antifungal (Sanjenbam & Krishnan, 2016), antibacterial, antioxidant (Kiran et al., 2018)
4	18.29	0.77	2-Propenamide	C_3H_5NO	71.1	-
5	19.43	0.46	(2-Piperidin-3-ylethyl)amine	$C_7H_{16}N_2$	128.2	Antifungal (Kunzler et al., 2013)
6	19.58	0.82	Palmitic acid, methyl ester	$C_{17}H_{34}O_2$	270.5	Antifungal (Pinto et al., 2017), antibacterial (Shaaban et al., 2021)
7	20.04	2.07	Nonadecane, 3-methyl-	$C_{20}H_{42}$	282.5	Antibacterial (Kumari et al., 2019)
8	20.32	68.75	Eicosane	$C_{20}H_{42}$	282.5	Antifungal (Awan et al., 2023), antibacterial (Wijayanti and Dewi, 2022)
9	20.72	0.40	3-Amino-2-ethyl-butyric acid	$H_{13}NO_2C_6$	131.17	-
10	20.89	0.47	Sarcosine, n-hexanoyl-, hexadecyl ester	$C_{25}H_{49}NO_3$	411.7	-
11	21.27	0.39	Cyanoacetylurea	$C_4H_5N_3O_2$	127.1	-
12	22.12	10. 30	Stearic acid, ethyl ester	$C_{20}H_{40}O_2$	312.5	Antifungal (Tay & Chong, 2016), antioxidant. antiinflammation (Ganesh & Mohankumar, 2017)
13	23.94	0.57	Sarcosine, N-isobutyryl-, tetradecyl ester	$C_{21}H_{41}NO_3$	355.6	-
14	24.38	0.61	3,6-Bis-dimethylaminomethyl-2,7-dihydroxy-fluoren-9-one	$C_{19}H_{22}N_2O_3$	326.4	-

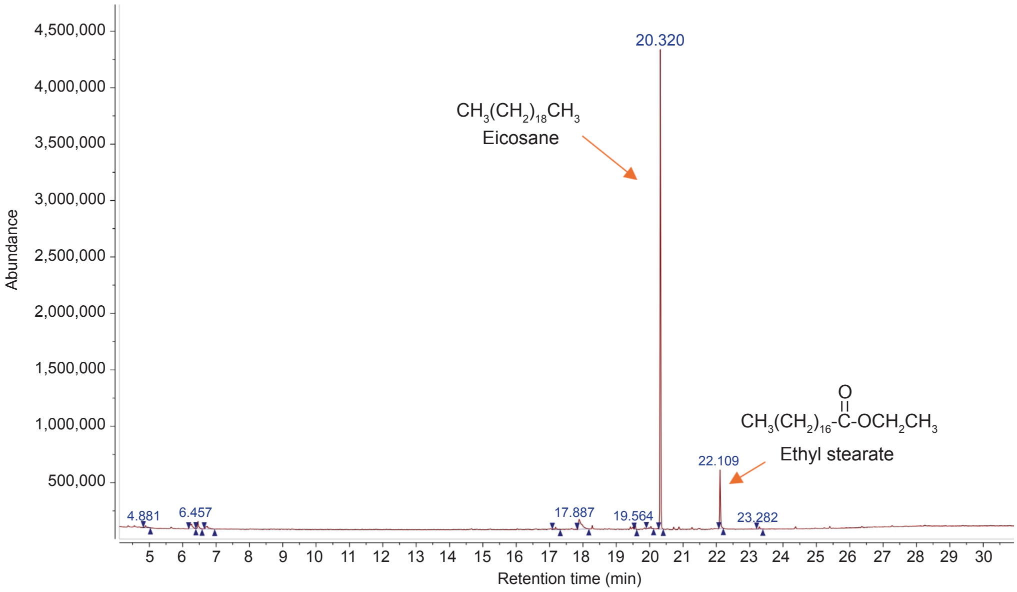
Note: MW - molecular weight.

(Huang et al., 2010). Pinto et al. (2017) have asserted that fatty acid esters, particularly against fungi, possess antimicrobial activity. These esters, identified through GC-MS analysis, were also found in the bacterium *B. subtilis* strain SVUNM4 (Sreenivasulu et al., 2017). Furthermore, Said et al. (2015) have reported that fatty acid esters, identified via GC-MS analysis, are most concentrated in healthy oil palm plants that are resistant to the pathogenic fungus *G. boninense*. These elevated levels of metabolite compounds in *G. boninense*-resistant oil palm plants may serve as potent antimicrobial agents.

Ethyl stearate is considered a primary metabolite, produced during the logarithmic phase in the bacterium *B. subtilis* strain MN704394.1, essential for its growth. Nevertheless, ethyl stearate may result from a reaction between the fatty acids produced by the bacteria and the ethanol solvent. This aligns with Da Silva et al. (2019) study, which obtained ester compounds with an 80% conversion rate after adding ethanol to the fermentation product containing stearic acid. Further research is required to ascertain whether ethyl stearate originates from bacterial production or is a result of a reaction with the solvent. Nonetheless, the method employed in this study yielded ethyl stearate abundantly and displayed promising antifungal activity.

The primary antifungal mechanism of fatty acids involves their insertion into the lipid bilayer membrane of the fungus, disrupting membrane integrity, which leads to the uncontrolled release of intracellular electrolytes and proteins, ultimately resulting in the disintegration of the fungal cell cytoplasm. Additionally, compounds can penetrate the fungal membrane and interfere with the synthesis of essential components like ergosterol, glucan, chitin, proteins, and glucosamine (Tay & Chong, 2016). Furthermore, exposure to bacterial compounds has been shown to alter fungal morphology, inhibit enzyme activities, and modulate gene expression. One such affected enzyme activity is laccase activity (Schmidt et al., 2015). Laccase, along with other ligninolytic enzymes such as manganese peroxidase and lignin peroxidase, is secreted by white-rot fungi (Yang et al., 2017). *G. boninense* also produces lignolytic enzymes capable of degrading lignin components in plant cell walls, leading to basal stem rot disease in oil palm (Ho et al., 2020).

(a)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20.320
CH3(CH2)18CH3
Eicosane
O
CH3(CH2)16-C-OCH2CH3
Ethyl stearate
22.109
4.881
6.457
17.887
19.564
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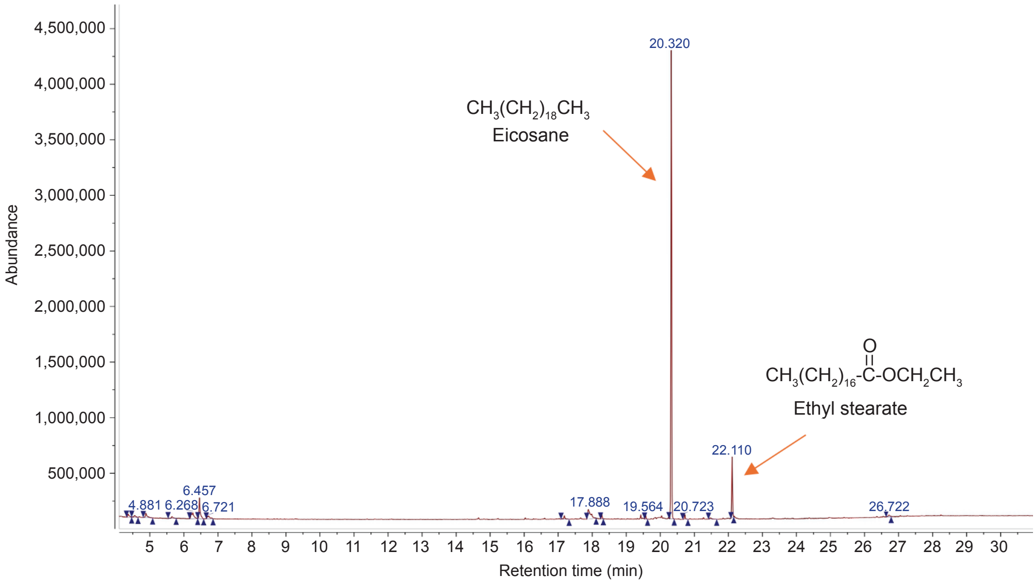
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Retention time (min)

(b)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Eicosane
O
CH3(CH2)16-C-OCH2CH3
Ethyl stearate
22.110
6.457
4.881
6.268
6.721
17.888
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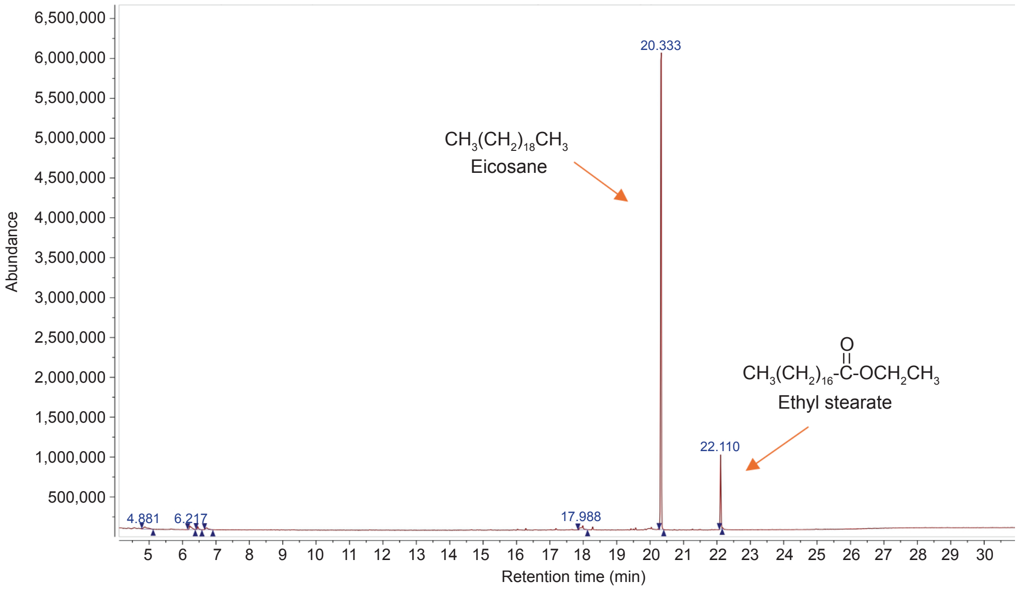
Retention time (min)

(c)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500,000
20.333
CH3(CH2)18CH3
Eicosane
O
CH3(CH2)16-C-OCH2CH3
Ethyl stearate
22.110
4.881
6.217
17.988
5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Retention time (min)

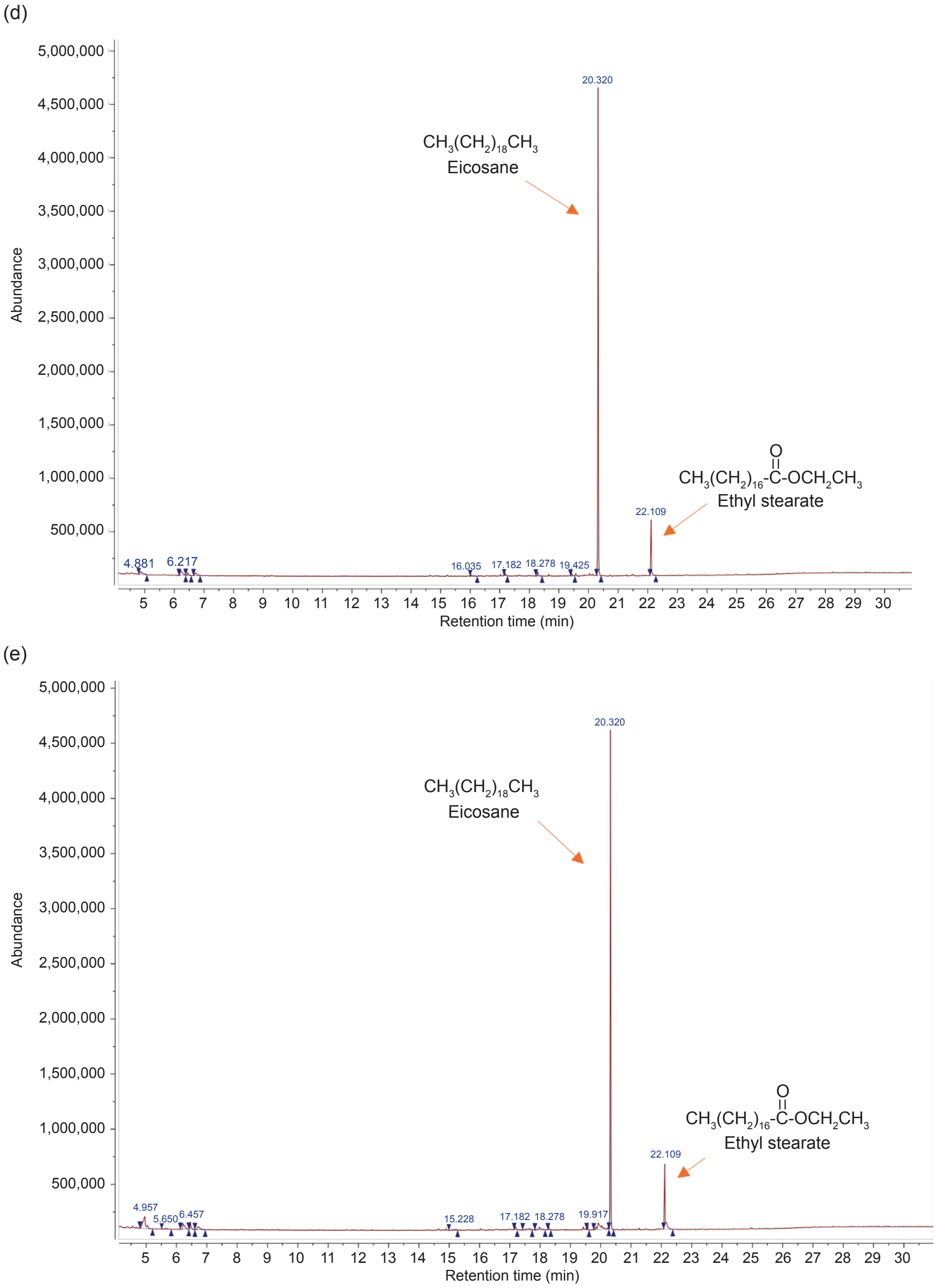

Figure 6. The chromatogram of GC-MS analysis of the ethanol extract at the (a) 8th, (b) 12th, (c) 16th, (d) 20th and (e) 24th hr.

CONCLUSION

The oil palm pathogenic fungus *G. boninense*'s growth can be effectively controlled using *B. subtilis* strain MN704394.1 as a biocidal agent. Ethanol extract from the supernatant culture of *B. subtilis* strain MN704394.1 demonstrated potent antifungal activity, with the highest inhibitory effect (96.44%) observed at the 8th hr when employing the preventive method. GC-MS analysis identified key compounds, including eicosane (68.75% area, retention time 20.32) and ethyl stearate (10.30% area, retention time 22.12), in the crude extract. Eicosane, a long-chain alkane with a high flash point, faces storage challenges at room temperature, limiting its exploration as an antifungal agent. Notably, ethyl stearate, a fatty acid ester, plays a pivotal role in inhibiting *G. boninense*. Utilising *B. subtilis* strain MN704394.1 as a biocidal agent holds significant promise, not only due to its capacity to produce bioactive compounds but also because these compounds exhibit robust antifungal properties.

This approach effectively suppresses *G. boninense*, the causative agent of basal stem rot disease in oil palm plants. The study underscores the potential of harnessing microbial resources for sustainable biological pest control in agriculture, with *B. subtilis* strain MN704394.1 as a promising candidate for eco-friendly fungal disease management.

ACKNOWLEDGEMENT

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THE USE OF FACTORIAL MATING DESIGN FOR ESTIMATION OF COMBINING ABILITIES IN COMMERCIAL OIL PALMS

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ABSTRACT

This study aimed to identify the parental palms with high mean and general combining ability (GCA), and the progenies with high mean and specific combining ability (SCA) for bunch yield and bunch component traits. A total of 30 crosses were made from the elite palms of six duras *and five* pisiferas *using a factorial mating design. The progenies were transplanted in a strip-plot experimental design with three replicates. Each plot had six palms of a cross, thus making a total of 540 palms. The data on bunch yield and components were collected from 2012 to 2022. The results showed that R10/1D and R5/21P had good means and GCA for fresh fruit bunches (FFB) and bunch number (BNO) while R8/9D showed significant GCA for oil to bunch, oil yield and kernel yield. R10/5D and KA17/2P expressed high GCA for kernel to bunch and kernel yield. The cross R10/1D × R9/8P showed significant SCA for FFB (mean 262.97 kg palm^{-1} yr^{-1}) and BNO (mean 20.81 bunches palm^{-1} yr^{-1}), while R15/14D × KA17/2P for average bunch weight (mean 18.57 kg bunch^{-1}). A43/9D × R9/8P had the best SCA for oil yield (mean 82.24 kg palm^{-1} yr^{-1}), and R10/5D × KA17/2P for kernel yield (mean 18.15 kg palm^{-1} yr^{-1}).*

Keywords: *dura*, general combining ability, *pisifera*, specific combining ability, *tenera.*

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INTRODUCTION

Oil palm is the most significant oil crop worldwide. It originally came from Africa but has been widely grown in Southeast Asia, mainly in Indonesia, Malaysia, and Thailand (Corley & Tinker, 2016). Palm oil is derived from the oil palm fruits and is the primary source of vegetable oil globally. USDA (2023) estimated that the vegetable oil production and consumption would reach 88.60 and 86.87 million tonnes by 2023, while the demand for vegetable oil would increase to 240 million tonnes by 2050. The oil palm industry has expanded by increasing planted areas from 10.57 to 21.03 million hectares (FAOSTAT, 2023), causing deforestation and air pollution. However, high-yielding oil palm planting materials have been developed and used to reduce the expansion of oil palm planted areas (Arolu et al., 2016; Gingold et al., 2012; Rajanaidu et al., 2000). Oil palm germplasm is classified based on the shell gene, which controls the thickness of the shell and the presence of a fibre ring in the mesocarp. The *dura* type has a thick shell and thin mesocarp (genotype sh^+sh^+), the *pisifera* type has no shell (genotype sh^-sh^-), and the *tenera* type with a thin shell and thick mesocarp with a fibre ring (genotype sh^+sh^-). Several studies have supported

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that the *tenera* type is more productive than the other two types (Arolu et al., 2016; Corley & Tinker, 2016; Soh et al., 2017).

The ability of a parent (*dura* or *pisifera*) that can give relatively superior or inferior progenies, when crossed with the other parents, is termed as general combining ability (GCA). A parent considered as having high GCA for a certain trait, can also have low GCA for the other traits. Another ability to be considered during the parental selection for hybrid production is specific combining ability (SCA). It is the ability of two parents that produce a cross with superior or inferior performance, when compared with the other crosses of the same experiment (Sprague & Tatum, 1942). GCA is conditioned mainly by the additive gene action, while SCA is conditioned by the non-additive ones (Griffing, 1956). Determining the GCA of each clone is a valuable tool for the plant breeder to select the *dura* or *pisifera* parents that yield superior progenies in general, whereas determining SCA helps to identify the crosses that produce desirable *tenera* for the trait.

There are several ways to estimate GCA and SCA of the genotypes and hybrids. However, plant breeders often used mating designs, especially the nested, factorial, and backcross designs (Comstock & Robinson, 1952). These designs are also known as the North Carolina Mating Design, NCM I, II, and III, respectively. The mating designs are not commonly used in oil palm breeding, partly due to the difficulty in preparing the progenies for testing. Arolu et al. (2016) used NCM I to estimate the GCA of 10 *pisifera* palms by pollinating each palm to 2-3 *duras*. Clearly that nested design can be used to estimate GCA and variance in only one side of the parents, such as the male side (*pisifera*) above. To estimate GCA (as well as genetic variation) for both male and female parents, the factorial mating design (NCM II) is usually employed. The factorial design can also reveal information for SCA of each cross combination and lead to the selection of particular parents for commercial production of superior *tenera* palm.

This study is set up to estimate the GCA of the selected *dura* and *pisifera* palms, as well as the SCA of their progenies (*tenera* palms) for bunch yield and bunch components. The parental palms would be chosen for further improvement and the best cross combination would be used for commercial production.

MATERIALS AND METHODS

Plant Materials

Thirty oil palm crosses (T11-T65) were created using a factorial mating design (North Carolina Mating Design II, NCM II) (Comstock & Robinson, 1952). There were six *dura* (female) parents (D1-D6) and five *pisifera* (male) parents (P1-P5) as shown in *Table 1.* The parents were elite palms developed during 1985–2005 by Golden Tenera Company Limited located in Krabi Province, Thailand. To obtain the clean crosses, the pollination was rigorously controlled as in the seed production steps. The female inflorescences from the *dura* palms were bagged and the male inflorescences were removed before flowering. The pollen was collected from the *pisifera* palms and refrigerated, awaiting for hand-pollination onto the intended female flowers. The pollinated inflorescence was then covered by a pollination bag, and sprayed with an insecticide to prevent possible insect pollination. The mature fruits from each bunch were processed for seeds and kept refrigerated. Germination was done when seeds from all 30 crosses were available. Before transplanting, the SSR markers were determined on the seedlings to confirm the parental genotypes were inherited in the *tenera*, by using the method described by Taeprayoon et al. (2015, 2016). The ripening fruits of the progenies were also regularly observed for shell thickness and the presence of a fibre ring during the study.

The location of the breeding farm is around 8° 14′ 0.16″ N latitude and 98° 47′ 28.69″ E longitude. The progenies were transplanted in a strip-plot (split-block) experimental design with three replications (LeClerg et al., 1966; Petersen, 1994). Each plot comprised six palms of a cross, resulting in a total of 540 palms in the experiment. All palms in the trial were transplanted in May 2009 using an equilateral triangular pattern (9 × 9 × 9 m) in a total experimental area of slightly over 4 ha.

Data Collection

Phenotypic data were collected from bunch yield and bunch components. Bunch yield comprised fresh fruit bunches (FFB) (kg palm^{-1} yr^{-1}), bunch number (BNO) (bunches palm^{-1} yr^{-1}), and average bunch weight (ABW) (kg bunch^{-1}). These traits were observed from individual palms beginning from two years and eight months after planting, twice a month for 11 consecutive years, from 2012 to 2022. Each bunch was harvested when a detached mature fruit from the bunch was found on the ground. Bunch components were determined following the method described by Rao et al. (1983). The bunches were taken for component analyses when the fruit skin was changing from black to reddish or orange during the ripening stage, generally 20–22 weeks after pollination. The data were collected by sampling a single bunch from each plot to determine the oil to bunch (OTB) (%) and the kernel to bunch (KTB) (%). Oil content was presented as oil yield

(OY) and kernel yield (KY) recorded in kg palm^{-1} yr^{-1}. Bunch analyses were performed between May 2017 to October 2018, when the palms were 8–9 years old. The data were collected on two bunches per cross, totaling 180 bunches in the experiment.

Statistical Analysis

The statistical model of an observation depicting the effects of *dura*, *pisifera*, and *tenera* (*dura* × *pisifera*) is given as Equation (1):

$$Y_{ijk} = \mu + d_i + p_j + (dp)_{ij} + e_{ijk} \qquad (1)$$

where, Y_{ijk} is the k^{th} observation on the $(i \times j)^{th}$ *tenera*, μ is the overall mean, d_i is the effect of the i^{th} *dura* parent, p_j is the effect of the j^{th} *pisifera* parent, $(dp)_{ij}$ is the interaction (specific) effect of the cross $d_i \times p_j$ and e_{ijk} is the error associated with each observation.

Each trait was subjected to an analysis of variance (ANOVA) as shown in *Table 2*. The trait means were compared by Duncan's multiple range test (DMRT). The GCA of each parent and SCA of each cross were determined and tested for significance against their standard error of estimates (Griffing, 1956). All the analyses were accomplished using the R-Stat Program (R Core Team, 2022).

RESULTS AND DISCUSSION

Analysis of Variance

The analysis of variance (ANOVA) revealed significant differences among the crosses in all traits as shown in *Table 3*. The significance of the parental effects (D and P) and their progenies (D × P) were also detected in all traits, except only for BNO in the progenies. This information revealed that the elite parental palms used in this experiment were diverse and should be investigated for combining abilities.

TABLE 1. FACTORIAL MATING USING SIX *Dura* PALMS (D1-D6) AND FIVE *Pisifera* PALMS (P1-P5) EXPRESSED AS CODE NAMES OF *Tenera*s

D×P	P1: R5/21P	P2: R3/8P	P3: R16/7P	P4: R9/8P	P5: KA17/2P
D1: R15/14D	T11	T12	T13	T14	T15
D2: A43/9D	T21	T22	T23	T24	T25
D3: R10/5D	T31	T32	T33	T34	T35
D4: R10/1D	T41	T42	T43	T44	T45
D5: A1/2D	T51	T52	T53	T54	T55
D6: R8/9D	T61	T62	T63	T64	T65

TABLE 2. AN ANALYSIS OF VARIANCE FOR FACTORIAL PROGENIES GROWN IN STRIP PLOT IN A RANDOMISED COMPLETE BLOCK DESIGN

Sources of variation (SOV)	Degrees of freedom (df)	Mean squares (MS)
Replications	r-1	MS_R
Crosses	dp-1	MS_C
Dura	d-1	MS_D
Pisifera	p-1	MS_P
Dura×*Pisifera*	(d-1)(p-1)	MS_{DP}
Error	(r-1)(dp-1)	MS_E
Total	**rdp-1**	

Notes: r - number of replication; d - number of *dura* parents; p - number of *pisifera* parents.

TABLE 3. MEAN SQUARES OF FFB, BNO, ABW, OTB, KTB, OY, AND KY OF 30 OIL PALM FACTORIAL PROGENIES, OBSERVED DURING 2012-2022

Sources of variation (SOV)	df	FFB	BNO	ABW	OTB	KTB	OY	KY
Replications	2	1588.60*	9.27**	0.53^{ns}	0.35^{ns}	0.281^{ns}	156.00*	7.01^{ns}
Crosses	29	1509.10**	21.28**	12.71**	15.73**	5.142**	169.30**	27.53**
Dura (D)	5	2385.90**	60.74**	18.67**	7.86*	7.478**	276.30**	25.16**
Pisifera (P)	4	2358.90**	68.78**	46.93**	48.77**	18.905**	133.00*	92.99**
D×P	20	1120.00**	1.92^{ns}	4.37**	11.09**	1.806**	149.80**	15.03**
Error	58	324.80	1.73	0.48	2.43	0.693	37.20	4.64
CV (%)		**8.14**	**8.04**	**5.01**	**5.16**	**17.97**	**9.14**	**20.98**

Note: FFB - fresh fruit bunches; BNO - bunch number; ABW - average bunch weight; OTB - oil to bunch; KTB - kernel to bunch; OY - oil yield; KY - kernel yield; * - significant difference at $p \leq 0.05$; ** - highly significant difference at $p \leq 0.01$; ns - non-significant.

Estimated Mean and GCA for Bunch Yield of the Parental Palms

Among the parental *dura*, R10/1D showed the highest contribution to FFB of the *tenera* at 235.67 kg palm^{-1} yr^{-1}, with a significant GCA of 14.28 kg palm^{-1} yr^{-1} (*Table 4*). Thus R10/1D showed the potential in increasing FFB in the *tenera* by the average of 14.28 kg palm^{-1} yr^{-1} when crossed with this elite *pisifera* set. In contrast, the *dura* A1/2D showed the lowest contribution to FFB of 198.09 kg palm^{-1} yr^{-1} and a negative GCA of -23.30 kg palm^{-1} yr^{-1}, meaning that this *dura* would give the progenies with the lowest FFB yield when crossed with this set of *pisifera*. R10/1D also gave the highest average BNO of 18.61 bunches palm^{-1} yr^{-1} and a significant GCA of 2.25 bunches palm^{-1} yr^{-1}. However, R10/1D had the smallest bunch of 12.76 kg bunch^{-1} with the GCA of -1.07 kg bunch^{-1}, implying that this *dura* would contribute to relatively smaller bunches to the progenies when crossed by this *pisifera* set. This is possibly due to the negative relationship between BNO and ABW (Tanya *et. al.*, 2013) as generally observed in most oil palm estates. Another promising *dura* is A43/9D which contributed to high BNO, giving the mean and GCA of 17.85 and 1.49 bunches palm^{-1} yr^{-1}, respectively.

For the *pisifera* side, R5/21P showed a contribution to the FFB of 233.82 kg palm^{-1} yr^{-1} with the GCA of 12.43 kg palm^{-1} yr^{-1}. It also contributed to high mean and GCA for BNO (17.63 and 1.27 bunches palm^{-1} yr^{-1}, respectively) (*Table 4*). However, R5/21P would give slightly smaller bunches with a negative GCA (13.40 and -0.43 kg bunch^{-1}) when crossed to this *dura* set. Another promising *pisifera* is R3/8P with the contribution to the average FFB yield of 232.19 and GCA of 10.80 kg palm^{-1} yr^{-1}. This male parent showed no contribution to BNO as its GCA for the trait was not significant. However, it would increase ABW on the average of 0.45 kg bunch^{-1} if crossed to these elite *duras*. Among the rest three *pisiferas*, R16/7P gave poor progenies for all three traits, R9/8P is a good contributor for high BNO, and KA17/2P is good for increasing the bunch size of the progenies.

Mean and SCA for Bunch Yield of the Crosses

The progenies showed the mean values for FFB, BNO, and ABW of 221.39 kg palm^{-1} yr^{-1}, 16.36 bunches palm^{-1} yr^{-1}, and 13.83 kg bunch^{-1}, respectively (*Table 5*). The crosses produced FFB from 179.82 kg palm^{-1} yr^{-1} in A1/2D × KA17/2P (T55) to 262.97 kg in R10/1D × R9/8P (T44). T44 also gave the highest SCA of 28.95 kg palm^{-1} yr^{-1}. The second highest yielding cross was R8/9 × R5/21 (T61) with an average yield of 252.40 kg palm^{-1} yr^{-1}. Another good cross giving high FFB was R10/1D × R16/7P (T43) (yield 245.18, SCA 23.23 kg palm^{-1} yr^{-1}). The FFB of these *teneras* were all higher than the selection criteria set by Malaysian Standard (SIRIM) MS 157 at 170 kg palm^{-1} yr^{-1} (Kushairi et al., 2011). The range for average BNO was from 10.29 bunches palm^{-1} yr^{-1} in T55 to 20.81 in T44. Whereas T43 gave 19.79 bunches palm^{-1} yr^{-1} with a significant SCA of 1.41 bunches palm^{-1} yr^{-1}. ABW of the crosses ranged from 10.75 kg bunch^{-1} in R10/5D × R9/8P (T34) to 18.57 kg bunch^{-1} in R15/14D × KA17/2P (T15). T15 also had a significant SCA of 1.52 kg bunch^{-1}, while T34 gave a negative SCA of -1.06 kg bunch^{-1} that contributed to the cross (*Table 5*).

TABLE 4. ESTIMATED MEAN AND GCA OF THE *Dura* AND *Pisifera* PARENTS FOR FFB, BNO AND ABW, OBSERVED DURING 2012-2022

Dura	**FFB**	**GCA**	**BNO**	**GCA**	**ABW**	**GCA**
R15/14D	222.08	0.69	15.58^{c}	-0.78*	14.53^{b}	0.70*
A43/9D	225.19	3.80	17.85^{a}	1.49**	12.68^{d}	-1.15**
R10/5D	220.25	-1.14	16.61^{b}	0.25	13.54^{c}	-0.29
R10/1D	235.67	14.28*	18.61^{a}	2.25**	12.76^{d}	-1.07**
A1/2D	198.09	-23.30**	12.87^{d}	-3.49**	15.61^{a}	1.78**
R8/9D	227.07	5.68	16.65^{b}	0.29	13.86^{c}	0.03
F-test	**ns**		**		**	
Pisifera	**FFB**	**GCA**	**BNO**	**GCA**	**ABW**	**GCA**
R5/21P	233.82	12.43*	17.63^{a}	1.27**	13.40^{c}	-0.43*
R3/8P	232.19	10.80*	16.50^{b}	0.14	14.28^{b}	0.45*
R16/7P	207.67	-13.72*	16.13^{b}	-0.23	13.02^{c}	-0.81**
R9/8P	219.75	-1.64	18.31^{a}	1.95**	12.10^{d}	-1.73**
KA17/2P	213.51	-7.88	13.23^{c}	-3.13**	16.36^{a}	2.53**
F-test	**ns**		**		**	

Note: FFB - fresh fruit bunches; GCA - general combining ability; BNO - bunch number; ABW - average bunch weight; within a column, means followed by the same letters are not significantly different according to DMRT ($p \leq 0.05$);* - significant at $p \leq 0.05$; ** - highly significant at $p \leq 0.01$; ns - non-significant.

TABLE 5. TRAIT MEANS AND SCA OF 30 *Teneras* FOR FFB, BNO AND ABW, OBSERVED DURING 2012-2022

Crosses	*Dura* × *Pisifera*	FFB	SCA	BNO	SCA	ABW	SCA
T11	R15/14D × R5/21P	244.28^{a-e}	9.77	17.54^{b-g}	0.70	13.92^{e-i}	-0.17
T12	R15/14D × R3/8P	235.34^{a-f}	2.46	15.09^{g-l}	-0.63	15.60^{c}	0.63
T13	R15/14D × R16/7P	185.33^{hij}	-23.03*	14.28^{j-m}	-1.07	12.99^{h-m}	-0.73
T14	R15/14D × R9/8P	211.63^{e-j}	-8.82	18.36^{a-e}	0.83	11.55^{nop}	-1.25**
T15	R15/14D × KA17/2P	233.81^{a-f}	19.62	12.61^{lmn}	0.16	18.57^{a}	1.52**
T21	A43/9D × R5/21P	216.25^{c-i}	-21.36*	19.13^{a-d}	0.02	11.30^{op}	-0.95*
T22	A43/9D × R3/8P	221.91^{b-g}	-14.07	18.83^{a-e}	0.84	11.82^{m-p}	-1.31**
T23	A43/9D × R16/7P	226.72^{b-g}	15.25	16.97^{d-i}	-0.65	13.38^{f-k}	1.52**
T24	A43/9D × R9/8P	247.80^{a-d}	24.25*	18.67^{a-e}	-1.12	13.27^{f-k}	2.32**
T25	A43/9D × KA17/2P	213.23^{d-j}	-4.07	15.64^{f-k}	0.92	13.62^{f-j}	-1.59**
T31	R10/5D × R5/21P	249.24^{abc}	16.56	17.72^{b-f}	-0.15	14.06^{d-h}	0.96*
T32	R10/5D × R3/8P	237.86^{a-f}	6.82	16.89^{d-i}	0.14	14.08^{d-h}	0.10
T33	R10/5D × R16/7P	204.54^{f-j}	-1.99	17.26^{b-h}	0.88	11.91^{l-p}	-0.81
T34	R10/5D × R9/8P	182.41^{ij}	-36.2**	17.69^{b-f}	-0.87	10.75^{p}	-1.06*
T35	R10/5D × KA17/2P	227.17^{b-g}	14.81	13.46^{klm}	-0.01	16.87^{b}	0.81
T41	R10/1D × R5/21P	226.23^{b-g}	-21.86*	19.57^{abc}	-0.31	11.55^{nop}	-0.77
T42	R10/1D × R3/8P	230.93^{a-g}	-15.53	18.14^{b-f}	-0.61	12.73^{i-n}	-0.47
T43	R10/1D × R16/7P	245.18^{a-e}	23.23*	19.79^{ab}	1.41*	12.42^{j-o}	0.48
T44	R10/1D × R9/8P	262.97^{a}	28.95*	20.81^{a}	0.25	12.62^{i-n}	1.59**
T45	R10/1D × KA17/2P	213.00^{d-j}	-14.78	14.74^{i-l}	-0.74	14.45^{c-g}	-0.84*
T51	A1/2D × R5/21P	214.51^{c-j}	4.00	14.05^{j-m}	-0.08	15.28^{cd}	0.10
T52	A1/2D × R3/8P	218.22^{b-h}	9.34	12.99^{lm}	-0.01	16.81^{b}	0.76
T53	A1/2D × R16/7P	181.76^{ij}	-2.60	12.05^{mn}	-0.59	15.10^{cde}	0.3
T54	A1/2D × R9/8P	196.10^{g-j}	-0.35	14.94^{h-l}	0.13	13.19^{g-l}	-0.69
T55	A1/2D × KA17/2P	179.82^{j}	-10.38	10.29^{n}	0.56	17.67^{ab}	-0.47
T61	R8/9D × R5/21P	252.40^{ab}	12.90	17.73^{b-f}	-0.18	14.25^{d-h}	0.82
T62	R8/9D × R3/8P	248.85^{a-d}	10.98	17.06^{c-i}	0.27	14.59^{c-f}	0.29
T63	R8/9D × R16/7P	202.49^{f-j}	-10.86	16.44^{e-j}	0.03	12.28^{k-o}	-0.76
T64	R8/9D × R9/8P	217.60^{b-h}	-7.83	19.37^{a-d}	0.78	11.22^{op}	-0.91*
T65	R8/9D × KA17/2P	213.98^{c-j}	-5.20	12.62^{lmn}	-0.89	16.95^{b}	0.57
Mean		**221.39**		**16.36**		**13.83**	
%CV		**8.14**		**8.04**		**5.01**	

Note: FFB - fresh fruit bunches; SCA - specific combining ability; BNO - bunch number; ABW - average bunch weight; within a column, means followed by the same letters are not significantly different according to DMRT ($p \leq 0.05$); * - significant different at $p \leq 0.05$; ** - highly significant different at $p \leq 0.01$.

Estimated Mean and GCA for Bunch Components of the Parental Palms

In bunch components including OTB, KTB, OY, and KY, the *dura* parents were less diverse than the *pisifera* parents as they were significantly different in only KTB. R8/9D had the highest OTB of 31.59%, with a significant GCA of 1.39%. It gave an average OY of 71.29 and KY of 11.47 kg palm^{-1} yr^{-1}, with positive GCA contributing to both traits (*Table 6*). Another promising *dura*, R10/5D, was a good contributor for KTB and KY as it showed significant positive GCAs in both traits. The *pisifera* palms were more diverse as they were different in all bunch components, except OY. R16/7P and R9/8P performed well in OTB but not in OY, due mainly to their low FFB. KA17/2P was the best combiner for improving KTB and KY on the *pisifera* side.

Mean and SCA for Bunch Components of the Crosses

The means and ranges of OTB, KTB, OY and KY of the crosses were 30.20% (25.94%–33.89%), 4.63% (2.71%–7.98%), 66.74 kg palm^{-1} yr^{-1} (50.41–82.24 kg palm^{-1} yr^{-1}) and 10.26 kg palm^{-1} yr^{-1} (5.73–18.15 kg palm^{-1} yr^{-1}), respectively (*Table 7*). The crosses A43/9D × R16/7P (T23) and A43/9D × R9/8P (T24) were superior in bunch components as they showed

TABLE 6. ESTIMATED MEAN AND GCA OF THE *Dura* AND *Pisifera* PARENTS FOR OTB, KTB, OY AND KY, OBSERVED DURING 2012-2022

Dura	OTB	GCA	KTB	GCA	OY	GCA	KY	GCA
R15/14D	29.63	-0.58	4.19^{b}	-0.45*	65.59	-1.15	9.50	-0.77
A43/9D	30.28	0.08	3.76^{b}	-0.87**	68.38	1.65	8.46	-1.81**
R10/5D	30.14	-0.07	5.21^{a}	0.58*	66.25	-0.49	11.75	1.49*
R10/1D	29.72	-0.49	4.10^{b}	-0.53*	69.79	3.05	9.54	-0.72
A1/2D	29.86	-0.34	5.51^{a}	0.88**	59.14	-7.61**	10.88	0.61
R8/9D	31.59	1.39**	5.02^{a}	0.39	71.29	4.55*	11.47	1.20*
F-test	ns		**		ns		ns	
Pisifera	**OTB**	**GCA**	**KTB**	**GCA**	**OY**	**GCA**	**KY**	**GCA**
R5/21P	27.57^{c}	-2.63**	4.89^{b}	0.26	64.47	-2.27	11.49^{b}	1.23*
R3/8P	30.23^{b}	0.03	4.46^{b}	-0.17	70.28	3.54*	10.41^{b}	0.14
R16/7P	31.69^{a}	1.49**	4.36^{b}	-0.27	65.81	-0.94	9.03^{c}	-1.24*
R9/8P	31.50^{a}	1.29*	3.31^{c}	-1.32**	68.92	2.18	7.24^{d}	-3.03**
KA17/2P	30.02^{b}	-0.18	6.14^{a}	1.51**	64.24	-2.50	13.17^{a}	2.91**
F-test	*		**		ns		**	

Note: OTB - oil to bunch; GCA - general combining ability; KTB - kernel to bunch; OY - oil yield; KY - kernel yield; within a column, means followed by the same letters are not significantly different according to DMRT ($p \le 0.05$); * - significant difference at $p \le 0.05$; ** - highly significant difference at $p \le 0.01$; ns - non-significant.

well-balanced in all four components with mainly positive SCA effects. R8/9D × R16/7P (T63), although gave the highest mean and SCA in OTB (33.89% and 0.82%), showed low means and/or negative SCA for the other components. Thus, T23 and T24 are good choices for commercial production of *tenera* clones with superior bunch components. Some crosses can be chosen to produce specific components such as the cross R10/5D × KA17/2P (T35) that gave the highest mean in both KTB (7.98%) and KY (18.15 kg palm^{-1} yr^{-1}). T35 expressed high positive SCA in both components.

Discussion

To the available information, this study was the first to use a factorial mating design (also known as North Carolina Mating Design II, NCM II) to create a population of *dura* × *pisifera* (D × P) in oil palm. Although Constantin et al. (2016) evaluated combining ability and genetic variance in introgressed Cameroon oil palms using NCM II, the authors used six *dura* female palms and six *tenera* male palms to produce only 21 (rather than 36) progenies. This was considered an incomplete factorial mating with limited use in a breeding program. They managed to analyse for GCA of the parents and SCA of the crosses without demonstrating the mean trait values for us to compare. Unlike these D × P, their D × T progenies were still segregating in shell thickness giving 1/2 $sh^{+}sh^{+}$ (thick shell *dura*) and 1/2 $sh^{+}sh^{-}$ (thin shell *tenera*), thus requiring further selection and could not be used as a new cultivar.

Noh et al. (2012) used a nested mating design (NCM I) to investigate GCA in a set of *pisifera* parents. This mating design led to the investigation of trait means and GCA on one side of the parents (*pisifera* in their case) because the other parents (*dura*) were randomly taken from the population. The data collected from their progenies during 1998–2004 showed that the estimated values of FFB in their *pisifera* ranged from 121.93–143.5 kg palm^{-1} yr^{-1} with the mean of 131.62 kg palm^{-1} yr^{-1}, BNO ranged from 8.00–9.46 bunches palm^{-1} yr^{-1} with the mean of 8.66 bunches palm^{-1} yr^{-1}, and ABW ranged from 14.40–17.27 kg bunch^{-1} with the mean 15.60 kg bunch^{-1}. The estimated values of five *pisiferas* used in this study (*Table 4*) were higher in FFB and BNO but less ABW than those reported by Noh et al. (2012). The *pisifera* OTB in the current study had an estimated average of 30.20%, ranging from 27.57%–31.69% (*Table 6*), which was higher than their report at the average of 24.91% and range of 23.33%–26.50%. The *pisifera* in this study tended to have less KTB at the range of 3.31%–6.14% compared to their range of 4.86%–6.25%. The *pisifera* in this study had the highest GCA for FFB at 12.43 kg palm^{-1} yr^{-1} (*Table 4*), similar to the highest GCA of Noh et al. (2012) at 12.28 kg palm^{-1} yr^{-1}. The highest GCA for OTB were also comparable, 1.49% in the current study vs. 1.59% in the study by Noh et al. (2012). The highest GCA for KTB in this experiment was 1.51% (*Table 6*) compared to their GCA at 0.57%. Noh et al. (2012) could not analyse the GCA of the *dura* parents nor the SCA of the crosses as the mating design does not allow them to do so.

Arolu et al. (2016) used NCM I to help select good combiners in a *pisifera* set based on data collected from 2001–2014. The palm progenies were obtained from 10 Nigerian *pisiferas* randomly crossed to 24

TABLE 7. TRAIT MEANS AND SCA OF 30 *Teneras* FOR OTB, KTB, OY AND KY, OBSERVED DURING 2012-2022

Crosses	*Dura* × *Pisifera*	OTB	SCA	KTB	SCA	OY	SCA	KY	SCA
T11	R15/14D × R5/21P	25.94^{l}	-1.05	$4.56^{c\text{-}j}$	0.12	$63.31^{d\text{-}h}$	0.00	$11.17^{b\text{-}g}$	0.45
T12	R15/14D × R3/8P	$30.60^{b\text{-}i}$	0.95	$4.39^{d\text{-}j}$	0.38	$72.03^{a\text{-}d}$	2.90	$10.33^{b\text{-}h}$	0.70
T13	R15/14D × R16/7P	$30.25^{c\text{-}i}$	-0.86	3.12^{jk}	-0.79*	56.06^{hi}	-8.60*	5.80^{i}	-2.46*
T14	R15/14D × R9/8P	$30.81^{b\text{-}h}$	-0.11	3.17^{ijk}	0.31	$65.29^{c\text{-}h}$	-2.47	6.73^{hi}	0.26
T15	R15/14D × KA17/2P	$30.51^{c\text{-}i}$	1.07	5.68^{bcd}	-0.01	$71.26^{a\text{-}e}$	8.17*	13.45^{bcd}	1.05
T21	A43/9D × R5/21P	$28.37^{g\text{-}l}$	0.73	3.17^{ijk}	-0.85*	$61.22^{d\text{-}i}$	-4.88	6.90^{ghi}	-2.78*
T22	A43/9D × R3/8P	$29.03^{e\text{-}k}$	-1.27	3.19^{ijk}	-0.40	$64.41^{c\text{-}h}$	-7.51*	7.10^{ghi}	-1.50
T23	A43/9D × R16/7P	33.63^{ab}	1.87*	$4.21^{d\text{-}k}$	0.73	76.25^{abc}	8.80*	$9.61^{d\text{-}i}$	2.40*
T24	A43/9D × R9/8P	33.25^{abc}	1.69*	3.42^{ijk}	0.98*	82.24^{a}	11.68**	$8.51^{e\text{-}i}$	3.08**
T25	A43/9D × KA17/2P	27.07^{jkl}	-3.02**	$4.81^{c\text{-}i}$	-0.46	57.79^{ghi}	-8.09*	$10.17^{b\text{-}h}$	-1.19
T31	R10/5D × R5/21P	$30.71^{b\text{-}h}$	3.21**	$5.32^{b\text{-}g}$	-0.14	76.49^{abc}	12.53**	13.21^{bcd}	0.24
T32	R10/5D × R3/8P	$28.60^{f\text{-}l}$	-1.57*	5.71^{bcd}	0.67	$67.98^{b\text{-}h}$	-1.80	13.59^{bcd}	1.70
T33	R10/5D × R16/7P	$28.99^{e\text{-}k}$	-2.63**	$3.92^{f\text{-}k}$	-1.02*	$59.36^{e\text{-}i}$	-5.95	8.06e-i	-2.45*
T34	R10/5D × R9/8P	$32.33^{a\text{-}d}$	0.91	3.11^{jk}	-0.78*	$59.23^{e\text{-}i}$	-9.19*	5.73i	-2.99*
T35	R10/5D × KA17/2P	$30.04^{d\text{-}j}$	0.08	7.98^{a}	1.26**	$68.15^{b\text{-}h}$	4.42	18.15a	3.50**
T41	R10/1D × R5/21P	$27.61^{i\text{-}l}$	0.53	$4.21^{d\text{-}k}$	-0.14	$62.57^{d\text{-}h}$	-4.94	9.64d-i	-1.13
T42	R10/1D × R3/8P	$30.62^{b\text{-}i}$	0.88	$3.63^{h\text{-}k}$	-0.29	$70.72^{a\text{-}f}$	-2.60	8.39e-i	-1.28
T43	R10/1D × R16/7P	$30.94^{a\text{-}g}$	-0.25	$4.39^{d\text{-}j}$	0.57	75.71^{abc}	6.86*	$10.70^{b\text{-}h}$	2.41*
T44	R10/1D × R9/8P	$27.81^{h\text{-}l}$	-3.19**	2.71^{k}	-0.06	$72.75^{a\text{-}d}$	0.79	7.16^{ghi}	0.64
T45	R10/1D × KA17/2P	$31.57^{a\text{-}f}$	2.04*	$5.53^{b\text{-}f}$	-0.07	$67.16^{c\text{-}h}$	-0.12	$11.81^{b\text{-}f}$	-0.64
T51	A1/2D × R5/21P	26.19^{kl}	-1.04	6.45^{b}	0.69	56.21^{hi}	-0.64	13.84^{bcd}	1.74
T52	A1/2D × R3/8P	$30.48^{c\text{-}i}$	0.59	$4.59^{c\text{-}j}$	-0.75	$66.63^{c\text{-}h}$	3.96	$9.96^{c\text{-}i}$	-1.05
T53	A1/2D × R16/7P	$32.40^{a\text{-}d}$	1.05	6.51^{b}	1.28**	$58.95^{f\text{-}i}$	0.75	$11.89^{b\text{-}e}$	2.26*
T54	A1/2D × R9/8P	$32.34^{a\text{-}d}$	1.18	$3.86^{g\text{-}k}$	-0.32	$63.46^{d\text{-}h}$	2.15	$7.60^{f\text{-}i}$	-0.25
T55	A1/2D × KA17/2P	$27.90^{g\text{-}l}$	-1.79*	6.12^{bc}	-0.90*	50.41^{i}	-6.22	$11.08^{b\text{-}g}$	-2.70*
T61	R8/9D × R5/21P	26.58^{kl}	-2.38**	$5.59^{b\text{-}e}$	0.32	$66.95^{c\text{-}h}$	-2.06	14.17^{bc}	1.48
T62	R8/9D × R3/8P	$32.04^{a\text{-}e}$	0.42	$5.23^{b\text{-}h}$	0.39	79.87^{ab}	5.05	13.03^{bcd}	1.43
T63	R8/9D × R16/7P	33.89^{a}	0.82	$3.98^{e\text{-}k}$	-0.76	$68.48^{b\text{-}g}$	-1.87	$8.06^{e\text{-}i}$	-2.16*
T64	R8/9D × R9/8P	$32.41^{a\text{-}d}$	-0.47	3.56^{ijk}	-0.13	$70.50^{a\text{-}f}$	-2.96	$7.70^{e\text{-}i}$	-0.74
T65	R8/9D × KA17/2P	26.58^{kl}	1.61*	6.71^{ab}	0.19	$70.62^{a\text{-}f}$	1.84	14.35^{b}	-0.02
Mean		**30.20**		**4.63**		**66.74**		**10.26**	
%CV		**5.16**		**17.97**		**9.14**		**20.98**	

Note: OTB - oil to bunch; SCA - specific combining ability; KTB - kernel to bunch; OY - oil yield; KY - kernel yield; within a column, means followed by the same letters are not significantly different according to DMRT ($p \leq 0.05$); * - significant difference at $p \leq 0.05$; ** - highly significant difference at $p \leq 0.01$.

Deli *duras*. They found FFB, BNO, ABW, OTB, KTB, OY and KY ranging from 173.80–211.46 kg palm^{-1} yr^{-1} (average 191.92 kg palm^{-1} yr^{-1}), 15.29–18.88 bunches palm^{-1} yr^{-1} (average 16.71 bunches palm^{-1} yr^{-1}), 10.28–12.79 kg bunch^{-1} (average 11.53 kg bunch^{-1}), 25.15%–29.41% (average 27.58%), 3.00%–5.24% (average 3.94%), 44.06–60.24 kg palm^{-1} yr^{-1} (average 53.72 kg palm^{-1} yr^{-1}) and 5.94–9.39 kg palm^{-1} yr^{-1} (average 7.62 kg palm^{-1} yr^{-1}). The range and average values of their traits were mainly lower than those in this research (*Table 4* and *6*). As usual, the nested mating design allowed them to only examine the merits of the male parents. Arolu et al. (2016) finally identified four *pisifera* palms based on their performances and GCA of FFB, OY and palm height. More comparisons on the results from this experiment with those from the NMC I experiment conducted by Rafii et al. (2002) showed that the calculated values for their *pisifera* parents in OTB, KTB, OY, and KY ranged from 22.25%–23.77% (average 22.73%), 4.67%–5.51% (average 5.15%), 22.10–42.55 kg palm^{-1} yr^{-1} (average 34.37 kg palm^{-1} yr^{-1}) and 5.27–9.74 kg palm^{-1} yr^{-1} (average 7.69 kg palm^{-1} yr^{-1}), respectively. Their OTB, OY and KY were lower than those reported in this study, while KTB was rather comparable (*Table 6*).

In the current study, the *dura* and *pisifera* parents were obtained from selfing a diverse set of commercial *tenera* oil palms. Lim et al. (2003) chose the female parents from D × T and the male parents from T × T based on bunch and fruit traits. Their *dura* and *pisifera* parents showed lower OTB (26.50%) but higher KTB (7.00%) as compared to the materials used in this study (*Table 6*). Teo et al. (2004) conducted progeny testing using a diverse source of *pisifera* originating from Binga (Congo), Ekona (Cameroon), and URT (Ulu Remis *tenera*) hybridised with Deli, African, and African × Deli *dura*s. They reported that FFB, BNO, ABW, OTB, KTB, and OY of the progenies were in the range of 93.0–146.0 kg palm^{-1} yr^{-1}, 12.0–20.2 bunches palm^{-1} yr^{-1}, 6.1–9.3 kg bunch^{-1}, 20.60%–26.40%, 3.70%–6.90% and 21.8–38.6 kg palm^{-1} yr^{-1}, respectively. Their *duras* showed FFB, BNO, and ABW in the range of 101.7–143.3 kg palm^{-1} yr^{-1}, 11.9–19.4 bunches palm^{-1} yr^{-1}, and 6.4–8.5 kg bunch^{-1}, respectively. These values, except for KTB, were generally lower than those of the *dura*s in this study (*Table 6*). Junaidah et al. (2011) evaluated D × P progenies for eight years to measure FFB, BNO, ABW, OTB, KTB, OY and KY. They reported the ranges and means of the traits at 165.74–175.34 kg palm^{-1} yr^{-1} (average 172.54 kg palm^{-1} yr^{-1}), 11.27–13.43 bunches palm^{-1} yr^{-1} (average 12.80 bunches palm^{-1} yr^{-1}), 13.16–14.88 kg bunch^{-1} (average 13.64 kg bunch^{-1}), 26.45%–29.50% (average 28.72%), 3.58%–4.95% (average 4.60%), 6.22–7.20 t ha^{-1} yr^{-1} (average 6.95 t ha^{-1} yr^{-1}) and 0.87–1.15 t ha^{-1} yr^{-1} (average 1.11 t ha^{-1} yr^{-1}), respectively. Their values are generally lower than those of D × P progenies in the current study (*Table 5* and *7*). Marhalil et al. (2013) recorded bunch yield and bunch components of 11 D × P for seven consecutive years. They found the maximum BNO of 16.10 bunches palm^{-1} yr^{-1} which was similar to this study. However, their FFB, ABW, OTB, OY and KY were less than those of the progenies in this study, except for only KTB (*Table 5* and *7*). Arolu et al. (2017) tested 34 D × P progenies for six consecutive years, their FFB and ABW were lower than the current study with rather similar values in BNO. Their results for OTB, KTB, OY and KY were less than, but the maximum of OTB (31.00%) and KTB (5.21%) were similar to the current results. Swaray et al. (2020) performed a study on 24 D × P crosses developed from 10 origins and found that Deli Ulu Remis × Nigeria recorded the highest FFB (184.62 kg palm^{-1} yr^{-1}) and BNO (22.91 bunches palm^{-1} yr^{-1}), while Deli Banting × AVROS had the highest ABW (10.36 kg bunch^{-1}). Their materials had lower FFB and ABW but more BNO as compared to this study. The yield components positively determining FFB are BNO and ABW, however BNO is negatively correlated with ABW (Tanya et al., 2013).

Based on the above results, the current study has made full use of trait means, GCA, and SCA in choosing suitable parents for production of superior *tenera* hybrids and for further genetic improvement. This D × P factorial population has a major advantage over the populations obtained from the other mating designs as it can be used to concurrently estimate the means of traits, GCA of both *dura* and *pisifera* parents and SCA of the progenies. Pictures of fruits and palms of some selected elite parents and crosses are shown in *Figure 1*.

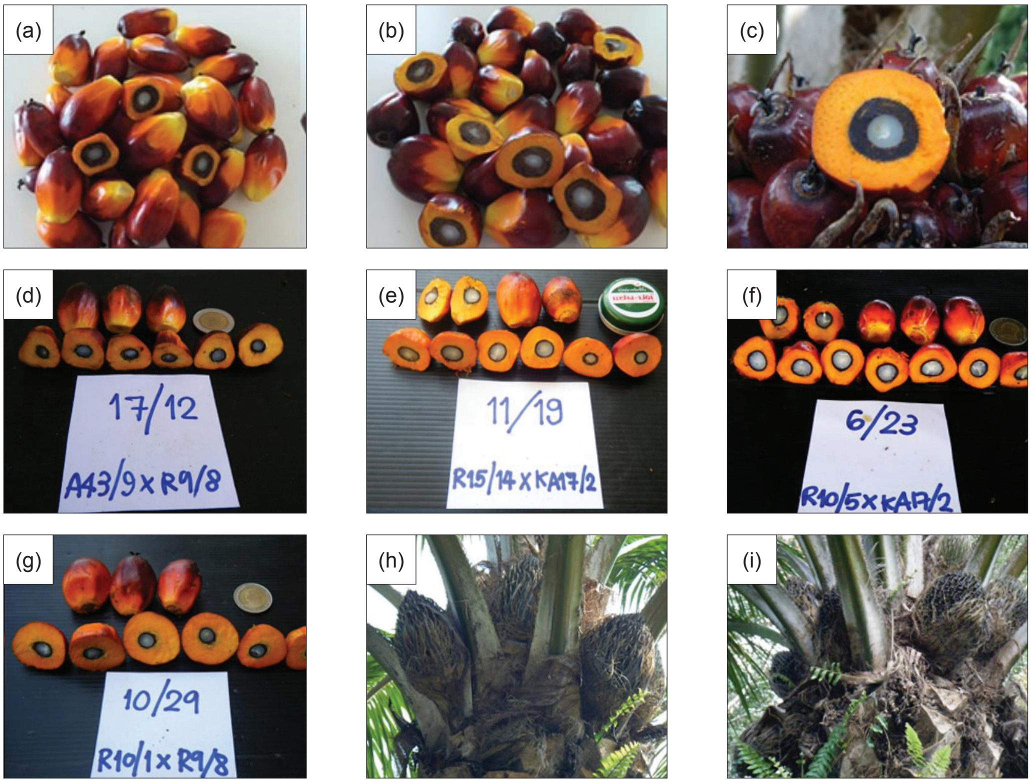

Figure 1. Fruit cross-section of (a) R10/1D, (b) R10/5D, and (c) A43/9D; four elite teneras *(d) A43/9D × R9/8P, (e) R15/14D × KA17/2P, (f) R10/5D × KA17/2P, and (g) R10/1D × R9/8P. Two types of* pisifera *palms were used in this experiment, (h) female sterile type (R5/21P, R16/7P, KA17/2P), and (i) female infertile type (R3/8P, R9/8P).*

CONCLUSION

The use of factorial mating design (NCM II) has several advantages over the other mating designs in oil palm breeding, as it can help select the desirable parental palms based on trait means and GCAs. Yet, it can earmark the superior cross combinations for commercial seed production based on trait means and SCAs of the progenies. The elite parents R10/1D and R5/21P were identified as having good GCA for FFB and BNO, while R8/9D showed high GCA for OTB, OY and KY. R3/8P showed good GCA for FFB and ABW, while A43/9D and R9/8P for BNO. R10/5D and KA17/2P were high in GCA for KTB and KY. These parents were chosen by the company for further improvement. Among the crosses, R10/1D × R9/8P showed significant SCA for FFB, BNO, and ABW, whereas R15/14D × KA17/2P gave high SCA for ABW and OY. A43/9D × R9/8P and R10/5D × KA17/2P had the best SCA for OY and KY, respectively. A43/9D × R9/8P also showed significant SCA in all traits under study, except only for BNO. As the result, superior parental palms were successfully identified for government registration and commercial production.

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EVALUATION OF DIFFERENT SEED DORMANCY BREAKING METHODS INCLUDING ENZYMATIC ASSAYS FOR GERMINATION IMPROVEMENT IN OIL PALM (*Elaeis guineensis* Jacq.)

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ABSTRACT

The oil palm (Elaeis guineensis) *is propagated by seeds for establishment in nurseries and commercial plantations. The seed is naturally dormant; thus, heat treatment is commercially used to alleviate this problem. This study evaluated eight seed dormancy breaking methods (operculum removal, 60 days storage, 60 days 40°C heat treatment, 120 days storage, 60 days storage + 40°C heat treatment, 180 days storage, 120 days storage + 40°C heat treatment and control) on seed germination, based on physical, morphological and physiological dormancy characteristics. Imbibition test indicated that less than 7% mass increment was recorded in all treatments. Germination of more than 82% was obtained for all heat-treated seeds with less than 13 days of mean germination time. The embryo was fully developed at 20 weeks after pollination, but applying heat treatment has accelerated its growth. The heat and storage treatments cause up to 36% reduction in peroxidase and 13% in catalase activities, with 9% (endosperm) and 26% (embryo) increment of α-amylase. It can be suggested that oil palm seeds exhibit a non-deep physiological dormancy, with heat treatment of 40°C as the most effective and practical dormancy-breaking method for commercial seed production.*

Keywords: enzymatic activity, germination improvement, heat treatment, oil palm.

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INTRODUCTION

Oil palm cultivations are mainly propagated through *dura* × *pisifera* hybrid (D×P) seeds (Corley & Tinker, 2015). The seeds are known to be highly dormant and recorded less than 25% germination after eight months of storage (Norsazwan et al., 2016). The use of heat treatment was first suggested by Hussey (1958) and Rees (1962). To date, the technique adopted by the seed producer to break dormancy and induce germination is by using heat treatment, at 40 ± 2°C for 60 days before the germination process (Corley & Tinker, 2015; Department of Standards Malaysia [DOSM], 2017). Since 1962, various alternative dormancy-breaking methods have been attempted, including the adoption of accelerated aging treatment (Murugesan et al., 2005) and extended heat treatment duration (Martine et al., 2009). However, the response does not surpass the standard heat treatment germination record of an average of 68% within 4 months. Recent studies, such as the use of neonicotinoid solution to trigger germination, gave 86% germination (Chanprasert et al., 2012), and stimulation with the electromagnetic field resulted

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in more than 90% germination (Sudsiri et al., 2017). However, these methods were not reproducible and were not practical for implementation at a commercial scale. Darkwah et al. (2021) suggested that there were variations in the germination percentage of oil palm seeds from different progenies, however, the role of heat treatment was found to be crucial in achieving more than 80% germination. The influence of heat on germination is intriguing as to what type of dormancy the oil palm has, and the mechanism that triggers germination. With an average of 68% germination upon heat treatment (Norsazwan et al., 2024), it appears that dormancy is not fully alleviated, or some seeds are not in the right stage to respond accordingly. In addition, germination after heat treatment takes up to 60 days with sporadic germination and spread across this duration. This raises the question of whether the heat treatment was effective in breaking dormancy or, whether a higher germination percentage and speed could be achieved. Seed dormancy is a complex process and can be influenced by various factors. The most systematic dormancy classification system used to date was developed by Baskin and Baskin (2004), where it can be divided into five main categories; physical dormancy (PD), morphological dormancy (MD), physiological dormancy (PYD), morpho-physiological dormancy (MPD) and combinational dormancy. According to Baskin and Baskin (2014), palms normally have MPD, however, dormancy mechanisms and the germination process have not been fully understood in oil palm seeds. To achieve the highest germination percentage, understanding these two processes is vital, since they are closely interrelated and regulated by various factors (Nautiyal et al., 2023).

Seed germination and dormancy are vital components of seed quality; hence, understanding these processes is essential for a sound seed production system. The two processes are closely interrelated and regulated, both by genetic as well as environmental factors. While dormancy provides an inherent mechanism aimed at the survival of the plant species to withstand adverse external conditions by restricting the mature seed from germinating, the ability of the dehydrated seed to remain viable and produce a vigorous seedling upon hydration under favourable conditions is the key to the survival and perpetuation of the plant species. In addition, quality seeds are expected to result in a timely and uniform germination, under favourable field conditions after sowing to establish a healthy crop stand. Therefore, in seed technology, dormancy is not considered a desirable trait to be monitored in the seed lots used for sowing. Thus, to achieve the highest germination percentage, understanding the factors controlling these two interlinked and contrasting processes are vital. In this study, several selected effective dormancy-breaking treatments were used to understand and elucidate the type of dormancy in oil palm seed with the heat treatment as the benchmark.

MATERIALS AND METHODS

Seed Source and Study Site

Freshly harvested *dura* × *pisifera* (D×P) seeds from four controlled pollinated bunches (replicates) of CALIX 600 *dura* mother palm sources were collected from Field PT 100, Sime Darby Research, Banting, Malaysia, at 20 weeks after pollination (WAP). The samples were subjected to standard seed processing methods as shown in *Figure 1*. All the laboratory experiments were conducted at the Seed Technology Laboratory, Faculty of Agriculture, Universiti Putra Malaysia (2° 59′ 15.4″ N, 101° 44′ 03.7″ E).

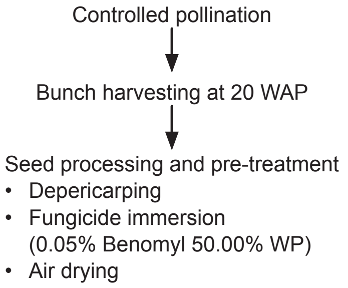


Figure 1. Flow of pollination and processing methods before seed dormancy evaluation.

Seed Treatments

Eight dormancy-breaking treatments, which include the fresh seed as follows: Treatment A – untreated control; Treatment B – operculum removal; Treatment C – ambient temperature (27°C) storage for 60 days; Treatment D – heat treatment (40 ± 2°C) for 60 days; Treatment E – storage at 20°C for 120 days; Treatment F – storage at 20°C for 60 days + heat treatment for 60 days; Treatment G – storage at 20°C for 180 days; and Treatment H – storage at 20°C for 120 days + heat treatment for 60 days.

The operculum removal was performed following Murugesan et al., (2008). The treatments were blocked according to their respective harvesting periods to analyse variations from the blocks.

Seed Imbibition

Four replicates of 20 seeds from each treatment were placed onto a sand medium inside a plastic container and completely submerged in water for 10 days, at 27°C. The seed weight (g) was measured after 0, 6, 12, 24, 48, 96, 192 and 240 hr. The percentage of mass increase over the imbibition period was calculated to determine the water uptake by seeds. To further assess the effect of imbibition on different seed components, the change in moisture levels for both endosperm and embryo tissues were evaluated in two phases; at 0 hr (pre-imbibition) and 240 hr of imbibition. The moisture content was determined gravimetrically as a percentage of fresh weight basis, using the Low Constant Temperature Oven Method (103°C for 17 ± 1 hr) as described by the International Seed Testing Association (ISTA, 2024).

Embryo Growth

Four replicates of 10 seeds were dissected and the embryo was excised on respective days after imbibition. The embryo length (mm), width (mm), and embryo-to-seed length ratio (E:S) were recorded using a stereomicroscope attached to a digital camera (Leica Microsystems, Germany) EZ4D.

Peroxidase (POD) Assay

One hundred mg of excised embryo and endosperm from all the treatments were ground into powder and homogenised with 1 mL of 100 mM potassium phosphate buffer (pH 7.0). The samples were centrifuged at 12,000 g at 4°C for 20 min. The reaction mixture (3.00 mL) consisted of 0.05 mL of 20 mM guaiacol, 2.83 mL of 10 mM phosphate buffer (pH 7.0), 0.10 mL enzyme extract, and 0.02 mL of 40 mM H_2O_2. The enzyme activity was determined spectrophotometrically at 470 nm. The oxidation of guaicol was measured by the increase in absorbance at 1 min. Peroxidase (POD) enzyme activity was expressed per mg of extractable fresh tissue using Equation (1) and Equation (2).

Catalase (CAT) Assay

Excised embryos and endosperms from all treatments were homogenised and extracted by using a similar extraction protocol as mentioned in the POD (EC1.11.1.7) assay. The reaction mixture (3.00 mL) consisted of 1.50 mL of 100 mM potassium phosphate buffer (pH 7.0), 0.50 mL of 75 mM H_2O_2, 0.05 mL of enzyme extract and 0.95 mL of distilled water. The unit of catalase (CAT) activity was recorded based on absorbance value at 240 nm after 2 min of reaction time. The calculation was done based on the following Equation (3), using an extinction coefficient of 43.6 M^{-1} cm^{-1} (Thant et al., 2017). CAT activity was expressed per mg of extracted fresh tissue (µmol min^{-1} mg fresh weight $[FW]^{-1}$) using Equation (4).

α-Amylase Assay

One hundred fifty mg of the ground powder (embryo and endosperm) from all treatments were homogenised with 1.5 mL of cold 20 mM sodium phosphate with 6.7 mM sodium chloride, pH 6.9 extraction buffer. The samples were centrifuged at 12,000 g at 4°C for 15 min. The α-amylase activity was determined based on the formation of reducing sugars using a colour reagent. A 100 µL of 10% starch solution was pipetted into a 2.0 mL microcentrifuge tube. Then 100 µL of the supernatant for analysis was added and incubated for 3 min at room temperature. Next, 100 µL of colour reagent that consisted of 5.3 M potassium sodium tartrate tetrahydrate, and 96 mM 3,5-dinitrosalicylic acid was added. The microcentrifuge tube was placed in a boiling water bath for exactly 15 min. The tube was then allowed to cool at room temperature. Distilled water (900 µL) was added and mixed by inversion. The absorbance value was measured at 540 nm using a Multiskan GO (Version 1.00.40) spectrophotometer (Thermo Fisher Scientific, USA).

$$\text{POD (nmol/min/mL)} = \frac{\left(\Delta\,\frac{470\text{A}}{\text{min}}\right) \times \text{Total volume} \times 1000}{26.6 \times \text{Sample volume}} \tag{1}$$

$$\text{POD (nmol/min/mg FW)} = \frac{\text{nmol/min/mL}}{\text{mg/mL enzyme}} \tag{2}$$

$$\text{CAT (µmol/min/mL)} = \frac{\left(\Delta\,\frac{240\text{A}}{\text{min}}\right) \times \text{Total volume} \times 100}{43.6 \times \text{Sample volume}} \tag{3}$$

$$\text{CAT (µmol/min/mg FW)} = \frac{\text{µmol/min/mL}}{\text{mg/mL enzyme}} \tag{4}$$

Seed Germination

The treated seeds were imbibed in water for 10 days, separated into 15 × 10 cm plastic bags containing four replicates of 100 seeds, and placed inside the germination room at 30°C. Seeds were monitored daily by recording the number of seeds showing the emergence of radicles from the fibre plug, according to Sime Darby Seed Production Unit (SPU) standard evaluation procedures (Norsazwan et al., 2024). Germinated seeds were removed from the bag and the remaining non-germinated seeds were kept until the end of the germination test period (day 60). The germination was determined as a percentage at time intervals throughout the germination period using the following Equation (5):

$$\text{Germination (\%)} = \frac{\text{Total number of seeds germinated}}{\text{Number of seeds used}} \times 100 \quad (5)$$

Statistical Analysis

Data were analysed using Microsoft Excel and Statistical Analysis Software, SAS 9.4 (SAS Institute, Cary, USA). Significant levels of $p \leq 0.05$ were used for Duncan's Multiple Range Test (DMRT) for analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Seed Imbibition

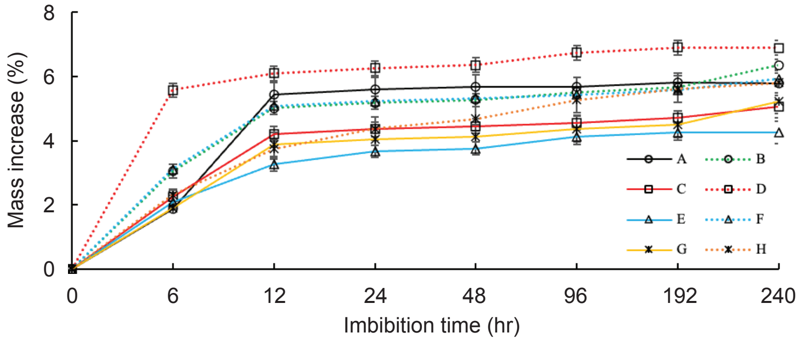
All treatments showed a gradual increase in seed mass from 0 to 240 hr after imbibition (*Figure 2*). Treatment D (heat treatment) showed the highest increment with 6.9%, whilst Treatment E (120 days storage) had the lowest overall mass increase (4.3%). Within the first 12 hr, a rapid mass increase was observed; ranging from 3.3% (Treatment E) to 6.0% (Treatment D). Thereafter, all the treatments showed no significant changes in the mass increase from 48 to 240 hr of imbibition, as the water uptake reached plateau.

Moisture Content in Endosperm and Embryo

In general, both endosperm and embryo tissues recorded a significant increase in moisture content due to imbibition, however, the magnitude of the increment differed markedly due to the treatments. Before imbibition, the endosperm tissue recorded moisture levels ranging from 15.4% (Treatment F) to 18.8% (Treatment A). After the 10 day imbibition period, all the treatments showed equal endosperm moisture content increment, with an average of 4.5% increase in comparison to the pre-imbibed stage (*Table 1*). In contrast, the embryo's initial (pre-imbibed) moisture content was significantly higher than in the endosperm, which ranged from 26.3% (Treatment C) to 30.7% (Treatment A). After 10 days of imbibition, the embryo showed an average of 11.4% increment in moisture. Interestingly, treatments that were heat treated or stored before heat treatment (Treatments D, F and H) recorded significantly higher embryo moisture content after imbibition (44.0%–48.8%), in comparison to other treatments. The lowest moisture values after imbibition were recorded by Treatments A, B and G, which showed no differences in moisture within the 35.0%–39.8% range.

Embryo Growth

Initially (day 0, immediately after imbibition), high variation was observed in embryo length with values ranging from 2.89–3.48 mm. The highest embryo length was recorded by Treatment H (3.48 mm), which differed significantly from most other treatments, except with Treatment F (3.35 mm). At day 20 after imbibition, a significant



Note: A - Treatment A; B - Treatment B; C - Treatment C; D - Treatment D; E - Treatment E; F - Treatment F; G - Treatment G; H - Treatment H.

Figure 2. The percentage of seed mass increases at 0, 6, 12, 24, 48, 96, 192, and 240 hr after imbibition from the initial seed mass.

TABLE 1. PERCENTAGE OF MOISTURE CONTENT FOR ENDOSPERM AND EMBRYO OF SEED TREATMENTS BEFORE AND AFTER IMBIBITION

Treatment	Moisture content (%)			
	Endosperm		Embryo	
	Pre-imbibition	Imbibed	Pre-imbibition	Imbibed
A	18.80b	21.20a	30.70d-f	39.80bc
B	18.60b	21.30a	30.20ef	36.60cd
C	17.80bc	21.50a	26.30f	38.10bc
D	16.18d	22.10a	29.03ef	48.80a
E	17.60b-d	23.20a	28.80f	37.20c
F	15.40e	22.10a	30.80d-f	46.30a
G	18.20bc	21.40a	28.80f	35.30c-e
H	16.60c-e	22.30a	30.50d-f	44.10ab

Note: Different letters (a-f) indicate significant differences within each column at a 5% probability level.

increase in embryo length was recorded only for Treatment D (heat treatment), and F (60 days storage + heat treatment), with 0.45 and 0.51 mm increments, respectively. From day 40 to 60 after imbibition, the pattern of embryo length remains the same, where Treatments D, F and H (120 days storage + heat treatment) recorded significantly higher embryo length, within the 3.23 to 3.82 mm range, in comparison to Treatment A, B, C, E and G (3.16–3.25 mm). The effect of each treatment on embryo length is summarised in *Figure 3a*. The embryo width showed less variation among the treatments, in comparison to the length. In general, Treatments A, C, E and G had no change in width throughout the 60-day evaluation period, with values ranging from 1.24–1.34 mm. On day 20 after imbibition, only Treatment D showed a significant width increment from 1.31–1.50 mm. From day 40 to 60 after imbibition, Treatments D, F and H showed higher embryo width in comparison to other treatments, with 1.62, 1.58 and 1.69 mm, respectively (*Figure 3b*). The embryo-to-seed length ratio (E:S) ranged between 0.10–0.16. Treatments A, B, C, E and G showed no significant change in the E:S (less than 0.12) within the 60-day evaluation period. Treatments D, F and H recorded a marked increase in ratio from day 0 to day 60 after imbibition, with 15%, 11% and 29% increments, respectively (*Figure 3c*).

POD Activity

All treatments recorded similar initial (pre-treated) activity levels, ranging from 184.7–205.9 nmol min^{-1} mg FW^{-1} of POD value. The application of heat treatment as well as storing the seeds before the heat treatment (Treatments D, F and H) resulted in more than 30% reductions in POD values. Treatments D, F and H recorded post-treatment POD activity of 130.4, 126.4 and 126.6 nmol min^{-1} mg FW^{-1} of POD value, respectively (*Figure 4*). Treatment A (204.0 nmol min^{-1} mg FW^{-1}), Treatment B (187.9 nmol min^{-1} mg FW^{-1}), Treatment C (193.3 nmol min^{-1} mg FW^{-1}), Treatment E (180.4 nmol min^{-1} mg FW^{-1}) and Treatment G (170.4 nmol min^{-1} mg FW^{-1}) showed no significant changes in POD activities in comparison to respective pre-treatment values.

CAT Activity

CAT showed a similar activity pattern as showed by the POD enzyme. The initial value for pre-treated seed ranged from 6.37–6.54 mmol min^{-1} mg FW^{-1} (*Figure 5*). Application of heat treatment and storage with heat treatments recorded a significant reduction in CAT activity, with 5.81 mmol min^{-1} mg FW^{-1} (Treatment D), 5.57 mmol min^{-1} mg FW^{-1} (Treatment F), 6.26 mmol min^{-1} mg FW^{-1} (Treatment G) and 5.84 mmol min^{-1} mg FW^{-1} (Treatment H) of post-treatment values. In contrast, Treatments A, B, C and E indicated no changes with respective pre-treatment CAT activity levels.

α-Amylase Activity

Endosperm. At the initial stage (0-hr of imbibition), Treatment D (heat treatment) indicated significantly higher α-amylase activity at 85.34 units uL^{-1}, in comparison to other treatments, which varied within the 56.20–59.00 units uL^{-1} range (*Figure 6a*). From 0 to 48 hr after imbibition, all treatments showed significant enzyme activity increment, however, some treatments (Treatments D, F and H) indicated a more rapid pattern than the others. Treatment D, Treatment F (60 days storage + heat treatment) and Treatment H (120 days storage + heat treatment) recorded high amylase activity; with 155.30, 132.10 and 135.90 units uL^{-1}, respectively. In contrast, Treatment A (untreated control), Treatment B (operculum removal), Treatment C (storage for 60 days), Treatment E (storage for 120 days) and Treatment G (storage for 180 days) indicated similar enzyme activity within 76.30–87.10 units uL^{-1} range. From 96 to 240 hr after imbibition, there were notable

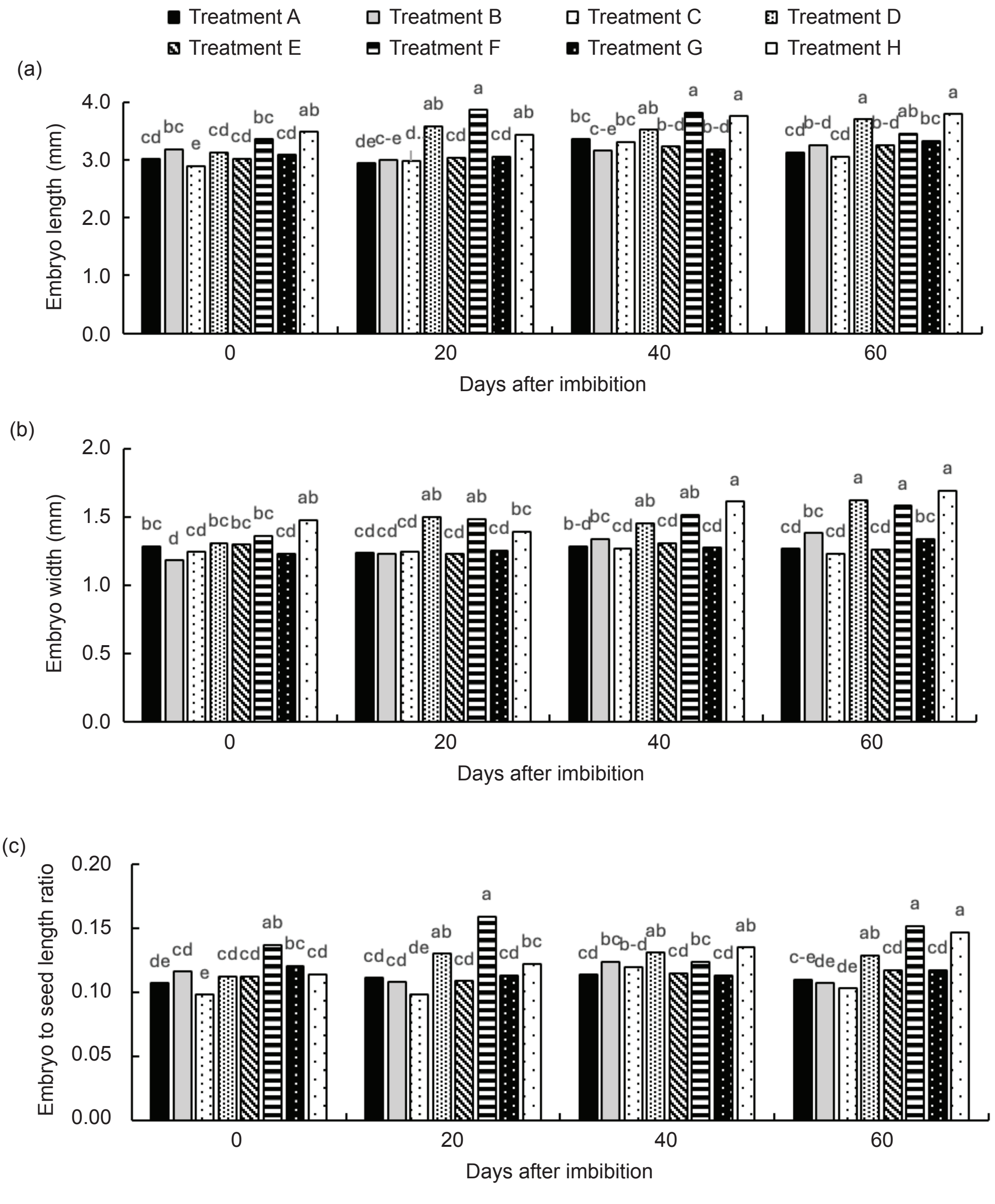


Figure 3. (a) Embryo length, (b) embryo width and (c) embryo-to-seed length ratio (E:S) at 0, 20, 40 and 60 days after imbibition.

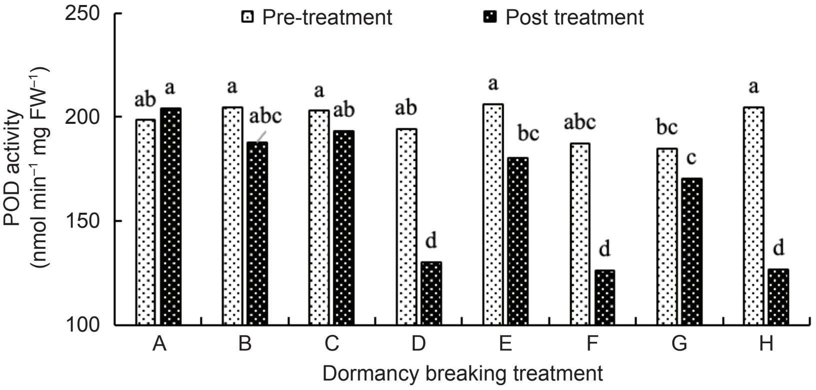


Figure 4. Peroxidase (POD) activities before and after dormancy-breaking treatments. Letters (a-d) indicate significant differences at 5% probability level.

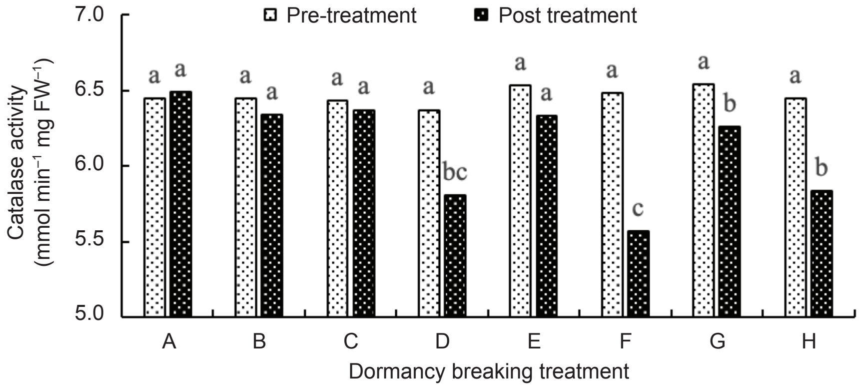


Figure 5. Catalase (CAT) enzyme activity before and after dormancy-breaking treatments. Different letters (a-c) indicate significant differences at a 5% probability level.

fluctuations in amylase activity; with a general gradual increment towards the end of the imbibition period. At 240 hr after imbibition, Treatment F (150.14 units uL^{-1}) recorded the highest α-amylase activity, followed by Treatments D, H, E, B and G (ranging within 125.00–135.70 units uL^{-1}), while the lowest activity was recorded by Treatment C (95.20 units uL^{-1}).

Embryo. In general, the pattern of α-amylase activity in the embryo showed fewer fluctuations as compared to the endosperm tissue. At 0 hr after imbibition, no differences were recorded irrespective of treatments; within the 26.0–36.6 units uL^{-1} range (*Figure 6b*). From 0–48 hr after imbibition, a rapid increase in amylase activity was observed. The highest activity was recorded by Treatment F (115.0 units uL^{-1}), followed by Treatment D (108.9 units uL^{-1}) Treatment H (99.0 units uL^{-1}), Treatment E (89.5 units uL^{-1}), Treatment A (92.7 units uL^{-1}), Treatment B (86.3 units uL^{-1}), Treatment G (81.9 units uL^{-1}) and Treatment C (71.8 units uL^{-1}). From 48–96 hr after imbibition, all treatments indicated a more gradual amylase activity increment. Towards the end of the imbibition period (from 96–240 hr), only a slight activity increment was recorded by the treatments. Treatments D, F and H showed the highest final α-amylase activity (125.3, 129.3 and 114.0 units uL^{-1}, respectively), followed by Treatments G, E, A, B and C (within 90.2–100.3 units uL^{-1} range).

Seed Germination

All treatments that were heat-treated and stored before heat-treatments (Treatments D, F and H) recorded significantly higher final germination percentage (FGP) as well as normal seeds, in comparison to other treatments (*Table 2*). Treatment F showed the highest mean FGP at 89.8%, similar with Treatment H (87.3%) and Treatment D (82.8%), followed by Treatment G (12.5%), Treatment E (9.8%), Treatment A (7.5%), Treatment B (6.5%) and Treatment C (4.5%). A similar trend was observed for the percentage of normal seeds, since a majority of the germinated seeds indicated normal development, with less than 2.0% abnormalities and disease occurrence throughout all treatments. In terms of mean germination time (MGT), Treatments D, F and H recorded significantly faster germination (within 12–13 days), whilst other treatments showed more than 50 days of MGT.

Seed Water Uptake

According to the imbibition and moisture content analysis, it is evident that regardless of the treatment used water is still being absorbed into the embryos. Since the size of the embryo was significantly smaller in comparison to the whole seed, the imbibition test (which was conducted based on the whole seed weight) only showed less than 7% mass increment. Nevertheless, the application of heat treatment as well as storage before heat treatment was proven to increase the rate of water entry into the seeds. The role of heat treatment in increasing imbibition has been reported in several studies. In *Geranium carolinianum*, the application of dry-heat treatment could increase the seed mass by 95.0% within 10 hr of imbibition, and it is only 0.3% in the case of untreated seeds. Similarly, the application of hot-water treatment in *Adenanthera pavonina* seeds resulted in a 90.0% increase in seed mass with no change recorded for the control group. The increase in water imbibition upon heat treatment could be due to the heat causing dislodgement of the palisade layer on the seed surface, thus creating a 'water gap' in the embryo (Gama-Arachchige et al., 2010; Jaganathan et al., 2018). Therefore, based on the observation of imbibition, moisture content and germination data, it can be concluded that oil palm seed does not exhibit PD. This is because even in the untreated (control) sample, the seed still imbibed water like those treated, with heat-treated seeds having an accelerated rate.

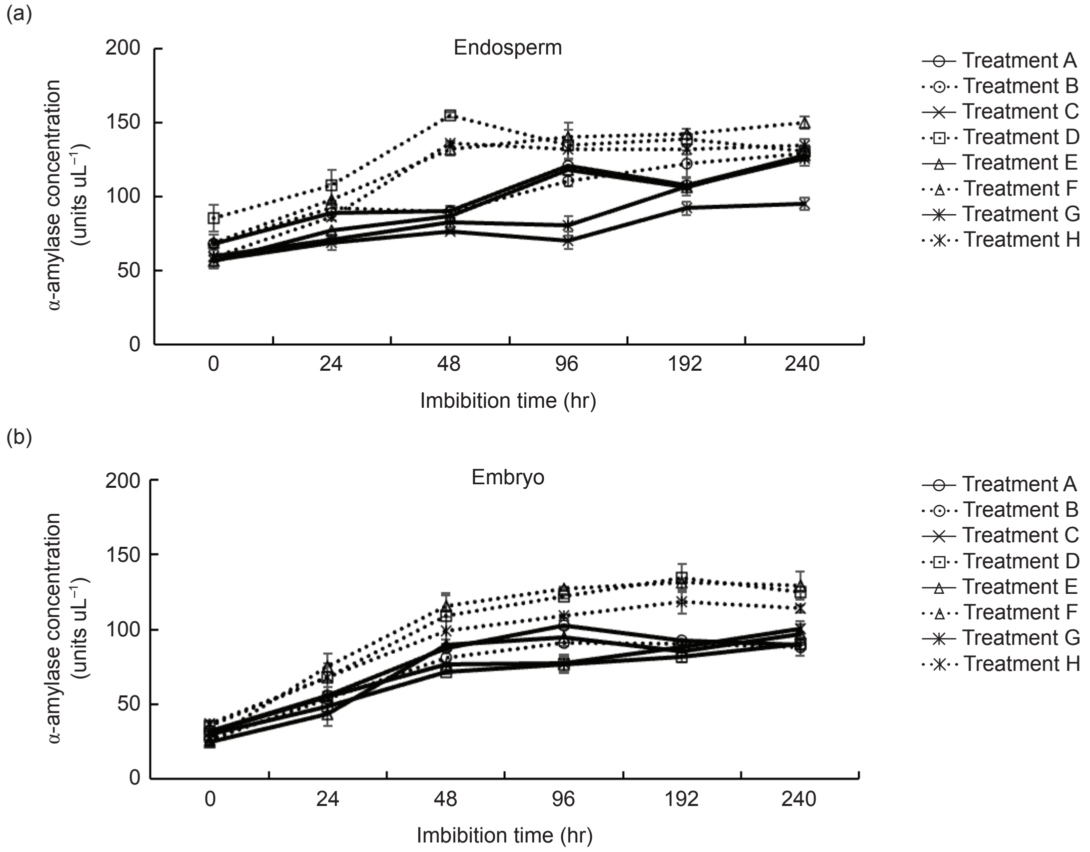


Figure 6. α-amylase activities for (a) endosperm and (b) embryo tissues from 0 to 240 hr after imbibition.

TABLE 2. MEAN VALUES OF FINAL GERMINATION PERCENTAGE (FGP), PERCENTAGE OF NORMAL, ABNORMAL, DISEASED SEEDS AND MEAN GERMINATION TIME (MGT)

Treatment	FGP (%)	Normal (%)	Abnormal (%)	Diseased (%)	MGT (days)
A	7.50bc	7.00bc	0.50ab	0.00b	50.08a
B	6.50c	6.50bc	0.00b	0.00b	50.50a
C	4.50c	3.00c	0.00b	0.50b	49.80a
D	82.80a	82.00a	0.25b	0.50b	12.30b
E	9.80bc	8.00c	0.25b	1.50a	50.00a
F	89.80a	87.80a	1.75a	0.25b	11.60b
G	12.50b	10.50b	0.50ab	1.50a	53.50a
H	87.30a	86.00a	1.25ab	0.00b	11.70b

Note: Letters (a-c) indicate significant differences within each column at a 5% probability level; FGP - final germination percentage; MGT - mean germination time.

Embryo Growth

Baskin and Baskin (2004) suggested seeds that exhibit MD simply require time for the embryo to develop and germinate. In this study, evaluation of embryo length, width and E:S showed that the application of heat treatment, and seeds that were stored before the heat treatment (Treatments D, F and H) indicated similar effects on embryo growth. On average, there was a 10% average increment in embryo length, 18% in width, and a 15% overall increase in E:S length ratio, shown by these treatments. Untreated (Treatment A), operculum removed (Treatment B) and seeds that were stored without any heat treatments (Treatments C, E and G) showed no significant changes within the 60 days evaluation period. Thus, this suggests that heat treating the seeds (with or without prior storage) accelerated the embryo growth. Earlier studies by Suranthran et al. (2011) and Kok et al. (2015) found that the embryo had completed the development and was able to resume growth into a normal seedling once excised from the seed. In contrast, morphologically dormant seeds would show a higher increment in embryo growth upon dormancy alleviation. For example, the underdeveloped *Podocarpus costalis* embryo showed a 54% increment in length before being able to germinate (Chen et al., 2013). Therefore, this suggested that oil palm does not exhibit a standard MD.

Enzymatic Changes

The enzymatic assays of α-amylase, POD and CAT enzyme activities showed coherent results with the germination data. Three of the Treatments; Treatment D (heat treatment), Treatment F (60 days storage + heat treatment), and Treatment H (120 days storage + heat treatment) consistently showed significant increases in α-amylase, along with reductions in both POD and CAT activities in comparison to other treatments. The effect of heat treatments on α-amylase activities has been reported in *Hordeum vulgare, Cicer arietinum* L. and *Sorghum bicolour* seeds. In *H. vulgare* L. seed, high temperature (of more than 35°C) causes higher degradation of endosperm structure, particularly by reducing the crushed cell layer (CCL) thickness, thus increasing amylase production and embryo growth in comparison to non-heat treated seeds (Wallwork et al., 1998). Increasing heat treatment duration was also found to cause a decrease in alpha amylase inhibitor activity (AIA) in *C. arietinum* L., and *S. bicolour* seeds, thus directly increasing the amylase production and faster germination (Mulimani & Rudrappa, 1994). In terms of dormancy release, α-amylase was mainly associated with the balance of gibberellin (GA) and abscisic acid (ABA) productions in the endosperm and embryo tissues. Weiss and Ori (2007) suggested that seed germination can be characterised by transcriptional induction of hydrolytic enzymes including α-amylase, which are needed for the degradation of starch and the subsequent mobilisation into the embryo. Therefore, the changes in α-amylase activity were an indirect measure of the changes in the balance of seed ABA/GA levels, which is the known central regulatory mechanism underlying the maintenance and release of seed dormancy from the physiological perspective (Finch-Savage & Footitt, 2017; Shu et al., 2016). Zhang et al., (2022) reported that the genes related to ABA production negatively regulate the synthesis of GA and are strongly up-regulated in the mid-late stage of oil palm embryonic development. From *Figure 6*, it is clear that within the first 48 hr of imbibition, a marked increase in amylase activity was recorded, particularly for Treatment D, F and H. Seeds of *Melanoxylon brauna* recorded a significant increment of 60%–80% in α-amylase activity after four days of imbibition, which corresponds to more than 93% of germinated seeds (Ataíde et al., 2016). In *Oryza sativa* seed, α-amylase activity in the endosperm was shown to be positively correlated to GA synthesis in the embryo, which in turn hydrolysed the starch for energy supply during the germination process (Kaneko et al., 2002). Changes in endogenous signalling factors such as reactive oxygen species (ROS) were also reported to influence the balance between ABA and GA, thus affecting dormancy and germination in seeds (Ishibashi et al., 2017; Izydorczyk et al., 2017). The reduction of CAT and POD in this study suggested that the enzymes were utilised to protect the seeds from the accumulation of ROS resulting from heat treatment and storage. The resulting hydrogen peroxide (H_2O_2) from the initial reaction of superoxidase dismutase (SOD) was then converted to water and oxygen (Farooq et al., 2009). This is following findings from Kaushal et al. (2011), that the activities of antioxidants enzyme including CAT and SOD in chickpeas decreased under high-temperature stress between 40°C to 45°C. Similar findings were reported in soybean seeds, where both CAT and POD activities declined significantly when subjected to natural field weathering (Yadav, 2003). In this study, the concept of after-ripening was applied for Treatment E, F, G and H, where the seeds were stored at varying durations for up to 180 days to further alleviate the PYD in oil palm seed. Theoretically, if the seed does exhibit PYD; then an extended period of after-ripening could potentially assist in decreasing the ABA concentration along with higher GA sensitivity, thus decreasing the 'depth' of the dormancy (Finch-Savage & Footitt, 2017). However, it was found that the dormancy-breaking capacity remain after the storage period based on similar germination percentage and speed with seeds that were only heat-treated. Therefore, this suggested that oil palm seed may exhibit a non-deep PD since the dry storage period did not improve seed germination, which would be the case for intermediate PD (Baskin & Baskin, 2004). Nevertheless, the application of heat treatment was indeed proven to be crucial in alleviating the non-deep PD of oil palm seeds.

CONCLUSION

In this study, different dormancy-breaking treatments were employed in oil palm to understand the dormancy mechanism in this species, based on physical, morphological, and physiological perspectives and concluded that oil palm seeds exhibit non-deep PYD. It was observed that the seeds were able to imbibe water and have a fully developed embryo requires PYD alleviation. Application of heat treatment to fresh or stored seeds, resulted in improved seed germination of more than 82% and a low mean germination time of less than 10 days; indicating the necessity of heat treatment. The treated seeds had reduced CAT and POD enzyme activities but with increased α-amylase production in the embryo. Heat treatment for 60 days at 40°C is confirmed as the most efficient and practical dormancy-breaking method in alleviating non-deep PD for commercial hybrid seed production.

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PRICE TRANSMISSION IN THE SUPPLY CHAIN OF INDEPENDENT OIL PALM SMALLHOLDERS IN WEST SUMATERA, INDONESIA: A CASE STUDY IN DHARMASRAYA DISTRICT

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ABSTRACT

This study employs the price relationship equation statistical test and the Error Correction Model (ECM) regression to analyse the transmission of crude palm oil (CPO) prices at the exporter level and its effects on the selling price of fresh fruit bunches (FFB) at the smallholder's level in Dharmasraya Regency, Indonesia. Data were collected through both quantitative and qualitative surveys, involving 155 respondents, including independent smallholders (117), small collectors (25), large collectors (10) and palm oil mills (3). Results revealed a price transmission elasticity of less than 1 (ET < 1), indicating asymmetric price transmission where small and large collectors, as well as palm oil exporters, significantly influence the FFB selling prices for independent smallholders. Furthermore, the extended supply chain contributes to narrow profit margins for farmers. To enhance sustainability in palm oil production, it is essential to establish cooperative-based mini-CPO and red palm oil (RPO) factories and implement an integrated information technology system within farmers' institutions to provide real-time data on selling and buying prices, as well as FFB quantities. This study contributes to sustainable practices in the palm oil sector by addressing price asymmetry, ultimately aiming to improve the economic well-being of smallholders and strengthen the overall sustainability of the palm oil supply chain.

Keywords: ECM regression, independent smallholders, palm oil, supply chain, sustainability.

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INTRODUCTION

Palm oil production per unit area which is higher when compared to other vegetable seed oils is an economic advantage that contributes to the community's economy. Furthermore, the Sustainable Development Goals (SDGs) specifically, Goals 1 (No Poverty), 2 (Zero Hunger), and 8 (Decent Work and Economic Growth), are significantly impacted by the production of palm oil. Expanding palm oil production in developing countries, which coincidentally have low wages, is seen to be essential in order to meet future demand for reasonably priced palm oil. Due to an increase in per capita income and global population growth, the demand for palm oil is expected to double from 120 to 240 t in 2050. The additional need for oil palm is estimated to reach around 7 to 25 million hectares in the next 40 years (Ayompe et al., 2021; Corley, 2009; Khatun et al., 2017; Srisawasdi et al., 2023).

Indonesia is the biggest exporter of palm oil worldwide (Observatory of Economic Complexity [OEC], 2023). West Sumatra Province has enormous potential for the development of

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palm oil commodities, which are a subsector of plantation crops. In comparison to rubber and cocoa plantations, oil palm ranks first in West Sumatra in terms of planting area and production of plantation crops. With an average planting area of 32,947 ha (Statistics of Sumatera Barat Province, 2023a) and a total production of 103,637 t (Statistics of Sumatera Barat Province, 2023b). Dharmasraya Regency is home to some of the greatest plantation areas for oil palm. Even though oil palm is the plantation crop in West Sumatra Province that contributes the most to GDP, there is frequently an excess of fresh fruit bunches (FFB) from independent smallholders. Nesti et al. (2018) stated that palm oil marketing practices in West Sumatra are not yet efficient because the purchase price received by oil palm smallholders is low, inadequate and monopsony. Furthermore, the lengthy supply chains diminish profit margins, hindering the economic sustainability of local smallholders.

Several factors indicate an effective supply chain, including the ability to offer goods to customers at a low cost and the ability to fairly distribute resources so that each party is satisfied to the same extent (The meaning of fair: A comparison between the sacrifices made and the profits obtained for each component) (Emhar, 2014). Since the concept is hard to quantify, other indicators are required, including supply chain flow. A shorter supply chain is more efficient than a long one since it will involve higher expenditures for storage, shipping, transaction, packing, and damage, which will reduce the amount that smallholders receive (Zuraida & Wahyuningsih, 2015).

The efficiency indicator can be seen from the value of "price transmission elasticity equal to one (ET = 1)" which is formed between two markets that interact with each other, both vertically and spatially (Arnade et al., 2017; Bekker et al. 2017; Bergmann et al., 2016; Ceballos, 2017; Darbandi, 2018; Juliaviani et al., 2017; Pejman, 2017; Vavra & Goodwin, 2005). The elasticity of price transmission (ET) can be used to find out the market structure formed where a low ET is one of the indicators that reflects the strength of monopsony/oligopsony power, leading to unequal benefits across the supply chain (Hutabarat & Rahmanto, 2004).

The two most crucial components of the pricing connection in the value chain are marketing margin analysis and market integration, often known as price transmission (Bekkers et al., 2017). According to Juliaviani et al. (2017) and Kumala et al. (2015), price transmission in agricultural commodities tends to be asymmetric (if there is a price increase at the consumer level, the information is not passed on to smallholders quickly and transparently, but the other way around). According to Tan (2005), an oligopolistic market structure and competitive features are reflected in asymmetric price transmission. Usually, inefficient allocation of resources caused by monopolistic practices in trading groups, intervention by outside parties, market failures and cost adjustments are some sources of asymmetric price transmission (Hassouneh et al., 2012; Rajendran, 2015).

Regarding the asymmetric price transmission of palm oil, Nakajima (2012) analysed and compared the asymmetric price transmission of palm oil between Malaysia and Indonesia. The author highlights that the palm oil industry plays an important role in employment, income generation and foreign exchange earnings in both countries. Understanding the nature of price transmission is considered important for policymakers, producers and consumers, as asymmetric price transmission can have significant implications for the distribution of benefits and risks along the supply chain. Asymmetric price transmission can affect the distribution of profits, industry competitiveness, and the final prices paid by consumers. Nesti et al. (2018) stated that one player in the oil palm supply chain has the power to regulate the selling price of FFB, enabling him to limit the selling price of FFB producers. The vertical integration market's inefficiency stems from this circumstance. Furthermore, Bergmann et al. (2016) analyse the price transmission and volatility between the European and global butter markets, as well as between butter, palm oil and crude oil. They found that changes in European Union policies have caused a sharp increase in the price volatility of dairy commodities, so it is necessary to develop appropriate risk management tools. Their analysis using multivariate VAR and GARCH models shows there are strong price transmission and volatility between European and global butter, as well as the impact of European butter price shocks on the volatility of palm oil; additionally, there is evidence that crude oil prices also impact the price and volatility of global butter.

Several studies on price transmission have been done by several academics, among others: Sukiyono and Asriani (2020), conducting research on the transmission and volatility of chilli prices in Bengkulu, Indonesia, have found that there is low volatility at the consumer level but high at the wholesaler and producer level. Furthermore, McLaren (2015) who has explored the asymmetry of price transmission in global markets found that monopsony power is the core cause of asymmetric price transmission. Acharya et al. (2011) used continuous testing methods for marketing fresh strawberries in the US to prove that price transmission is asymmetric with the finite mixture model; where the finding is that price transmission conditions depend on the harvest season. Acharjee et al. (2023) reported on "Price transmission asymmetry of selected fishes in Bangladesh: An econometric and value chain analysis" found that the retail price leads the farmer and wholesale price. In Indonesia, Ridha et al. (2022) have researched the asymmetric price transmission in the cocoa supply chain and the findings indicate that there is a negative asymmetric price transmission between the global cocoa market and the price of cocoa paste to the price of cocoa farmers in Indonesia. For further details, *Table 1* below shows some previous studies related to price transmission in the agricultural market.

Reflecting on previous research, this study occurred intending to analyse how CPO prices are transmitted at the exporter level and how that affects FFB sales prices at the independent smallholder level in West Sumatra's Dharmasraya Regency, Indonesia. It also offers suggestions to strengthen the negotiating position of independent oil palm growers. This study examines the vertical price relationship between each player in the oil palm supply chain using the price relationship equation, formula, and error correction model (ECM) regression. Thus, through this approach, this study will provide a deeper understanding of the price mechanism in the palm oil industry and provide recommendations that can improve the welfare of independent oil palm smallholders in Dharmasraya Regency, West Sumatra, Indonesia.

The urgency of addressing the issue of price asymmetry in Dharmasraya stems from the fact that this study builds upon the author's previous studies. In 2018, a study titled "The efficiency of palm oil fresh fruit bunches in West Pasaman, Indonesia (2010–2017)" (Nesti et al., 2018) was conducted. This was followed by the 2019 study, "Analysis of prospects for CPO in West Sumatra Province" (Nesti et al., 2019) and in 2020, "Competitive analysis of CPO in West Sumatra province compared to other provinces on Sumatra Island in the domestic market" (Nesti et al., 2020).

The topic of price asymmetry is particularly relevant given that West Sumatra, and specifically Dharmasraya Regency, is a major palm oil production hub in Indonesia. Addressing this issue is crucial as it can provide valuable insights for policymakers at both the provincial and national levels to tackle price asymmetry and enhance the competitiveness of the palm oil industry. Price asymmetry, where the prices of raw materials (FFB) and the final product (CPO) do not move in tandem, is a common issue in the palm oil industry. This imbalance can affect the profitability and competitiveness of smallholder and other stakeholders throughout the value chain.

Given the critical nature of palm oil production in Dharmasraya Regency, West Sumatra, this study provides valuable insights for policymakers at both provincial and national levels to tackle price asymmetry and improve the industry's competitiveness. Understanding the mechanisms behind price transmission is essential for formulating effective strategies that promote the welfare of industry participants and ensure a sustainable palm oil supply chain. By focusing on these issues, this study aims to foster informed decision-making that enhances both economic and environmental sustainability within Indonesia's palm oil sector.

MATERIALS AND METHODS

Data collection was conducted using quantitative and qualitative methods. The quantitative method was conducted by distributing questionnaires to respondents randomly selected from the target population; while the qualitative method examined the description of the characteristics of oil palm smallholders in Dharmasraya district based on field visits and direct questions and answers with respondents. This research employed the purposive sampling method, selecting respondents based on specific characteristics relevant to the study. Respondents were deliberately chosen for their ability to provide pertinent information, including palm oil smallholders, the Palm Farmers' Cooperative, the West Sumatra Provincial Plantation Office and the Indonesian Palm Oil Farmers' Association (GAPKI). Purposive sampling was utilised to ensure that the sample represented all key actors in the supply chain, from smallholders and small collectors to large collectors, processing industries, and exporters. This approach allows for selecting respondents with relevant knowledge and experience related to the research topic.

The study's sample included 155 respondents from the oil palm supply chain in Dharmasraya Regency: 117 independent smallholders, 25 small collectors, 10 large collectors and 3 palm oil mills that also serve as exporters. This sample size was chosen as it was deemed representative of the population, whose exact number was unknown due to incomplete records by the Dharmasraya Regency government. To analyse price transmission in the palm oil commodities of Dharmasraya Regency, the study utilised ECM regression. Data sources included primary data from direct observations (using questionnaires and interviews) and secondary data from Statistics Indonesia. Observations covered all stages of the supply chain, from smallholders and small collectors to large collectors, processing industries and exporters. The data collected included roles and characteristics of supply chain players, profiles of independent smallholders, buying and selling processes of FFB, collection and sales activities, and the processing of FFB into CPO. The flow of information and price transmission at each supply chain level were also documented. *Figure 1* illustrates the study's flowchart.

TABLE 1. PREVIOUS STUDIES RELATED TO PRICE TRANSMISSION IN AGRICULTURAL MARKETS

No.	Title	Publication and year	Author	Variable	Tools and analysis	Result	Gap
1	Analysis of Price Transmission Along the Food Chain.	OECD food, Agriculture and Fisheries Working Papers 3, OECD Publishing.	Vavra and Goodwin (2005)	Assessment of price transmission along the supply chain (prices at the farmer level to prices at the consumer level).	Qualitative descriptive (literature study) and quantitative descriptive (ECM Regression).	• The process of price transmission through supply chains has attracted the attention of agricultural economists, as well as policy makers. • Imperfect price transmission is thought to be caused by market power and oligopolistic behaviour. • Market power is an important explanation for evidence of asymmetry in price transmission but may not be the only causal factor.	The study did not observe the supply chain in an integrated manner regarding the flow of goods, information, and money.
2	*Efisiensi Harga Pada Vertical Integrated Market Studi Tentang Pasar Produk Industri Karet Alam Indonesia.*	Jurnal Manajemen dan Pembangunan, Vol. 4(1).	Tan (2005)	SIR 20 export volume per company in the observed province, total exports each year, buying price of Slabs at broker level and selling price of SIR 20 on the world market.	Using the Ordinary Least Square (OLS) regression model to explain the relationship between the purchase price of slabs at the collector's dealer and the selling price of SIR in the international market, concentration ratio, H index, and R index to measure market strength.	The operation of the imperfect competition market in the domestic natural rubber market and price competition in purchasing slabs are inefficient in vertically integrated marked natural rubber products in Indonesia. Efficiency is relatively good in North Sumatra, less so in South Sumatra and very poor in Jambi.	The study used a static OLS regression model did not include government policy variables in the model and did not carry out analysis down to the farmer level.
3	Vertical Price Transmission Between Market Operators in Hungarian Agricultural Product Chains.	Studies in Agricultural Economics (106).	Varga (2007)	The input supplier/ agricultural producer price, retailer price.	ECM	Within a few months, it is necessary to restore balance. Prices are transmitted more quickly and efficiently from processors to retailers.	The study explained the existence of asymmetric market conditions, the greatest market concentration is in retailers, while the role of exporters was not explained.

TABLE 1. PREVIOUS STUDIES RELATED TO PRICE TRANSMISSION IN AGRICULTURAL MARKETS (continued)

No.	Title	Publication and year	Author	Variable	Tools and analysis	Result	Gap
4	*Fluktuasi Harga, Transmisi Harga dan Marjin Pemasaran Sayuran dan Buah.*	Analisis Kebijakan Pertanian, 5(4).	Irawan (2007)	Prices of vegetables, prices of fruit.	Price transmission of vegetables is relatively low (49%–55%) compared to fruit and other food commodities (65%–81%). This shows that the vegetable market at the farmer level tends to be monopsony/oligopsony.	An imbalance between supply volume and consumer needs occurs more often in vegetables. Vegetable marketing margins are also relatively high. On the other hand, the prices received by farmers and price transmission from consumer areas to producer areas are low. The vegetable market at the farmer level is monopsony/oligopsony.	It did not explain the causes of the supply and demand imbalance and the government's role in it.
5	*Analisis Tataniaga dan Elastisitas Transmisi Harga CPO Internasional Terhadap Harga TBS Kelapa Sawit.*	Skripsi. Depertemen Sosial Ekonomi Pertanian, Universitas Sumatera Utara.	Bisuk (2004)	Sales costs, purchasing costs, commerce costs, and consumer purchasing prices.	Using the calculation of trading margin, share margin, trading system efficiency and price transmission elasticity using the cob doughlas model.	There are two FFB marketing channels, Palm oil marketing in the research area is efficient. The CPO price coefficient is 0.98, meaning that a change in the international CPO price of 1.00% results in an FFB price change of 0.98%.	The determining factors for marketing channel efficiency were only based on trade margins and elasticity.
6	Testing International Price Transmission Under Policy Intervention. An Application to the Soft Wheat Market.	Associazione Alessandro Bartola, Phd Studies Series, 6.	Listorti (2008)	Wheat price.	Vector Error Correction Model.	Integration in grain markets has improved after 1993 because of CAP reforms and trade liberalisation reforms.	Market concentration was not analysed and the role of exporters in influencing prices in the domestic market was not explained in the model.
7	Price Transmission Along the Food Supply Chain in the European Union.	EAAE 113th Seminar Chania, Crete, Greece.	Bukeviciute, et al. (2009)	Fragmentation, concentration and consolidation, market power along the supply chain.	ECM	Supply chain approach, possible causes of transmission are fragmentation, concentration and consolidation, market forces along the supply chain; institutional arrangements and business practices.	The title relates to food supply chains in Europe, but it was not empirically tested.

TABLE 1. PREVIOUS STUDIES RELATED TO PRICE TRANSMISSION IN AGRICULTURAL MARKETS (continued)

No.	Title	Publication and year	Author	Variable	Tools and analysis	Result	Gap
8	*Analisis Harga Minyak Sawit, Tinjauan Kointegrasi Harga Minyak Nabati dan Minyak Bumi.*	Jurnal Manajemen dan Agribisnis, 7(1).	Arianto, et al. (2010)	Prices of vegetable oil, prices of palm oil, prices of soybean oil.	Variance decomposition.	In the long term, CPO is the most influential variable in the vegetable oil market.	It was not clear whether CPO had an effect in the short term.
9	*Analisis Transmisi Harga Jagung sebagai Bahan Pakan Ternak Ayam Ras di Sumatera Barat.*	Jurnal Peternakan Indonesia, 14(2).	Rahmi and Arif (2012)	prices at the corn farmer level, prices at the collector level, and prices at the consumer level.	To see market integration, look at the elasticity value of price transmission using simple linear regression.	The sensitivity to price changes at the corn farmer level is smaller than the price at the consumer level, so the market is less efficient. Price transmission from consumers to producers and vice versa does not work well because of the accumulation of margins on collecting traders as market players who control the market and hinder price transmission.	The study still used the OLS regression model.
10	*Transmisi Harga Asimetri dalam Rantai Pasok Bawang Merah dan Hubungannya dengan Impor di Indonesia: Studi Kasus di Brebes dan Jakarta.*	Buletin Ilmiah Litbang Perdagangan, 10(1).	Ruslan and Firdaus (2016)	The price of shallots at the farmer level, the price at the wholesale level and the price at the retail level, as well as the price of imported shallots.	Using the Houck model and ECM as well as long-term cointegration and causality tests.	The farmer-wholesaler relationship experiences price asymmetry in the short term because it is related to adjustment costs, while the wholesaler-retailer relationship occurs asymmetry in the long term. The import price of shallots plays an important role in determining the price of shallots at the producer and consumer levels. It is hoped that the ceiling price and floor price policies will prevent exploitation by intermediary traders.	The study did not examine the market structure that occurs in the shallot supply chain, making it difficult to make policies.

TABLE 1. PREVIOUS STUDIES RELATED TO PRICE TRANSMISSION IN AGRICULTURAL MARKETS (continued)

No.	Title	Publication and year	Author	Variable	Tools and analysis	Result	Gap
11	An Analysis of Price and Volatility Transmission in Butter, Palm Oil and Crude Oil Market	Agricultural and Food Economics, 4(23).	Bergmann, et al. (2016)	European Union butter prices and world butter prices as well as world prices of palm oil and crude oil	A vector autoregression (VAR) model is applied to capture the effects of price transmission between these markets. This is combined with a multivariate GARCH model to account for potential volatility transmission.	EU prices are transmitted to World butter prices. The European Union butter shock affected palm oil prices. Crude oil prices are transmitted to world butter prices and world butter volatility.	The study did not analyse price transmission upstream [suppliers (farmers)].
12	Study of Transmission of Price from Farm to Retail Shop in Saffron Market	American Scientific Research Journal for Engineering, Technology and Science, 32(1).	Pejman, et al. (2017)	Marketing margin, the price at the supplier level and the price at the consumer level	ECM regression	Shows symmetrical price transmission from producers to wholesalers in the short term, and wholesalers have more power than producers.	It has not explained the role of the government in determining price policy.
13	*Transmisi Harga Kopi Arabika Gayo di Provinsi Aceh*	Jurnal Agribisnis Indonesia, 5(1).	Juliaviani, et al. (2017)	The price of Arabica coffee at the farmer level, the price at the export level	Using the ECM to see the speed of price transmission in the short term and long-term transmission	The ECM shows that the speed of price transmission in the short term is asymmetric, while in the long term, the transmission is symmetrical, indicating that in the long term, the Gayo Arabica Coffee market in both areas is efficient.	The study did not analyse the causes of asymmetric transmission and government policies in dealing with this.
14	Price Transmission Analysis for the Nicaragua Rice Market	International Journal of Food and Agricultural Economics, 6(1).	Darbandi (2018)	Analysing the relationship between wholesale and retail level prices of the Nicaraguan rice market	The autoregressive distributed lag (ARDL) approach is used to check the stability of long run coefficients, cumulative sum of recursive residuals (CUSUM), and Granger causality test.	There is significant evidence supporting asymmetry between the wholesale and retail sectors, indicating market inefficiencies and distortions between the two markets.	The study did not analyse the relationship between prices at the wholesale and retailer levels to prices at the farmer level.

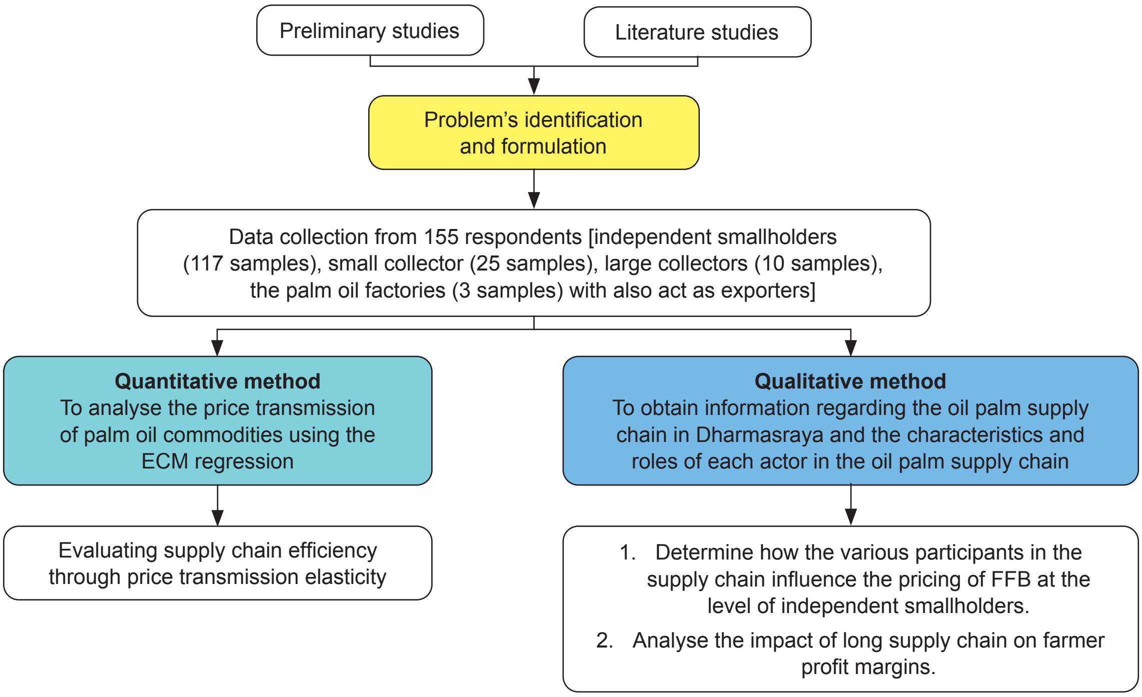


Figure 1. Flowchart of the study.

Research Hypothesis

H_0 = There is an indication that the price transmission in the supply chain of independent oil palm smallholders in Dharmasraya is symmetrical.

H_1 = There is an indication that the price transmission in the supply chain of independent oil palm smallholders in Dharmasraya is asymmetrical.

Regression Assumption Testing

Regression assumption testing criteria for price transmission are outlined as follows (Arnade et al., 2017; Bekker et al., 2017; Bergmann et al., 2016; Ceballos, 2017; Darbandi, 2018; Juliaviani et al., 2017; Pejman et al., 2017). When the elasticity of transmission (ET) equals 1 (ET = 1), a 1% change in prices at the buyer level results in a 1% change at the producer (smallholders) level, indicating a perfectly competitive market where the marketing system is efficient and symmetrical, with complete price information passed along. If ET is less than 1 (ET < 1), a 1% price change at the buyer level leads to a less than 1% change at the producer level, reflecting an imperfect competition market where the marketing system is inefficient and asymmetrical, with incomplete transmission of price information. Conversely, if ET is greater than 1 (ET > 1), a 1% price change at the buyer level results in a greater than 1% change at the producer level, signifying an imperfect competition market with an inefficient and asymmetrical marketing system where price information is not fully transmitted.

Classical Assumption Testing

Classical assumption testing involves several criteria to ensure the validity of a regression model, as outlined by Mardiatmoko (2020). Multicollinearity is assessed by examining the variance inflation factor (VIF); if no VIF value exceeds 10 or 5, multicollinearity is considered absent between the independent variables. Autocorrelation is tested using the Durbin-Watson method, where the Durbin upper (DU) and Durbin lower (DL) values from the Durbin-Watson Table are compared with the Durbin-Watson (DW) statistic. No autocorrelation is indicated if DW and 4-DW both exceed DU, or equivalently, if DU < DW < 4-DW. Heteroscedasticity is evaluated using the Breusch-Pagan test, with the presence of heteroscedasticity indicated by a significance < 0.05. For normality, the residuals of the data are considered normally distributed if they closely follow a diagonal line in a point distribution plot. Lastly, the linearity test checks the relationship between the predictor variable (X) and the response variable (Y), with a significance value greater than 0.05 suggesting a linear relationship.

Data Stationarity Testing and Cointegration Testing

To analyse data stationarity, the unit root test and the Augmented Dickey-Fuller (ADF) model are utilised. If the unit root test indicates that the time series data is non-stationary, the next step involves performing a degree of integration test to determine the level at which the data becomes stationary. As outlined by Hadri (2000), Kao (1999) and Pedroni (1999), the process includes conducting a cointegration test to examine if the data in the model are cointegrated. The Engle-Granger test is commonly employed for this purpose and requires the data to be equally integrated. A negative result for the null hypothesis (H_0) from this test indicates cointegration. If cointegration is not found, the next step is to analyse the regression form of the estimation model. Conversely, if cointegration is present, the price relationship can be modelled using an ECM.

The price connection equation model with ECM was used to further evaluate the model if the test results indicate cointegration. Using four independent variables and the ECM, the functional connection was simultaneously examined and analysed in this ECM regression to determine the role that exporters play in short- and long-term price regulation of FFB:

$$\Delta log\ Pp_t = \alpha_0 + \alpha_1\ \Delta log\ Pk_t + \alpha_2\ \Delta log\ Pa_t + \alpha_3\ \Delta log\ Pe_t + \alpha_4\ D_t + \alpha_5\ EC_t + \varepsilon_t \qquad (1)$$

with:

$$EC_t = log\ Pp_{t-1} - \beta_0 - \beta_1\ log\ PK_{t-1} - \beta_2\ log\ Pa_{t-1} - \beta_3\ log\ Pe_{t-1} - \beta_4\ D_{t-1} \qquad (2)$$

where, *Pa* = the actual price of palm oil sales at the small collector level, *Pb* = the actual price of palm oil sales at the large collector level, *Pe* = the actual price of CPO export sales, *Pk* = the actual price of kernel export sales, ΔPp_t = difference from FFB price realisation of sales at the level of self-sufficient smallholders, α_1, α_2, α_3 = short term coefficient, β_1 = long term coefficient, α_4 = unbalance correction coefficient, $\Delta log\ Pe_t$ = difference log of the actual price of CPO export sales (FOB), $\Delta log\ PK_t$ = difference log of the actual price of kernel export sales (FOB), $\Delta log\ Pa_t$ = difference log of the actual price of FFB sales at the collector level, ε_t = error term.

The efficiency value (elasticity) was shown on each regression coefficient in the equation.

Assumption Testing

Multicollinearity test. *Table 2* displays data from the multicollinearity test findings. *Table 2* demonstrates that the independent variables' partial correlation coefficient, which is less than 0.8, is comparatively low. There are no indications of multicollinearity between the independent variables, according to this figure.

Heteroscedasticity test. *Table 3* presents the findings of the heteroscedasticity test for this regression model. Upon obtaining an Obs*R-squared value of 7.749412 from the Breusch-Pagan-Godfrey test, which was larger than α = 5%, it was possible to infer that the regression model employed did not exhibit heteroscedasticity.

Autocorrelation, normality, and linearity. *Table 4* shows the findings of the autocorrelation test used in this investigation. Based on *Table 4,* Chi-Square probability value of 0.1680 > 5% significance threshold, it can be said that autocorrelation is absent from the model.

TABLE 2. MULTICOLLINEARITY TESTING (α = 5%)

Item	Log (Pb)	Log (Pa)	Log (Pe)	Log (Pk)
Log (Pb)	1.000	0.766	0.767	0.727
Log (Pa)	0.766	1.000	0.788	0.722
Log (Pe)	0.767	0.788	1.000	0.704
Log (Pk)	0.727	0.722	0.704	1.000

TABLE 3. HETEROSCEDASTICITY TESTING (α = 5%)

Heteroskedasticity test: Breusch-Pagan-Godfrey			
F-statistic	2.700890	Prob. F (3,86)	0.0506
Obs*R-squared	7.749412	Prob.Chi-Square (3)	0.0515
Scaled explained SS	66.90308	Prob.Chi-Square (3)	0.0000

TABLE 4. AUTOCORRELATION TESTING (α = 5%)

Breusch-Godfrey Serial Correlation LM Test			
F-statistic	1.733815	Prob. F (2,84)	0.1829
Obs*R-squared	3.568026	Prob. Chi-Square (2)	0.1680

Figure 2 illustrates the regularly distributed data. The distribution of the data points is mostly close to the straight line, so it can be said that the regression model has met the assumption of normality.

Table 5 displays the outcomes of the linearity test with an F statistic value of 0.6704, surpassing the significance level of 5%. This indicates that the model accurately captures the relationship between the actual selling price of FFB at the independent smallholder level in Dharmasraya District and the actual prices of FFB sales at the collector level, CPO export sales (FOB), and kernel export sales (FOB) at the local level.

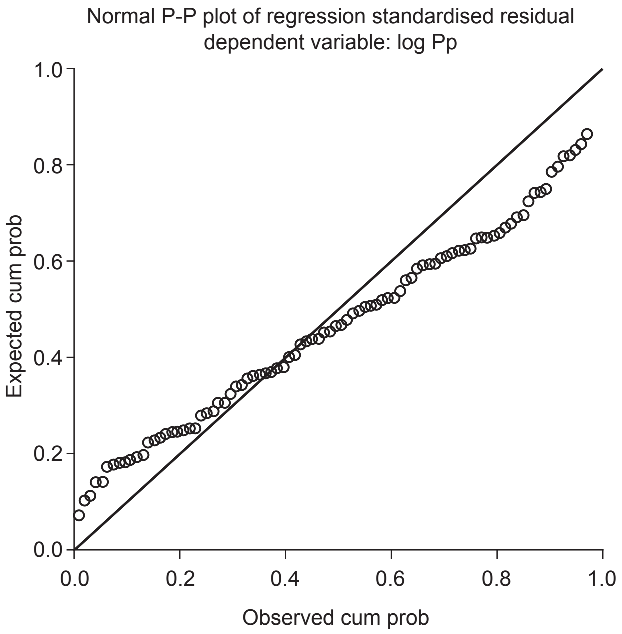


Figure 2. Normal distribution test.

TABLE 5. LINEARITY TESTING (α = 5%)

Ramsey RESET Test			
	Value	**df**	**Probability**
t-statistic	0.427028	85.00	0.6704
F-statistic	0.182353	(1.85)	0.6704
Likelihood ratio	0.192872	1.00	0.6605

Unit Root Test and Integration Degree Test

Based on the unit root test with the ADF method starting at the level, it was found that all variables were not stationary (*Table 6*). Therefore, the stationary test was repeated for the first differentiation and stationarity of the data was achieved after performing the first differentiation at a 5% significance level.

TABLE 6. UNIT ROOT TEST RESULTS FOR EACH MODEL VARIABLE (α = 5%)

Variable	Unit root test			
	Level		1st difference	
	ADF	Prob.	ADF	Prob.
Log (Pa)	-2.59	0.0996	-9.31	0.0000
Log (Pe)	-2.76	0.0690	-4.61	0.0000
Log (Pk)	-5.29	0.0000	-4.61	0.0003
Log (Pp)	-2.57	0.1023	-9.11	0.0000
Log (Pb)	-2.63	0.0912	-9.50	0.0000

Cointegration Test

Table 7 shows the outcomes of the unit root test at the error correction term (ECT) level. It is possible to conclude that the data is cointegrated since the unit root test for ECT results shows that the stationary residual at the level has a probability value of 0 < 5%. If it is established that ECT is stationary at levels, then work on creating the ECM regression equation may proceed.

Price Relationship Equation Statistical Test between Pp and Pa, Pb, Pe, Pk

This model examines the degree to which the real selling price of FFB at the independent smallholders' level in Dharmasraya District is influenced by the actual price of FFB sales at the collector level, the actual price of CPO export sales (FOB), and the actual price of kernel export sales (FOB).

In *Table 8* the prob value (F-statistic) of 0.0000 demonstrates that the relationship model between the price of Pp with Pa and Pb is good; however, partial testing reveals that the regression coefficient of the actual price of kernel export sales is not significant at the 5% significance level.

The following is a regression model using ECM (Equation 3):

$$D[\log\log(Pp)] = 0.00627 + 0.657202*D[\log\log(Pa)] + 0.205981*D[\log\log(Pb)] + 0.156501*D[\log\log(Pe)] + 0.003630*D[\log\log(Pk)] - 0.865389*ECT(-1) \tag{3}$$

The regression coefficient in the ECM indicated the price transmission elasticity value, or the efficiency of the FFB supply chain system in Dharmasraya Regency. With positive independent variable regression coefficient values less than one and all independent factors having a substantial influence on the dependent variable, the regression equation showed a short-term equation. The pricing elasticity value (ET) of less than 1 (ET < 1) indicates that various factors influence the selling price of FFB at the independent smallholder level. The constant value of 0.00627 suggests that if smallholders do not sell their FFB to small collectors, the FFB selling price would be 0.63%. The coefficient Pa of 0.657202 indicates that 65.70% of the FFB price from small collectors is transmitted to independent smallholders. The Pb coefficient of 0.205981 shows that 20.60% of the price from small collectors reaches the smallholders, while the Pe coefficient of 0.156501 demonstrates that 15.70%

TABLE 7. ECT UNIT ROOT TEST RESULTS AT LEVEL ($\alpha = 5\%$)

Null hypothesis: ECT has a unit root				
			t-statistic	**Prob.**
ADF test statistic			-8.514757	0.0000
Test critical values: 1% level			-3.505595	
5% level			-2.894332	
10% level			-2.584325	
Variable	**Coefficient**	**Std. error**	**t-statistic**	**Prob.**
ECT (-1)	-0.906101	0.106415	-8.514757	0.0000
C	4.28E-05	0.000518	0.082598	0.9344
R-squared	0.454549	Mean dependent var		5.42E-05
Adjusted R-squared	0.448280	S.D. dependent var		0.006583
S.E. of regression	0.004890	Akaike info criterion		-7.781057
Sum squared resid	0.002080	Schwarz criterion		-7.725133
Log likelihood	348.2570	Hannan-Quinn criter		-7.758516
F-statistic	72.50109	Durbin-Watson stat		1.989814
Prob (F-statistic)	0.000000			

TABLE 8. ECM REGRESSION COEFFICIENT WITH FOUR INDEPENDENT VARIABLES

Dependent variable: D [Log (Pp)]				
Variable	**Coefficient**	**Std. error**	**t-statistic**	**Prob.**
C	0.00627	0.000525	0.119551	0.9051
D [Log (Pa)]	0.657202	0.035352	18.59035	0.0000
D [Log (Pb)]	0.205981	0.030358	6.785038	0.0000
D [Log (Pe)]	0.156501	0.041872	3.737605	0.0003
D [Log (Pk)]	0.003630	0.003201	-1.134192	0.2600
ECT (-1)	-0.865389	0.111106	-7.788838	0.0000
R-squared	0.977241	Mean dependent var		0.001766
Adjusted R-squared	0.975870	S.D. dependent var		0.031814
S.E. of regression	0.004942	Akaike info criterion		-7.717067
Sum squared resid	0.002027	Schwarz criterion		-7.549293
Log likelihood	349.4095	Hannan-Quinn criter		-7.649442
F-statistic	712.7835	Durbin-Watson stat		2.031256
Prob (F-statistic)	0.000000			

of the price from CPO exporters is transmitted to them. Additionally, the Pk coefficient of 0.003630 reveals that only 0.36% of the price from palm kernel oil (PKO) exporters is transmitted to smallholders. The ECT coefficient of 0.865389 indicates that 86.50% of the adjustment is needed to achieve efficient price transmission. These findings highlight that small collectors, large collectors and palm oil exporters play a significant role in determining the FFB selling price at the independent smallholder level.

RESULTS AND DISCUSSION

Roles and Characteristics Description

In the oil palm supply chain of Dharmasraya District, various sector plays distinct roles. Independent oil palm smallholders are smallholders who cultivate their land and manage the entire process from planting to harvesting. Typically owning around 2 ha, these smallholders produce 1.5–2.0 t of FFB per ha every 20 days. However, they cannot sell their FFB directly to palm oil processing mills. Instead, their produce must first go-through small collectors who act as intermediaries. Small collectors buy FFB from these smallholders, managing about 200 independent smallholders each, and transport the FFB to large collectors. They use motorised carts and scales to facilitate this process, conducting transactions and weighing the FFB directly at the smallholders' holdings or homes.

Large collectors, purchase FFB from small collectors, then sell it to palm oil processing mills. Typically, each large collector works with three small collectors, handling an average daily production of 40 t, equivalent to five trucks with a capacity of 8 t each. The FFB is sent to various factories; some of which have their plantations and some that do not. Mills without plantations generally offer higher prices but enforce stricter FFB quality standards, whereas those with plantations offer lower prices and have more lenient sorting criteria.

In the export sector, there are 15 CPO exporters. Among them, 12 operate their palm oil mills, and nine of these also own palm oil plantations. Three exporters focus solely on exporting without having mills or plantations. Consequently, many exporters fulfil dual roles, both processing FFB into CPO and handling its export. Additionally, two CPO companies exclusively distribute their product domestically, sending it to Java and other Indonesian islands.

Respondent Data

Independent smallholders' data. Data obtained from 117 independent smallholders showed that 60% of independent smallholders had primary school education, 20% had junior high school education and 20% had senior high school education. Meanwhile, based on gender, 75% are male. Almost all independent smallholders in Dharmasraya Regency live around their holdings, so they can manage their plantations at any time and most of their main livelihood relies solely on the results of their oil palm plantations. As many as 76% of respondents in Dharmasraya Regency stated that the FFB seeds they used produced good quality FFB. 80% of respondents stated that the time they harvested FFB was in accordance with the standard time for a good harvest. As many as 62% of independent smallholders stated that the FFB that had been harvested was not completely sold to collectors. As many as 64% stated that the FFB selling price set by collectors was far below the current standard price. Furthermore, as many as 64% stated that FFB pricing was not transparent. As many as 94% stated that the price set by the collector could not be negotiated. Furthermore, 70% of independent smallholders stated that the equipment used for harvesting was adequate and 30% stated that capital for production and harvest was considered adequate.

Small collectors' data. A summary of questionnaires from small collectors in Dharmasraya Regency reveals several insights into their operational challenges. About 60% of small collectors find their transportation means for distributing FFB adequate, yet the same percentage reports that the distance for collecting FFB from smallholders is quite far. Road infrastructure is considered poor by 80% of the small collectors. In terms of FFB quality, 70% of them believe the quality is substandard. Transparency in the selling price to large collectors is a concern for 80%, and 60% report that not all FFB sold to large collectors is sold. Regarding equipment, 50% consider it adequate, but 60% indicate that the FFB weighing equipment has never been calibrated. Most notably, 90% of small collectors feel that the benefits they receive are insufficient. The primary issues faced by these collectors include the instability of FFB prices and inadequate wages for transport workers.

Large collectors' data. From the large collector respondents interviewed in Dharmasraya Regency, the characteristics of large collectors were obtained, namely that all respondents were 100% male. Judging from the level of education, 50% had a junior high school education and 50% had an elementary school education. The location of large collectors is not far from the plantations of independent smallholders and small collectors. On average, the respondents' livelihood only works as a large collector and only a small portion also work as smallholders.

A summary of questionnaires from large collectors in Dharmasraya Regency provides several key insights into their operations (*Table 9*). About 60% of large collectors believe that their transportation means for delivering FFB to the mill are adequate, but 80% find the distance to the processing mill quite far. Similarly, 80% report that road infrastructure is poor. The quality of FFB from small collectors is deemed unsatisfactory by 60% of the large collectors. Regarding pricing, 80% of large collectors say that the selling price to mills is not transparent, with prices frequently fluctuating between mills. Furthermore, 100% of large collectors report that they are unable to negotiate the selling price, having to accept the price set by the mill. As for the FFB delivered to mills, 20% is reportedly sold, while 70% is not sold due to poor quality. Despite these challenges, 83% of large collectors consider their FFB collection equipment adequate, and 80% feel that the profits from their activities are sufficient.

TABLE 9. INDEPENDENT SMALLHOLDERS' DATA

No.	Questionnaire output	(%)
1	Education	
	Primary school	60
	Junior high school	20
	Senior high school	20
2	Gender	
	Male	75
	Female	25
3	FFB quality	
	Good	76
	Not good	24
4	Harvest time	
	According to standard	80
	Not according to standard	20
5	Number of FFB harvests sold to collectors	
	All FFB harvests sold to collectors	38
	Not entirely sold to collectors	62
6	Selling price of FFB at collector	
	According to standard	36
	Below standard price	64
7	Offer selling price of FFB from smallholders to collectors	
	Price negotiations cannot be carried out	94
	Price negotiations can be carried out	6
8	Equipment for harvesting FFB	
	Adequate	90
	Inadequate	10
9	Capital for FFB production and harvest	
	Adequate	70
	Inadequate	30

Note: Standard price means the selling price of fresh fruit bunches that is in accordance with the price set by the plantation service, so 36% is standard, meaning independent smallholders receive the selling price of fresh fruit bunches from collectors according to the price set by the plantation service, 64% is below the standard, meaning smallholders receive a price from collectors below the price set by the plantation service.

Palm oil processing mills. A recapitulation of questionnaires from palm oil processing mills in Dharmasraya Regency reveals several key points. About 60% of the factories indicated that the quality of FFB sent by large collectors did not meet company standards, and 80% reported that many FFB deliveries were rejected due to non-compliance with these standards. Additionally, 60% of the mills noted that the FFB selling price was determined by the mill's set price at the time, and 80% stated that large collectors could not negotiate this price, having to accept whatever was set by the mills. Regarding government policy on FFB prices, 70% of the mills rated it as unsatisfactory. Furthermore, 20% of the mills mentioned that FFB deliveries from collectors occur daily, though the quantity is limited because 80% of the mills operate at maximum production capacity.

Exporter. Some palm oil processing mills' owners also act as exporters, so that with their monopsony power they can regulate the amount of FFB supplies belonging to independent smallholders that enter the mill, thus exporters can regulate the selling price of FFB from large collectors so that this directly impacts the low selling price of FFB received by independent smallholders so that on the one hand large profits are obtained by exporters from selling CPO abroad, while on the other hand exporters with their monopsony power are able to reduce the selling price of FFB to independent smallholders.

An Overview of the Oil Palm Supply Chain in Dharmasraya District

Direct interviews were conducted to gather information on the FFB supply chain flow in Dharmasraya Regency. The process started with the delivery of FFB owned by independent smallholders to collectors and subsequently transported to the ramp, which serves as a collection point for FFB to be loaded onto trucks. Once the truck is fully loaded, it is taken to the cooperative, and then transported to the palm oil processing mill. *Figure 2* and *3* illustrate the autonomous smallholders' supply chain flow in Dharmasraya Regency.

Figure 3 illustrates the general flow pattern of the oil palm supply chain in Dharmasraya, which consists of three main flows: Physical, information, and money. The physical flow moves from upstream to downstream, starting with FFB harvested by independent smallholders. These are collected and distributed by small collectors using land transportation to large collectors, who then transport the FFB directly to palm oil mills. The information flow is reciprocal, with independent smallholders notifying small collectors about the FFB harvested and receiving information on the selling price in return. Small collectors inform large collectors of the FFB quantities and prices, while large collectors update the palm oil mills on the FFB volume and relay the CPO production figures to exporters for international distribution. The money flow involves payments for the FFB purchased along the supply chain, starting from exporters, and moving towards the independent smallholders. Payments for FFB are made directly in cash by small collectors to the farmers, providing immediate capital and meeting daily needs.

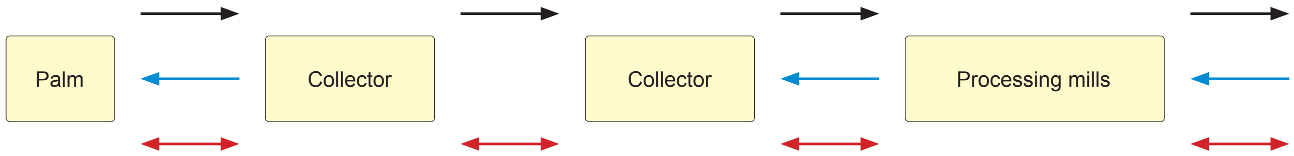
Specific Findings

The findings of this study reveal significant inefficiencies in the oil palm supply chain in Dharmasraya District, highlighting how these inefficiencies adversely affect not only the profitability of independent smallholders but also the sustainability of the agricultural system. The extended supply chain results in increased transportation, storage, packaging and damage costs, which diminish profit margins for all players, particularly smallholders. This economic strain can lead to unsustainable agricultural practices, as limited financial resources prevent smallholders from investing in environmentally friendly techniques and technologies.

Furthermore, as illustrated in *Figure 3*, the flow of information regarding palm oil prices in the Dharmasraya District is still somewhat not transparent. A key aspect of sustainability is transparency, both in economic transactions and in the flow of information. The study illustrates that the lack of transparent pricing information limits smallholders' ability to negotiate fair prices, forcing them to accept predetermined rates set by small collectors. This issue aligns with the findings of Rahayu et al. (2021), which indicate that insufficient transparency restricts smallholders' access to equitable pricing structures. By contrast, Saragih (2015) underscores that transparent communication among supply chain partners fosters trust, which is essential for building sustainable relationships within the agricultural sector.

Another significant issue in selling FFB to other supply chain members is the fluctuation in FFB prices. This volatility often causes financial difficulties for smallholders. To obtain higher prices, smallholders would need to bypass the intermediaries and sell directly to palm oil mills (Pratama, 2020). However, as shown in *Figure 2*, FFB from independent smallholders cannot be sold directly to palm oil processing mills but must go through small collectors and then large collectors. Consequently, the margins earned by independent smallholders are divided between small and large collectors. Independent smallholders are generally unable to meet the pre- and post-harvest handling requirements set by the mills. Due to limitations in capital, human resources, and technology, independent smallholders have limited bargaining power, leading to a significant portion of their FFB being rejected by the mills. As a result, the extended distribution process by independent smallholders contributes to an inefficient palm oil supply chain. Collectors act as distributors or intermediaries, facilitating the delivery of FFB from independent smallholders to the mills.

From the price relationship model [Equation (3)] stated before, indicates that the role of small collectors, large collectors and exporters of palm oil is influential in determining the selling price of FFB at the independent smallholder level. The functional relationship between the selling price of palm oil end products (CPO and PKO) in the world market and the purchasing price of FFB at the



Note: ⟶ - physical flow; ⟵ - money flow; ⟷ - information flow.

Figure 3. Physical flow, information flow and money flow of oil palm supply chain.

domestic market in West Sumatra shows that price information is not fully transmitted from mills to collectors and from collectors to smallholders; the price is completely set by the millers which is also the exporter, so the oil palm supply chain in West Sumatra is not yet efficient. Since FFB price is determined at every level of the supply chain flow from exporters to smallholders, it is proven that monopsony market power is in operation. These dynamics highlight the need for reforms that not only enhance economic equity but also promote sustainability. For instance, fair pricing structures can incentivise smallholders to adopt sustainable agricultural practices, aligning their economic interests with environmental stewardship.

Given the information above, to foster a more sustainable supply chain, the development of an integrated information system for smallholder organisations is paramount. Such a system would facilitate the transparent dissemination of information regarding selling prices, purchase prices, and FFB quantities. Increased transparency would empower smallholders to negotiate better terms, promoting economic resilience and fostering sustainable practices. Additionally, government support through outreach programs, access to high-quality seeds, and education can enhance crop quality and productivity, leading to sustainable agricultural growth.

Furthermore, as suggested by Teten Masduki, Minister of Cooperatives and SMEs, the government could assist by establishing cooperatively run small CPO and red palm oil (RPO) facilities to process smallholders' FFB. This is particularly important since oil palm smallholders face challenges in selling their harvests even after the ban on CPO and its derivatives was lifted (VOA Indonesia, 2022). Establishing cooperatively run mini-CPO and red palm oil (RPO) facilities would not only reduce dependency on intermediaries but also enhance local value-added processing. This shift can contribute to sustainable development goals (SDG), such as promoting decent work and economic growth (SDG 8) and fostering sustainable agriculture (SDG 2). By providing smallholders with the tools and resources to process their produce, we can create a more equitable and environmentally sustainable supply chain.

An increase in the supply of FFB from more productive trees will likely boost CPO production from the downstream sector and meet the demands of the cooking oil industry, both domestically and for export. This could positively impact the Indonesian economy and enhance the bargaining power of independent oil palm smallholders. Achieving supply chain efficiency will enable these smallholders to compete with or surpass plasma smallholders in terms of FFB prices.

The research recommends establishing smallholder cooperatives and mini-CPO mills to eliminate intermediaries and integrate them into the smallholder institutions. An integrated information system within these cooperatives would facilitate access to crucial data, such as palm oil prices and availability, thus improving transparency and efficiency in the supply chain.

CONCLUSION

This study provides a comprehensive analysis of the oil palm supply chain in Dharmasraya District, uncovering the intricate challenges faced by independent smallholders, small collectors, large collectors, and exporters. Our findings reveal that the price transmission elasticity (ET) is below 1, indicating that the real sales prices received by independent smallholders for FFB are significantly influenced by the pricing dynamics at both small and large collector levels, as well as by CPO and kernel export prices. This situation underscores the substantial impact that palm oil exporters, large collectors, and small collectors have on FFB pricing, highlighting the urgent need for structural reforms to empower independent smallholders and ensure they receive fair compensation for their produce. Moreover, the study illustrates how the lack of pricing transparency and the monopsony power held by larger market players restrict smallholders' bargaining power, jeopardising their economic viability. To promote sustainability within this supply chain, it is essential to establish independent smallholders' institutions and integrated information systems. These initiatives would empower smallholders by enhancing their access to market information and improving their negotiation capabilities, ultimately fostering a more equitable and resilient supply chain. By increasing transparency and advocating for fair pricing practices, smallholders will be better positioned to invest in sustainable agricultural methods, which are critical for ensuring long-term environmental health and economic stability. This research is still limited to one district so the results need to be studied in further research for a wider area. The advantage of this research is that the topic studied discusses the characteristics of actors in the oil palm supply chain and important problems in the oil palm supply chain so that it can be studied comprehensively. This study can be a starting point for further research that explores the relationship between actor characteristics and oil palm supply chain performance and the research model needs to be developed by adding other factors, such as financial access, technology and policy, to provide deeper insights.

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MATERIAL AND CURVATURE OF THE OIL PALM HARVESTING KNIFE AND THEIR EFFECTS ON FORCE AND ENERGY REQUIREMENTS

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ABSTRACT

Oil palm is an economically important crop, and the process of harvesting its fruit constitutes a major proportion of the production cost. Using an efficient harvesting knife can reduce the cultivation cost and ensure a high yield. In this study, the effect of the material and curvature of the knife for cutting fronds and fresh fruit bunches (FFB) was investigated. Three different materials, namely, EN-42J (M_1), EN-9 (M_2), and hardened and tempered (H&T) (M_3) steel and five different curvatures namely, Malaysian model (B_1), Andhra model (B_2), Kerala model (B_3), FMD-1 (B_4) and FMD-3 (B_5) were examined. A total of 15 harvesting knives were developed and evaluated in the field using a load cell indicator attached between the pole and the harvesting knife. The parameters of maximum cutting force (CF_c) , maximum specific cutting force (SCF_c), and maximum specific cutting energy (SCE) were calculated. The findings indicated that the material of the knife exhibited a negligible effect, but the curvature had a significant effect. Of the various models tested, the values of the CF_c (30.56, 54.50 kgf), SCF_c (0.26, 0.95 kg cm^{-2}), and SCE (5.08, 4.78 kg·cm cm^{-2}) were the least for the Malaysian model followed by FMD-3 model for cutting the frond and FFB.

Keywords: harvesting knife, load cell indicator, oil palm harvesting, specific cutting energy, specific cutting force.

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INTRODUCTION

The oil palm is an economically important crop and contributes to ensuring world food security; moreover, it has emerged as a renewable energy source (Hashim et al., 2012; Henson, 2012; Khatun et al., 2017; Zahari et al., 2015). Globally, oil palm production reached 416.39 million tonnes from 28.91 million hectares of plantation, with an average production of 14.41 t ha^{-1} (Food and Agriculture Association [FAOSTAT], 2023). In India, the area under oil palm is 0.37 million hectares, with a production of 16.89 million tonnes. Among the various states in India, the area under oil palm (32,982 ha) is the highest in Tamil Nadu, followed by Andhra Pradesh and Karnataka, amounting to a production of 3,038 t (Anonymous, 2021). Across all the cultivation stages of oil palm after its maturity, the harvesting stage is associated with the highest production cost (Law et al., 1992). This stage involves important activities, viz., cutting the fronds and fresh fruit bunches (FFB), stacking the fronds, collecting the loose fruits, and carrying the harvested fruits to the collection point (Henson, 2012; Jelani et al., 1998). The harvesting of oil palm constitutes approximately 32% of the overall production cost, followed by 33% for applying fertilisers (Law et al., 1992).

Efficient harvesting of FFB is crucial for ensuring the quantity and quality of FFB. A minimum ripeness standard of five detached fruits per bunch at 10-day harvesting intervals has been reported to be suitable and effective for producing FFB of the desired quality (Teo & Tan, 1992). Although some standards are available, no single standard is appropriate for different scenarios. Even within the same plantation, varied standards must be adopted based on changes in palm age, height, topography and weather. A good harvesting standard provides the best compromise among oil yield, oil quality and harvesting cost and involves the accurate determination of minimum ripeness standard, harvesting interval, harvesting techniques and procedures.

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A sickle attached to an elongated pole made from long bamboo sticks was previously used as the oil palm harvesting knife. Since 1986, aluminium poles have been used for harvesting the oil palm as a substitute for bamboo poles as they became scarce and expensive (Hassan et al., 1988). Chisels were employed widely for cutting fruits from young and short oil palms, and sickle-shaped knives with an aluminium pole were used for cutting fruits from tall oil palms. As it was lightweight and allowed telescopic adjustments, the aluminium pole-attached sickle was easier to operate than the bamboo pole-attached sickle. The harvesting capacity of the sickle attached to the aluminium pole was in the range of 0.4–1.0 man-day t^{-1} of FFB. This value depends on the age of the oil palm, harvesting season, skill of the harvester, and field topography (Omereji, 1994). The reaction force and energy requirement of harvesting knives determine their efficiency. These parameters were tested using spring-powered sickle cutters with cutting angles of 90°, 60°, and 45° at three levels of frond maturity. The findings indicated that the cutting angle rather than frond maturity exerted considerable effects on the specific reaction force and energy requirement. The cutting angle increased from 45° to 90°, and the specific reaction force increased to approximately 72% (Jelani et al., 1999). The Malaysian Palm Oil Board (MPOB) developed a motorised cutter for harvesting FFB at < 4.5 m from the ground level (Jelani et al., 2008). Field trials revealed that the harvesting capacity of the cutter was 560–750 bunches per day. Furthermore, the use of this cutter reduced the labour cost by 50%. In addition, Abbood (2020) experimented to determine the minimum cutting force requirement for cutting date palm fronds. A triangle-type knife with an edge angle of 20°, a width of 0.05 mm, and a length of 12 cm was tested with a hydraulic machine at three levels of moisture, namely, 5%, 10% and 40% and three cutting angles, namely, 0°, 45° and 90°. The cutting angle of 90° resulted in the lowest cutting force of 6.04 KN at 10% fronds water content. Shokripour et al. (2012) developed a harvesting mechanism for oil palm FFB, which involved the design of a blade tooth for rapid and clean cutting. In designing the blade, the parameters of metal type, width, blade set, thickness, tooth form, and blade length were considered. The contact angle between the saw tooth and the material was a key factor in ensuring the effective cutting performance of the saw blade. The energy consumption of the circular saw-cutting process of oil palm fronds was measured (Sun & Zaidi, 2023). A theoretical model of work and power was developed and applied to optimise the cutting process of an oil palm frond with a base width of 100 mm. A minimum cutting energy of 330 J for a rotational speed of 1,000 rpm and a feed rate of 10 mm s^{-1} was measured during the harvesting process. Although promising results were obtained in developing an effective cutting edge, economically, the device could not compete with existing manual tools.

Conventionally, harvesting knives are fabricated without considering the physical properties of the material, reaction force, cutting method, cutting angle, and cutting speed, which leads to the inefficient operation of such harvesting knives. The cutting of fronds and harvesting of FFB is a labour-intensive operation, and skilled labour is required. Moreover, the harvesting operation is time-bound as the FFB should be processed within 24 hr after harvest to obtain edible quality of raw palm oil (Arumugan & Sundaresan, 1992). The migration of labourers from rural areas to various scholastic jobs in the urban sector has made oil palm harvesting a tiresome process for the planters. This situation has necessitated the introduction of improved knives for harvesting the oil palm. The pertinent factors that influence the performance of oil palm harvesting knives are the material and the curvature of the knife (Jelani et al., 1998). In this study, the effect of the material and shape of the knife on the force and energy required for oil palm harvesting was investigated.

MATERIALS AND METHODS

Preliminary information was collected on various types of harvesting tools used in different parts of the world. Areas within Tamil Nadu and other states were visited to gain knowledge about oil palm grooves, knives, and methods used for harvesting. Among the world's countries, Malaysia is one of the major producer of palm oil. In India, Andhra Pradesh, Tamil Nadu, and Kerala are the leading states that cultivate oil palm intensively. Hence, details were collected on conventional tools used in Malaysia, Andra Pradesh, and Kerala. In Tamil Nadu, the Department of Farm Machinery and Power Engineering, Tamil Nadu Agricultural University (TNAU), conducted study on the knife used to harvest oil palm. Six different knife curvatures, namely, FMD-1, FMD-2, FMD-3, FMD-4, FMD-5 and FMD-6 were considered, and two curvatures, i.e., FMD-1 and FMD-3 were optimised based on the minimum force required to harvest oil palm. Hence, five different curvatures were selected for the investigation, which included Malaysian model (B_1), Andhra model (B_2), Kerala model (B_3), FMD-1 (B_4) and FMD-3 (B_5) (*Figure 1*).

The five knives differed in their shape, width and height. The detailed specifications of oil palm harvesting knives are presented in *Table 1*, and the dimensions are illustrated in *Figure 2* (Singh, 2012).

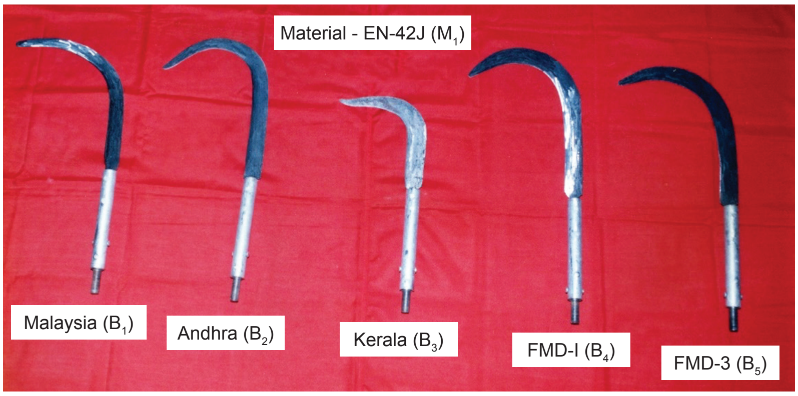

Figure 1. A view of the five types of knife curvatures selected.

TABLE 1. DIMENSIONS OF THE STUDIED OIL PALM KNIVES

Parameters	**Dimensions of sickles (mm)**				
	Malaysia (B$_1$)	**Andhra (B$_2$)**	**Kerala (B$_3$)**	**FMD-1 (B$_4$)**	**FMD-3 (B$_5$)**
Maximum width of the knife (B)	38	39	50	44	45
Knife thickness (C)	4	4	4	4	4
Cutting surface (D)	450	516	302	472	456
Outer length of the knife (E)	555	608	418	631	589
Concavity of the knife (F)	136	182	98	157	135
Sickle length (G)	634	664	535	676	651
Maximum handle length (H)	300	300	300	300	300
Maximum handle diameter (I)	31.25	31.25	31.25	31.25	31.25
Size of the sickle (L)	388	352	281	380	403

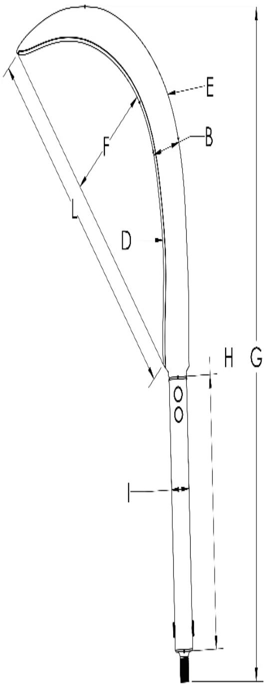

Figure 2. Dimensions of the oil palm knife.

The hardness of the knife material plays a vital role in determining the energy consumed for harvesting the FFB and cutting the fronds (Zhang et al., 2023). Material hardness is mainly based on the chemical composition and carbon content present in it (Trzaska & Sitek, 2023). Three different materials commonly used for manufacturing the harvesting knife namely EN-42J (M$_1$), EN-9 (M$_2$) and hardened and tempered (H&T) steel (M$_3$) were selected for the investigation in which the percentage composition of carbon, silicon, and manganese was studied (Singh et al., 2022). The details of the chemical composition analysis of the abovementioned four materials are described in *Table 2*.

TABLE 2. METALLURGICAL COMPOSITION OF THE SELECTED MATERIALS

Material	**Carbon %**	**Silicon %**	**Manganese %**
EN-42J	0.58	0.20	0.69
EN-9	0.55	0.05	0.77
H&T Steel	1.10	0.09	0.76

Hence, this study was conducted using five different curvatures, viz., B$_1$, B$_2$, B$_3$, B$_4$ and FB$_5$ made of three different materials, namely, M$_1$, M$_2$ and M$_3$. Using all possible combinations of the materials and curvatures selected, a total of 15 knives were developed and evaluated in the field.

Measurement of the Force Using Load Cell Indicator

The harvesting knives were evaluated in one of the largest private oil palm plantations located in Upparpatti, which is approximately 6 km from Theni toward Cumbam, India. This plantation was established in 1994 with 600 oil palms over an area of 10 acres. Presently, the total area under oil palm in this plantation is approximately 160 acres. The assessment was performed on a 10-years-old oil palm having an average height of 4.3 m.

The handle of the knife was made by inserting a wooden roller in an aluminium pipe 31.25 mm in diameter, 2.50 mm in thickness, and 270.00 mm in length. The knife handle was inserted into it and riveted using aluminium rivets at four different positions. The aluminium handle was provided with a threaded collar, which allowed the attachment and detachment of the sickle to the aluminium pole. The handle of the knife was threaded to one end of the load cell, and the other end was threaded to the pole. Subsequently, the load cell was connected to the load cell indicator with a long cable (*Figure 3*). The strain gauge-type load was used to measure the cutting force (Kathirvel et al., 2009, 2011). The knife was attached to or detached from the aluminium poles of various lengths as per the requirement, i.e., the height of the oil palm. The pole was of three different lengths, i.e., 3.60, 2.10 and 1.50 m. A combination of poles was also used depending on the height of the oil palm.

After setting up the harvesting knife with the instrument, the cutting operation was performed by the operator. Harvesters with comparable skill and experience (10 years) in oil palm harvesting were used in this study. The harvesting of oil palm fruits involves two activities: The first one is to lift the pole upright and the second is to cut the fronds and fruit bunches. The readings were noted from the load cell indicator when the operator cut the fronds in a single stroke. Trained operators usually cut the fronds and FFB in the first attempt itself. After cutting the frond, its exposed cut surface was traced on a plain sheet and the area of the cut surface was measured using a polar planimeter and its depth using a vernier calliper. With the same setup, the knife was used to harvest the FFB of the oil palm. The readings were noted from the load cell indicator when the operator cut the FFB in a single stroke. A total of 3–4 fronds must be cut to reach the peduncle of the FFB. The operational view of harvesting FFB is shown in *Figure 4*. The exposed area of the peduncle and the depth of the cut were measured using a polar planimeter and vernier calliper, respectively. After cutting the fronds and FFB with the selected knife, the same procedure was repeated for all developed knives. Each treatment was replicated eight times for cutting the fronds and four times for harvesting the FFB for statistical analysis to obtain accurate results.

Evaluation of the Parameters

The parameters used to evaluate the selected knives were maximum cutting force (CF_c), maximum specific cutting force (SCF_c) and maximum specific cutting energy (SCE) (Jelani et al., 1998; Persson, 1987; Prasad & Gupta, 1975).

Maximum cutting force (CF_c). CF_c between the pole and the knife is defined as the maximum force exerted by the operator for cutting the fronds or harvesting the FFB. The value of this force directly affects the cutting performance and the drudgery involved in harvesting.

The average of the readings recorded from the load cell indicator was calculated and compared

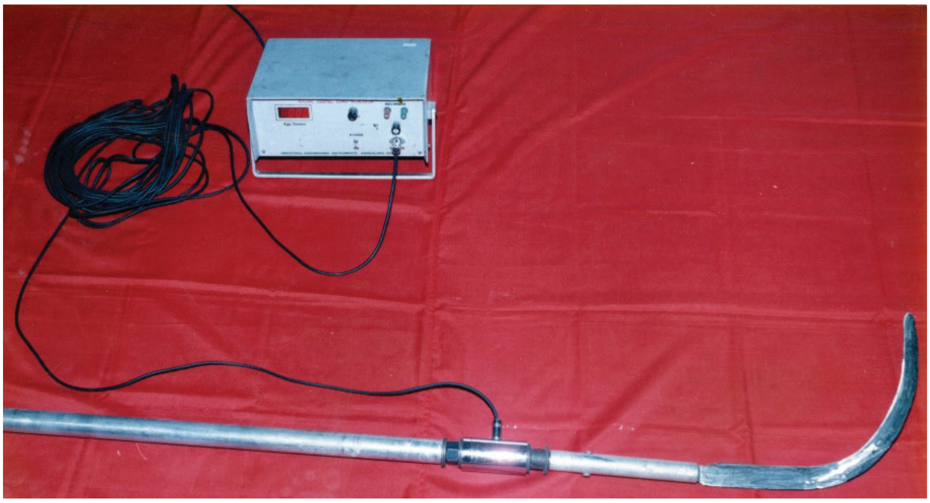

Figure 3. A view of the load cell and indicator connected between the pole and the knife.

Figure 4. The harvesting of FFB.

with the calibration chart. The corresponding load cell indicator readings from the calibration chart directly provided the maximum cutting force required by the operator for cutting the FFB.

Maximum specific cutting force (SCF_c). The SCF_c is defined as the maximum value of the cutting force per unit cross-sectional area of the material under the knife [Equation (1)]. The area of the cut varies with the approach angle for cutting the FFB, which in turn affects the cutting performance directly through the specific cutting force.

$$SCF_c = F/A \quad (1)$$

where, SCF_c is the maximum specific cutting force (kg cm^{-2}), F is the force (kg), and A is the area of the cut (cm^2).

The exposed cut area of the frond was calculated based on the recorded dimensions. Similarly, the area of the cut surface of the peduncle of FFB was calculated from the diameter of the peduncle.

Maximum specific cutting energy (SCE). The SCE is defined as the cutting energy required to cut per unit cross-sectional area [Equation (2)]. The depth of the cut for cutting the FFB also varies with the angle at which the operator cuts the FFB. The energy required for cutting directly affects the performance of the harvesting operation.

$$SCE = (F/A) \times D \quad (2)$$

where, SCE is the maximum specific cutting energy (kg·cm cm^{-2}), F is the force (kg), A is the area of the cut (cm^2), and D is the depth of the cut (cm).

The results were analysed statistically using analysis of variance (ANOVA) with the MINITAB software. A completely randomised design was used to assess the effects of the variables, namely, material (M) and knife curvature (B), on CF_c, SCF_c and SCE.

RESULTS AND DISCUSSION

The ANOVA for CF_c, SCF_c and SCE is shown in *Table 3*. The "F" value was significant at a 1% level of probability for all treatments for CF_c, SCF_c and SCE. The main effect of the type of knife curvature (B) was significant at a 1% level of probability, which signifies that this variable influenced the CF_c, SCF_c and SCE. On the contrary, the effect of knife material (M) was insignificant.

TABLE 3. ANOVA FOR MAXIMUM CUTTING FORCE (CF_c), MAXIMUM SPECIFIC CUTTING FORCE (SCF_c) AND MAXIMUM SPECIFIC CUTTING ENERGY (SCE)

SV	DF	F		
		CF_c	SCF_c	SCE
Treatment	14	6.61**	5.87**	5.92**
Knife material (M)	2	1.82 ns	2.21 ns	< 1
Knife curvature (B)	4	15.7**	12.36**	15.51**
M×B	8	3.25**	3.55**	2.55*
Error	105			
Total	119			
CV		**29.0%**	**38.0%**	**34.5%**

Note: ** - Significant at 1% level; * - significant at 5% level; ns - not significant.

The interaction effects of material and curvature of the knife on CF_c, SCF_c and SCE are listed in *Table 4*. M_3 did not exert a significant effect on CFc, SCFc and SCE with different types of knife curvatures as the force required remained the same at all levels of the variables. The interaction effects of the treatments from the other two materials M_1 and M_2 were significant. The CF_c was the least for the combination of M_1B_1 (30.56 kgf), M_2B_1 (31.81 kgf) and M_2B_5 (32.56 kgf). The specific cutting force was the least for the combination of M_1B_1 (0.26 kg cm^{-2}), M_2B_1 (0.29 kg cm^{-2}) and M_2B_5 (0.26 kg cm^{-2}). Similarly, the maximum specific cutting energy was the lowest for the combination of M_1B_1 (5.08 kg·cm cm^{-2}), M_2B_1 (5.31 kg·cm cm^{-2}) and M_2B_5 (5.05 kg·cm cm^{-2}).

Effect of the Material

The effect of the material on CF_c, SCF_c and SCE for cutting fronds and FFB is represented in *Figure 5*. The material of the knife exerted a negligible effect on all three parameters for cutting oil palm FFB as the cutting force for each material was almost the same for the different curvatures of the knife. Hence, the material had negligible influence on the CF_c, SCF_c and SCE. This finding substantiated the results obtained from the statistical analysis that the effect of curvature of the knife on CF_c, SCF_c and SCE was significant.

Effect of Knife Curvature

The effect of knife curvature on CF_c, SCF_c and SCE for cutting fronds and FFB is represented in *Figure 6*. The knife curvature B_1 resulted in the least value (30.56, 54.5 kgf) of CF_c irrespective of the material. Apart from B_1, knife B_5 (32.56, 55.0 kgf) required the least CF_c for cutting oil palm fronds and FFB when compared with other curvatures. The knife curvatures B_1 (0.26, 0.95 kg cm^{-2}) and B_5 (0.26, 1.15 kg cm^{-2}) required the least SCF_c when compared with other curvatures. Moreover, the curvature of the knife influenced the SCE, which was predominant for knife B_5 (5.05, 4.50 kg·cm cm^{-2}) as it exhibited the least value for the SCE, followed by knife B_1 (5.08, 4.78 kg·cm cm^{-2}). All other knives were ineffective as their curvatures made the operation tedious. This difficulty could be attributed to the radius of the knife curvature matching the frond structure, which ensured easy cutting. For example, knife curvature B_3 slipped during the cutting operation owing to its right-angle curvature. This finding validated the results obtained from statistical analysis that the effect of knife curvature on CF_c, SCF_c and SCE was significant.

Optimisation of Parameters for the Selected Knives

Based on the above analysis and the results obtained, the optimum combination of the knife material with the least value for the selected parameters, viz., CF_c, SCF_c and SCE is presented in *Table 5*.

The harvesting productivity of the knives was evaluated, and the performance of the optimised values of different variables were determined. FFB harvested per day using the conventional knife was only 96 bunches, whereas those harvested per day with the modified harvesting knives were 144–147 bunches, which was 53% more than that of conventional tools.

TABLE 4. M×B TABLE OF MEANS FOR MAXIMUM CUTTING FORCE (CF_c), MAXIMUM SPECIFIC CUTTING FORCE (SCF_c) AND MAXIMUM SPECIFIC CUTTING ENERGY (SCE)

Curvatures	CF_c			SCF_c			SCE		
	EN-42J (M_1)	EN-9 (M_2)	H&T (M_3)	EN-42J (M_1)	EN-9 (M_2)	H&T (M_3)	EN-42J (M_1)	EN-9 (M_2)	H&T (M_3)
Malaysia (B_1)	**30.56 c**	**31.81 c**	44.25 a	**0.26 b**	**0.29 c**	0.34 a	**5.08 b**	**5.31 c**	6.89 a
Andhra (B_2)	46.81 b	50.06 b	45.31 a	0.36 b	0.50 b	0.38 a	7.88 b	8.83 b	7.71 a
Kerala (B_3)	48.88 b	35.13 c	37.94 a	0.40 b	0.56 b	0.38 a	7.78 b	9.46 b	7.52 a
FMD-1 (B_4)	72.94 a	67.63 a	49.81 a	0.65 a	0.76 a	0.43 a	12.81a	13.21a	9.28 a
FMD-3 (B_5)	45.44 b	**32.56 c**	47.38 a	0.39 b	**0.26 c**	0.47 a	6.93 b	**5.05 c**	8.61 a

TABLE 5. OPTIMISED PARAMETERS FOR THE SELECTED KNIVES

Knife	CF_c		SCF_c		SCE	
	Frond	FFB	Frond	FFB	Frond	FFB
EN-42J (M_1) and Malaysia (B_1)	30.56	54.5	0.26	0.95	5.08	4.78
EN-9 (M_2) and FMD-3 (B_5)	32.56	55.0	0.26	1.15	5.05	4.50

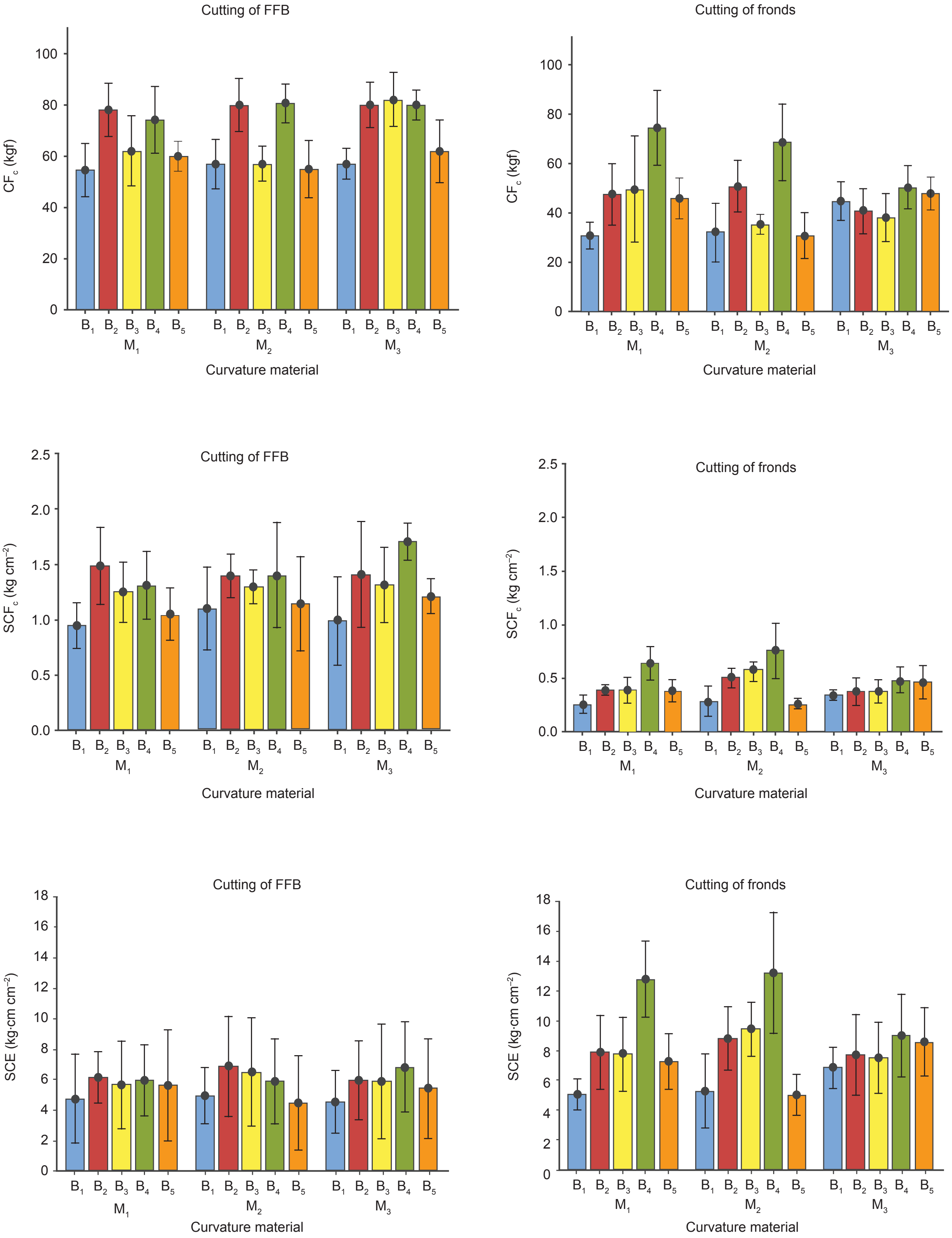

Figure 5. The effect of the material on CF_c, SCF_c and SCE for cutting fronds and fresh fruit bunches.

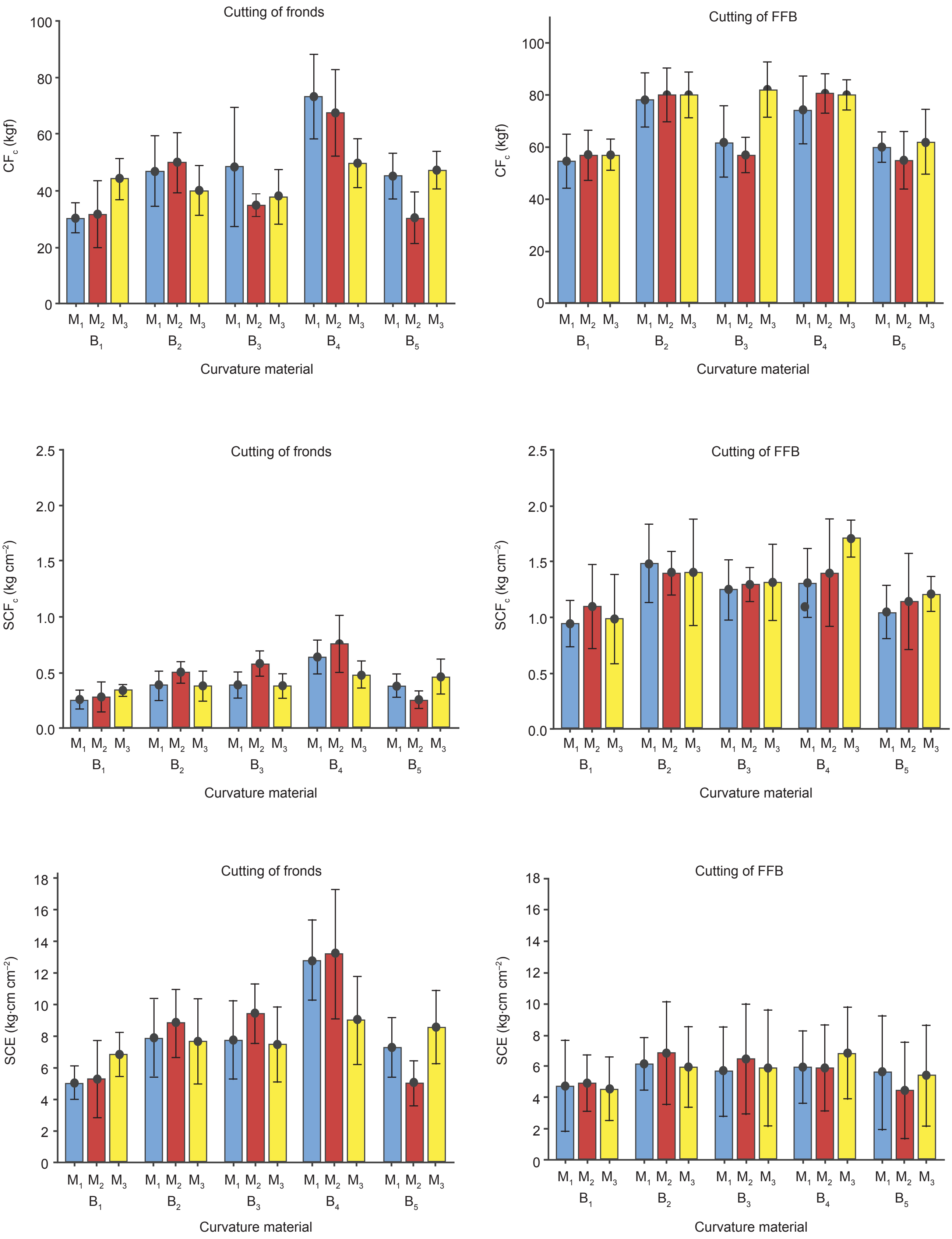

Figure 6. The effect of knife curvature on CF_c, SCF_c and SCE for cutting fronds and FFB.

CONCLUSION

The material used to construct the knife had negligible effect on CF_c, SCF_c and SCE for cutting oil palm FFB. The least values for CF_c (30.56, 54.5 kgf), SCF_c (0.26, 0.95 kg cm^{-2}) and SCE (5.08, 4.78 kg·cm cm^{-2}) were recorded for the Malaysian model, which was followed by the FMD-3 model (32.56, 55.0 kgf; 0.26, 1.15 kg cm^{-2} and 5.05, 4.50 kg·cm cm^{-2} respectively), for cutting the fronds and FFB.

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A PRELIMINARY EVALUATION OF A RADIO-CONTROLLED HYDROSTATIC TRANSMISSION MOWER FOR OIL PALM PLANTATION

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ABSTRACT

Maintaining effective weed control is essential for oil palm fields. To address sustainability concerns and the increasing cost of chemicals, a cutting-edge mechanical mower has been recommended as an alternative solution. Integrating mechanical, fluid mechanics and electronic systems in a mower is a solution to enhance manoeuvrability in the field. The development and testing of an integrated hydrostatic transmission (iHST) mower with a teleoperation system to improve operational efficiency were discussed. The time-motion study on a 3 ha plot showed that the prototype could achieve an effective field capacity of 0.44 ha/hr at RM255.50/ha, an annual cost for path and palm circle mowing operation in the test area of flat terrain, mild weed infestation and in a dry condition. A 5.8 GHz FPV camera enabled the machine's teleoperation, which provides real-time feedback for improved control during operations. Although its productivity was inferior to the current mechanised spraying approach, the machine could be a sustainable alternative with benefits such as attracting skilled local workforces in plantation operations and environmental friendliness.

Keywords: mechanisation, teleoperation, weed management.

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INTRODUCTION

Palm oil plays a critical role in global food and other industries. Malaysia alone has a total planted area of 5.67 million hectares with an average fresh fruit bunch (FFB) yield of 15.49 t/ha in 2022 (Parveez et al., 2023). Productivity and planted areas experienced fluctuations due to various factors, including environmental impacts, operational practices, and other influences. The availability of workers is one of the primary determinants of productivity. Unfortunately, a situation such as the COVID-19 pandemic has further strained the availability of workers for tasks such as FFB harvesting and other fieldwork. To address this issue, there is a growing need to modernise field practices to minimise reliance on foreign workers and attract local participation within the industry.

Maintaining field conditions in oil palm plantations is crucial for smooth operation, particularly in fruit evacuation and loose fruit collection. Herbicides such as glyphosate, metsulfuron-methyl and triclopyr are commonly used for weed control in oil palm plantations (Rusli et al., 2014). However, their application comes with environmental implications. In this respect, mechanical mowing could be an alternative to minimise environmental impact (Darras et al., 2019). Due to its cost-effectiveness, mechanical mowing technology, such as zero turn and push-over mower, is not widely practised in matured palm oil. Besides, the operator's onboard mechanical drive is unsuitable for palm circle mowing. Thus, integrating a mechatronic system on a mower could alleviate the situation through autonomous or teleoperation navigation.

In autonomous function, various sensors, actuators, processors and system interfaces must be configured to work seamlessly through specific algorithms. Autonomous agriculture vehicles have faced challenges in heterogeneous field conditions, with systems such as RTK-GNSS and vision-based

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obstacle avoidance showing limitations in accuracy and complexity (Villemazet, et al., 2022). Fusion of sensors in autonomous vehicles is challenging due to the unpredictable nature of agricultural fields, especially in crop-type agriculture.

Among the appealing methods to increase automation for machinery navigation in the field is the teleoperation system. Teleoperation uses human interaction as the central controller with machines as executors, known as the Human-Machine Interaction (HMI) method (Vasconez et al., 2019). Three teleoperation modes have been identified and described in *Table 1* (Al-Razgan et al., 2016; Murakami et al., 2008).

Figure 1 shows the basic configuration of the teleoperation system. Feedback varied depending on the selection of the principle. However, a field of view (FOV) system is commonly utilised in direct control mode (Saputra & Mirdanies, 2015), with feedback for decision-making sent through visualisation.

Most of the innovations in radio control mowers available in the market are based on battery-powered systems. The battery could only last for a specific time, even with a rechargeable system (Azwan et al., 2017). Limited operation time is not favourable for plantation operation as it could lower productivity. Longer operation time, wireless control and adaptation to oil palm field conditions are critical parameters for oil palm direct control teleoperation systems. On the other hand, a direct mechanical drive is not suitable for long-range control in agricultural conditions due to the complexity of simultaneous control of the vehicle's mechanical system. Incorporating hydrostatic transmission and electronic systems can control internal combustion engine vehicles through teleoperation.

Hydrostatic transmission (HST) is a system that transmits engine power through hydraulic fluids. HST machine is excellent for crop-type agriculture conditions as it provides greater torque, speed and control. HST with track-type tyres has a better-floating ability to reduce soil compaction due to its larger weight distribution area. HST could be easily integrated with a radio-controlled system and provide longer operational time. The advanced HST technology is the integrated hydrostatic transmission (iHST) system, where the hydrostatic motor and pump are integrated as one component and no external connection is required. It provides flexibility in terms of space and effectiveness to agriculture machinery. Thus, this study evaluated an iHST machine's technical and economic viability with teleoperation function in oil palm plantations for weed management operation. A cost comparison

TABLE 1. TELEOPERATION MODE

No.	Principle types	Description
1	Direct control teleoperation	Direct control is a short-distance communication between the operator and the machine. Typically, a traditional controller such as a joystick will be the primary input of the machine or actuators for navigation. It is also known as a forced or master-slave control system. The feedback system could comprise several methods, including real-time video streaming.
2	Supervisory teleoperation	The system involves two control methods: Direct control and autonomous action. The fusion of sensors helps the system to make certain decisions, such as turning, collision avoidance, automatic tracking, swarm control, and others. The command centre could be placed at a distance for direct control. Thus, a good communication network is required.
3	Multimodal teleoperation	The system incorporates systems such as augmented reality (AR) and virtual reality (VR) as the interface for the operator. The multi-sensory system offers a variety of options for machine navigation and could lead to higher precision.

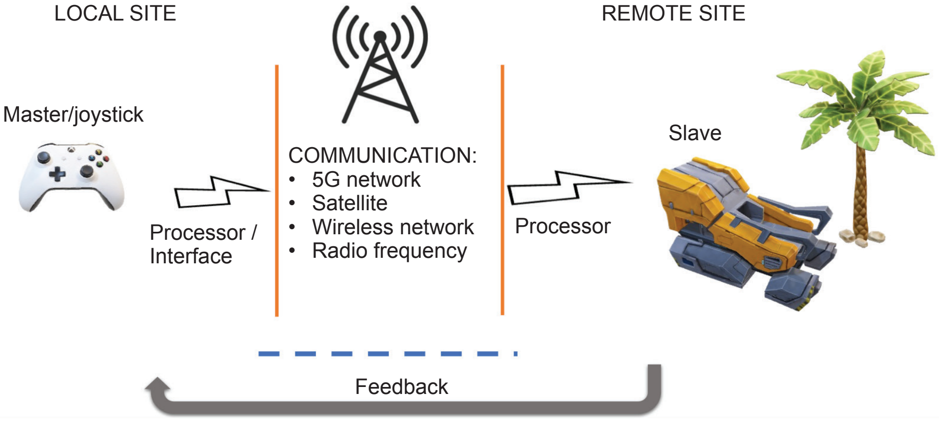

Figure 1. General configuration of the teleoperation system.

of the proposed system with chemical herbicide spraying was analysed in this study, as it is the current practice in the industry. The study's novelty is integrating electronic control with the iHST system in oil palm plantation conditions.

MATERIALS AND METHODS

Prototype of Teleoperation Hydrostatic Transmission Machine

A prototype with a teleoperation function was developed and utilised in the performance test (*Figure 2*). The prototype was built based on a commercial push-over flail mower chassis with its drivetrain modified with an iHST and transaxle. The specifications of the prototype are shown in *Table 2*. The machine was compact and had zero turning radius, and it had a flail mower with a maximum cutting width of 700–800 mm attached to it.

TABLE 2. SPECIFICATIONS OF THE PROTOTYPE

No.	Components/ Parameters	Specification
1	Dimension (W x L)	950 x 1,450 mm
2	Weight	200 kg
3	Flail cutting height	50–80 mm
4	Engine	Water-cooled petrol 10 hp (Mitsubishi GB290PN)
5	Working engine speed	3,000 rpm
6	Hydrostatic	10 cc Integrated HST

A customised electronic control board was specially designed to control the machine. The board was configured to manage a set of linear 12V-DC servo actuators connected to it. Two servo actuators were designated to manoeuvre the machine's HST for steering, while another actuator was responsible for regulating the fuel throttle. In addition to directional control, the iHST could also adjust the speed of the machine. By setting the HST knob lever to a specific position, the vehicle's maximum speed could be limited to ensure safety. The machine manipulated three 2.4 GHz radio transmitter channels directly integrated into the controller board. A standard-resolution first-person view (FPV) camera of 720 × 576 @ 25 fps is attached to the prototype.

Time Motion Study of the Prototype in Actual Field Environment

The prototype's capabilities were evaluated in a 3 ha plantation in Bagan Datuk, Perak, Malaysia. The study selected the site for a preliminary study to validate its performance in an actual scenario. The prototype was anticipated to manoeuvre in tight spaces and perform in terrace areas due to its small size and comparable engine power with existing machinery in the terrace area (10 hp engine). The research plot had 10 year old palm trees. A maximum weed height of about two feet was available during the test along the harvesting or mechanical paths, and the topography was predominantly flat with inland-type soil. The paths were undisturbed and connected, making them ideal for the test. The

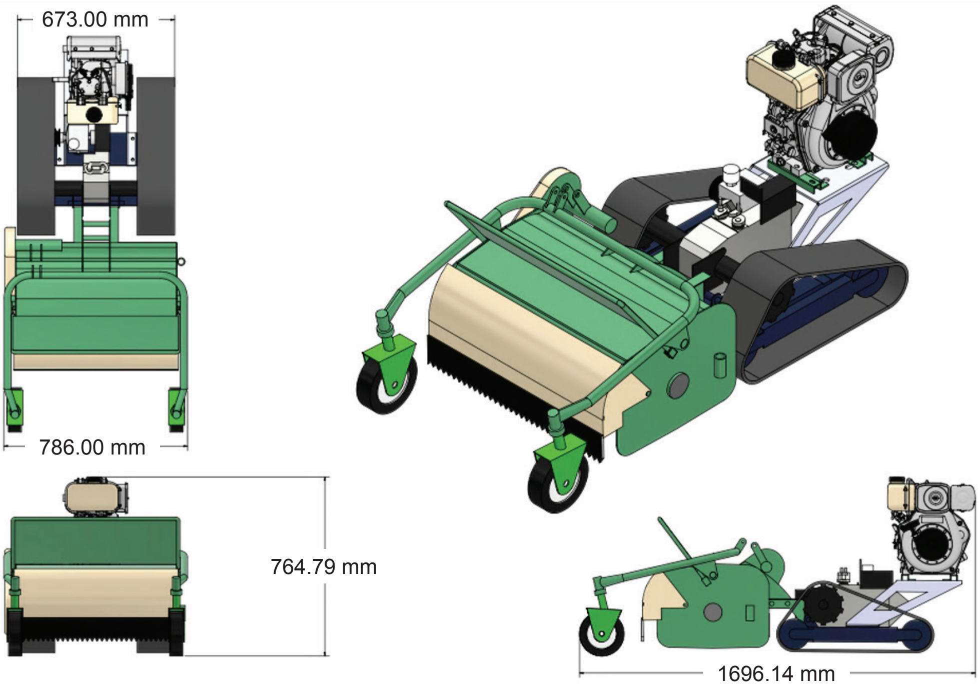

Figure 2. CAD drawing of the prototype.

weeds consisted mainly of grass with narrow leaves and fibrous root systems. Tests were conducted under dry conditions.

The machine could cut grass along the harvesting paths and around the palm circles. Thus, two time-motion studies were conducted: Mowing on the harvesting path in a straight line and circling palm trees.

Mowing on the harvesting path (Straight line movement). The prototype traversed in a straight line on the harvesting paths or between palm tree rows. The machine was controlled at a low speed (about 3 km/hr), and all obstacles, such as fallen fronds, were removed. The distance between the palms was about 9.0 m apart and planted in a triangle formation. The width of the machine is 0.8 m and requires to be traversed several times to cover the entire harvesting path. In this study, the machine traversed three trips back and forth to cover 2.4 m of the harvesting path width, where the area was heavily infested with the mentioned grass. Sectional zones were created and named alphabetically for every 10 palms in a row, as depicted in *Figure 3*. Besides, the operator could get better views when operating the machine within a zonal area (about 100 m range). The area covered by the machine was about 192 m^2 for traversing the zone thrice. The assessment was conducted for 15 zone areas.

Mowing around oil palm trees (Circle movement). The palm circle mowing was carried out by circling the machine around the palm to clear weeds. Each circle was timed, and three repetitions were carried out equivalent to almost a 2 m radius distance from each palm. The time-motion study was carried out for ten palms. Thus, an average timing could be calculated.

The data collected was sufficient to simulate the performance of the prototype. The average data for each category would be used to estimate the machine's average field capacity. This process is depicted in Equation (1).

$$\text{Effective field capacity (ha/hr)} = \frac{\text{Area (ha)}}{\text{Time (hr)}} \qquad (1)$$

Operational Cost Estimation

The study adopted the American Society of Agriculture and Biological Engineering (ASABE) standard to assess the economic feasibility of the proposed system. This standard enabled the economic justification of agricultural machinery to be evaluated during the study. Thus, a cost comparison with current practice could be made. A detailed description of the cost parameters is shown in *Table 3*.

All costs associated with its operation (*Table 3*) were divided by the estimated number of operating hours and the total value determined as hourly operational costs (HOC) (Azwan et al., 2017). The metric refers to the total costs incurred to operate a machine for 1 hr (RM/hr). It also refers to the economic efficiency of machine operating. Meanwhile, the total effective cost (TEC) was assessed based on the HOC rate divided by the machine's effective field capacity (EFC), as depicted in Equation (2).

$$\text{TEC (RM/ha)} = \frac{\text{HOC (RM/hr)}}{\text{EFC (ha/hr)}} \qquad (2)$$

RESULTS AND DISCUSSION

Prototype of the iHST Mower with Teleoperation Function

Integrated hydrostatic transmission (iHST), connected to the engine via a pulley, is an essential component of the system (*Figure 4*). The pulley

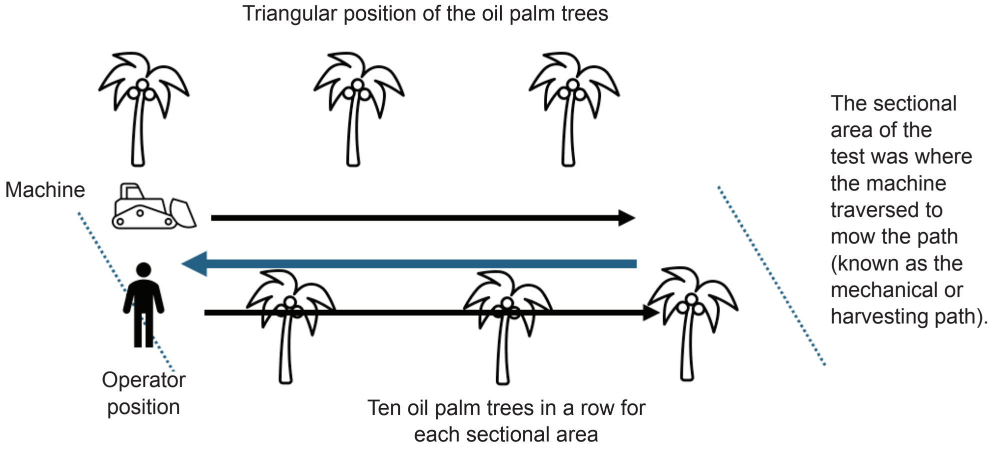

Figure 3. Illustration on weed clearing time-motion study on paths.

TABLE 3. COST PARAMETER

No.	Cost parameters	Description
1	Depreciation	Depreciation covers the decline in the machine's value over its life.
2	Interest on investment	Interest covers the cost of investing funds in the machine.
3	Shelter and insurance	The parameter covers the requirement to minimise the machine's liabilities and prolong its functionalities.
4	Repair and maintenance	The parameters cover parts replacement, installation charges and general maintenance.
5	Fuel and lubrication	Fuel charges were based on current fuel prices. Lubrication costs were figured at a certain percentage of fuel cost.
6	Labour	Labour charges were based on Malaysia NUPW (National Union Plantation Workers) per hour.

and belt system becomes crucial as it provides a mechanism to split the power transmission in case of unexpected obstacles. This ensures the machine can function safely and effectively under diverse operating conditions. Power from the engine drives the hydraulic flow within the iHST. Another pulley connects the engine to the flail cutter. A servo attached to the iHST allows precise control over the hydraulic flow, facilitating forward and reverse movements. This hydraulic power is then converted into mechanical output to operate a gear system in the transaxle. The gear selection component in the gearbox provides the capability for left and right movements. The comprehensive system is detailed in *Figure 5*. A FPV camera is integral to the system, whereby a 5.8 GHz FPV camera is positioned on the vehicle, offering real-time feedback to the machine's operator, enhancing their field of view and operational control.

Infield Performance Test

The time-motion studies assessed the prototype's performance in carrying out the intended operation. The tests took place in an oil palm field with predefined parameters and were operated by a skilled research technician. The operator controlled the machine operation from a distance; no other tasks were required. It was noted that weed infestation was faster in mechanised paths than in shaded palm circle areas, likely due to direct exposure to the sun. Thus, separate time-motion studies were conducted for grass-cutting activity on the mechanised paths and within the palm circle.

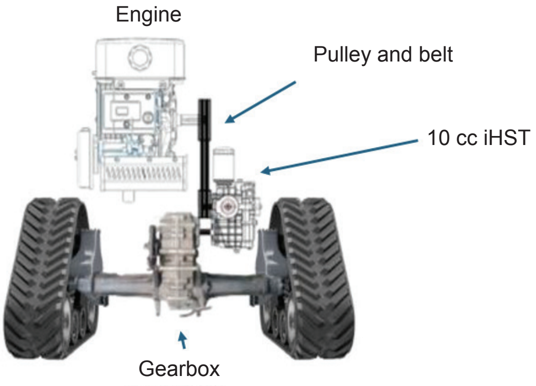

Figure 4. Location of the main components on the prototype.

Preliminary evaluation of weed mowing was carried out on a plot of land exceeding 3 ha along the mechanised paths. The findings from this evaluation are depicted in *Figure 6.* The prototype had to traverse the middle area of the roughly 2,500 mm path three times due to its 800 mm flail width. As the machine traversed along the paths, it cut the weed 50–80 mm in height from the ground. A statistical data analysis yielded valuable insights, as shown in *Table 4*. This analysis offered valuable information on the variation and consistency of the data obtained from these repeated measurements.

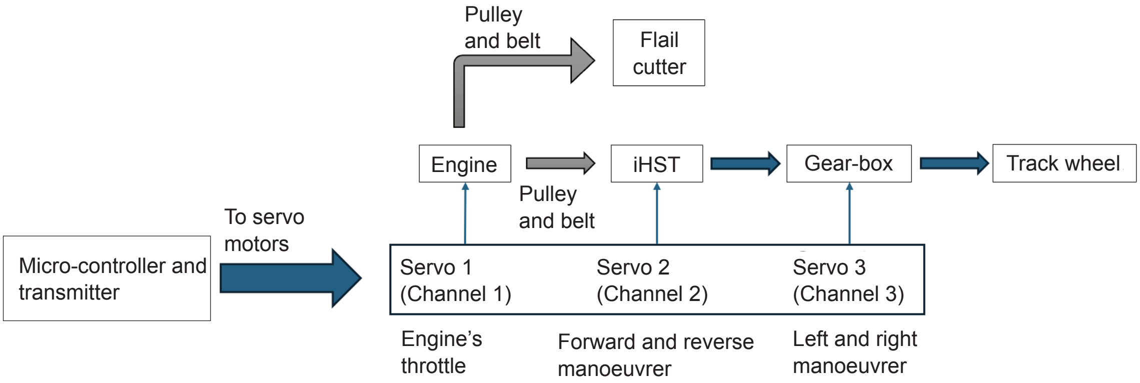

Figure 5. Control flow diagram.

The time-motion study conducted on the weed mowing process provides significant insights into the efficiency and consistency of the prototype being tested. As observed in *Figure 6*, the mowing time varies across different zone areas, with the time to mow decreasing progressively from Zone A to Zone O. This variation indicates the influence of uncontrolled factors, possibly due to the operator's unfamiliarity with the operations. However, the operator becomes more comfortable controlling the machine over a certain period, and the efficiency gains towards the end of the test.

Table 4 presents descriptive statistics, offering an understanding of the data collected. The mean time for path clearing was 663.600 s, with a standard deviation of 7.707 s. This low standard deviation indicates that the prototype's performance was relatively consistent across multiple trials, with only minor fluctuations in time. The minimum time recorded was 653.000 s, while the maximum time reached 676.000 s, demonstrating a range of 23.000 s between the fastest and slowest trials. The consistency in these results suggests that the prototype is reliable in maintaining a steady performance level, which is crucial for practical applications where time efficiency is paramount.

The data indicated that the average time required to mow a zonal area of the path was about 663.6 s. Based on the average value, it was estimated that for a 1 ha palm-planted area, the prototype required about 4,645.2 s or 77.4 min to carry out the mechanical or harvesting path mowing task. The value obtained by multiplying the average value by seven as seven zonal areas were equivalent to almost one ha of the planted area.

The prototype cut weeds in palm circle areas during the second testing. The test consisted of three repetitions involving weeds cutting in 10 palm circles. The test area had minimal weed infestation. Despite these conditions, the data collected was sufficient to analyse the prototype's performance and estimate its effective field capacity for maintaining palm circles. The test results are shown in *Figure 7*, while detailed statistical information is shown in *Table 5.*

Significant consistency in mean values and range across three repetitions was observed, suggesting consistency in measurement. While there were minor differences in standard deviation, they did not significantly impact the overall reliability and consistency of the measurements overall. Thus, the time-motion study provided valuable information on the capability of the prototype to undertake the palm circle maintenance activity within an average of 24 s for each palm. Thus, for a hectare of land with 150 palms, it was estimated that the time required for palm circle activity was 3,600 s.

The prototype's effective field capacity (EFC) to carry out the tasks is depicted in *Table 6* and explained by Equation (1). The EFCs were 0.78 and 1 ha/hr, respectively. However, the EFC was 0.44 ha/hr if the machine needed to cover both path and palm circle operations.

The prototype was estimated to cover only 4 ha of a path and palm circle mowing operation daily (9 hr of working time a day). On the other hand, a mechanised spraying system with a three-wheeler vehicle could cover more than 10 ha/day (Azwan et al., 2016). However, a teleoperated mechanical mower could benefit from high-speed

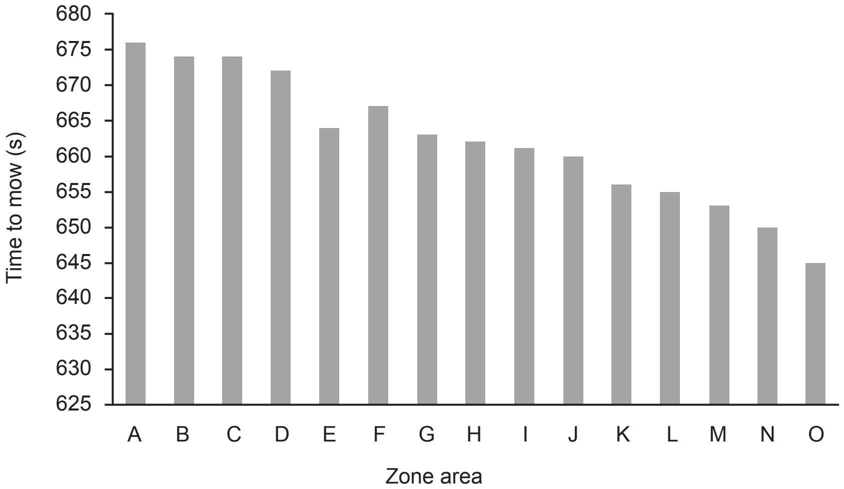

Figure 6. Results of the time motion study for the path mowing.

TABLE 4. DESCRIPTIVE STATISTICS FOR PATH CLEARING TIME (s)

	N	Minimum (s)	Maximum (s)	Mean (s)	Std. deviation (s)
Time-motion (s)	15	653	676	663.60	7.707

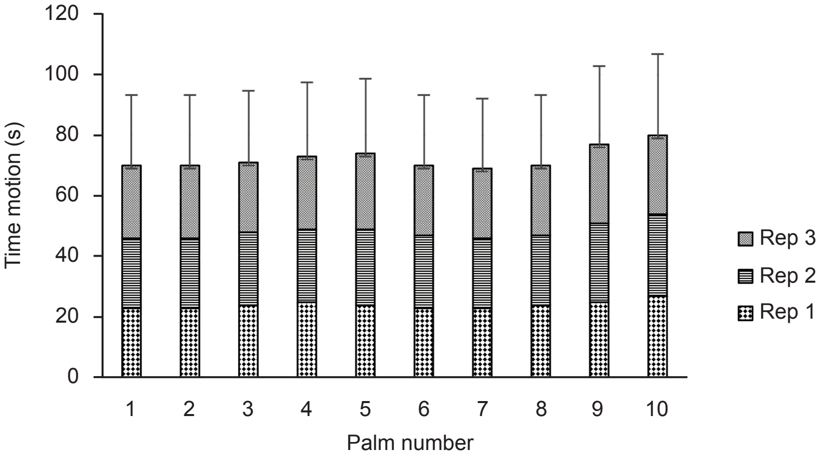

Figure 7. Results of the time motion study for the palm circle test.

TABLE 5. DESCRIPTIVE STATISTICS OF PALM CIRCLE TEST

	N	Minute (s)	Maximum (s)	Mean (s)	Std. deviation (s)
Rep 1	10	23	27	24.10	1.287
Rep 2	10	23	27	24.20	1.398
Rep 3	10	23	26	24.20	1.135

communication networks in the plantation if available, allowing long-range control for improved performance. This long-range communication would allow the operator to control the machine from the office or centre (remote operation), potentially increasing its working time and availability. Furthermore, the prototype could be enhanced by increasing its dimensions, specifically by expanding its width from 800 to 1,300 mm. This modification would allow it to cover a larger area and improve its operational reach.

Another observation found that the soft soil and uneven topography will affect the cutting performance. The machine could traverse those conditions, but the flail angle impacted the consistency of the cutting. Thus, it limits the prototype functionality.

TABLE 6. EFFECTIVE FIELD CAPACITY OF THE PROTOTYPE

No.	Field mowing task	Effective field capacity (EFC)
1	Path	1/4,645 ha/s or 0.78 ha/hr
2	Palm circle	1/3,600 ha/s or 1 ha/hr
3	Path and palm circle	1/6,915 ha/s or 0.44 ha/hr

Operational Cost Analysis

Operational cost analysis was done by determining the prototype's hourly operating expense. Based on the materials purchased during its development, the cost of the prototype was estimated to be RM30,000. Another assumption, detailed in *Table 7*, was made to facilitate the analysis. The data was referenced from previous studies on internal combustion engine machinery in oil palm plantations, which are almost consistent in their infield application and power source with the studied prototype.

The prototype's hourly operating cost (HOC) was RM11.24/hr, as depicted in *Table 8*. The values in *Table 8* were obtained from the initial assumption in *Table 7* and divided by the estimated operating hours of the machine per its entire economic life. Thus, the corresponding values of the operational cost could be obtained for the prototype's entire economic life at the time of study. Standards were made based on references from previous studies that assumed the current scenario while the future scenario, such as inflation rates, were not incorporated.

TEC could be obtained by dividing the HOC (RM11.24/hr) by the EFC (0.44 ha/hr) as in Equation (2). Thus, TEC was about RM25.55/ha. Assuming 24 working days per month and nine working hours daily, the ratio is one machine per 100 ha area. The machine is expected to visit the same area every month for ten months in a year. Only 10 months a year was anticipated for the operation due to the seasonal monsoon period. Therefore, the prototype's total effective cost per year was RM255.50/ha. The cost of herbicide applications was reported to be almost RM214.00 ha/hr at the current herbicide cost of RM30.00/L (Wahyu et al., 2010). However, the current

TABLE 7. INPUT TABLE

Parameter	Values	Reference
Bill of materials (RM/unit)	30,000	
Operating hours (hr/yr) - *Assume an availability factor of 50%*	4,380	Azwan et al. (2021)
Assume economic life (yr)	5	Azwan et al. (2021)
Salvage value @ 5th yr	10% from the initial value	Johari et al. (2020)
Shelter and insurance	2% from the initial value	Johari et al. (2020)
Interest on investment	10% from the initial value	Johari et al. (2020)
Fuel consumption (L/hr)	0.95	Based on the observation
Fuel price (RM/L)	2.10	Price at the time of study
Lubricant cost	15% of the fuel cost	Shuib et al. (2020)
Repair and maintenance costs	Total 5% of the initial cost	Azwan et al. (2021)
Operator wages per month	1,500	Azwan et al. (2021)

cost of glyphosate has increased to more than RM50.00/L, and the total effective cost of glyphosate application was estimated to be more than RM250.00 ha/hr. Thus, the TEC of the prototype application is almost similar to or slightly higher than that of the herbicide application for path and palm circle mowing operations.

TABLE 8. OUTPUT OF THE ECONOMIC ANALYSIS

Operational cost	Values
Salvage value (RM/hr)	0.14
Tax, shelter and insurance (RM/hr)	0.03
Interest on Investment (RM/hr)	0.14
Fuel cost (RM/hr)	2.00
Repair and maintenance cost (RM/hr)	0.07
Lubricant cost (RM/hr)	0.30
Operator cost (RM/hr)	8.57
Total hourly operating cost (RM/hr)	**11.24**

A combination of herbicide and mechanical mowing in oil palm plantations could lead to improved cost-effectiveness. Herbicide should be applied in areas where it is difficult for the machinery to operate, such as muddy and sloped areas, to achieve better cost-effectiveness. Furthermore, applying herbicide under the palm canopy or within the circle of mature palm trees where the weed infestation growth is slower could reduce the need for frequent mechanical mowing, subsequently increasing the TEC per year.

Mechanical mowing may require more frequent visits than chemical applications, but the work will be less arduous and safer. This, along with other advantages, can attract local operators. Working efficiency could also be enhanced by having multiple machines working simultaneously or using the swarm technique (Moniruzzaman et al., 2022; Peña et al., 2018; Vasconez et al., 2019). Establishing a local ground network is a potential solution for improved connectivity (remote operation). Localisation, big data transmission and low latency could enhance teleoperation performance in oil palm plantations.

CONCLUSION

The study showcased the successful implementation and validation of a hydrostatic transmission machine within the oil palm plantation with a specific terrain and other conditions mentioned in the test. This machine operated precisely along its intended path through teleoperation control and delivered the anticipated outcomes. Based on blanket area operation (path and palm circle), the machine's effective field capacity was 0.44 ha/hr, with an annual operating cost of nearly RM255/ha. Improvement could be achieved via hybrid applications involving mechanical mowers and herbicides. While its productivity was lower than mechanised spraying, integrating an advanced teleoperation system with an effective communication network in the oil palm field could lead to further enhancements (remote operation). These advanced systems offer enhanced distance control, reduced labour intensity, and extended work hours. Appropriate modifications could also benefit plantation operations, such as loose fruit collection, FFB evacuation and palm harvesting. Benefits such as attracting skilled local workforces into the industry, improved sustainability and modernisation in the oil palm field practice could be realised.

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BIO-OIL AND BIO-HYDROGEN PRODUCTION USING RED MUD CATALYST FROM OIL PALM BIOMASS WASTE

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ABSTRACT

Indonesia produces solid waste from oil palm plantations that have the potential to produce bio-oil and bio-hydrogen in the context of sustainable energy development. This article reports on the utilisation of biomass as bio-oil and bio-hydrogen producer by catalytic pyrolysis using solid waste from the aluminium industry as a catalyst. Catalytic pyrolysis was carried out in a fixed-bed reactor with varying temperatures. Three types of oil palm biomass were subjected to pyrolysis and catalytic pyrolysis to obtain hydrogen-rich gas products at various temperatures and flow rates. The products were characterised using gas chromatography-mass spectrometry (GC-MS). The results showed that the yields of bio-oil and bio-hydrogen obtained from oil palm biomass varied in the range of 18–40 wt% and 23–37 wt% respectively. The liquid product from catalytic pyrolysis of palm biomass using red mud (RM) catalyst contains primary oxygenate compounds such as acids, aromatics and esters.

Keywords: biomass waste, bio-hydrogen, bio-oil, oil palm, red mud.

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INTRODUCTION

The oil palm industry produces abundant biomass waste, both solid and liquid waste. Oil palm plantations globally generate over 300 million tonnes of annual waste. The oil palm plantation generates solid waste, including biomass residues such as trunks and fronds. Meanwhile, palm kernel shells and fibre are by-products of the milling process. The oil palm plantations produced a total of 75% of biomass waste which is mostly left to rot in the plantations for composting and fertilisation purposes such as for mulching and nutrient recovery, while the remaining 25% is from the milling process (including empty fruit bunches, kernel shells and fibre) (Dungani et al., 2018). Direct combustion of biomass waste can generate energy.

Lignocellulosic biomass, an abundant renewable resource can be efficiently converted into energy and various chemicals at biorefinery facilities. Oil palm trunks are biomass produced from replanting activities after oil palm reaches an economic age of 20–25 years. Production of hydrogen from the oil palm biomass is a breakthrough procedure as it can lower deforestation rates and preserve the environment. Oil palm trunks are available abundantly because oil palm plantations replant when oil palm trees are no longer productive (Loh, 2017).

The frond consists of a mature leaf and a leaf stem, which produces leaflets. The fronds are left to rot in the oil palm plantation area. The oil palm plantation produces approximately 11 t of fronds per hectare after the harvest containing around 44%–45% cellulose, 23%–24% hemicellulose and 17%–18% lignin. The physical and chemical properties of oil palm fronds and trunks are influenced by plant age, planting location and soil conditions. Oil palm plantations will experience a decline in fruit yield and oil production after 25 years. Therefore, the plantation management replants the oil palm and leaves the trunk as biomass waste. In general, 1 ha of oil palm plantation can

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generate around 75 t of trunks. The trunk contains high levels of lignocellulosic substances such as cellulose, hemicellulose and lignin (Loh, 2017).

Gasification, pyrolysis and supercritical processes are thermo-chemical techniques performed on biomass involving a series of chemical reactions to produce liquid, gaseous and solid products (Ong et al., 2020). Pyrolysis is a thermo-chemical conversion method in the absence of oxygen (O) and releases the embodied energy of biomass. The main gaseous pyrolysis products are hydrogen (H_2), carbon monoxide (CO), carbon dioxide (CO_2) and methane (CH_4). Thermal gasification of biomass produces gas that contains synthesis gas in addition to charcoal and tar. The main composition of the synthesis gas is CO and H_2 with small amounts of CO, N_2, CH_4 and H_2O. The composition varies depending on the type of feedstock, gasification method, operating conditions etc. The percentage of hydrogen yield in synthesis gas can be increased by applying water gas shift (WGS) technology using an appropriate catalyst. Several types of catalysts including zeolite, carbon, metal oxide, alumina and silica have been reported for reforming and improving biomass pyrolysis vapour. The catalytic properties of these different types of catalysts were enhanced by depositing various metals, including nickel (Ni), cobalt (Co), copper (Cu), cerium (Ce), platinum (Pt), lanthanum (La), and ruthenium (Ru) (Ho & Wu, 2020; Jiang et al., 2015; Liu et al., 2020; Santamaria et al., 2020a; 2020b).

Red mud (RM) is generated from the refining of bauxite to produce alumina by the Bayer process. In the bauxite industries, the processing of 1 t of bauxite will produce 500–600 kg of RM. RM contains mostly metal oxides that can be used as catalysts in several chemical reactions (Hidayat et al., 2020, 2021). RM released into the environment will threaten environmental safety and human health such as heavy metal pollution, soil pollution and groundwater pollution. Therefore, the utilisation of RM will provide an alternative solution to the solid waste problem from the bauxite processing industry.

RM impregnated with nickel was prepared as a catalyst for ammonia decomposition. The test results showed good catalytic activity performance (Cao et al., 2014). The RM modified by embedding Ru metal showed good performance for ammonia decomposition reaction and had high catalytic efficiency and stability. The RM-based catalysts have demonstrated economic advantages and technological feasibility in the production of hydrogen. The catalytic efficiency has improved through high-temperature calcination, an acid treatment, impregnation of active metals and a reduction process (Kurtoğlu and Uzun, 2016). RM can be used for hydrogen production via steam reforming tar from biomass waste at 200°C–850°C. Besides, catalysts with different iron (Fe) and Ce weight percentages were prepared using co-precipitation (Dulger Irdem et al., 2014; Kurtoğlu & Uzun, 2016). The RM can be modified by acid treatment followed by calcination at high temperatures (> 500°C). The tar conversion and hydrogen yield increased when the temperature increased in the non-catalytic tar steam reforming process (Das & Mohanty, 2019). The tar conversion is almost complete when operating temperatures are above 700°C. At high temperatures, hydrogen production increases by the thermal cracking of tar using steam as the gasifying agent. The addition of active metals can further enhance water gas shift reactions and steam reforming, leading to a significant increase in tar conversion and hydrogen production. The modified RM catalyst resulted in an increase in hydrogen yield compared to the Fe-Ce catalyst due to decreased activity. The reduced process efficiency was observed when modified RM was present due to its limited activity at lower steam reforming temperatures (Duman et al., 2014). The water gas shift reaction catalysed by iron oxide will be inhibited by the sodium aluminium silicate hydrate contained in the RM. Upgrading bio-oil by enhancing various long-chain hydrocarbon compounds from the pyrolysis of hemp seeds can be carried out by using RM as a catalyst at high pressure. The co-processing of bio-oil produced a bio-oil with low acidity and oxygen content.

Several catalysts such as alkali metals, transition metals, calcium-based and nickel-based have been intensively developed for hydrogen production from biomass (Adnan et al., 2017; Dang et al., 2020; Ebadi et al., 2019; Zhang et al., 2014). The physicochemical characteristics of catalysts required to support hydrogen production are high porosity, large surface area, wide pore size distribution, high activity and good thermal stability. High-activity catalysts will increase the reaction rate and enhance the yield of hydrogen production. Synthesis gas production from pyrolysis of pine wood chips catalysed by Ni/CaAlOx has been investigated by Chen et al. (2016). The catalyst was synthesised by the co-precipitation method by varying calcium to aluminium ratio (Ca:Al). The results showed abundant hydrogen content, and the synthesis gas reached 90% by volume. CO selectivity and H_2 concentration increased with the addition of Ca to the catalyst, thereby increasing synthesis gas production. The catalyst decreased in activity due to the formation of coal deposits on the surface of the material. The bifunctional Fe/CaO catalyst can be prepared by the impregnation method (Yang et al., 2017). Catalyst activation was investigated during biomass pyrolysis which was carried out in a fixed-bed reactor consisting of two-stage. The tar catalytic cracking occurs in the presence of $Ca_2Fe_2O_5$ on the bifunctional catalyst (Valle et al., 2020). Catalytic pyrolysis of rice husk biomass using a catalyst in a fixed bed system efficiently produced synthesis gas with high hydrogen content (Liu et al., 2020). The study found that the hydrogen production reached a maximum temperature of 800°C and a steam to carbon dioxide ratio of 0.8. The catalyst has high activity due to the good dispersion of active sites and its ability to release oxygen for carbon decomposition.

The advanced technology to produce hydrogen from biomass needs to be developed to gain renewable and sustainable energy (Sukiran et al., 2017). Natural gas which is the conventional source for hydrogen production requires a long process route, is expensive, inefficient in energy use and not environmentally friendly. Biomass is a potential and important candidate as a renewable energy source. Edible oil, which is mostly produced from oil palm fruits, is abundantly available in Indonesia. A palm oil mill generates 55 t of solid waste for every hectare of oil palm plantations. The abundant amount of solid waste requires the utilisation of palm oil solid waste to produce hydrogen by the pyrolysis process. The palm oil industry continuously maintains the productivity of yield and quality of palm fruit without opening new areas for plantation. It is necessary to replant oil palm trees by cutting down the trees that are over 20 years old to maintain sustainability. This situation will increase the amount of solid waste and provide opportunities for the utilisation of oil palm solid waste for various applications including renewable energy. Pyrolysis of oil palm solid waste can produce bio-oil and bio-hydrogen which will reduce the negative impacts of waste disposal. Utilisation of palm solid waste as a renewable energy source would create a carbon-neutral process and be more environmentally friendly due to low sulphur (S) and nitrogen (N) content.

Based on the literature search, it is still rare for palm oil solid waste pyrolysis research to produce hydrogen and bio-oil using the pyrolysis process using RM as a catalyst. This study aims to study the production of bio-oil and bio-hydrogen using RM catalysts from oil palm/palm oil solid waste. The pyrolysis process was operated under different operating conditions (catalyst concentration, temperature and catalyst addition), to obtain a higher yield of hydrogen and bio-oil.

MATERIALS AND METHODS

Catalyst Preparation and Characterisation

Red mud (RM) was obtained from the bauxite refinery industry in West Kalimantan Province, Indonesia. First, the RM was ground and screened to obtain a uniform particle size. Then, the washing process is carried out using tap water. RM was dried at 120°C for 8 hr in an oven. The RM was calcined in an electric furnace at 800°C for 2 hr. The porosity properties of RM were characterised using nitrogen gas sorption analysis. The crystalline structure was determined by X-ray diffraction (XRD) instrument.

Characterisation of the Oil Palm Biomass

The oil palm biomass was collected during the replanting at plantations of PT Perkebunan Nusantara (PTPN) XXIII, Central Kalimantan Province, Indonesia. The oil palm biomass was washed to remove any dust and impurities before being cut into pieces to reduce their size and dried in the sun until dry. The remaining moisture was removed by drying in an oven for 24 hr at 105°C. After that, the oil palm biomass was ground into small pieces using a grinder and sieved to a size of less than 1 mm. The proximate analysis was applied to calculate the content of volatile matter, fixed carbon, moisture and ash. Meanwhile, the ultimate analysis was to calculate the elemental content of oxygen (O), hydrogen (H), carbon (C), nitrogen (N), sulphur (S) and ash.

Catalytic Pyrolysis Experiments

Figure 1 shows the apparatus applied for catalytic pyrolysis of oil palm biomass using RM as a catalyst in a fixed bed system. The reactor was heated in a tubular furnace equipped with an electric heater and a thermocouple was deployed to monitor the operating temperature. The catalyst and oil palm biomass in each test were placed into the reactor. The nitrogen gas flow rate was 150 mL min^{-1} to remove the remaining oxygen from the reactor. Gas samples were collected and analysed using a gas analyser. The liquid and solids formed were collected after the reactor reached room temperature. The liquid was analysed for the content of the constituent compounds using gas chromatography mass spectrophotometry. The functional groups were determined using Fourier transform infrared (FTIR).

RESULTS AND DISCUSSION

Catalyst Characterisation

Analysis of porosity. The porosity characteristics of materials can be predicted by analysing the nitrogen gas (N_2) isotherm adsorption-desorption curve. *Figure 2* exhibits the N_2 isotherm adsorption-desorption of RM before and after activation. Based on *Figure 2(a)*, the N_2 uptake pattern in RM before activation has type II referring to the Brunauer, Deming, Deming and Teller (BDDT) classification. In type II, N_2 absorption occurs in non-porous or

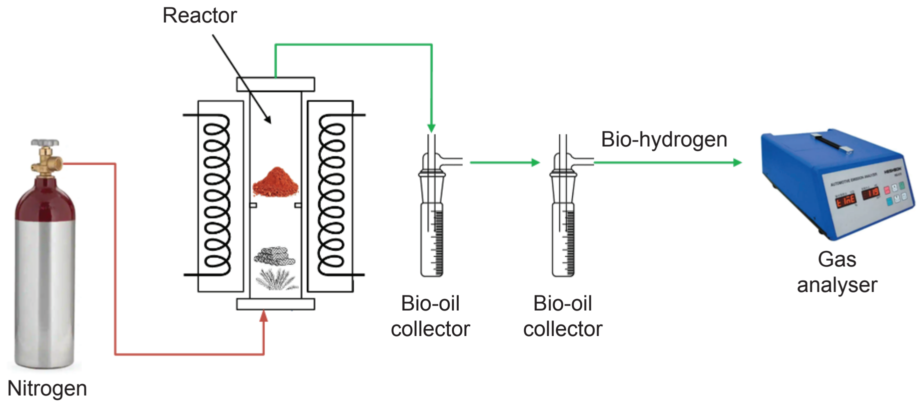

Figure 1. The catalytic pyrolysis apparatus.

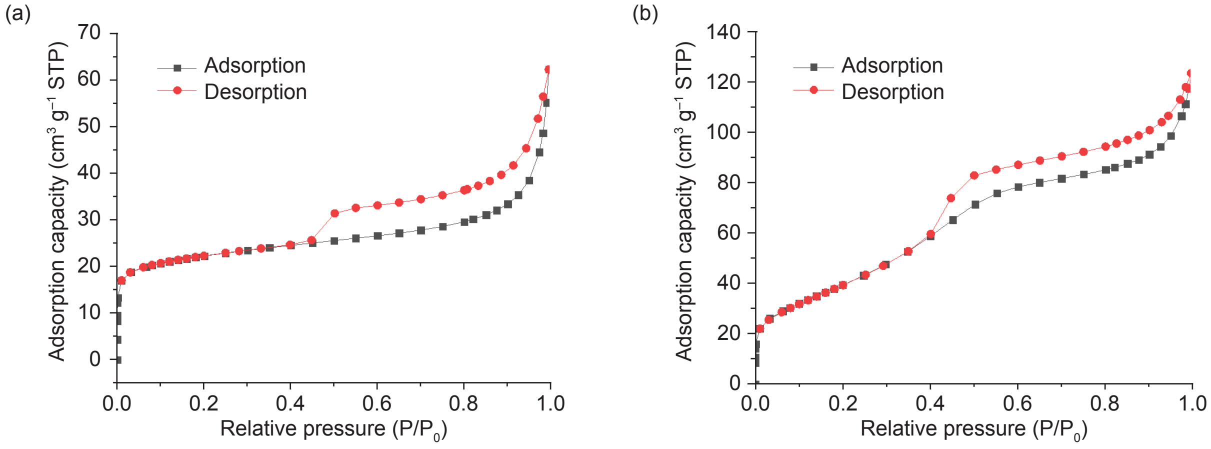

Figure 2. The nitrogen isotherm adsorption-desorption of RM (a) before and (b) after activation.

large-porous media which indicates monolayer and multilayer absorption properties. Adsorption in the monolayer occurs at a relative pressure P/P_0 smaller than 0.2. Multilayer adsorption will occur in the pores of the solid at relative pressures above 0.2. This is characterised by the slope which tends to level out until the relative pressure approaches 1.0. Generally, solids with a mixture of micropores and mesopores show an N_2 uptake pattern similar to type II with H3 or H4 type hysteresis. The H3 or H4 type hysteresis indicates that the material has lamellar or slit-shaped pores. The adsorption capacity increases significantly at relatively high pressures, which indicates the presence of mesopores in the material. As shown in *Figure 2(b)*, the curve of RM after activation showed the existence of type IV with H1 or H3 type hysteresis, which indicates that the pores are cylindrical or lamellar.

The Brunauer-Emmett-Teller (BET) specific surface area is an important parameter of material characteristics. A catalyst that has a high surface area will provide more active sites that are anchored on the surface to enhance catalytic activity. *Table 1* shows the porosity characteristics of RM before and after calcination.

TABLE 1. THE POROSITY CHARACTERISTICS OF RM BEFORE AND AFTER CALCINATION

Characteristics	RM	Activated RM
Specific surface area ($m^2 g^{-1}$)	114.53	203.04
Pore volume ($cm^3 g^{-1}$)	0.2106	0.2402
Average pore radius (Å)	22.93	21.32

Note: RM - red mud.

Table 1 shows that the specific surface area and total pore volume increase due to the diminishing impurities in the internal pore of RM. Meanwhile, the average pore radius is reduced from 22.93–21.32 Å. During the calcination process, new pores are likely to form due to the release of compounds on the surface at high temperatures.

Analysis of X-ray diffraction (XRD). The XRD aims to determine the mineral crystal structure content in RM (*Figure* 3). Based on *Figure 3*, the diffractogram shows the characteristic peaks, which indicate a high presence of gibbsite, the largest component contained in bauxite, followed by Al_2O_3, hematite (Fe_2O_3), sodium oxide (Na_2O), quartz (SiO_2) and anatase (TiO_2). This means that the RM is mostly composed of the Al_2O_3

and Fe_2O_3. The RM diffractogram patterns do not show significant differences between before and after calcination. The mineral from the RM does not decompose significantly after the calcination process. This can be due to the favourable calcination process of RM before and after activation in opening the pores (Dong et al., 2023; Liu et al., 2023).

Chemical composition. The X-ray fluorescence (XRF) results in *Table 2* confirmed that the RM sample contained several metal oxides and alkali metals impurities such as Na_2O and SiO_2, with percentages of 17.39% and 10.3% respectively. Trace components of impurities were also detected such as phosphorus (P), magnesium (Mg), calcium (Ca), potassium (K) and titanium (Ti). The unusual amount of Al_2O_3 in this RM sample was so high as compared to other worldwide refinery plants which commonly ranged from 14%–28%. In ratio, the iron to aluminium ratio (Fe:Al) was calculated at 0.34 while the other impurities ratio (Fe:Na and Fe:Si) are at 1.02 and 1.72 respectively. There was a slight change in the chemical composition of milled RM which assumed would not affect the reaction.

Characterisation of the Palm Oil Trunks

Based on *Table 3*, the composition of oil palm biomass comprises lignin, hemicellulose, and cellulose. Compared to fossil fuels, oil palm biomass has a lower N content (less than 1% mass), S content (less than 0.2% mass) and O content (40%–50% mass). The quality of bio-oil is influenced by the variation in the physicochemical properties of biomass feedstock.

A higher cellulose content from oil palm biomass is needed to achieve a high production efficiency of liquid products (bio-oil). Decomposition of cellulose components produces more volatile matter which can be condensed into bio-oil during pyrolysis. The empty fruit bunches have the highest cellulose content (59.70% mass) followed by the trunk (34.50 wt%), and fronds (20.80% mass).

The proximate analysis showed a low water content (< 10 wt%) which is affordable as feedstock for the pyrolysis process. The water content in raw materials influences the yields and bio-oil quality. The heat required for pyrolysis will increase when the biomass used has a high moisture content because extra heat is needed to evaporate the water

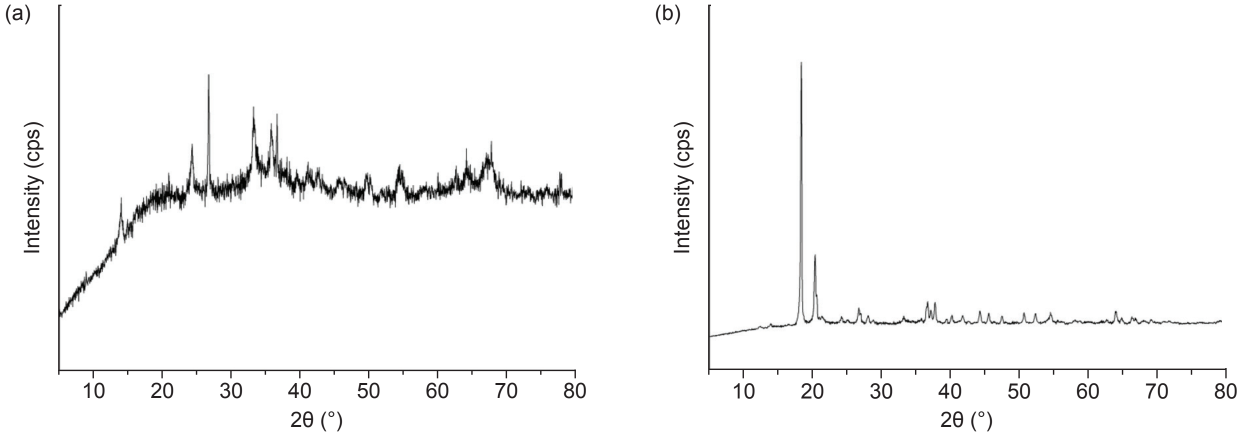

Figure 3. X-ray diffraction (XRD) analysis of red mud (RM): (a) raw RM and (b) after calcination.

TABLE 2. CHEMICAL COMPOSITION OF RED MUD (RM) BEFORE AND AFTER CALCINATION OBTAINED BY XRF

Sample	Major component (%)					Ratio		
	Al_2O_3	Fe_2O_3	Na_2O	SiO_2	TiO_2	Fe:Al	Fe:Na	Fe:Si
RM Parent	51.8	17.73	17.39	10.3	1.67	0.34	1.02	1.72
RM 8 hr-mill	49.47	17.60	17.45	11.93	1.55	0.36	1.01	1.48

Note: RM - red mud; Al_2O_3 - Aluminium oxide; Fe_2O_3 - hematite; Na_2O - sodium oxide; SiO_2 - quartz; TiO_2 - anatase.

TABLE 3. ANALYSIS OF THE COMPONENT OF OIL PALM BIOMASS

Type of biomass	Cellulose (% mass)	Hemicellulose (% mass)	Lignin (% mass)
Trunks	59.70	22.10	18.20
Fronds	40.00	20.00	30.00
Empty fruit bunches	34.50	31.80	25.70

vapour and increase the temperature of the vapour to the setting temperature. Therefore, pyrolysis using feedstock containing a high-water content will provide bio-oil with a high-water content with more energy consumption during the pyrolysis. In the pyrolysis process, higher volatile matter content in biomass is favourable for bio-oil production. The bio-oil formation reaction is dependent on the content of volatile materials. Meanwhile, the fixed carbon content is the element that can be transformed into char. Biomass with high fixed carbon content is favoured to produce charcoal. The ash content of the fronds is lower than that of empty fruit bunches and trunks. Low biomass ash content will improve the quality and quantity of bio-oil by avoiding secondary reactions from condensed steam and reducing solid content in bio-oil (Stefanidis et al., 2015).

The elemental analysis shows that the contents of C, H, O, N and S in oil palm trunks, fronds and empty fruit bunches are in the range of 43.00%–52.00%, 6.00%–7.00%, 40.00%–50.00%, 0.20%–0.70% and 0.00%–0.16% mass. The relatively high C and H content provides a higher heating value (HHV). The oxygen content in the biomass should be low during the pyrolysis process because the oxygen will be bound to the hydrocarbon molecules as oxygenated compounds to reduce the quality of the bio-oil. Meanwhile, biomass pyrolysis with high oxygen content tends to produce bio-oil with high water content. The formation of aromatic compounds in bio-oil can be enhanced by the presence of C and H atoms in biomass. Low S and N content in biomass would prevent the formation of SOx and NOx (Abu Bakar & Titiloye, 2013).

Thermogravimetric analysis (TGA) of trunks, fronds and empty fruit bunches of oil palm is to observe the thermal decomposition pattern, and the results are presented in *Figure 4*. The thermal decomposition covers three stages due to the different lignocellulosic components. The first stage takes place at the temperature range of 50°C–120°C where the mass of biomass decreases slightly due to evaporation of water content. The second stage occurs at the temperature range of 120°C–250°C where the mass of palm oil biomass is relatively constant as the amount of light volatile matter that evaporates is relatively low. The biomass receives most of the heat energy supplied to it to raise the temperature. The following step is the primary thermal breakdown of oil palm biomass, which takes place at temperatures between 250°C and 500°C. The major volatile organic matter (hemicellulose and cellulose) undergoes a decomposition process to become condensable or non-condensable gas. In the final stage at temperatures above 500°C, thermal degradation of biomass occurs slowly due to lignin decomposition. The thermal decomposition pattern of palm biomass is consistent with the results of proximate analysis. Thermal degradation of hemicellulose primarily occurs in the temperature range of 220°C–350°C, accompanied by the degradation of cellulose in the temperature ranging from 325°C–400°C (Wang et al., 2022).

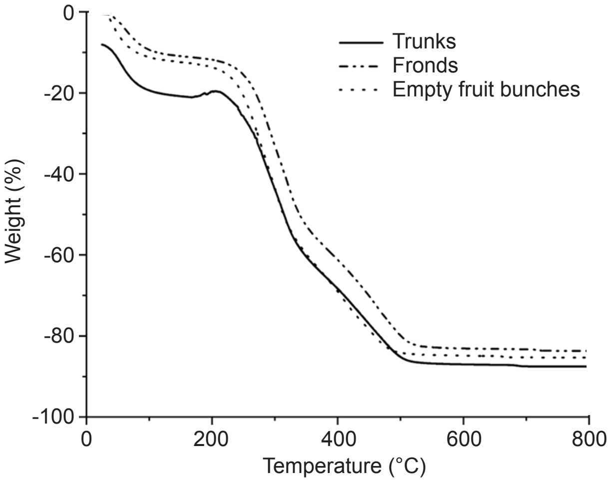

Figure 4. *Thermogravimetric analysis (TGA) of oil palm biomass waste (trunks, fronds and empty fruit bunches).*

Catalytic Pyrolysis Experiments

Effect of the addition of RM on distribution of pyrolysis products. To determine the effect of the addition of RM on the pyrolysis of palm biomass, pyrolysis experiments were conducted as shown in *Figure 5*. Both catalytic and non-catalytic pyrolysis show the same trend i.e., the percentage of liquid and gas increases with the increase of pyrolysis temperature. Meanwhile, the percentage of solid products reduces with increasing temperature. The gas yield in catalytic pyrolysis is higher than in non-catalytic pyrolysis, implying that the addition of a catalyst contributes to the addition of gas products during the pyrolysis process. While the solid product does not have a significant difference between non-catalytic and catalytic pyrolysis.

Effect of temperature on distribution of catalytic pyrolysis products. The distribution of catalytic pyrolysis products obtained from the pyrolysis of trunks, fronds, and empty fruit bunches at different temperatures can be seen in *Figure 6*. The yield of liquid, char and gas products from pyrolysis at 400ºC, 500ºC and 600ºC is in the range of 21.21–36.85, 10.75–25.40 and 41.60–68.05 wt% respectively. The type of oil palm or palm oil biomass and pyrolysis temperature affects the product yields. Pyrolysis of oil palm fronds provides higher liquid yields than from empty fruit bunches and trunks. The highest amount of charcoal was obtained from the pyrolysis of empty fruit bunches. Pyrolysis of biomass with high volatile matter and low ash content produces large amounts of liquid products. The yield of pyrolysis products is in accordance with the lignocellulosic content of the biomass reported by Lu and Gu (2022).

Based on *Table 4*, empty fruit bunches have high hemicellulose and cellulose content but low lignin and extractives content. Thus, pyrolysis of empty fruit bunches provides the highest amount of liquid product. Increasing the pyrolysis temperature has a small influence on the yield of products obtained from the trunks and empty fruit bunches. It is because most of the components of oil palm trunks and empty fruit bunches are cellulose and hemicellulose which can decompose completely at temperatures below 500°C. However, increasing the pyrolysis temperature for fronds affects product yields, especially liquid products because they contain high levels of lignin, which can decompose over a wide temperature range of 200°C–900°C. The biochar yield decreased with increasing pyrolysis temperature due to greater primary decomposition of biomass (Claoston et al., 2014).

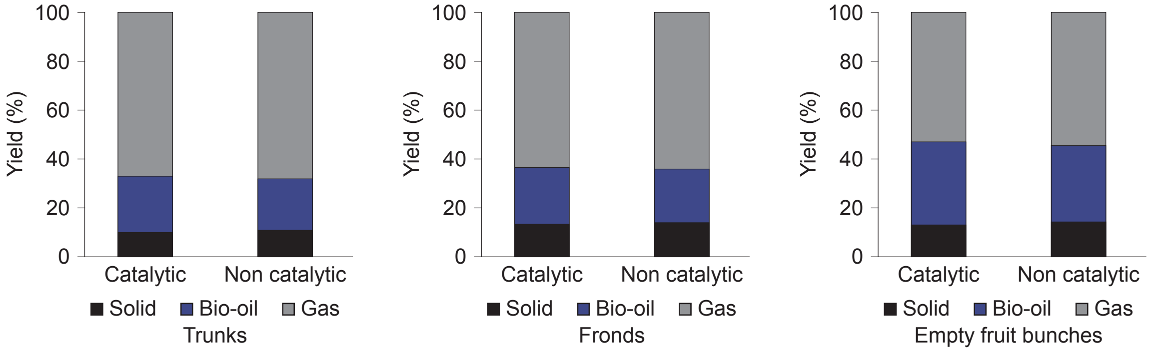

Figure 5. Effect of red mud (RM) catalyst on distribution of pyrolysis products.

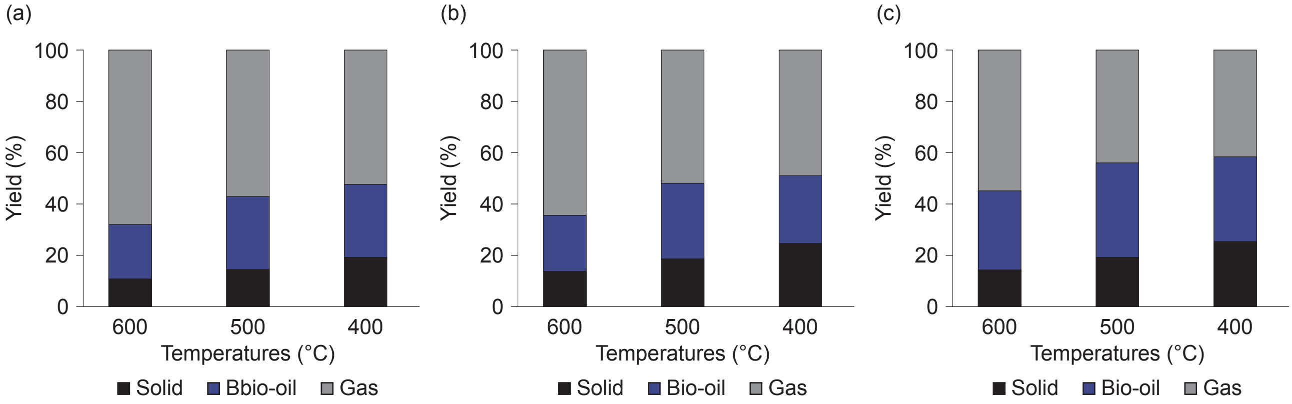

Figure 6. Effect of temperature on distribution of catalytic pyrolysis products from oil palm biomass waste: (a) trunks, (b) fronds and (c) empty fruit bunches.

TABLE 4. PROXIMATE AND ULTIMATE ANALYSIS OF OIL PALM BIOMASS WASTE

Analysis proximate (% mass)	**Percentage (% mass)**		
	Trunks	**Fronds**	**Empty fruit bunches**
Moisture	7.60	7.50	7.38
Volatile matter	74.06	73.06	76.41
Fixed carbon	14.92	16.55	11.57
Ash	3.42	2.89	4.64
Analysis ultimate (% mass)	**Percentage (% mass)**		
	Trunks	**Fronds**	**Empty fruit bunches**
Carbon	43.68	43.44	51.77
Hydrogen	6.07	6.08	7.04
Oxygen	49.84	50.28	40.31
Sulphur	0.40	0.19	0.72
Nitrogen	0.0	0.01	0.16

Effect of catalyst concentration on distribution of pyrolysis products. The concentration of catalyst was varied from 5% to 15% in order to study the impact of varying the catalyst concentration on the distribution of catalytic pyrolysis products. *Figure 7* shows the effect of catalyst concentration on distribution of pyrolysis products. As shown in *Figure 7*, the liquid yield increases as the catalyst concentration increases. Increasing the amount of RM catalyst leads to the cracking rate of non-volatile oligomeric compounds into monomers, thus promoting the yield of volatile products which will be condensed into liquid. The bio-char yield decreases with the increase of catalyst concentration. An increase in temperature will generate more carbon monoxide (CO) and hydrogen (H_2) which consequently reduces the yield of the solid product. The decrease in bio-char yield can be explained as the decomposition of tar formed during pyrolysis into gaseous and liquid products.

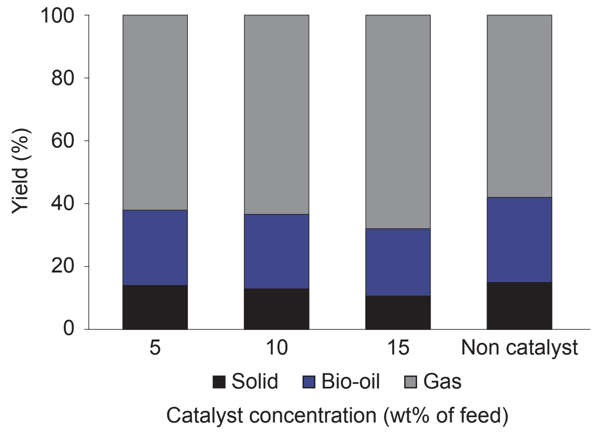

Figure 7. Effect of catalyst concentration on distribution of pyrolysis products from oil palm trunk biomass waste.

Composition of Liquid Products from Catalytic Pyrolysis of Oil Palm Biomass

Table 5 shows the liquid product compounds produced from the empty fruit bunches, trunks, and fronds of oil palm at the temperature of 500°C. Based on GC-MS analysis, oxygenated compounds are the main composition present in the liquid product such as acids, aromatics, phenols, aldehydes, ketones, alcohols and esters. The high content of these compounds contributes to the low calorific value of the liquid product. The composition of the compounds contained in the liquid product depends on the type of oil palm biomass and its constituent atoms, especially the lignocellulosic components. The biomass composition greatly influences the compounds in bio-oil or liquid products. The decomposition of cellulose and hemicellulose into anhydrosugars and heterocyclic compounds such as furan and furan derivatives and acetic acid. The lignin fraction of biomass undergoes devolatilisation resulting in oxygenated aromatic compounds such as phenol and phenolic derivatives. Besides lignin content, biomass alkaline mineral metals will influence the content of the liquid product (Bensidhom et al., 2021).

Gas Product Distribution from Catalytic Pyrolysis of Oil Palm Biomass Waste

Figure 8 presents the main composition of the gas product from pyrolysis of oil palm biomass waste. It can be seen from the table that the pyrolysis gas products obtained contain low hydrogen (H_2), carbon monoxide (CO), carbon dioxide (CO_2) and methane (CH_4) components. Gas products resulting

TABLE 5. COMPOUND COMPOSITION OF LIQUID PRODUCTS FROM OIL PALM BIOMASS WASTE

Compound	Composition (%)		
	Trunks	Fronds	Empty fruit bunches
Furan, 2,5-diethoxytetrahydro-	0.17	0.22	0.24
Furan, 2-ethyl-	0.07	0.05	0.04
Acetic acid	13.39	0.32	14.49
2-Furan-carboxaldehyde	4.41	6.63	3.34
2-Furanethanol	-	-	1.78
3-Furanethanol	2.93	1.23	-
Phenol	9.32	8.17	3.32
Phenol, 2-methoxy-	1.65	2.23	2.78
Phenol, 2,6-demethoxy-	2.96	0.11	3.85
Phenol, 2,6-demethoxy-4-(2-propenyl)-	0.15	0.13	0.28
2-Propanone, 1-hydroxy-	6.69	5.36	4.65
4-Proyl-syringol	0.10	0.10	0.15
2-Cyclopenten-1-one, 3-ethyl-2-hydroxy-	0.61	0.52	0.35
Octanoic acid	-	-	0.33
Benzenemethanol	-	0.63	0.43
3,5-Dimethoxy-4-hydroxytoluene	1.01	1.18	1.88
Benzene, 1,2,3-trimethoxy-5-methyl-	-	0.54	-
Benzaldehyde, 4-hydroxy-3-methoxy-	0.10	0.29	0.42
2-Methoxy-4-vinylphenol	0.21	-	0.46
2,6-Dimethoxy-4-(prop-1-en-1yl)phenol	0.08	0.06	0.15

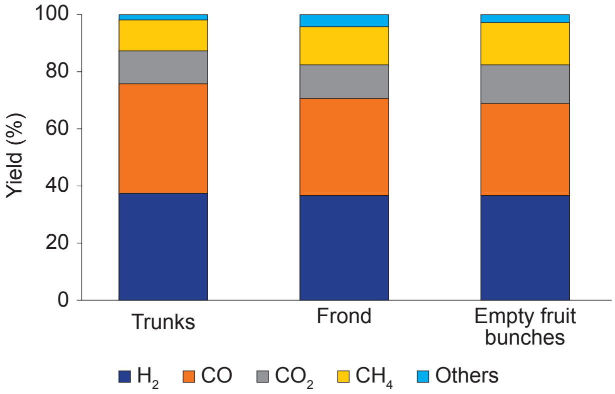

Figure 8. Gas product distribution from catalytic pyrolysis of oil palm biomass waste.

from pyrolysis from oil palm trunks contain higher concentrations of H_2, CO, CO_2 and CH_4 compared to products obtained from oil palm fronds and empty fruit bunches. Oil palm trunks contain high levels of cellulose and hemicellulose but low levels of lignin. Cellulose and hemicellulose can decompose and be released as vapour at lower temperatures compared to lignin. Empty fruit bunches contain high levels of lignin so the pyrolysis gas produced is low (Sembiring et al., 2015).

CONCLUSION

The three types of oil palm biomass can produce bio-oil and bio-hydrogen as a renewable energy source by pyrolysis process using catalysts from RM. The yield of liquid and gaseous pyrolysis product increases as temperature increases. The addition of catalyst also promotes the increase of liquid and gas pyrolsis products yield. Based on GC-MS analysis, the presence of RM catalyst enhanced the amounts of hydrocarbons in bio-oils. The results showed that the liquid product from catalytic pyrolysis of palm biomass using RM catalyst contains primary oxygenated compounds such as acids, aromatics and esters. RM is a solid waste and is widely available, therefore it is a plausible alternative to be produced on an industrial scale. RM catalysts were tested for catalytic pyrolysis of oil palm biomass under different operating conditions. The RM catalyst showed good activity to produce higher bio-oil yields.

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DUAL APPLICATION OF PALM OIL MILL EFFLUENT (POME) AND BENEFICIAL MICROBE ISOLATES FOR *Oryza sativa* GROWTH UNDER GLASS HOUSE CONDITION

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ABSTRACT

Oryza sativa *is the primary staple food in Asia. However, the high demand for rice leads to the overuse of chemical fertilisers and requires an alternative. Thus, this study aimed to investigate the impact of the combination of palm oil mill effluent (POME) and plant growth-promoting rhizobacteria (PGPR) on the growth of paddy cultivation. PGPR was isolated from lemongrass-cultivated soil and tested on nitrogen fixation, phosphate solubilisation and potassium solubilisation plates. POME, a byproduct from the palm oil milling process, has been combined with PGPR in powder form with different ratio combinations. The combination of POME: PGPR treatments was used in the following ratios: T0 = (0 g: 0 g), T1 = (5 g: 0 g), T2 = (0 g: 5 g), T3 = (5 g: 10 g) and T4 = (10 g: 5 g) and applied it alternately once in a week for 30 days in glasshouse condition. Treatment T4 produced the highest result in rice plant height (42.6 cm), rice plant dry weight (0.777 g) and root dry weight (0.700 g). Specifically, these values represent a 4.39 fold increase in plant height, 1.17 fold increase in plant dry weight and 3.70 fold increase in root dry weight compared to control (T0). These findings show that the combination of POME and PGPR may be a potential organic biofertiliser as an alternative to chemical fertilisers.*

Keywords: biofertiliser, *Oryza sativa*, palm oil mill effluent, plant growth-promoting rhizobacteria.

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INTRODUCTION

Rice is the primary food source in Malaysia and the government prioritises it. However, unequal distribution of fertiliser is contributing to the decline in paddy production despite extensive cultivation. Previous study show that farmers are unable to afford costly fertilisers, pesticides and herbicides, leading to reduced productivity. Othman et al. (2022) confirm that rice is the primary staple food in many developing countries, with approximately 90% of the world's rice production concentrated in 15 countries. Malaysia's rice cultivation area in Southeast Asia is approximately 0.70 million hectares, the smallest in the region. According to Dohlman et al. (2022), Indonesia, Vietnam, and Thailand produced 11.50, 7.54 and 10.83 million hectares of rice, respectively.

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Paddy crops require essential nutrients to flourish and achieve high yields. Chemical fertilisers gradually diminish soil fertility, leading to groundwater pollution and reduced soil productivity, which negatively impacts rice cultivation. Organic fertilisers, while contributing to soil health, are insufficient in providing necessary nutrients for growth. Saputro and Kurniawati (2024), found that there are intricate relationships among soil, plants and microbes that regulate the movement of micronutrients, leading to enhanced soil fertility. Several studies indicate that biofertilisers play a crucial role in integrated nutrient management and the promotion of sustainable agriculture.

In general, many countries rely on palm oil products, which will produce palm oil mill effluent (POME), a form of waste. Thus, managing POME may enhance the quality of water, air and the environment. According to Wu (2009), it is recommended to utilise POME as a cost-effective organic fertiliser as an alternative to the excessive use of chemical fertilisers. POME enhances soil structure and contains essential plant-growth nutrients such as phosphorus, potassium, magnesium and calcium, thereby enhancing root health, soil productivity and food yield. It is non-toxic due to its chemical-free composition. Also, beneficial bacteria are crucial for the well-being and growth of plants. Plant rhizospheres Harbour plant growth-promoting rhizobacteria (PGPR), which engage with root crops to influence their growth and yield. According to Andy et al. (2020), PGPR refers to biofertilisers, biocontrol chemicals, or microbial inoculants. Rhizobia are beneficial microorganisms that facilitate the growth and flourishing of plants. Nazma et al. (2023) highlighted that investigating advantageous PGPR as the symbionts in the symbiotic relationship in rhizospheres of plants that possess the ability to impede infections and enhance plant growth is a more convenient, expeditious and cost-effective approach. This study aims to investigate the effects of POME and beneficial PGPR on the growth of *Oryza sativa* as it is a cost-effective and environmentally friendly alternative to chemical fertilisers.

MATERIALS AND METHODS

Isolation of Beneficial Microbes

Approximately 5 g of soil sample obtained from lemongrass cultivation was placed into a Falcon tube, to which 10 mL of distilled water was added (Othman et al., 2022). Three replicates were prepared. The samples were left undisturbed for 24 hr. Bacterial isolation was performed using nutrient agar plates.

Characterisation of Plant Growth-Promoting Bacteria

Nitrogen fixation activity. The soil sample was agitated on a nutrient agar medium using a glass spreader, and then placed in an incubator for 24 hr. A nitrogen fixation test was done to observe the nitrogen fixation activity from the isolated PGPR. This activity required to prepare nitrogen-free malate plate with the following composition, per litre of distilled water containing K_2HPO_4 (5.00 g), NaCl (0.50 g), $MgSO_4·7H_2O$ (0.10 g), $CaCl_2·2H_2O$ (0.20 g), micronutrient solution (0.02 g), bromothymol blue solution (2 mL), FeEDTA solution (2 mL), KOH (4.50 g), agar (15.00 g). The solution was adjusted to 6.5 of pH and autoclaved in 20 min with 120°C. The solution then was poured into four replicates of petri dishes and set aside until it cool and harden. The pure culture of PGPR was inoculated onto the nitrogen-free malate plate, using the streak plate method under sterilised condition. The plates were then incubated for 24 hr, at 30°C in the incubator.

Phosphate solubilising activity. Phosphate solubilising activity was tested to ensure that the PGPR isolated can provide phosphate to the plant. This activity was inoculated on National Botanical Research Institute's (NBRIP) phosphate growth medium, with the composition prepared per litre of distilled water – glucose (10.00 g), $(NH_4)_2SO_4$ (0.10 g), KCl (0.20 g), $Ca_3(PO_4)_2$ (5.00 g), $MgCl·6H_2O$ (5.00 g), $MgSO_4·7H_2O$ (0.25 g) and agar (15.00 g). The solution was homogenised and autoclaved. The solution was then standardised and sterilised using an autoclave. The selected bacteria were introduced on the phosphate plate and allowed to incubate for 48 hr.

Potassium solubilisation. The potassium solubilising test was conducted by using Alexandrov medium agar. Mica powder is provided as insoluble potassium. The autoclaved media was then poured onto petri dishes and set aside to harden. Then, the selected PGPR was placed on the potassium plates and cultured for 48 hr.

Indole compound content. A colourimetric technique was used to quantify the synthesis of indole-3-acetic acid (IAA) in each isolate. The Van Urk Salkowski reagent was employed for this purpose, following Salkowski's method as originally described by Ehmann in 1977. A total of 1.0 mL of PGPR culture was added to 100 mL of nutrient broth, and the solution was enhanced with 5.0 mL of L-tryptophan. The solution was then incubated at 28°C. After one day of incubation, 1.5 mL of PGPR culture was added into the microcentrifuge tube, and then centrifuged at 7,000x g for 7 min. About 1.0 mL of the supernatant was transferred carefully into a cuvette. To detect the indole component's presence, four mL of Salkowski reagent was added, and a pink colouration was observed within 20–30 min. The concentration of the indole compound was determined using a spectrophotometer.

Encapsulation of Plant Growth-Promoting Rhizobacteria

About 13 g of nutrient broth powder was mixed with 1 L of distilled water. The solution was thoroughly mixed using a stirrer and vigorously shaken. Then, the solution was transferred into a bottle and autoclaved at 121ºC for 2 hr. After that, the pure PGPR culture was introduced into a nutritional broth using a sterilised inoculating loop in a sterilised laminar airflow. The PGPR were cultured for 24 hr at 30ºC in the incubator. Then 1 g of sodium alginate, and 50 mL of PGPR culture with 100 mL of sterilised distilled water were mixed in a Schott bottle. Then, a calcium chloride solution was prepared by mixing 30 g of $CaCl_2$ with 100 mL of distilled water. The sodium alginate solution was injected into the calcium chloride solution to catalyse its transformation into gel beads. After filtration, the gel beads were positioned on top of a tray. A layer of encapsulated beads was thinly spread on a tray and placed in an incubator set at 121ºC. The PGPR was allowed to dry for 3–4 days. Subsequently, the desiccated beads were pulverised into a fine powder using a grinding apparatus and a mortar and pestle. The procedure was repeated until the gel beads achieved a refined texture.

Preparation of POME in Powder Form

The POME powder was prepared by first collecting the liquid effluent and allowing it to undergo sedimentation for 48 hr to reduce the solid content. This method was adjusted based on Ismail et al. (2014). The supernatant was then placed in aluminium trays in batches and subjected to drying at 100°C for 24 hr in an oven. The dried POME flakes obtained were manually ground using a commercial grinder to achieve a fine powder that could pass through a 100 micron sieve. This powder was stored in air-tight containers.

Dual Application of PGPR and POME on Rice Cultivation

POME was obtained from a palm oil mill in Perak, Malaysia. Rice seeds were obtained from Batu Merah Agrofarm in Perak. Rice seedlings come in packets of 50 seeds. The variety used was MRIA1. The seedlings were then soaked in water for two days, which speeds up germination and boosts the percentage of seeds that germinate successfully. The moist tissue approach was used to germinate the seeds, which took around a week to see the results. After the seeds germinated, the uniformed size seedlings were selected to be transferred into 16′ x 16′ pots. The rice cultivation was monitored for 30 days after sowing (DAS) under nursery conditions. The PGPR and POME were alternately applied once a week during the cultivation period. Plants were watered twice a day. The design of this experiment was a complete randomised design (CRD), with three replications, as the plant requirements are uniform. The treatments involved; T0 – control, T1 – 5 g of POME, T2 – 5 g of PGPR, T3 – 5 g of POME + 10 g of PGPR and T4 – 10 g of POME and 5 g of PGPR.

Vegetative Growth Measurements

Growth parameters of rice plants were taken during the cultivation period. Plant height, fresh weight, dry weight and root length were measured using tape and digital weighing scales, respectively. The chlorophyll content was measured using a SPAD meter (SPAD-502 plus) (Konica Minolta, Japan).

Statistical Data Analysis

All the collected data was analysed using SPSS (Version 2021). At a confidence level of 95%, the differential of the data was examined using one-way ANOVA, followed by Tukey's B test.

RESULTS AND DISCUSSION

Isolation of Bacterial Isolates as Plant Growth-Promoter

Four isolates of PGPR were successfully isolated from lemongrass soil and roots and cultured in triplicate on nutrient agar plates (*Figure 1*). Prior investigations by Halimursyadah et al. (2022), have shown that distinct colonies of rhizobacteria can be isolated and differentiated by their varied densities (e.g., hard and dry, butter, slime or dull). In this study, the isolates showed a slimy appearance, milky white and accompanied by an unpleasant odour. This observation is consistent with the findings of Silva et al. (2021), where PGPR has been isolated from chromium-contaminated, noting similar characteristics. These PGPR can fix nitrogen and enhance nutrients in maize, highlighting the potential of these bacteria to thrive under extreme conditions. The milky white and slimy appearance in this study may suggest that these PGPR consist of similar adaptive traits that enable them to support plant growth. This similarity underscores the potential of the isolates from lemongrass soil to contribute as an effective biofertiliser.

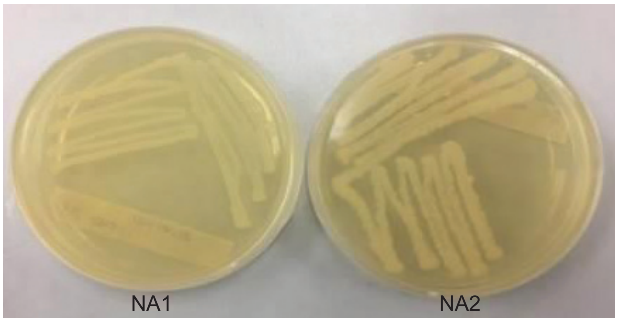


Figure 1. Two plates of microbial isolates were obtained from the soil in which lemongrass was planted, namely NA1 and NA2.

Characterisation of Plant Growth-Promoting Rhizobacteria

The positive results were achieved from the nitrogen fixation test, phosphate solubilising test and potassium solubilising test for the PGPR isolates derived from the lemongrass soil and lemongrass root (*Figure 2*). After 24 hr of incubation, the PGPR activity transformed the nitrogen-free malate agar from green to blue, validating its characterisation. The outcome is indicative of nitrogen fixation which transformed nitrogen-free malate agar from green to blue which confirms the ability of these isolates to convert nitrogen in the atmosphere into ammonia, making it available to plants (Yilihamu et al., 2020). The findings are in concurrence with (Wang et al., 2020), who reported similar nitrogen-fixing capabilities in a variety of soil bacteria, by emphasising the significance of these microorganisms for long-term and sustainable agriculture.

The phosphate solubilisation test showed the presence of a halo zone around the colonies on the NBRIP plate which indicates effective phosphate solubilisation. Purwaningsih et al. (2022) found that different PGPR isolates may exhibit varying degrees of phosphate solubilisation efficiency. Some of the isolates produced large zones of solubilisation. This variability highlights the potential for selecting specific strains that can optimise phosphate availability in soils because this is crucial for root growth. Furthermore, behind phosphate solubilisation, lies a mechanism where the production of organic acids can cause low soil pH, which facilitates the release of bound phosphates (Halimursyadah et al. 2022). This is aligned with the study by Othman et al., 2022, who demonstrated that *Acinetobacter* sp. effectively solubilised phosphate through organic acid production which increases the phosphate availability in soil.

The potassium solubilisation activity of PGPR was evidenced by the formation of transparent zones (*Figure 2c*) which surround the colonies of isolates on Aleksandrive media, indicative of positive and effective potassium solubilisation. Potassium solubilising microorganisms play an important role in enhancing plant growth and mitigating the environmental effects in agriculture practice as highlighted by Ashfaq et al. (2020). Similar to these findings, previous studies have shown that potassium solubilising bacteria, which was isolated from the rhizosphere, may produce soluble zones on the silicate media, with diameters ranging from 0.65–1.50 cm, depending on the specific isolates (Sood et al., 2023). This further supports that potassium solubilising bacteria can leverage the potassium availability in soil and can encourage plant development (Joshi et al., 2023)

Moreover, a qualitative IAA production test was conducted by using the Salkowski reagent. The IAA test results indicated that indole compounds have been successfully exhibited on the test, by investigating the transformation of solution, from yellow pale to pink colour (*Figure 2d*). This result is consistent with Adhyaningtas et al. (2023) who reported that IAA concentrations in a specific range, suggest that the isolates from lemongrass may have varying IAA production capabilities. However, the findings were contradictory to the previous study in which the IAA concentrations, in the range of 21.66–83.38 ppm created by rhizobacteria were indicated by a light-yellow colony (Halimursyadah et al., 2022).

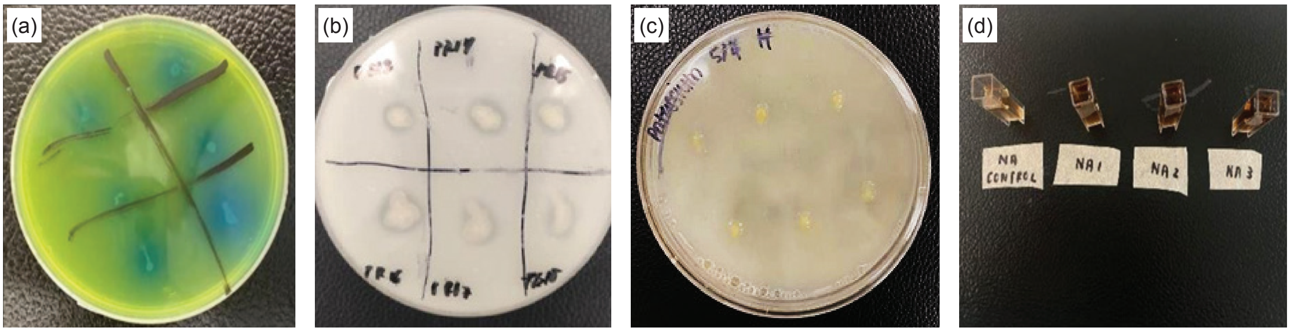


Figure 2. The compilation of plant growth-promoting rhizobacteria characteristics tests for positive results of (a) nitrogen fixation, (b) phosphate solubilisation, (c) potassium solubilisation and (d) IAA production.

Effect of POME and PGPR Combination on Plant Height

The results of plant height data indicate that Treatment T4, consisting of a 10 g: 5 g mixture of POME and PGPR, produced the most substantial plant growth after a four-week period of observation at $p < 0.05$ (*Figure 3*). The weekly measurements were 9.700, 17.800, 4.367 and 42.600 cm respectively. Statistically significant analysis confirmed significant differences were identified across the five treatment groups. Treatment T4 resulted in the highest plant height, with a 4.39 fold increase compared to the control (T0). This indicates synergistic effects of POME and PGPR can lead to enhanced plant growth compared to treatment lacking in this combination. The application of 10 g POME introduces a rice source of organic matter and essential nutrients such as potassium, phosphorus and magnesium, which are important for plant height. Palm oil mill effluent is known for its high nutrients contents, which support plant growth, a finding in concurrence with Alam et al. (2022) who reported improved growth and performance in Brazilian spinach (*Alternanthera sissoo*) following POME application. In addition to POME, the inclusion of PGPR enhances the nutrient profile by introducing beneficial microbes that can fix nitrogen, solubilise phosphate and potassium and produce IAA (Othman et al., 2022). This aligns with the findings by Ray et al. (2024), which demonstrated the synergistic effects of PGPR and organic matter which promoted spinach growth. The ratio of 10 g of POME to 5 g of PGPR appears to be optimal for PGPR to effectively colonise plant roots and enhance nutrients and growth. Puri et al. (2020) also found that the presence of PGPR can convert unavailable form of organic matter into available forms, as observed in Pinaceae trees under nutrient-limited conditions. Similarly, the findings of this study show that the 10 g: 5 g of POME and PGPR ratio provides a balanced nutrient supply from POME while ensuring sufficient microbial inoculants to stimulate plant growth effectively.

Effect of POME and PGPR Combination on Chlorophyll Content

The mean value of chlorophyll content was similar to all treatments at $p < 0.05$ (*Figure 4*). It is important to note that although the combination of POME and PGPR has improved plant height, it does not necessarily lead to an increase in chlorophyll content. Ghasemi et al. (2020) found that, the presence of nutrients alone does not guarantee increase of chlorophyll content as other physiological processes may contribute to the increment.

Effect of POME and PGPR Combination on Plant Fresh Weight and Dry Weight

The fresh and dry weight of rice plants across the different treatments indicate an obvious trend, with Treatment T4 (10 g POME: 5 g PGPR) obtaining the highest plant fresh and dry weight. The mean value of plant fresh weight was not significantly different from the other treatments ($p > 0.05$) (*Table 1*). In contrast, Treatment T4 produced the highest dry

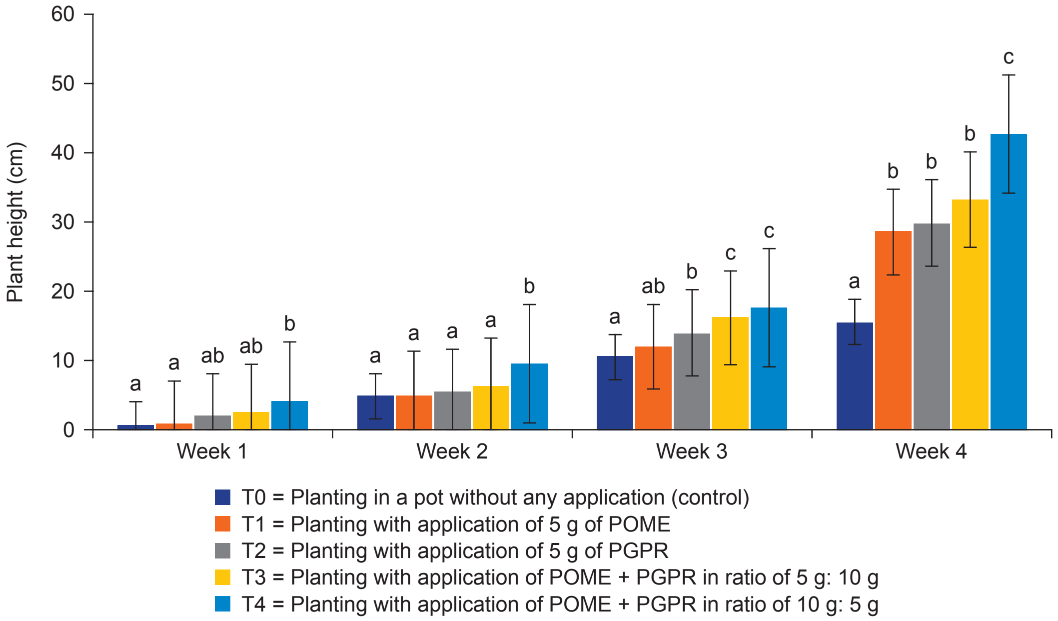
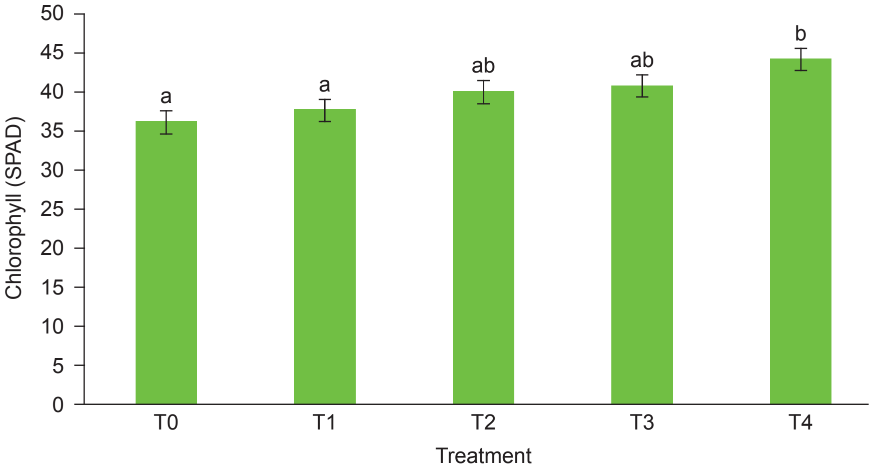


Figure 3. Plant height measurements of different treatments application based on weekly progress at p < *0.05.*



Note: T0 - control, T1 - Application of 5 g POME; T2 - Application of 5 g PGPR; T3 - Application of POME + PGPR in ratio of 5 g: 10 g; T4 - Application of POME + PGPR in ratio of 10 g: 5 g.

Figure 4. Chlorophyll content of all treatments rice plant differences at 95% of significance level.

TABLE 1. THE MEAN VALUE OF PLANT FRESH WEIGHT AND PLANT DRY WEIGHT OBSERVATION AFTER FOUR WEEKS AT 95% SIGNIFICANCE LEVEL

Growth Parameter	Treatment T0	Treatment T1	Treatment T2	Treatment T3	Treatment T4
Fresh weight (g)	0.577 ± 0.07a	1.343 ± 0.55ab	1.890 ± 0.33ab	2.713 ± 0.35bc	3.933 ± 1.12c
Dry weight (g)	0.667 ± 0.08a	0.210 ± 0.04b	0.343 ± 0.05c	0.470 ± 0.06d	0.777 ± 0.09e

Note: T0 - control; T1 - Application of 5 g POME; T2 - Application of 5 g PGPR; T3 - Application of POME + PGPR in ratio of 5 g: 10 g; T4 - Application of POME + PGPR in ratio of 10 g: 5 g.

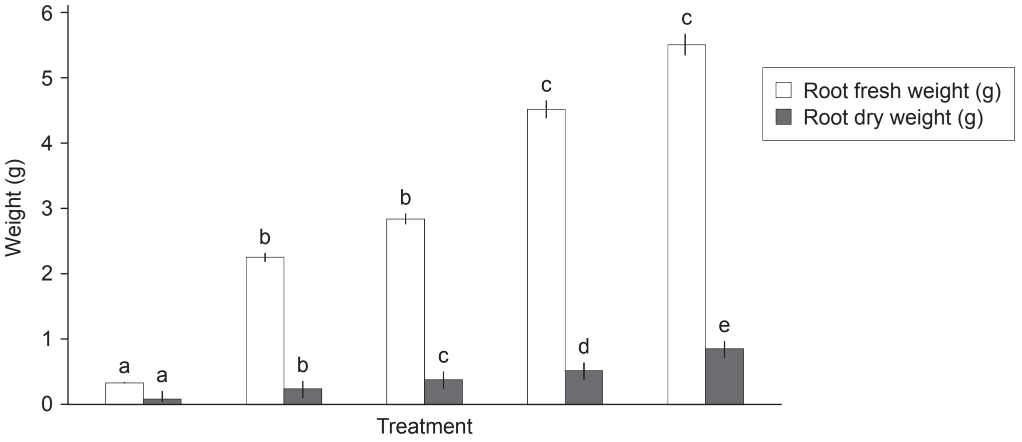
weight at 0.777 g ($p < 0.05$) compared to the other treatments, with a 1.17 fold increase compared to the control (T0). The observed increment in biomass, especially dry weight can be attributed to the synergistic effects of nutrient management in POME and the application of PGPR. This is consistent with findings from Midya et al. (2021) who found that integrated nutrient management, involving both microbial inoculants and organic amendments has led to improved biomass production which is dry weight in the lower rice field of Indo-Gangetic Plain, India while also increasing the soil fertility. The enhancement of biomass in Treatment T4 is most likely resulting from the improved nutrient availability and improved soil structure due to organic matter content in POME.

Effect of POME and PGPR Combination on Root Fresh Weight and Root Dry Weight

The combination of POME and PGPR significantly enhanced root dry weight only (*Figure 5*). For root fresh weight, Treatment T4 showed similar results to Treatment T3 and both were higher than other treatments (*Figure 5*). In terms of root dry weight, Treatment T4 recorded the highest dry weight (0.700 g), with a 3.70 fold increase compared to control (T0) ($p < 0.05$). This outcome aligns with Astuti et al. (2021), who reported that the nutritional composition in POME can support the growth of beneficial microorganisms especially PGPR, which in turn promotes *Lantara camara* root growth by *Bacillus thuringiensis*-induced carrier composition. Similarly, Hastuti et al. (2022) found that the application of PGPR in conjunction with POME significantly improved the growth of oil palm seedlings, with PGPR alone showing fewer effective results for growth and root parameters. Our findings further suggest that PGPR can mitigate some of the potential negative effects of POME's nutrient load, improving root weight and biomass and overall helping plant growth.

CONCLUSION

In conclusion, the isolation of beneficial microbe (PGPR) from the lemongrass soil and crop was successful. Two isolates show positive results for the nitrogen fixation test, phosphate and



Note: T0 - control; T1- Application of 5 g POME only; T2 - Application of 5 g PGPR only; T3 - Application of POME + PGPR in ratio of 5 g:10 g; T4 - Application of POME + PGPR in ratio of 10 g: 5 g.

Figure 5. The mean value of root fresh weight and root dry weight observation after 4 weeks at 95% difference of significance level.

potassium solubilisation test and IAA production test. Significant enhancements were noted in multiple parameters such as rice plant height, rice plant dry weight and root dry weight with 4.39, 1.17, and 3.70 fold increases compared to control. The dual application of POME and PGPR presents a promising approach to promote sustainable rice cultivation and sustainable oil palm waste production for reuse and recycle. The findings also support the potential of this combination as an alternative that reduces the reliance on chemical fertilisers for environmental sustainability.

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PERFORMANCE OF B10/B20 USAGE IN HEAVY-DUTY DIESEL VEHICLES

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ABSTRACT

Palm oil biodiesel has been a sustainable and renewable alternative to petroleum since the 1980s. Palm biodiesel was gradually blended with petroleum diesel to introduce environmentally friendly fuels into the Malaysian market. Consumers sought additional information to assess the potential risks of higher palm biodiesel blends. Six diesel trucks under actual on-road conditions were involved in using palm B20 and B10. Fuel economy for the B20 vehicles was 2.020 km L^{-1} *and for B10 vehicles was 1.910 km* L^{-1}*. Idling adjusted fuel economy for the B20 vehicles was 2.107 km* L^{-1} *and for B10 vehicles was 2.055 km* L^{-1}*. Statistical analysis indicated no significant difference in fuel economy, vehicle payload affecting fuel economy, or maintenance costs between the B20 and B10 groups. Engine oil samples, taken at 5,000 km intervals, were tested to determine the impact of using the B20 blends. All engine oil samples were found to remain within operational service limits. B20 usage positively impacted engine oil quality with higher total basic number and lower iron content than B10. Statistical analysis demonstrated insignificant differences between the two fuel groups in fuel economy, idling adjusted fuel economy, payload, service cost, viscosity, zinc, sodium, soot in oil and magnesium, with a high degree of confidence.*

Keywords: engine oil quality, heavy-duty diesel vehicles, on-road fleet testing, palm biodiesel.

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INTRODUCTION

Biodiesel, a sustainable and eco-friendly diesel alternative derived from vegetable oil through transesterification (Mosarof et al., 2015), has been identified as a viable substitute for petroleum since the 1980s. In Malaysia, palm oil serves as the primary raw material for biodiesel production, and as one of the world's largest palm oil producers, Malaysia produced over 19 million tonnes of crude palm oil in 2017 (Kushairi et al., 2018). Palm biodiesel production in the country commenced in 2006, reaching over 720,000 t in 2017 (Kushairi et al., 2018; Nambiappan et al., 2018). Palm oil's superior oxidation stability and year-round availability make it an abundant resource suitable for sustainable and environmentally friendly fuel production. The global shift away from fossil fuels due to finite reserves and rising carbon dioxide (CO_2) emissions has increased the significance of biofuels as a renewable and low-carbon footprint energy source. Biodiesel, as a prominent commercial fuel worldwide, is increasingly adopted by numerous countries as a substitute for fossil diesel in powering diesel vehicles.

Aligned with the National Biofuel Policy (NBP), Malaysia has introduced palm-based biodiesel blends with diesel, including B5, B7, B10 and B20 (in specific states) (Kushairi et al., 2018; Nambiappan et al., 2018). The National Agricommodity Policy 2021-2030 aims to implement increased B20 and B30 targets by 2022 and 2030, respectively. Despite these goals, there is a lack of operational and technical data for palm B20, hindering informed decisions and addressing concerns about its use in

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diesel vehicles. As diesel vehicle manufacturers' acceptance is crucial for the success of palm biodiesel in the commercial market, comprehensive fleet testing, laboratory analyses and chassis dynamometer investigations are essential for evaluating the impact of the palm B20 blend.

Most reported studies are focused on engine testing with biodiesel blends under controlled laboratory conditions, such as the single cylinder engine experimental setup to investigate the combustion efficiency of biodiesel blends (Sharma et al., 2022). There have been limited field studies on actual on-road operation, particularly regarding the impacts on extended engine durability and operating costs. Fleet tests in the U.S. indicated no significant differences in fuel economy, maintenance costs, or engine wear, although instances of fuel filter plugging were noted for B20 in some cases (Barnitt et al., 2008; Fraer et al., 2005; Lammert et al., 2010; Proc et al., 2006; Tang et al., 2016). However, for palm biodiesel, there remains a notable lack of B20 operational and technical in-use data under actual on-road conditions.

Engine lubricating oil (LO) plays a crucial role in diesel engines, performing functions such as reducing friction and wear, cooling, cleaning, sealing and protecting engine parts. However, the quality and performance of LO can degrade over time due to factors like oxidation, contamination, dilution and degradation. The use of biodiesel blends as alternative fuels for diesel engines is one factor that may influence LO quality and performance. *Table 1* illustrates the significance of LO parameters, along with their minimum and maximum limits.

TABLE 1. LIST OF LO QUALITY TESTING PARAMETERS SIGNIFICANCE AND LIMITS

Parameter	Limit
Viscosity	12.6–16.4 mm^2 s^{-1}
Total base number	Min 5.0 mg KOH g^{-1}
Wear metals	Iron: Max 100 ppm Others: Max 40 ppm
Oxidation and nitration	Max 2.0 A cm^{-1}
Sulphation	Max 2.0 A cm^{-1}
Water and coolant contamination	Water: Max 0.3% Coolant: Any detectable amount is a concern
Flash point	Min 220°C
Soot	Max 2.0 A cm^{-1}
Particle count	Max 20 ppm
Pour point	-24°C
Ash content	0.9%–1.5% by weight
Phosphorus and zinc	Phosphorus: 600–1,200 ppm Zinc: 800–1,400 ppm
Specific gravity	0.85–0.89 at 15°C

Hypothesis testing is a common practice in real-world vehicle trials to assess the significance of observed differences or relationships. Using a t-test or ANOVA after data collection helps test hypotheses. Inferential statistics, including techniques like confidence intervals and *P*-values, quantify the uncertainty associated with estimates and determine the statistical significance of findings, aiding in data-driven decision-making (Bietresato et al., 2019). These tests are employed to separate and isolate the considerable background disturbances present during actual on-road driving scenarios, which may significantly impact the results. This underscores the significance of statistical analyses in comparable trials.

Idling fuel consumption, a substantial source of energy waste and emissions, can be influenced by biodiesel blends. Biodiesel's characteristics, including oxygen content and a higher cetane number, may potentially reduce fuel consumption during idling. Real-world vehicle trials, using on-board diagnostics (OBD) systems for data retrieval, offer a practical approach to estimating idling fuel consumption, considering the rudimentary method when sophisticated methods are unavailable.

Payload weight's impact on fuel economy is well-established, with heavier payloads leading to increased fuel consumption. Optimising fuel economy with varying payload weights involves strategies like efficient route planning, load optimisation, vehicle maintenance and driver training. Proper maintenance practices are crucial for optimal fuel economy, as neglected maintenance can result in decreased engine efficiency and increased fuel consumption. Biodiesel's lubricity and impact on engine wear are factors to consider, making maintenance practices essential for achieving optimal fuel efficiency.

In summary, this project aims to acquire the necessary dataset for the understanding of extended palm B20 usage in heavy-duty diesel vehicles under actual on-road driving conditions. The 100,000 km road test, coupled with laboratory analyses, will assess on-road fuel economy, reliability, service maintenance and engine oil performance. The study's results aim to inform government decisions on higher biodiesel blends in Malaysia and instil confidence in the logistics sector regarding the applicability of biodiesel blends in their day-to-day operations.

MATERIALS AND METHODS

The study employs fleet testing to gather actual on-road data on the usage of palm B20 and B10 through operational fleet vehicles. While both fleet testing and stationary engine testing offer valuable insights into biodiesel blend performance, fleet testing presents several advantages, particularly in evaluating real-world applicability and performance. These advantages include exposure to diverse operating conditions, variability in ambient conditions, real-world emissions data, considerations for vehicle integration, assessment of durability and long-term impact, and obtaining direct feedback from drivers.

Fleet testing involves vehicles operating under actual driving conditions, encompassing a range of speeds, accelerations, decelerations, idling periods and more. This comprehensive assessment provides a realistic portrayal of biodiesel performance in various scenarios (McCormick & Westbrook, 2010). In contrast to the controlled environments typical of stationary tests, fleet tests subject biodiesel blends to different weather conditions and temperatures, revealing issues related to cold starts, fuel gelling, or volatility (Shrestha et al., 2005). Fleet tests offer a clearer understanding of real-world emissions and environmental benefits, potentially differing significantly from laboratory results (Durbin et al., 2008).

Additionally, fleet testing can unveil challenges related to the integration of biodiesel with vehicle systems, including potential issues with seals, hoses and compatibility with emission control systems (McCormick & Westbrook, 2010). It provides insights into the long-term impact of biodiesel blends on engine durability, maintenance requirements and overall vehicle lifespan, aspects challenging to replicate in stationary setups. Real-world fleet tests enable drivers to provide feedback on vehicle performance, drivability and potential issues, offering a holistic understanding of biodiesel's impact (Abed et al., 2019). Therefore, data obtained from fleet testing can provide more insightful information for logistics companies and government policymakers interested in the real-time implications of new fuel mandates.

The oil palm fleet company contributed six trucks serving as palm oil tankers transporting processed palm oil from refineries. The fleet company also provided the specifications of the diesel vehicles used in the fleet testing, as shown in *Table 2*.

TABLE 2. SPECIFICATIONS OF THE FLEET VEHICLES

Gross vehicle weight (GVW)	26,100 kg
Maximum power	400 hp (294 kW)
Maximum speed	125 km hr^{-1}
Axle configuration	6 × 2
Engine	D134
Emission norms	Euro 3
Engine cylinders	Inline 6/4/cycle
Displacement (cc)	12,800
Max power	400 hp @ 1,600–1,700 rpm
Max torque	2,000 Nm @ 1,000–1,300 rpm
Transmission	Manual with I shift
Gearbox	12 speed
Fuel tank (L)	400

Test Fuel

Both the B20 and B10 fuels were supplied to the depot in a pre-blended state by the petroleum supplier, Petronas Dagangan Berhad. The specifications of the provided fuel are detailed in *Table 3*.

Fleet Testing

For the fleet testing phase, an appropriate diesel fleet was initially identified for a 100,000 km road test spanning 18–24 months. This specific distance and duration were chosen to align with a similar extended-use fleet testing study conducted for the same purpose (Lammert et al., 2010). The targeted vehicles for the study were suitable trucks and tankers, with fleet type, vehicle specifications, service length and route selections determined based on distance travelled and similarity in vehicle models and functions. Driver behaviour was considered a potential factor that could influence vehicle monitoring, as the drivers were responsible for both driving and ensuring the trucks underwent necessary servicing. To mitigate this influence, the routes assigned to the trucks rarely deviated, ensuring consistent driving patterns whenever the truck engines were operational. The 100,000 km road test involved capturing detailed data on vehicle refuelling, workshop servicing, on-road fuel economy, scheduled maintenance and road call records through onboard vehicle fuel monitoring software and workshop written logs. Results were reported as monthly and cumulative averages, with the latter representing the average results from the study's initiation to any given point. The collected data underwent a two-tailed, paired t-test analysis to determine the statistical significance of differences between the B10 and B20 groups.

The selected vehicles for fleet testing were chosen based on their service length, constituting a significant portion of the fleet. *Table 4* shows the key information of the heavy-duty diesel vehicles used in this study. Vehicle specifications were provided by the fleet company and drivers were instructed to conduct operations without deviation from their assigned delivery routes. These vehicles were primarily engaged in transporting cargo, such as palm oil, between palm oil mills and refineries across Malaysia. The routes were dictated by the operations department of the fleet company and their evaluation was based on Global Positioning System (GPS) tracking data collected from on-board vehicle systems. The LO used in all six trucks was the Petronas Urania 3,000 SAE 15w/40.

TABLE 3. SPECIFICATIONS OF B20 AND B10 FUEL USED IN THE STUDY

Test	Method	Diesel specifications		B10	B20	Unit
		Min	Max			
Acid number	ASTM D664-18e1	-	0.25	< 0.05	< 0.05	mg KOH g^{-1}
Ash	ASTM D482-13	-	0.01	< 0.001	< 0.001	mass %
Carbon residue (on 10% bottom)	ASTM D4530-15	-	0.20	< 0.10	< 0.10	mass %
Cloud point	ASTM D2500-17a	-	19.0	10.0	8.0	°C
Copper corrosion (3 hr at 100°C)	ASTM D130-18	-	1	1A	1A	-
Colour (ASTM)	ASTM D1500-12 (2017)	-	2.5	0.5	L 1.5	-
Density at 15°C	ASTM D4052-18a	0.8100	0.8700	0.8461	0.8490	kg L^{-1}
Electrical conductivity	ASTM D2624-15	50	-	208	359	pS m^{-1}
Flash point	ASTM D93-18	60.0	-	67.0	77.0	°C
Sulphur	ASTM D4294-16e1	-	500	249	804	mg kg^{-1}
Physical distillation at 95% recovered volume	ASTM D86-18	-	370.0	364.3	368.4	°C
Sediment by extraction	ASTM D473-07 (2017)e1	-	0.01	< 0.01	< 0.01	% (m/m)
Water by distillation	ASTM D95-13 (2018)	-	0.05	< 0.05	< 0.05	% (V/V)

TABLE 4. KEY INFORMATION OF THE TRUCKS INVOLVED IN B10/B20 PROJECT

Truck identification	Categories	Year of purchase	Engine	Fuel used
B20-01	Palm oil tanker	2012	Euro3	B20
B20-02				
B20-03				
B10-01	Palm oil tanker	2012	Euro3	B10
B10-02				
B10-03				

The GPS data revealed that, on average, fleet vehicles covered short distances (2.310%), engaged in urban driving (30.515%) and conducted highway driving (67.175%). *Table 5* shows the description of these types of driving. These distinctions were crucial for understanding the driving patterns and conditions encountered during the fleet testing phase, contributing to a comprehensive assessment of biodiesel blend performance in diverse scenarios.

The fleet vehicles utilising B20 fuel were mandated to maintain a sufficient fuel level for deliveries and subsequent returns to the depot for B20 refuelling. Notably, during the fleet testing period, B20 fuel was exclusively available at the fleet company depots and was not commercially accessible at external service stations. Engine oil sampling, a critical aspect of the testing process, was conducted by the fleet company at specified intervals, aligning with each scheduled service interval. The planning of these service intervals adhered to the predetermined schedule established by the fleet company. In particular, the engine oil sampling service interval was set at every 5,000 km, allowing for a strategic balance between accumulating an adequate sample stockpile and obtaining sufficient data points to monitor the degradation of the diesel engine LO samples. This systematic approach ensured a comprehensive and meaningful assessment of the impact of B20 fuel usage on engine oil quality throughout the fleet testing period.

Laboratory Investigation

Fuel sampling, both from onboard and station sources, as well as used engine oil sampling, was carried out at 5,000 km intervals throughout the testing period. The collected samples underwent

TABLE 5. THE DISTINCTION BETWEEN SHORT DISTANCE DRIVING, URBAN DRIVING AND HIGHWAY DRIVING

Driving	Description
Short distance	Any instance of movement under 5 min.
Urban	Vehicle constantly varies in speed and/or does not have a sustained speed of above 50 km hr^{-1} for more than 3 min at a time within the instance. Also includes an instance of speeds below 50 km hr^{-1}. This is not indicative of the road type or environment in which the vehicle is travelling.
Highway	The vehicle has sustained speeds of above 50 km hr^{-1}, or smooth acceleration and deceleration throughout the instance. This does not indicate the vehicle is physically driving on a highway.

thorough analysis to assess various physicochemical properties, including biodiesel, diesel and water content, oxidation stability and other relevant characteristics, as outlined in *Table 3*. The primary focus of the analysis lay in comparing key parameters derived from the data collected in the diesel engine LO samples. These parameters encompassed viscosity, total basic number, wear metals and additives. The samples were sent to an external laboratory for comprehensive testing, providing valuable insights into the impact of B20 and B10 fuel usage on these crucial LO properties.

Statistical Analysis

The data collected was analysed using a two-tailed, paired t-test to assess the statistical significance of the difference between the B10 and B20 groups. With similar make and model of the test vehicles, statistical tests can be employed to ascertain the significance of the fuel blends used by isolating the other background factors that may impact the results obtained. More specifically, the two-tailed test examines if the difference between two sample means is statistically significant and considers the possibility of the difference occurring in either direction within the context of this study. The calculation involves determining the probability, known as the *P*-value, that the observed difference could have happened by chance. If the *P*-value is less than the chosen significance level (commonly set at 0.05), it can then be concluded that the difference between the means is statistically significant. The versatility of a two-tailed t-test lies in its ability to evaluate differences in both directions, assessing whether one mean is either greater or less than the other. The significance level helps control the probability of a Type I error (false positive). Even with small sample sizes, the t-test remains applicable, making it suitable for real-world datasets where collecting large amounts of data might be impractical. Given its adaptability, control, and applicability to smaller datasets, the two-tailed t-test proves to be a fitting statistical analysis method for this study, which focuses on evaluating the impact of B20 and B10 usage in diesel fleet vehicles.

RESULTS AND DISCUSSION

The results presented here are derived from the fleet test data collected by both the local logistic company and the Malaysian Palm Oil Board (MPOB). All the vehicle trials successfully completed at least the second cycle of servicing. This comprehensive dataset provides insights into the extensive utilisation of palm B20 in fleet vehicles over the course of the study.

Fuel Economy

At the conclusion of the trial period, the vehicles collectively utilised 392,259 L of fuel, covering a total distance of 777,479 km. Within this same trial period, the B20 vehicles consumed 174,924 L of B20 and travelled 354,702 km. In comparison, the B10 vehicles consumed 217,335 L of B10 and covered 422,776 km. This difference can be attributed to the greater flexibility of B10 vehicles, which could refuel at conventional petrol stations supplying B10 and make multiple stops on their delivery routes. In contrast, the B20 vehicles had to return to the depot after deliveries to refuel with B20, available exclusively at the depot. It's worth mentioning that the B20 group's lower fuel consumption is partially attributed to one B20 vehicle being taken off service for five months due to transmission repair work, unrelated to the fuel and/or engine systems which may be caused by the fuel used. Additionally, there was a temporary B20 fuel stock depletion in August 2020 due to the petroleum company's supply disruption. Prompt communication between the fleet company's procurement manager and the petroleum company's supply executive ensured a swift resolution, allowing the study to resume without any significant interruption.

Fuel economy is pivotal for logistics companies, allowing them to assess potential profits from deliveries, given that fuel costs constitute a significant portion of operational expenditures alongside maintenance. Over the 18 month period, both groups exhibited similar average fuel economy values of around 2.00 km L^{-1} for each vehicle. While the figure suggests a slight decline in fuel economy when using B10 compared to B20, statistical analysis using a two-tailed test shows that the calculated *P*-value is 0.6577, indicating a high degree of confidence that the observed difference in fuel economy between the two groups is statistically insignificant. Therefore, based on the available data, there is no significant evidence to suggest a difference in fuel economy between the B20 and B10 groups.

To obtain a more accurate representation of fuel economy in this study, the impact of idling on fuel consumption is taken into consideration. For logistics companies, idling time during queues at delivery sites or rest stops during long-haul trips can significantly affect fuel economy as vehicles consume fuel without making progress on deliveries. To address this, idling time data for the fleet vehicles was filtered from the GPS movement data. Any period where the vehicle's engine was on without speed input (indicating idling) was accumulated. Idling fuel consumption was first calculated using Equation (1).

$$\text{Idling fuel consumption (l)} = \text{Total idling time (hr)} \times \text{Vehicle base idling rate} \left(\frac{1}{\text{hr}}\right) \qquad (1)$$

The idling fuel consumption was determined and subtracted from the total fuel consumption to get the adjusted fuel consumption using Equation (2).

$$\text{Adjusted total fuel consumption (l)} = \text{Total fuel consumption (l-) [Idling fuel consumption percentage (\%)} \times \text{Total fuel consumption (l)]} \qquad (2)$$

Then, the adjusted fuel consumption rate was acquired by dividing total mileage covered by the adjusted total fuel consumption using Equation (3).

$$\text{Adjusted fuel consumption rate} \left(\frac{\text{km}}{\text{l}}\right) = \frac{\text{Total mileage covered (km)}}{\text{Adjusted total fuel consumption (l)}} \qquad (3)$$

After adjusting for idling fuel consumption, the fuel economy of both the B20 and B10 groups improved, with the B20 fuel economy at 2.10 km L^{-1} and B10 at 2.05 km L^{-1} over the 18 month period. The adjustment aimed to provide a more accurate representation of fuel economy by excluding the fuel consumed during idling periods. *Figure 1* illustrates the adjusted fuel economy of both groups. Despite the improvement in fuel economy after adjusting for idling, there is still no discernible difference between the B20 and B10 groups. A two-tailed, paired t-test, conducted on monthly individual datasets adjusted for idling fuel consumption, indicates that the difference in fuel economy between the two groups is statistically insignificant with a high degree of confidence ($P = 0.8958$). This statistical analysis further supports the conclusion that, when accounting for idling fuel consumption, there is no significant difference in fuel economy between the B20 and B10 groups.

While fuel economy remains consistent, the impact on logistics companies' profit margins may be influenced by other factors such as the increased frequency of servicing and maintenance associated with the use of higher blends of biodiesel, as discussed in the following section.

Vehicle Payload and Fuel Economy

The analysis of running fuel economy per running payload for both the B20 and B10 groups shows no discernible difference. A two-tailed, paired t-test on the available dataset suggests that the difference in running fuel economy per running payload is statistically insignificant with a high degree of confidence ($P = 0.2766$). This finding implies that, within the observed dataset and conditions, the choice between B20 and B10 does not significantly impact the running fuel economy per running payload. The payload weight appears to have a consistent effect on fuel economy for both biodiesel blends.

Service and Maintenance

The fleet vehicles using B20 underwent regular service, including the replacement of LO and the change of fuel and water filters. The

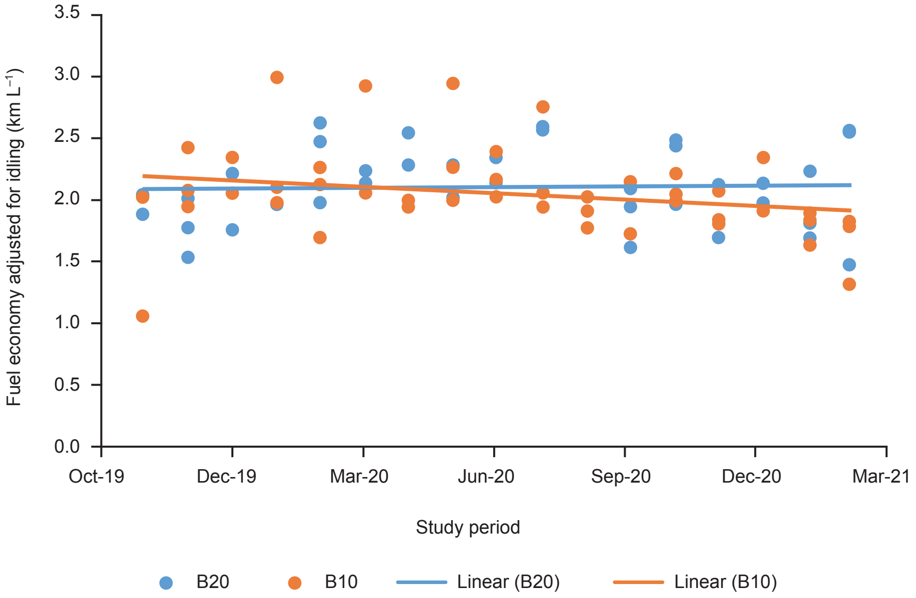

Figure 1. Fuel economy adjusted for idling fuel consumption of B20 and B10 groups of vehicles over 18 months of fleet testing.

service was performed by the in-house transport company technician before switching to B20 diesel. The service at 30,000 km, conducted at the start of the trial period, involved changing filters and engine oil according to the standard service procedure. The sampling intervals for engine oil were planned at 5,000, 10,000, 15,000, 20,000 and 25,000 km. However, real-world challenges such as scheduling conflicts between the maintenance and operations departments, and the nature of fleet operations, where vehicles need to be constantly on the road for deliveries, can make the scheduling of these intervals challenging. Despite these, continuous engine oil samples were collected, and at least one set of samples was obtained for each vehicle. It is important to highlight here that the crucial engine oil sample was collected at the required service interval of 30,000 km. This sample is of significance for data analysis, as it represents the condition of the engine oil after an extended period of usage. In summary, this provides context to the challenges in collecting continuous engine oil samples from fleet vehicles but ensures that key sampling points, especially at the 30,000 km service interval, were closely monitored and carried out.

Service Cost and Fuel Economy

The information provided outlines the breakdown of service costs for vehicles using different biodiesel blends of B10 and B20. The hypothesis under consideration is whether the usage of higher blends of biodiesel would reduce service intervals, consequently increasing operational costs. The service costs for the vehicles at three different service intervals include costs for LO, oil filters, water filters, and fuel filter changes. The total service intervals for each group were 13 times, all conducted at the depot. The basic service cost averaged RM2,194.67 per truck, covering both parts and labour but excluding warranty costs. Notably, there were no reported breakdowns in the engine or fuel system associated with the usage of either B10 or B20 during the vehicle trials. The data suggests that there is no significant difference in the total average service cost per distance travelled between both fuel groups. Similar trials on buses between B20 and diesel also showed no significant increase in maintenance costs across the mileage covered by the test vehicles during the trial period (Proc et al., 2006).

Figure 2 visualises the running average service cost per average mileage covered over each service interval for both groups of vehicles throughout the study. The graph indicates no evident correlation between service cost and mileage covered, suggesting that the type of fuel used did not significantly affect service interval costs. The two-tailed, paired t-test analysis conducted on the dataset further supports this observation, showing that the difference between the groups for running average service cost over running mileage covered is statistically insignificant with a high degree of confidence ($P = 0.1345$).

Engine Lubricating Oil Quality and Biodiesel Blends

To ensure homogeneity, the LO sample was taken after running the engine for at least 15 min. It's worth noting that a certain amount of wear metals, also known as trace metals, in used oil is expected due to normal engine wear. This analysis is crucial for evaluating the impact of biodiesel blends on engine lubrication and wear over the course of the fleet testing period.

Viscosity. The viscosity of LO is a crucial property, and changes in viscosity can indicate the condition of the oil. Viscosity is measured using ASTM D7042-21a or the standard test method for dynamic viscosity and density of liquids by Stabinger viscometer (and the calculation of kinematic viscosity). Higher viscosity may suggest deterioration from oxidation or contamination, while a decrease can indicate oil dilution. *Figure 3* illustrates the scatter plot of viscosity values in the B20 and B10 groups over the course of the fleet testing period, approaching the 30,000 km service interval. The plot displays a decreasing trend in viscosity as the vehicles accumulate mileage, with both groups consistently showing a decrease in viscosity. A two-tailed, paired t-test was performed, indicating that the difference in viscosity between the two groups is statistically insignificant with a high degree of confidence ($P = 0.1110$). The lower viscosity values in the B20 group may be attributed to the presence of inorganic acids, which are more prevalent in higher biodiesel blends. Despite this, the viscosity values for B20 remain within the accepted operational limits for the specific engine oil used in the study. Studies have shown that as engine oil ages, viscosity tends to deteriorate due to oxidation, and dilution of diesel and carbon black can further impact viscosity (Salehi et al., 2017). The results indicate that increased biodiesel content influenced the flowability of the LO, but this effect was not significant during the duration of the fleet test.

Total base number. The total base number (TBN) is a crucial parameter that measures the alkaline reserve or acid-neutralising capacity of diesel engine oil. It gauges the oil's ability to neutralise acids formed during combustion and maintain a

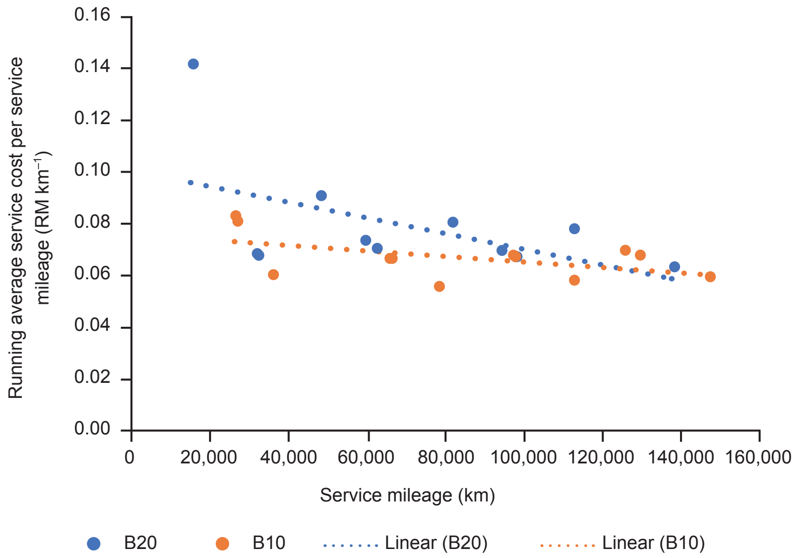

Figure 2. Running average service cost per service mileage at each vehicle service interval mileage.

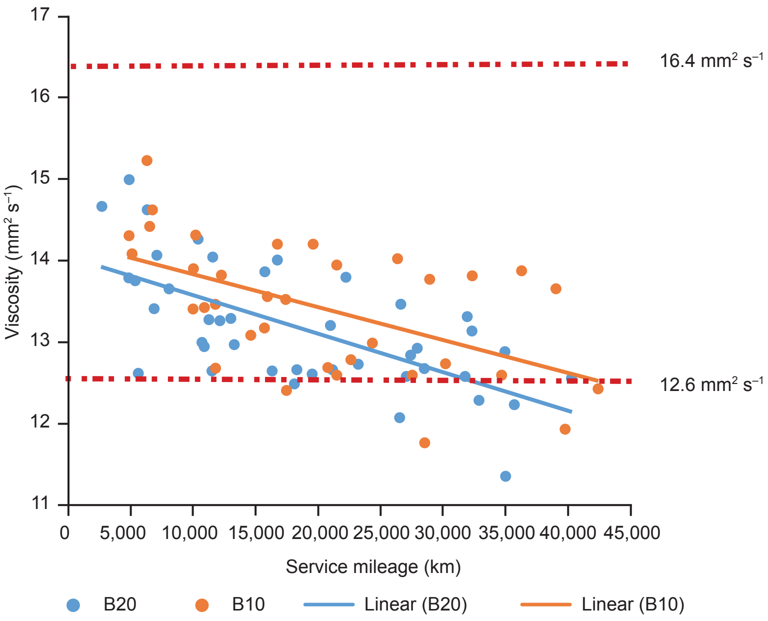

Figure 3. Scatter plot of viscosity at 100°C for B20 and B10 groups of vehicles.

stable pH level. As acidic by-products are generated during combustion, the TBN quantifies the oil's capacity to neutralise these acids, preventing potential damage to engine components. *Figure 4* depicts the scatter plot of TBN values for the B20 and B10 groups, revealing a descending pattern in trendlines for both groups with greater mileage. This could be attributed to incomplete combustion with longer operation, leading to the formation of acidic by-products that lead to TBN depletion. A two-tailed, paired t-test indicates that the difference in TBN values between the two groups is statistically significant with a high degree of confidence (P = 0.0156). The increased biodiesel content in the B20 group contributes to a significant difference in TBN as compared to the B10 group, with marginally lower TBN values. This contrasts with other reported work where elevated formation of acidic by-products from ester hydrolysis in the sump after incomplete combustion was observed in B20 blends, which increased the amount of weak acids in the engine oil (Gulzar et al., 2016). This suggests that monitoring TBN in both high and low blends of biodiesel is essential through timely oil changes to maintain optimal engine protection.

Metals contamination. Metals contamination in engine oil, such as iron, copper and chromium concentrations, provides insights into the wear

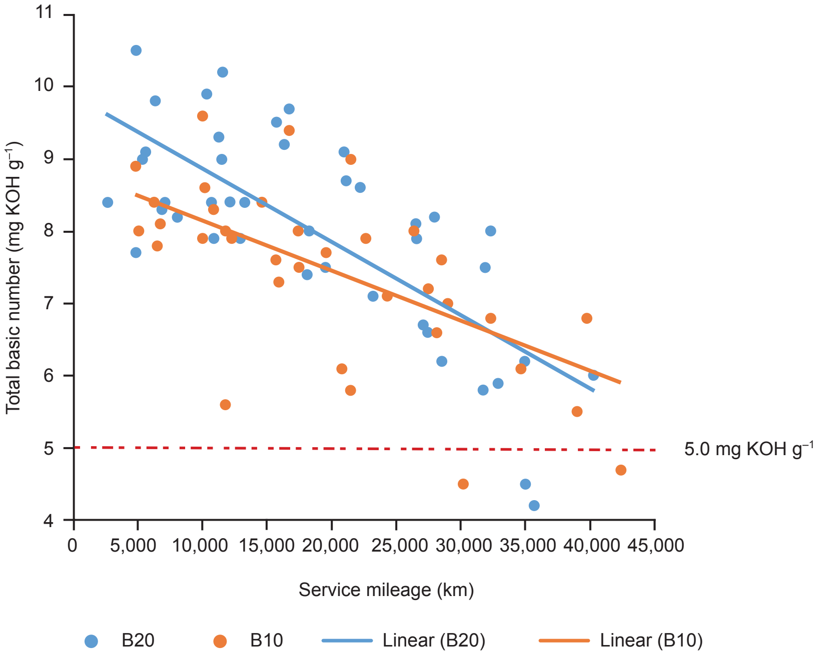

Figure 4. *Scatter plot of total basic number for B20 and B10 groups of vehicles.*

and tear of the metal engine components. Metals contamination is measured using ASTM D5185-18 or the standard test method for multielement determination of used and unused LO and base oils by inductively coupled plasma atomic emission spectrometry (ICP-AES). Higher concentrations of these metals in used LO after each round of service maintenance can indicate increased degradation due to wear and tear on lubricated engine components. *Figure 5* illustrates the trend of iron particles in both the B20 and B10 groups, showing an increasing pattern over mileage. A two-tailed, paired t-test indicates that the difference in iron content in engine oil between the two groups is statistically significant with a high degree of confidence ($P = 0.02346$). The average iron concentration in the engine oil increased to 11 ppm for B20 vehicles and 15 ppm for B10 vehicles, with the increased biodiesel content reducing the amount of iron wear in the engine oil due to its higher lubricity property, which is advantageous in high-friction areas of the engine such as the piston heads and sides. This observation aligns with previous studies that found palm oil blends used in engine operation to have lower concentrations of wear metals in the engine oil sump compared to conventional diesel. Additionally, the efficiency of the oil filter also plays a crucial role in removing solid particles, including iron, from the engine oil. A clogged or inefficient filter may allow higher iron levels in the oil, potentially causing increased wear and engine damage. In this study, copper and chromium values for both groups are in trace amounts, suggesting no significant increase in wear and tear. Overall, the results suggest that B20 does not adversely affect engine wear, supporting its viability as a fuel option without compromising engine longevity.

Engine oil evaporation. The metals analysis, particularly focusing on calcium, zinc and phosphorus concentrations in the engine oil, provides insights into the condition and performance of the LO. These metal concentrations can increase as engine oil evaporates during operation, potentially leading to lower oil levels and necessitating an oil change. However, in the case of this study, the metal concentrations showed a stable and consistent trend, indicating that there was no significant reduction that would warrant an oil change before the recommended limit of 30,000 km.

Zinc is a vital additive in diesel engine oil, usually present as zinc dialkyldithiophosphate (ZDDP). These multifunctional additives form a protective film on metal surfaces, reducing wear on critical engine components and acting as an antioxidant to inhibit oil oxidation. The statistical analysis comparing zinc concentrations between the B20 and B10 groups revealed that the difference is statistically insignificant ($P = 0.1153$), suggesting that the zinc content in the LO remained within acceptable limits for both fuel groups.

Phosphorus, often part of ZDDP additives, also contributes to forming a protective layer on metal surfaces, reducing wear, and preventing oxidation. The statistical analysis indicated that the difference in phosphorus concentrations between the B20 and B10 groups is statistically significant ($P = 0.0437$), but the recorded phosphorus content

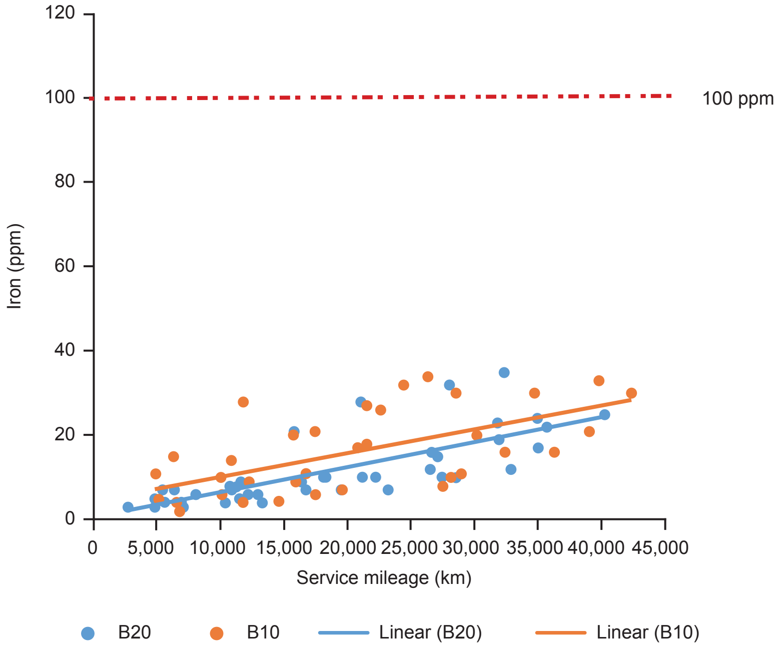

Figure 5. Scatter plot of iron concentration for B20 and B10 groups of vehicles.

from the LO samples did not fall below the minimum limit of 600 ppm. This suggests that the LO used still had remaining usage life for both fuel groups.

Calcium-based additives, such as calcium sulphonate or calcium phenate, serve as detergents and dispersants in diesel engine oil. They prevent the formation of deposits, sludge, and varnish, maintaining engine cleanliness. The statistical analysis showed a significant difference in calcium concentrations between the B20 and B10 groups ($P = 0.0056$), potentially indicating increased sludge build-up in the B10 group due to reduced calcium additives. However, it's important to note that the recorded calcium content from the LO samples did not fall below the minimum 600 ppm limit, indicating that the LO used still had remaining usage life for both fuel groups.

In summary, the metals analysis suggests that the LO used in both B20 and B10 vehicles remained within acceptable limits during the 30,000 km service interval, and there were no indications of excessive wear or degradation that would necessitate early oil changes for the B20 group.

CONCLUSION

This study aimed to assess the impact of utilising palm B20 and B10 on heavy-duty diesel vehicles under actual on-road driving conditions through a combination of fleet testing and laboratory investigations. Comparisons between the B20 and B10 groups focused on fuel economy and service maintenance of the vehicles. Both groups exhibited similar fuel economy, with the B20 group averaging 2.107 km L^{-1} and the B10 group averaging 2.055 km L^{-1}. With engine oil analysis conducted to examine the impact of B20 usage on oil degradation, all engine oil samples were found to remain within the acceptable service quality limits as recommended by the engine oil manufacturer. This indicates that an increased frequency of servicing is not required as commonly thought. The B20 samples displayed degradation trends comparable to those of the B10 samples. The significant statistical difference in TBN and iron content of the B20 group indicates the positive impact on the engine oil condition during on-road trials. Further in-depth investigation, particularly into the benefits of B20 on engine oil lifespan and fuel filter sedimentation, would contribute further valuable insights to the knowledge base of real-world usage of higher biodiesel blends. In conclusion, the study results suggest that the B20 blend is a viable choice for vehicle fleets in logistics companies, as it demonstrates no significant differences in fuel economy and service maintenance intervals as compared to when using the B10 blend.

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SYNTHESIS OF LAURIC-RICH MEDIUM-CHAIN TRIGLYCERIDES FROM PALM KERNEL OIL AND LAURIC ACID BY ENZYMATIC ACIDOLYSIS

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ABSTRACT

Oil palm fresh fruit bunches contain two types of oil: Palm kernel oil (PKO) from the palm kernel and crude palm oil from the fruit flesh. Enzymatic acidolysis of PKO and lauric acid can produce medium-chain triglycerides (MCT), which offer nutritional and health benefits. This study aimed to synthesise laurate-rich MCT through enzymatic acidolysis. The reaction used an immobilised NS 400190 lipase catalyst (Novozyme). Variables included reaction time (0–48 hr), temperature (44°C–104°C), substrate ratio of PKO to lauric acid (1:1–1:11, molar basis) and an enzyme load of 7% w/w. The MCT yield (%) was analysed by determining the triglyceride's fatty acid composition using gas chromatography (GC). Results indicated that lauric acid incorporation increased with treatment time. GC analysis revealed that the highest lauric acid triglyceride profile occurred at 24 hr, 94ºC and a substrate ratio of 1:9, achieving 81.4% lauric acid incorporation. The enzyme demonstrated thermophilic properties, with an optimal temperature of 94ºC. The NS 400190 lipase enzyme exhibited significant operational stability, with a half-life of 1,517 hr in the acidolysis reaction.

Keywords: acidolysis enzymatic, lauric acid, medium-chain triglycerides, palm kernel oil.

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INTRODUCTION

Native lipids, more than 80%-85% of lipids, are in the form of triglycerides (TG) with specific properties and characteristics (Kim & Akoh, 2015). The characteristics of natural lipids are not always suitable for producing lipids with desirable added value, such as the synthesis of specific TG, known as structured TG. Specific TG with optimal properties results from modification of the fatty acid composition or the positional of the acyl groups on the glycerol backbone (Devi et al., 2008; Huang & Akoh, 1996; Willis & Marangoni, 2002).

Medium-chain TG (MCT) themselves are a type of modified TG. The uniqueness of MCT is metabolised differently compared to the other saturated oils, suggesting the potential benefits of MCT. TG's nutritional value and biochemical properties are measured by the composition of fatty acids and their position in the TG molecule (Hasanah & Warnasih, 2020).

MCT consists of medium-chain fatty acids (C8-C12) esterified on its glycerol skeleton. MCTs are quickly metabolised to produce energy in the body, and the energy produced by MCTs is twice that of carbohydrates and proteins (Nainggolan & Sinaga, 2021). Due to the uniqueness of MCTs in their physicochemical and nutritional properties, MCTs are widely used as nutraceuticals in food and pharmaceuticals, for example treating malabsorption syndrome, cystic fibrosis, epilepsy, improving protein and fat metabolism, premature infant food formulations, increasing stamina, reducing and controlling body weight, improving cognitive function, reducing allergenicity, antiviral therapy

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drugs, antibacterial agents (synergy between free fatty acids and monoglycerides) MCTs (Dayrit, 2014; Lee et al., 2012, 2022).

Palm kernel oil (PKO) is an oil primarily made up of medium-chain fatty acids (MCFA), which is more than 50%. Of which, lauric acid (C12) is the major fatty acid (46%–52%), caprylic acid (C10) and capric acid (C8) are the less prominent component (Dayrit, 2014; McCarty & DiNicolantonio, 2016; Silalahi et al., 2018). As lauric acid is the most pervasive medium-chain fatty acid in PKO, it is a key representative of the oil's properties. The metabolic and physiological properties of lauric acid increasingly play important roles in food processing technology, pharmacology and clinical nutrition (Dayrit, 2014; Enig, 1996; Lieberman et al., 2006; Ubgogu et al., 2006).

MCTs are not found naturally and must be synthesised (Liang et al., 2019). Enzymatic synthesis of MCTs is more advantageous than chemical as it is an environmental-friendly reaction, taking place at lower temperatures, little or no reaction by-products, ease of product recovery and controlling the reaction and low-risk factors for consumer health (Nunes et al., 2012). Several papers have reported on the enzymatic synthesis of MCT, such as interesterification (Feltes et al., 2009) and esterification (Langone & Sant'Anna, 2002; Nandi et al., 2005; Wong et al., 2000). However, only a few studies reported the acidolysis of medium-chain fatty acid with lipids catalysed by non-specific enzymes (Gökçe et al., 2013; More et al., 2018; Sousa et al., 2018). Non-specific enzymes have catalytic interesterification properties, such as chemicals where the interesterification produces a complete randomisation of acyl groups in TG so that it will produce the highest yield of MCT (Huang & Akoh, 1996; Osborn & Akoh, 2002).

Furthermore, the important objective of this research is to produce a novel type of MCT that contains rich-lauric acid by enzymatic acidolysis using immobilised non-specific lipase NS 400190 with variation in time, temperature and substrate ratio treatments and the operational stability of the enzyme-based on half-life value was carried out.

MATERIALS AND METHODS

Materials

Crude palm kernel oil was obtained from PT. Perkebunan Nusantara IV. Immobilised nonspecific enzyme NS 400190 was provided by Novozymes Group Entity, Denmark. Lauric acid (> 99% purity) and Standard TG mix were purchased from Sigma Aldrich, Germany, for gas chromatographic (GC) analysis using Series 2010 Plus (Shimadzu, Japan). Thin-layer chromatography (TLC) and TLC silica gel 60 F254 Glass plates were also purchased from Sigma Aldrich, Germany. For chemicals such as N-hexane, 1-octanol, NaOH, Whatman filter paper No. 4, diethyl ether, glacial acetic acid, and other analytical chemicals were obtained from Sigma Aldrich, Germany and PT. Smart Lab Indonesia.

Experimental Methodology

Lipase-catalysed acidolysis reaction. The enzymatic acidolysis reaction used was a modified method (Abigor et al., 2003). A total of 10 g of the substrates (PKO and lauric acid with a molar ratio of 1:1 to 1:11) was transferred into a 50 mL Erlenmeyer flask. The substrate mixture was then added with NS 400190 lipase as much as 7% (of the total weight of the substrate). The reactions were conducted in a solvent-free system. They used seven different temperatures: 44°C, 54°C, 64°C, 74°C, 84°C, 94°C and 104°C. The reactions also varied across ten different time intervals: 0, 4, 8, 12, 16, 20, 24, 32, 40 and 48 hr. A heater shaker was used at a speed of 200 rpm. After the acidolysis reaction, the resultant mixture was separated from the enzyme using Whatman filter paper No. 4. The mixture was stored in a freezer (T, -4ºC) for further analysis. Each reaction in this research was carried out in duplicate. The residual activity of the recovered enzyme was calculated to assess its operational stability.

Separation and analysis of triglycerides using (TLC). This procedure was done using a modified method based on Nunes et al. (2011). The triglycerides, which are the primary products of the acidolysis reaction, were analysed through TLC silica gel 60 F254 20 x 20 cm glass plate. The TLC plates were developed with a solution of hexane: diethyl ether: glacial acetic acid (80:20:1, v/v/v). Iodine gas was used to visualise the spots on the TLC plate. TG were identified by comparing them with the standard. Spots corresponding to each lipid type were scraped from the plate and dissolved in hexane. The hexane fraction was used for fatty acid composition analysis using GC.

Fatty acid composition analysis by GC. The fatty acid composition was analysed based on MPOB (Kuntom, 2005) by GC capillary column with flame ionisation detector (FID) under operating conditions: Crossbond capillary column (carbowax polyethylene glycol) of 25 m length, 0.25 mm diameter, 0.25 µm film thickness. The initial column temperature of 120ºC and maintained for 7 min. It was then increased to 240ºC at a rate of 3ºC min^{-1} and held at this final temperature for

26 min. The detector temperature was also set to 240°C. Helium was used as the carrier gas with a flow rate of 0.8 mL min^{-1}, while nitrogen was used as the make-up with a flow rate of 30 mL min^{-1}. The injection mode was set to split with a ratio of 1:100. Fatty acid composition was identified by comparing the retention time of the sample peak with the standard solution. The concentration of fatty acid in triglycerides was calculated by using Equation (1).

$$\begin{array}{c}\text{The concentration}\\ \text{of fatty acid}\end{array} = \frac{\text{Area of fatty acid}}{\text{Total area}} \times 100\% \qquad (1)$$

Residual activity of NS 400190 lipase enzyme. Determination of the remaining activity of NS 400190 lipase enzyme was carried out by esterification reaction based on the modified method of Kuhn et al. (2010). The esterification reaction was chosen because the analytical method is very simple and gives a rapid response. The used enzyme must first be washed using a modification of the Aguieiras et al. (2016) method. The washed enzyme was then incubated using 15 mL hexane as a solvent for 2 hr at 25°C. Then the suspension was separated from the supernatant with the enzyme by filtration with Whatman filter paper No. 4. The separated enzyme was analysed for enzymatic activity. Esterification was carried out by reacting lauric acid substrate with 1-octanol with a molar ratio of 1:1 in a 50 mL Erlenmeyer flask. A 5% (w/w of total substrate) amount of lipase NS 400190 was added to the substrate. The reaction was carried out at 50°C for 60 min. After the reaction, the substrate was filtered to separate from the enzyme. The substrate mixture was then titrated with 0.01 M NaOH solution. In addition, a blank sample titration was also carried out which was a mixture of 1-octanol and lauric acid substrates without enzyme. Determination of lipase enzyme activity is based on the amount of lauric acid consumed. One unit of lipase activity (U) is defined as 1 µmol of lauric acid used in the esterification reaction (EA) per min per gram of lipase, according to the following Equation (2).

$$EA = \frac{VNaOH \times MNaOH \times 1000}{W \times t} \qquad (2)$$

where *VNaOH* is the volume difference of NaOH blank and after esterification reaction, *MNaOH* is the molarity of NaOH, *W* is the amount of enzyme (g), and t is the reaction time (min). The same procedure was used to calculate the initial esterification activity value of NS 400190 lipase enzyme (reaction hr 0/*EA0*). The remaining activity value of the enzyme (*EAΔ*) was calculated using Equation (3), where *EA1* indicates the esterification activity value of the enzyme after reaction at a certain time:

$$EA\Delta\ (\%) = \frac{EA1}{EA0} \times 100\% \qquad (3)$$

Statistical analysis. All data in this study were conducted in duplicate. Each data was presented as means ± standard deviation by using MS Excel. One-way ANOVA was performed to determine the difference between the means in the activity of temperature, hours and molar ratio of substrate. Half the lifetime of the lipase was predicted based on the linear regression model.

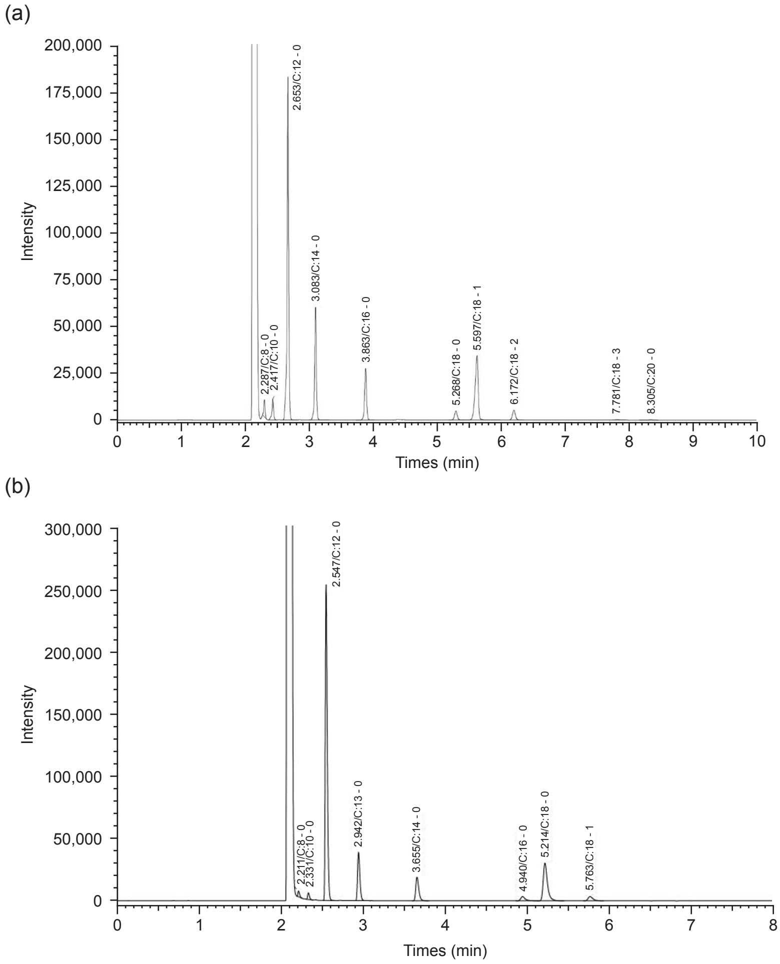
RESULTS AND DISCUSSION

In this study, enzymatic acidolysis between PKO and lauric acid is expected to produce lauric-rich medium-chain triglycerides with the treatment of time, temperature and substrate ratio. The lipase enzyme used was immobilised NS 400190 lipase

Effect of Reaction Time on Lauric Acid Incorporation into Palm Kernel Oil

Figure 1 shows the chromatogram profile of the fatty acid analysis before and after the acidolysis reaction. The incorporation of lauric acid (C12) before reaction (A) increases after the enzymatic acidolysis reaction of PKO with C12 (B). Peak characterised as C12 (peak #3) with a retention time of 2.653, increasing from 47.83 (area%) to 60.97 (area%).

Figure 2 shows that the reaction time is significantly affected by lauric acid concentration. At the beginning of the reaction (up to 4 hr), there was no change in lauric acid incorporation, which remained steady from 44.15%–44.30%. Between 4 and 8 hr, lauric acid incorporation increased substantially from 44.3%–62.9%, representing an 18.6% rise. In contrast, from 8–24 hr, the increase was modest, with incorporation rising from 62.9%–66.6%, or just 3.7%. This indicates that the reaction period from 4–8 hr is more effective than from 8–24 hr. Extending the reaction time to 48 hr showed no significant difference in lauric acid incorporation between 8–48 hr. Thus, it appears that the reaction has reached an equilibrium state at a reaction time of 8–48 hr. These results indicate that the reaction time influences the concentration of lauric acid incorporation. In this study, the concentration of lauric acid peaked at 66.6% after 24 hr. There was no significant difference in lauric acid concentration between 8–24 hr. During this period, the rate of acyl incorporation into TAGs either slowed down or slightly decreased.



Peak#	Ref. time	Area	Height	Area%	ID#	Cmpd Name
1	2.287	128,497	88,492	1.9307	1	C:8 - 0
2	2.417	198,206	110,069	2.9781	2	C:10 - 0
3	2.653	3,183,855	1,798,423	47.8380	3	C:12 - 0
4	3.083	1,091,092	592,487	16.3939	4	C:14 - 0
5	3.863	582,553	272,360	8.7530	5	C:16 - 0
6	5.268	141,671	47,782	2.1286	6	C:18 - 0
7	5.597	1,150,168	341,871	17.2815	7	C:18 - 1
8	6.172	165,292	51,600	2.4835	8	C:18 - 2
9	7.781	7,207	1,718	0.1083	9	C:18 - 3
10	8.305	6,949	1,364	0.1044	10	C:20 - 0
Total		6,655,490	3,306,166	100.0000		

Peak#	Ref. Time	Area	Height	Area%	ID#	Cmpd Name
1	2.211	6,318	4,550	0.9060	1	C:8 - 0
2	2.331	8,239	5,256	1.1814	2	C:10 - 0
3	2.547	425,230	249,888	60.9747	3	C:12 - 0
4	2.942	75,713	38,198	10.8567	4	C:13 - 0
5	3.655	48,051	18,703	6.8902	5	C:14 - 0
6	4.940	13,014	3,438	1.8661	6	C:16 - 0
7	5.214	106,891	29,936	15.3274	7	C:18 - 0
8	5.763	13,930	3,414	1.9975	8	C:18 - 1
Total		697,386	3,53,383	100.0000		

Figure 1. Fatty acid chromatogram (a) before and (b) after acidolysis reaction.

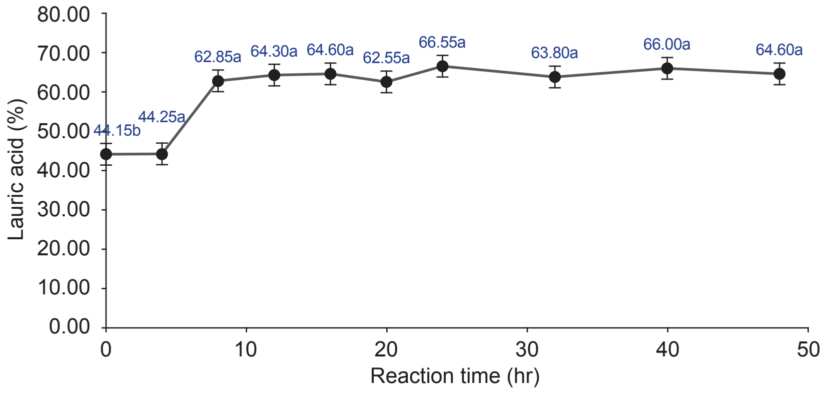


Figure 2. Effect of reaction time on lauric acid incorporation in PKO.

Effect of Reaction Temperature on Lauric Acid Incorporation into Palm Kernel Oil

In *Figure 3*, the temperature of the enzymatic acidolysis reaction affected the incorporation of lauric acid. The increasing incorporation of lauric acid at a reaction temperature of 44ºC–94ºC, from 61.8%–71.3%, is about 9.5%, then at a reaction temperature of 104ºC, the lipase NS 400190 began to denature, causing reduced and catalytic activity which was indicated by a decrease in lauric acid incorporation to 49.2% (Kosiyanant et al., 2018).

At 94ºC temperature, lauric acid incorporation was the highest at 71.3%, but there was no significant difference between 64ºC and 94ºC. The same research conducted by Langone and Sant'Anna (2002) showed that the highest trilaurin was 76% at 80ºC–90ºC after 26 hr of reaction. The temperatures of 44ºC and 94ºC showed a significant difference in lauric acid incorporation. The higher the reaction temperature, the solubility of the reactants increases and decreases the viscosity of the solution. It improves the interaction between the reactants and the enzyme's active sites, leading to increased enzyme activity and a higher rate of fatty acid incorporation (Abed et al., 2017; Kavadia et al., 2018; Langone & Sant'Anna, 2002; Lee et al., 2012; Li et al., 2021). The advantage of this study is that the lipase enzyme is non-specific, such that its activity is like that of a chemical reaction. Based on this study, the lipase NS 400190 is a high-temperature resistant enzyme, and the best reaction temperature is from 44ºC–94ºC.

Effect of Substrate-Molar Ratio on Lauric Acid Incorporation into Palm Kernel Oil

The substrate-molar ratio is a critical factor affecting the equilibrium point in the reversible reaction to produce optimum medium-chain TG during the enzymatic acidolysis reaction (Hu et al., 2011).

Figure 4 shows the effect of substrate molar ratio on lauric acid incorporation in TG. The results indicated that substrate molar ratio had a significant effect on lauric acid incorporation. When the molar ratio was increased, the concentration of lauric acid increased from 57.20%–80.00%. With increasing molar substrate, the reaction equilibrium will shift toward the synthesis direction. The concentration of lauric acid incorporation in triglycerides increased (57.2%–80.0%) as the molar ratio of substrate increased (1:1–1:11) (Kosiyanant et al., 2018; Langone and Sant'Anna, 2002). This study showed that the reaction reached equilibrium at a molar substrate ratio of 1:3 to 1:11. However, lauric acid incorporation decreased when the molar ratio shifted from 1:9–1:11. This reduction may be attributed to decreased activity of lipase NS 400190 at higher substrate molar ratio, which affects the equilibrium constant and may also impact the product purification stage (Abed et al., 2017; Li et al., 2021).

Operational Stability of Non-specific NS 400190 Lipase

The operational stability of non-specific lipase NS 400190 was measured by observing the esterification activity and residual activity after being used for a certain reaction time.

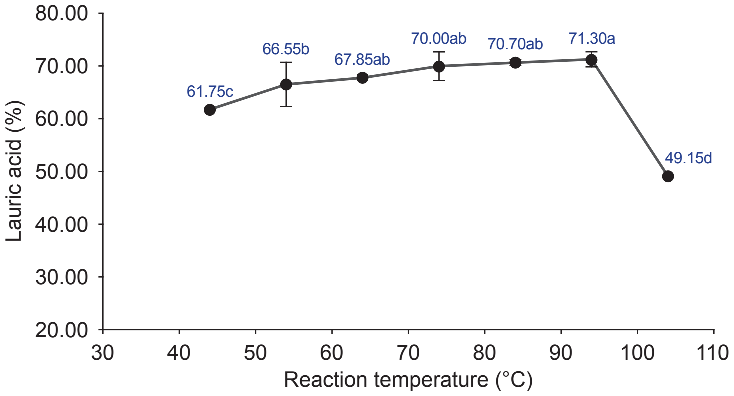


Figure 3. Effect of reaction temperature on lauric acid incorporation in PKO.

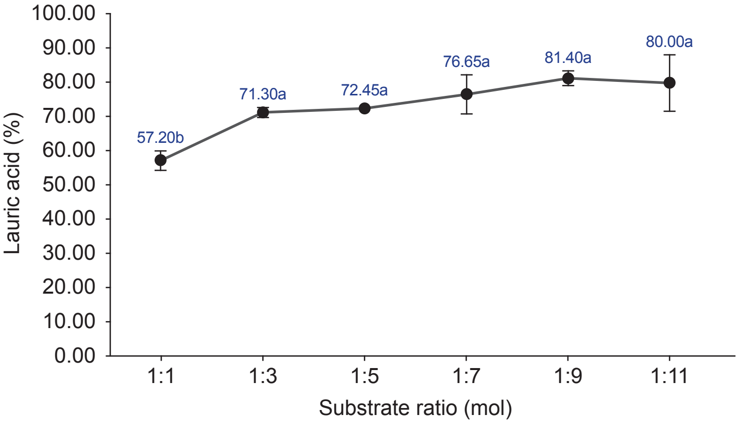


Figure 4. Effect of the molar ratio of PKO oil to lauric acid substrate on lauric acid incorporation into PKO.

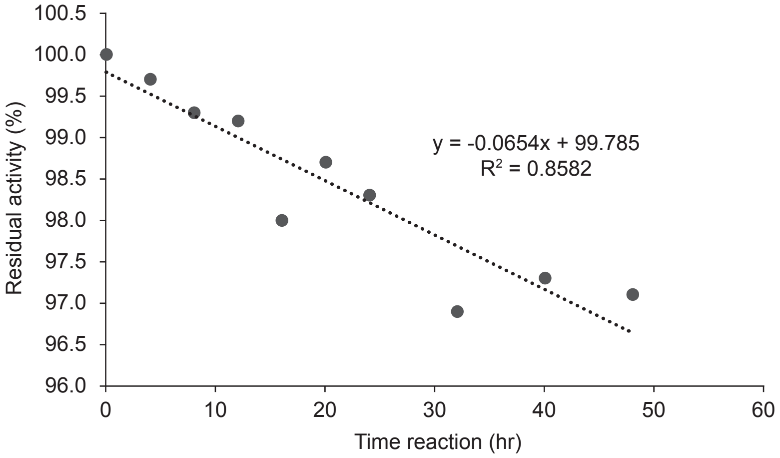


Figure 5. The remaining activity value of the lipase NS 400190 in acidolysis reactions.

In this study, esterification and residual activity of NS 400190 lipase enzymes were observed because of the different interesterification reaction times (acidolysis). Esterification activity shows the esterification ability of the enzyme after being used in the interesterification reaction (acidolysis) during a specific time interval. The remaining activity value is the value of the enzyme esterification activity that remains after being used in the interesterification reaction during a specific time interval. The remaining activity value can be obtained by comparing the esterification activity value of NS 400190 lipase enzyme after the interesterification reaction at a certain time to the initial esterification activity value of NS 400190 lipase enzyme (before being used in the interesterification reaction).

Figure 5 shows the stability value of the non-specific NS 400190 lipase enzymes based on the change of the enzyme's remaining activity value after being used in the acidolysis reaction. In this study, the initial (esterification) activity of NS 400190 lipase was 814.0 U after being used for 48 hr in the acidolysis reaction, the remaining activity value of NS 400190 lipase was 792.5 U. The remaining activity value of NS 400190 slightly decreased from 100%–97.1%. Based on the linear equation, the operational stability value of the non-specific NS 400190 lipase enzyme was obtained based on the 50% half-life time value of 1,517 hr in the acidolysis reaction.

CONCLUSION

The synthesis of lauric-rich medium chain triglycerides can be carried out by enzymatic acidolysis in a solvent free system with the best conditions at 94°C, 24 hr and a palm kernel oil to lauric acid substrate ratio of 1:9 which can incorporate 81.4% lauric acid. These conditions provided operational stability of the immobilised NS 400190 lipase for 1,517 hr in the acidolysis reaction.

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DELVING INTO THE SYNERGISTIC BEHAVIOURS OF TRIBOLOGICAL ADDITIVES IN MINERAL AND VEGETABLE OILS VIA THERMODYNAMIC ANALYSIS

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ABSTRACT

This study presents a comprehensive thermodynamic modelling and analysis to enhance lubrication assessment in tribotests, focusing on ASTM D2783 extreme pressure (EP) and ASTM D4172 anti-wear (AW) tests. The model facilitates the identification of optimal high-performance lubrication and synergistic additive combinations, even for lubricants yielding similar test results. A case study evaluates 36 lubricant samples, including mineral oil, vegetable oil, and blends. Rooted in the entropy balance, the model employs the dissipative coefficient U_w *to simulate friction-wear interplay, providing insights into wear conditions, lubrication mechanisms and efficacy. Both mineral-based and vegetable-based lubricants exhibit analogous friction-wear dynamics, but their compatibility with additives differs, resulting in distinct synergistic packages. Blended base oils' compositional complexity significantly influences additive synergies, challenging conventional tribotest evaluations. The* U_w *parameter-based approach empowers the selection of optimal lubricant formulations, aiding in designing effective lubricant solutions by considering wear conditions, lubrication mechanisms, and additive compatibilities.*

Keywords: additive, entropy dissipation, formulation, friction, lubricant.

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INTRODUCTION

Palm oil stands out as the most extensively utilised vegetable oil globally, contributing to about 40% of the total production of all vegetable oils combined (Gan et al., 2023). This places palm oil at the forefront as a promising candidate for use as a biolubricant base stock. According to Lee et al. (2022), palm is the most studied bio-lubricant, followed by castor oil. Its substantial production establishes its significance in the food market and as a commodity and positions it as a viable alternative in the biolubricant sector, offering the potential to replace traditional petroleum-based lubricants (Parveez et al., 2023). Biolubricants derived from renewable resources are generally perceived as environmentally friendly, biodegradable and non-toxic lubricants. Biolubricant products can be made up of blending through either partially or completely with bio-based oil. This depends on the requirements of international standards such as bio-based content, biodegradability and technical performance. In general, lubricant formulation development involves multi-objective optimisation of the types and compositions of base stocks and additives. Among the additives, tribological additives are one of the important elements to ensure optimum lubrication performance such as anti-wear (AW) and load-carrying capabilities (Bart et al., 2013). The investigation of the tribological behaviours of the additives is crucial in lubricant formulation development. Such investigation is usually conducted through

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a series of tribological assessments such as Four-ball tribo-testers and pin-on-disc tribo-tester. One of the flaws of these physical assessments is that they rely heavily on friction and wear parameters to assess lubrication performance, without considering the underlying fundamental mechanism of the sliding phenomenon in situ. As a result, the investigation of additives behaviours and synergy can be difficult and misleading if complex interaction of the additives is involved. Under these circumstances, a tribological model can be employed to support the tribological assessments.

Tribological modelling has evolved to support advancements in various applications, including lubrication, coating, and surface materials. Initially rooted in a basic contact mechanics approach, tribological modelling has progressed toward a more intricate multi-physical approach. This advanced approach encompasses different time scales, length scales, and conditional states, enhancing our understanding of tribological phenomena (Vakis et al., 2018). Indeed, tribological modelling plays a pivotal role in simulating and predicting the friction and wear behaviours of a tribology system. Traditionally, Reynolds Equation has been employed to analytically solve the classical lubricated contact problems considering the non-Newtonian effect, surface roughness effect (Allmaier et al., 2012), surface topography effect (Ripoll et al., 2011), thermal effect (Li et al., 2013; Ouyang et al., 2017), and boundary lubrication behaviours (Mukchortov et al., 2017). The capabilities mentioned above necessitate the integration of additional model frameworks (e.g., asperity contact model [Ripoll et al., 2011]) and advanced approaches (e.g., finite element method contacts [Hao & Meng, 2015; Ripoll et al., 2011]), molecular dynamic simulation (Ghaffari et al., 2018; Godlevskiy & Blinov, 2016), making them highly complex and tend to require long computational time. In addition to the Reynolds Equation and its variants, other lubrication models revolve around regression analysis based on statistical approaches (Baskar et al., 2016; Simonovic & Kalin, 2016; Weinebeck et al., 2017; Xiong et al., 2015), empirical model driven by experimental observations (Hu et al., 2017; Zhou et al., 2018), and models derived from first principles (Chong & Ng, 2016; Ghanbarzadeh et al., 2016; Lyashenko & Khomenko, 2012). Typically, these models offer "black box" simulations, where the intricacies of the contact phenomena are obscured or not fully captured.

Conversely, wear modelling typically follows friction and lubrication modelling in sequence (Gao et al., 2018; Li & Anisetti, 2017; Zhang et al., 2017), although it can be conducted independently provided that information on asperity contacts (or models), the mechanical properties of the surfaces, the wear mechanism, and the lubricant actions are known (Bosman & Schipper, 2011, 2012; Mishina & Hase, 2013). Due to the limited and incomplete understanding of wear mechanisms, even at the macroscopic level, wear modelling is primarily conducted using empirical approaches (Cao et al., 2015; Gao et al., 2018; Mishina & Hase, 2013; Tan et al., 2015; Zhang et al., 2017). Considering the aforementioned models, it's evident that most friction and wear models were developed for very specific systems, lacking the desired level of generality (Banjac et al., 2014). Undoubtedly, these models are useful for describing specific phenomena in tribology. However, there remains a lack of clarity and connection between friction and wear, as their mutual dependence and correlation among themselves are often unclear and disconnected (Banjac et al., 2014).

The correlation between the friction and the wear behaviours of a tribological system is possible to be established through thermodynamic analysis and modelling. In this model, the tribological system can be seen as a thermodynamic system that deteriorates irreversibly with the dissipation of energy (Abdel-Aal, 2010; Amiri & Khonsari, 2010; Fox-Rabinovich et al., 2007; Gershman et al., 2016; Ramalho & Miranda, 2006). This work demonstrated a method to assess and evaluate the synergistic behaviours of tribological additives in lubricants using thermodynamic analysis to further extend its use. The interaction of different additives in lubricant formulation is crucial to be investigated as it creates synergistic or antagonistic effects. Some synergistic effects are difficult to observe as the interactions are specific and only happen at certain contact conditions (Tomala et al., 2017; Yang et al., 2021) and temperature (Yang et al., 2021). For instance, the presence of zinc dialkyldithiophosphates (ZDDP) additive in PAO enhances the friction-reducing and AW effect of WS2 nanoparticles in the boundary lubrication regime (Aldana et al., 2016).

In this study, thermodynamic analysis is performed on Four-ball lubrication to map the friction-wear relationship of the lubrication. The mapping is then used to assess and evaluate the additive synergy of 36 lubricant samples comprising vegetable oil, mineral oil and their blends formulated with several AW and extreme pressure (EP) additives. Besides, the effect of different base oils (BASE) on the additive compatibility in the formulation is also investigated. Finally, an empirical approach to select the best lubricant formulations from the thermodynamic viewpoint is developed and demonstrated.

MATERIALS AND METHODS

Materials and Reagents

Mineral oil (M) i.e., G2 SN150 from H&R (Malaysia) Sdn. Bhd., and vegetable oil (V), i.e., palm olein from the local market were used as the BASE in this study. Two commercial AW additives (A1 and A2) and one commercial EP additive (A3) were obtained from a local supplier. A1 is a multifunctional complex ester designed to enhance lubricity, reduce wear, and prevent corrosion in milky, semisynthetic, and neat oil metalworking fluids. A2 is a high molecular weight polar polymer equipped with various functional groups, used to improve lubricity in rolling emulsions and in cutting and forming fluids. A3 is a sophisticated compound frequently used to boost the EP capabilities of working fluids, suitable for both ferrous metals and aluminium alloys.

Lubricant Formulations

In this study, 36 lubricant samples were prepared according to the following formulations in the format of BASE either M, V, or their blends together with additives (A1, A2, A3) as tabulated in *Table 1*.

TABLE 1. LUBRICANT SAMPLES FORMULATION

No.	Formulation of lubricant
1	BASE
2	BASE + A1 (2% w/w)
3	BASE + A2 (2% w/w)
4	BASE + A3 (2% w/w)
5	BASE + A1 (2% w/w) + A3 (2% w/w)
6	BASE + A2 (2% w/w) + A3 (2% w/w)
	BASE in the formulation
1	M
2	80% v/v M + 20% v/v V (M80 + V20)
3	60% v/v M + 40% v/v V (M60 + V40)
4	40% v/v M + 60% v/v V (M40 + V60)
5	20% v/v M + 80% v/v V (M20 + V80)
6	V

Note: BASE - base oil; M - mineral oil; V - vegetable oil.

Tribological Testing

The tribological behaviours of the 36 samples were assessed using a Four-ball test machine (DUCOM TR-30, Ducom Instruments, USA). In this configuration, a steel ball is rotated against three stationary steel balls under specific load and speed conditions. The employed test methods are the ASTM D2783 EP test (ASTM International [ASTM], 2019) and the ASTM D4172 AW test (ASTM, 2020). Given the test method, the EP test assesses the ability of the lubricant to work under extreme load conditions such as in bearing application. The EP ability is commonly determined by the weld point, which is the load at which the sliding surfaces seize and then weld, or the stationary balls having wear scar diameter above 4 mm. The EP test is first evaluated at 80 kg load, 1,760 rpm rotation speed and 10 s sliding time, and then it is repeated at incremental loads till the weld point. On the other hand, the AW test measures the wear-preventive characteristics of the lubricant sample based on the wear scar diameter and the average friction coefficient. This test is conducted at a load of 40 kg, rotation speed of 1,200 rpm and time of 3,600 s.

Thermodynamic Analysis

The EP and AW test results were analysed via the thermodynamic modelling framework obtained from our previous work (Chan et al., 2020). In the modelling framework, the rate of entropy generation due to friction from the sliding (or rotating activity) of the tribosystem can be expressed as Equation (1):

$$\frac{dS_i}{dt} = \frac{(\mu wv)^2}{A_c U T^2} \tag{1}$$

where, dS_i is the entropy production due to frictional sliding; μ is the friction coefficient; w is the normal load; v is the sliding speed; U is the heat transfer coefficient of the domain; A_c is the interfacial contact area; t is the time domain, and T is the lubrication temperature. Naturally, certain entropy dissipation mechanisms occur within the tribosystem to counteract the effects of entropy generation due to friction, ensuring the prevalence of sliding activity. The first entropy dissipation mechanism considered in this study is the wearing process, as described in Equation (2):

$$\frac{dS_w}{dt} = \frac{2\gamma}{T}(b \times k_w wv) \tag{2}$$

where, dS_w is the decrease in entropy as a result of the wearing process, γ is the surface energy of the worn material, b is the ratio of worn surface area to the worn volume, k_w is the specific wear rate (total wear volume divided by load and sliding distance). The ratio b depends entirely on the geometry of contact (e.g., flat-on-flat, ball-on-flat). Another entropy dissipation mechanism involving lubricant action is also present in the tribosystem. In this case, the rate of entropy dissipation due to lubrication is taken as a certain portion of the total frictional energy dS_i as Equation (3):

$$\frac{dS_f}{dt} = \phi \frac{dS_i}{dt} \tag{3}$$

where, dS_f is the decrease in entropy due to lubricant action, and ϕ is the dissipated portion of the total frictional energy due to the lubricant action. On the other hand, when the tribosystem operates at a stationary state with negligible heat loss to surrounding and insignificant wear particle formation, the entropy generation due to frictional sliding (dS_i/dt) is equal to the entropy dissipation due to wearing process (dS_w/dt) and lubrication (dS_f/dt). As such, equating Equation (1) with Equation (2) and (3) gives a simple equation correlating the friction and the wear of a tribological system [Equation (4)]:

$$k\mathrm{w} = \frac{\mu^2}{cU_w} \tag{4}$$

where, c is the physical characterisation parameter of the tribosystem [Equation(5)]:

$$c = 2A_c\gamma bTw^{-1}v^{-1} \tag{5}$$

and U_w is the dissipative coefficient of the tribosystem [Equation (6)]:

$$U_w = U\,(1 - \phi)^{-1} \tag{6}$$

In this expression, the parameter c can be calculated based on the physical property of the tribosystem and the operating conditions; whereas U_w is the proportionality constant between the entropy dissipation capability of the lubrication and that of the wear. In reality, both U and ϕ are difficult to measure since they are interfacial properties. Therefore, for simplicity, these parameters are lumped together as U_w to denote the tendency of the tribosystem to cause wear.

Model Parameters Calculation

The use of the friction-wear correlation in Equation (4) to study the Four-ball EP and AW test results of the 36 lubricant samples requires the calculation of the related model parameters. Knowing the distance from the centre of the contact surfaces of the underlying balls to the axis of rotation, x is about 3.67 mm according to the machine specification, the sliding speed, v (ms^{-1}) can be calculated as Equation (7):

$$v = 2\pi wx / 60{,}000 \tag{7}$$

where ω is the rotational speed of the top ball (1760 rpm for the EP test and 1,200 rpm for the AW test). For a given machine loading, L (kg), few contact parameters can be determined (Czichos & Kirschke, 1972; Sethuramiah, 2003).

The normal load on one bottom ball (kg). [Equation(8)]:

$$L_N = 0.408L \tag{8}$$

The contact diameter between the top ball and the bottom ball (mm) [Equation(9)]:

$$d_H = 0.0873L^{1/3} \tag{9}$$

The contact area between the top ball and the bottom ball (mm^2) [Equation(10)]:

$$A_c = \pi d_H{}^2/4 \tag{10}$$

In some machines, the friction data is reported as friction torque, F_t (kg-mm). To convert it to friction coefficient, the following formula from IP 239 (2014) is used [Equation (11)].

$$\mu = 0.22248F_T / L \tag{11}$$

In practice, the average wear scar diameter of the bottom balls, d_w (mm) is measured and reported. Based on works (I-Ming, 1962; Sethuramiah, 2003; Wright et al., 1989), d_w can be used to calculate the wear volume of one bottom ball (mm^3) [Equation(12)],

$$V_w = 1.55 \times 10^{-2}\mathrm{d_w}^4 - 1.07 \times 10^{-5}\,Ld_w \tag{12}$$

and the specific wear rate (mm^3 N^{-1} m^{-1})

$$k_w = V_w/(9.81L_Nvt) \tag{13}$$

where, t is the sliding duration (10 s for the EP test and 3,600 s for the AW test). On the other hand, the surface energy, γ of the AISI 52100 steel ball is about 1.95 J m^{-2} based on Fe as in (Inman & Tipler, 1963). For EP tests, the ratio of worn surface area to worn volume, b, is set to 18 mm^{-1}, while for AW tests, it is set to 322 mm^{-1}. The calculated model parameters, along with the experimental data for the 36 samples in the EP and AW tests, are presented in *Table 2* and 3, respectively.

TABLE 2. THE MODEL PARAMETERS FOR THE FOUR-BALL EP TEST

Sample	Load, L (kg) [a]	Wear scar diameter, d_w (mm) [b]	Average frictional torque, F_t (kg mm)	Friction coefficient, μ	Specific wear rate, k_w (mm^3 N^{-1} m^{-1})	Physical characterisation parameter, c (NKW^{-1})	Dissipative coefficient, U_w (W m^{-2} K^{-1})
M	80	2.52	105.10	0.292	2.88E-04	0.011	2.80E+13
	100	2.62	118.46	0.264	2.70E-04	0.010	2.60E+13
	126	4.00	456.98	0.807	1.16E-03	0.009	6.10E+13
M + A1	80	2.74	113.80	0.316	4.01E-04	0.011	2.30E+13
	100	2.74	134.88	0.300	3.23E-04	0.010	2.80E+13
	126	4.00	435.02	0.768	1.16E-03	0.009	5.50E+13
M + A2	80	0.42	31.60	0.088	5.67E-08	0.011	1.30E+16
	100	2.44	94.62	0.211	2.03E-04	0.010	2.20E+13
	126	4.00	428.70	0.757	1.16E-03	0.009	5.30E+13
M + A3	80	0.42	30.83	0.086	5.67E-08	0.011	1.20E+16
	100	0.46	27.59	0.061	7.46E-08	0.010	5.10E+15
	126	0.50	42.40	0.075	8.64E-08	0.009	7.00E+15
	160	0.54	48.09	0.067	9.09E-08	0.009	5.80E+15
	200	0.61	54.93	0.061	1.49E-07	0.008	3.20E+15
	250	4.00	63.77	0.057	1.30E-03 [c]	0.007	3.40E+11
M + A1 + A3	160	0.54	45.72	0.064	9.09E-08	0.009	5.20E+15
	200	0.59	48.66	0.054	1.14E-07	0.008	3.20E+15
	250	4.00	325.18	0.289	5.85E-03 [c]	0.007	1.90E+12
M + A2 + A3	160	0.54	39.92	0.056	9.09E-08	0.009	4.00E+15
	200	0.59	55.90	0.062	1.14E-07	0.008	4.30E+15
	250	4.00	59.93	0.053	1.67E-03 [c]	0.007	2.30E+11
M80 + V20	80	2.14	104.12	0.290	1.48E-04	0.011	5.30E+13
	100	2.31	71.41	0.159	1.62E-04	0.010	1.60E+13
	126	4.00	410.95	0.726	1.16E-03	0.009	4.90E+13
M80 + V20 + A1	80	2.41	86.14	0.240	2.39E-04	0.011	2.20E+13
	100	2.51	115.14	0.256	2.28E-04	0.010	2.90E+13
	126	4.00	409.09	0.722	1.16E-03	0.009	4.90E+13
M80 + V20 + A2	80	2.15	80.78	0.225	1.52E-04	0.011	3.10E+13
	100	2.54	81.14	0.181	2.39E-04	0.010	1.40E+13
	126	4.00	410.65	0.725	1.16E-03	0.009	4.90E+13
M80 + V20 + A3	80	2.33	111.86	0.311	2.12E-04	0.011	4.30E+13
	100	2.48	116.07	0.258	2.17E-04	0.010	3.10E+13
	126	4.00	409.60	0.723	1.16E-03	0.009	4.90E+13
M80 + V20 + A1 + A3	80	2.44	99.44	0.277	2.54E-04	0.011	2.80E+13
	100	2.91	133.81	0.298	4.10E-04	0.010	2.20E+13
	126	4.00	414.05	0.731	1.16E-03	0.009	5.00E+13
M80 + V20 + A2 + A3	80	2.28	109.53	0.305	1.93E-04	0.011	4.50E+13
	100	2.75	127.09	0.283	3.28E-04	0.010	2.40E+13
	126	4.00	419.47	0.741	1.16E-03	0.009	5.10E+13

TABLE 2. THE MODEL PARAMETERS FOR THE FOUR-BALL EP TEST (continued)

Sample	**Load, L (kg)** [a]	**Wear scar diameter, d_w (mm)** [b]	**Average frictional torque, F_t (kg mm)**	**Friction coefficient, μ**	**Specific wear rate, k_w (mm^3 N^{-1} m^{-1})**	**Physical characterisation parameter, c (NKW^{-1})**	**Dissipative coefficient, U_w (W m^{-2} K^{-1})**
M60 + V40	80	2.51	96.61	0.269	2.81E-04	0.011	2.40E+13
	100	2.79	94.38	0.210	3.45E-04	0.010	1.30E+13
	126	4.00	438.51	0.774	1.16E-03	0.009	5.60E+13
M60 + V40 + A1	80	2.41	80.73	0.225	2.41E-04	0.011	1.90E+13
	100	2.45	70.64	0.157	2.07E-04	0.010	1.20E+13
	126	4.00	387.77	0.685	1.16E-03	0.009	4.40E+13
M60 + V40 + A2	80	2.42	101.28	0.282	2.45E-04	0.011	3.00E+13
	100	2.72	95.73	0.213	3.12E-04	0.010	1.50E+13
	126	4.00	413.17	0.730	1.16E-03	0.009	5.00E+13
M60 + V40 + A3	80	2.11	90.63	0.252	1.41E-04	0.011	4.20E+13
	100	2.26	77.10	0.172	1.49E-04	0.010	2.00E+13
	126	4.00	413.35	0.730	1.16E-03	0.009	5.00E+13
M60 + V40 + A1 + A3	80	2.04	93.87	0.261	1.23E-04	0.011	5.20E+13
	100	2.76	146.97	0.327	3.30E-04	0.010	3.20E+13
	126	4.00	409.13	0.722	1.16E-03	0.009	4.90E+13
M60 + V40 + A2 + A3	80	1.46	51.62	0.144	3.20E-05	0.011	6.00E+13
	100	2.68	116.53	0.259	2.95E-04	0.010	2.30E+13
	126	4.00	429.57	0.759	1.16E-03	0.009	5.40E+13
M40 + V60	80	2.38	80.16	0.223	2.28E-04	0.011	2.00E+13
	100	2.20	63.11	0.140	1.34E-04	0.010	1.50E+13
	126	4.00	383.28	0.677	1.16E-03	0.009	4.30E+13
M40 + V60 + A1	80	0.82	27.78	0.077	2.92E-06	0.011	1.90E+14
	100	2.38	67.94	0.151	1.84E-04	0.010	1.20E+13
	126	4.00	390.72	0.690	1.16E-03	0.009	4.40E+13
M40 + V60 + A2	80	0.70	40.06	0.111	1.41E-06	0.011	8.20E+14
	100	2.48	79.51	0.177	2.16E-04	0.010	1.50E+13
	126	4.00	401.58	0.709	1.16E-03	0.009	4.70E+13
M40 + V60 + A3	80	2.48	102.96	0.286	2.68E-04	0.011	2.80E+13
	100	2.60	83.28	0.185	2.62E-04	0.010	1.30E+13
	126	4.00	367.74	0.649	1.16E-03	0.009	3.90E+13
M40 + V60 + A1 + A3	80	2.47	93.00	0.259	2.63E-04	0.011	2.40E+13
	100	2.91	105.27	0.234	4.10E-04	0.010	1.30E+13
	126	4.00	394.04	0.696	1.16E-03	0.009	4.50E+13
M40 + V60 + A2 + A3	80	2.27	115.42	0.321	1.91E-04	0.011	5.00E+13
	100	3.39	114.22	0.254	7.59E-04	0.010	8.50E+12
	126	4.00	388.33	0.686	1.16E-03	0.009	4.40E+13
M20 + V80	80	1.75	53.57	0.149	6.59E-05	0.011	3.10E+13
	100	2.22	59.96	0.133	1.38E-04	0.010	1.30E+13
	126	4.00	389.31	0.687	1.16E-03	0.009	4.40E+13

TABLE 2. THE MODEL PARAMETERS FOR THE FOUR-BALL EP TEST (continued)

Sample	Load, L (kg) [a]	Wear scar diameter, d_w (mm) [b]	Average frictional torque, F_t (kg mm)	Friction coefficient, μ	Specific wear rate, k_w (mm^3 N^{-1} m^{-1})	Physical characterisation parameter, c (NKW^{-1})	Dissipative coefficient, U_w (W m^{-2} K^{-1})
M20 + V80 + A1	80	2.33	91.83	0.255	2.12E-04	0.011	2.90E+13
	100	2.37	69.37	0.154	1.79E-04	0.010	1.30E+13
	126	4.00	390.11	0.689	1.16E-03	0.009	4.40E+13
M20 + V80 + A2	80	2.25	74.41	0.207	1.82E-04	0.011	2.20E+13
	100	2.37	88.03	0.196	1.79E-04	0.010	2.10E+13
	126	4.00	435.45	0.769	1.16E-03	0.009	5.50E+13
M20 + V80 + A3	80	2.23	80.35	0.223	1.77E-04	0.011	2.60E+13
	100	2.60	73.76	0.164	2.60E-04	0.010	1.00E+13
	126	4.00	288.39	0.509	1.16E-03	0.009	2.40E+13
M20 + V80 + A1 + A3	80	2.49	79.51	0.221	2.75E-04	0.011	1.70E+13
	100	2.51	97.26	0.216	2.26E-04	0.010	2.10E+13
	126	4.00	377.31	0.666	1.16E-03	0.009	4.10E+13
M20 + V80 + A2 + A3	80	2.38	78.95	0.220	2.30E-04	0.011	1.90E+13
	100	2.35	82.84	0.184	1.73E-04	0.010	2.00E+13
	126	4.00	423.27	0.747	1.16E-03	0.009	5.20E+13
V	80	1.89	42.63	0.119	9.12E-05	0.011	1.40E+13
	100	2.32	50.73	0.113	1.64E-04	0.010	7.80E+12
	126	4.00	356.27	0.629	1.16E-03	0.009	3.70E+13
V + A1	80	1.87	57.74	0.161	8.66E-05	0.011	2.80E+13
	100	2.63	68.30	0.152	2.73E-04	0.010	8.50E+12
	126	4.00	288.94	0.510	1.16E-03	0.009	2.40E+13
V + A2	80	2.32	71.59	0.199	2.08E-04	0.011	1.80E+13
	100	2.66	71.45	0.159	2.84E-04	0.010	8.90E+12
	126	4.00	370.08	0.653	1.16E-03	0.009	4.00E+13
V + A3	80	0.42	24.88	0.069	5.67E-08	0.011	7.80E+15
	126	0.50	37.85	0.067	8.64E-08	0.009	5.60E+15
	160	0.54	39.83	0.055	9.09E-08	0.009	4.00E+15
	200	4.00	266.06	0.296	7.32E-03 [c]	0.008	1.50E+12
V + A1 + A3	160	0.54	40.88	0.057	9.09E-08	0.009	4.20E+15
	200	4.00	56.88	0.063	7.32E-04	0.008	6.90E+11
V + A2 + A3	160	0.54	41.33	0.057	9.09E-08	0.009	4.30E+15
	200	0.74	43.69	0.049	5.58E-07	0.008	5.30E+14
	250	4.00	331.29	0.295	5.85E-03 [c]	0.007	2.00E+12

Note: [a] - The weld point corresponds to the heaviest load used in the test of each oil sample; [b] - Measured using the DUCOM imaging device for the non-welded ball. In the case of the welded ball, the wear scar diameter is treated as 4 mm and [c] - Calculated based on shorter sliding time since the ball welded before test duration: M + A3 (sliding time of 4.5 s); M + A1 + A3 (1.0 s); M + A2+ A3 (3.5 s); V + A3 (1.0 s); V + A2 + A3 (1.0 s).

TABLE 3. THE MODEL PARAMETERS FOR THE FOUR-BALL AW TEST

Sample	**Load, L (kg)** [a]	**Wear scar diameter, d_w (mm)** [b]	**Average frictional torque, F_t (kg mm)**	**Friction coefficient, μ**	**Specific wear rate, k_w (mm^3 N^{-1} m^{-1})**	**Physical characterisation parameter, c (NKW^{-1})**	**Dissipative coefficient, U_w (W m^{-2} K^{-1})**
M	40	0.73	14.03	0.078	1.58E-08	0.355	1.1E+15
M + A1	40	0.54	21.25	0.118	4.06E-09	0.355	9.7E+15
M + A2	40	0.51	25.78	0.143	3.21E-09	0.355	1.8E+16
M + A3	40	0.64	23.12	0.129	9.00E-09	0.355	5.2E+15
M + A1 + A3	40	0.52	23.64	0.131	3.33E-09	0.355	1.5E+16
M + A2 + A3	40	0.60	14.59	0.081	6.55E-09	0.355	2.8E+15
M80 + V20	40	0.51	25.85	0.144	3.10E-09	0.355	1.9E+16
M80 + V20 + A1	40	0.58	22.88	0.127	5.84E-09	0.355	7.8E+15
M80 + V20 + A2	40	0.54	45.97	0.256	4.06E-09	0.355	4.5E+16
M80 + V20 + A3	40	0.54	26.16	0.145	3.95E-09	0.355	1.5E+16
M80 + V20 + A1 + A3	40	0.42	26.26	0.146	1.19E-09	0.355	5.1E+16
M80 + V20 + A2 + A3	40	0.40	30.58	0.170	8.49E-10	0.355	9.6E+16
M60 + V40	40	0.51	26.28	0.146	3.21E-09	0.355	1.9E+16
M60 + V40 + A1	40	0.60	12.54	0.070	6.74E-09	0.355	2.0E+15
M60 + V40 + A2	40	0.56	25.40	0.141	4.91E-09	0.355	1.1E+16
M60 + V40 + A3	40	0.55	20.14	0.112	4.34E-09	0.355	8.1E+15
M60 + V40 + A1 + A3	40	0.54	17.77	0.099	4.20E-09	0.355	6.5E+15
M60 + V40 + A2 + A3	40	0.55	14.27	0.079	4.49E-09	0.355	3.9E+15
M40 + V60	40	0.60	23.75	0.132	6.74E-09	0.355	7.3E+15
M40 + V60 + A1	40	0.60	19.23	0.107	6.74E-09	0.355	4.8E+15
M40 + V60 + A2	40	0.58	27.52	0.153	5.54E-09	0.355	1.2E+16
M40 + V60 + A3	40	0.39	36.65	0.204	7.21E-10	0.355	1.6E+17
M40 + V60 + A1 + A3	40	0.34	13.31	0.074	2.63E-10	0.355	5.9E+16
M40 + V60 + A2 + A3	40	0.54	27.52	0.153	4.06E-09	0.355	1.6E+16
M20 + V80	40	0.61	27.56	0.153	7.15E-09	0.355	9.2E+15
M20 + V80 + A1	40	0.61	22.53	0.125	6.94E-09	0.355	6.4E+15
M20 + V80 + A2	40	0.63	27.69	0.154	7.95E-09	0.355	8.4E+15
M20 + V80 + A3	40	0.44	25.43	0.141	1.44E-09	0.355	3.9E+16
M20 + V80 + A1 + A3	40	0.39	21.36	0.119	7.59E-10	0.355	5.2E+16
M20 + V80 + A2 + A3	40	0.39	7.91	0.044	7.59E-10	0.355	7.2E+15
V	40	0.63	16.61	0.092	8.35E-09	0.355	2.9E+15
V + A1	40	0.55	20.74	0.115	4.45E-09	0.355	8.4E+15
V + A2	40	0.69	24.17	0.134	1.18E-08	0.355	4.3E+15
V + A3	40	0.66	21.57	0.120	9.75E-09	0.355	4.2E+15
V + A1 + A3	40	0.57	23.30	0.130	5.20E-09	0.355	9.1E+15
V + A2 + A3	40	0.66	22.34	0.124	9.75E-09	0.355	4.5E+15

RESULTS AND DISCUSSION

Mapping of Friction-wear Relationships in Four-ball Lubrications

The friction and the wear behaviours of a lubricated tribosystem are interrelated in such a way that the wear increases with the friction during sliding activity. The friction-wear relationship can be categorised and mapped into certain performance regions according to the thermodynamic framework as illustrated in *Figure 1*. The figure captures the friction-wear behaviours of lubrication in a Four-ball tribo-tester running at various conditions from mild-wear condition (e.g., 40–60 kg load) up to moderate/severe wear condition (e.g., 80–126 kg load) and finally weld point (e.g., 126–250 kg load). A glance at the figure clearly shows that both the mineral-based and vegetable-based lubrication behave similarly in the EP and AW tests, whereby the wear generally increases with sliding friction. As shown in *Figure 1*, one important parameter governing the performance regions is the dissipative coefficient U_w, which simply denotes the tendency of the tribosystem to cause wear at a specific sliding condition. For instance, the high value of U_w represents less tendency to cause wear in the tribosystem at the specific sliding conditions (i.e., load and speed). In other words, the change of U_w corresponds to the change in wear conditions of the triboparts and also that in the sliding conditions. At mild wear conditions ($k_w = 1 \times 10^{-8}$ to 1×10^{-6} $mm^3\ N^{-1}\ m^{-1}$) such as in the AW test (i.e., 40 kg load) and in low load EP test (i.e., < 63 kg load), the tribosystem was able to dissipate the frictional energy efficiently without inducing much wear. Within the mild wear region, the friction-wear relationship is bounded by U_w of the order of 10^{15} to 10^{17} $W\ m^{-2}\ K^{-1}$. Following that, when the load in the EP test increased further, the wear of the triboparts reached the severe wear region ($k_w > 1 \times 10^{-6}$ $mm^3\ N^{-1}\ m^{-1}$). This wear region is narrowly governed by U_w of the order of 10^{13} to 10^{14} $W\ m^{-2}\ K^{-1}$. Up to this point, the friction-wear relationship of the lubrication is mainly influenced by the base oil portion of the lubrication regardless of mineral-based or vegetable-based. Further, increase in load may result in welding of triboparts unless tribo-performance additives in the lubrications are present and active. The result in *Figure 1* shows that lubrications with active tribo-performance additives are characterised by U_w in the order of 10^{11} to 10^{13} $W\ m^{-2}\ K^{-1}$. Because of this region, it is interesting to note that there are two additive mechanisms in action at the extreme sliding conditions: one generated higher friction (U_w in the order of 10^{12} to 10^{13}) and the other one produced lower friction (U_w in the order of 10^{11} to 10^{12}). From the model perspective, the tribo-performance (EP or AW) additives provide an additional entropy dissipation mechanism on top of the base oil mechanism to balance out with the friction entropy generation from the sliding condition, especially at load beyond the weld point of the base oil.

In summary, the U_w parameter represents the tendency of the tribosystem to cause wear (Chan et al., 2020). Indirectly, it also indicates the state of the lubrication mechanism in action during the sliding activity. *Table 4* tabulates the characteristics of the lubrications at various U_w values and their significance according to the modelling framework. In practice, the classification of the magnitude order of the dissipative coefficient U_w determines the lubrication mechanism in action, which is beneficial to the lubricant formulation and the selection of suitable additives. In the next section, the individual effects of additive A1, A2 and A3 based on U_w parameter are assessed.

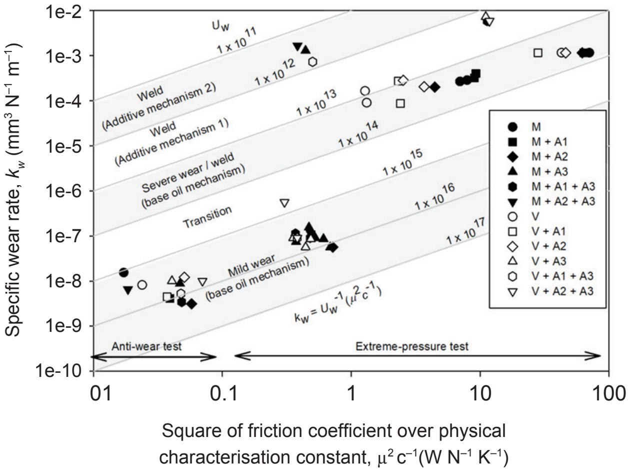

Figure 1. Friction-wear relationship of M and V with additives (A1, A2 and A3) in the anti-wear and extreme pressure tests. The highlighted regions are bounded by a certain range of dissipative coefficient U_w.

TABLE 4. THE DISSIPATIVE COEFFICIENT U_W AND ITS ENTROPY DISSIPATION MECHANISM

No.	The dissipative coefficient U_w (W m^{-2} K^{-1})	Wear condition	Possible lubrication mechanisms	Significance and implication
1	1×10^{15} to 1×10^{17}	Mild wear	BASE mechanism: Lubricant film thickness; viscosity effect; and lubrication regimes such as mixed, boundary lubrication	Both mineral-based and vegetable-based lubrications behave similarly
2	1×10^{14} to 1×10^{15}	Transition	BASE mechanism	The lubrication cannot be sustained in this unstable region
3	1×10^{13} to 1×10^{14}	Severe	BASE mechanism	Both mineral-based and vegetable-based lubrications behave similarly. (Weld point = 126 kg)
4	1×10^{12} to 1×10^{13}	Weld point	EP additive mechanism 1: Formation of a protective layer	The lubrication has a high weld point (> 126 kg). It generated higher friction
5	1×10^{11} to 1×10^{12}	Weld point	EP additive mechanism 2: Formation of a protective layer	The lubrication has a high weld point (> 126 kg). It generated lower friction

Additive Synergy to Enhance EP and AW Performances

The synergy of the additive A1, A2 and A3 in the mineral-based and vegetable-based lubrications in the EP test and the AW test were assessed experimentally. In view of the tribological additives, A1 and A2 are AW additives, whereas A3 is an EP additive. A glance at *Figure 2* shows that the additive synergy in the mineral-based and vegetable-based lubrication was distinct. Certain combinations of additives worked well in certain BASE as they offered greater advantages than the individual effect of the additives. In the case of mineral oil lubrication, *Figure 2a* illustrates that the additive A1 and A2 were able to reduce the friction of the contact slightly without changing the weld point of the lubrication in the EP test, while the additive A3 was able to increase the weld point of the mineral oil from 126–250 kg. These additives offer no synergistic advantage in terms of weld point when they are paired with A3 in the mineral oil formulation. From a conventional viewpoint, the lubrication M + A1 + A3 seemed to be antagonistic because of poorer frictional performance than M + A3 in the EP test. In *Figure 2b*, the additive A2 worked better than the additive A1 in terms of wear minimisation effect in the AW test, despite the formed incurring a higher friction coefficient. The EP additive A3 also gave a slight wear minimisation effect. In terms of additive synergy, in this case, no significant effect was observed in the mineral-based lubrication with any additive combination. On the other hand, looking at the effect of the additives in V lubrication in the EP test as in *Figure 2c*, the EP additive A3 enhanced the weld point of the lubrication to 200 kg. When the additive A2 was coupled with the additive A3, the weld point was boosted further to 250 kg. However, on individual additive effect, the additive A1 provided a positive effect in terms of frictional performance, while the additive A2 gave no significant positive effect. With regards to the additive performance in AW test, there is no conclusive remark that can be drawn based on the result in *Figure 2d*. This is because the result of the wear scar diameter and also the friction coefficient were span narrowly and they are not significantly different than each other. In this assessment of additive synergy, some interactions are difficult to identify and confirm, based on merely experimental parameters. A thorough and simplistic assessment using a modelling approach can be employed and will be demonstrated in the following section. To maintain a fair comparison, both the assessments through the experimental parameters and the modelling approach must be based on an identical dataset. Since the modelling approach prioritises the identification of trends over the precision of individual data points, incorporating error bars or data repetition in *Figure 2* is unnecessary for this comparison study.

Assessment of Lubricant Formulations via the Modelling Approach

This study demonstrated the assessment of the tribological performance of various lubricant formulations in the AW and the EP test based on the dissipative coefficient U_w. In this assessment, the lubrication with a higher value of U_w has better tribological performance in general. This is because it has a higher dissipation rate of frictional entropy and hence lesser tendency to cause wear. For fair assessment, the comparison between two lubrications must be made at the same operating conditions, especially at the same load and sliding speed. The modelling results presented in *Figure 3* illustrated the effect of blending the V into the M from 0%–100% in five formulations (BASE; BASE + A1; BASE + A2; BASE + A3; BASE + A1 + A3;

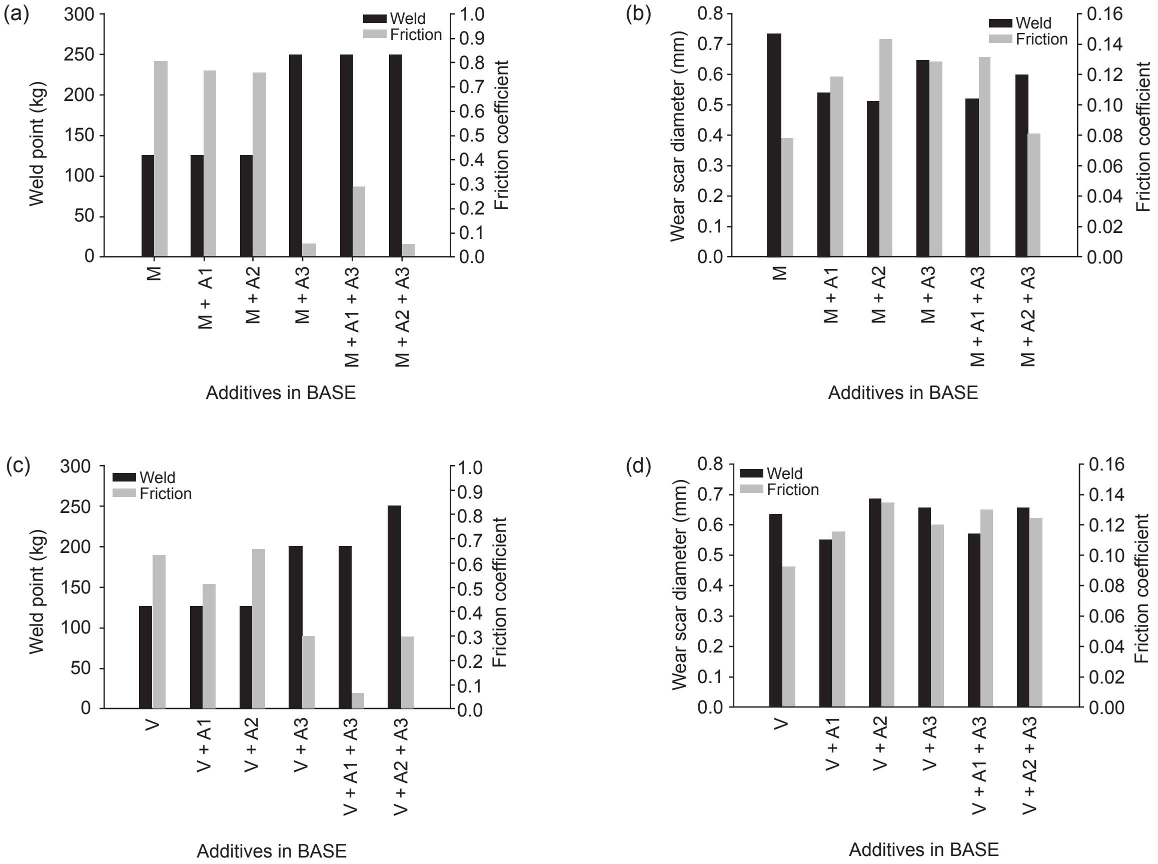

Figure 2. Tribological results of mineral-based lubrications in (a) the EP test and (b) the AW test; while vegetable-based lubrications in (c) the EP test and (d) the AW test.

BASE + A2 + A3) during the AW test. From the results, incorporating V in all five formulations was found to be beneficial probably due to the advantages of the oil in lubricity and friction performances as suggested in work (Reeves et al., 2015). The optimum BASE compositions for each of the lubricant formulations based on highest U_w value are: BASE (M80 + V20 and M60 + V40); BASE + A1 (M and V); BASE + A2 (M80 + V20); BASE + A3 (M40 + V60); BASE + A1 + A3 (M40 + V60) and BASE + A2 + A3 (M80 + V20). The above results suggest that the additive A1 and A3 have affinity to both the M and V, whereas the additive A2 favours mineral-rich formulations.

Overall, the formulation BASE + A3 (M40 + V60) gave the best AW performance from the thermodynamic perspective. Nevertheless, it generated 0.39 mm wear scar diameter, which is slightly larger than the 0.34 mm wear scar diameter from the formulation of BASE + A1 + A3 (M40 + V60). Because of that, the former is considered to be the optimum formulation from the thermodynamic point of view, whereas the latter is the best according to the experimental results. In practice, if few formulations achieved similar experimental results, the modelling approach emphasising the U_w parameter can be a great help in identifying the better formulation. This can be demonstrated in the case of the EP test in this study.

On the other hand, the modelling result of the lubrications in the EP test is plotted in *Figure 4*. Note that the effect of blending the V into the M from 0%–100% is not illustrated in the figure because they did not exhibit significant effects. The weld points of the blended oils, even in the formulations involving additives, were still at 126 kg as tabulated in *Table 2*. Based on the modelling results of the lubrication performances at the weld point of 250 kg, the lubrication V + A2 + A3 performed slightly better than the lubrication M + A1 + A3. However, in the load below the weld point such as at 200 kg, the lubrication M + A2 + A3 outperformed the lubrications V + A2 + A3 and M + A1 + A3. This observation suggests that lubricant with a higher weld point may not necessarily be the best choice for the application that requires a lower weld point lubricant. In this modelling approach, the U_w values of the lubrications serve as an additional parameter on top of the weld point, to assess and select the best lubrication formulations out from the list of lubrications that passed the EP test.

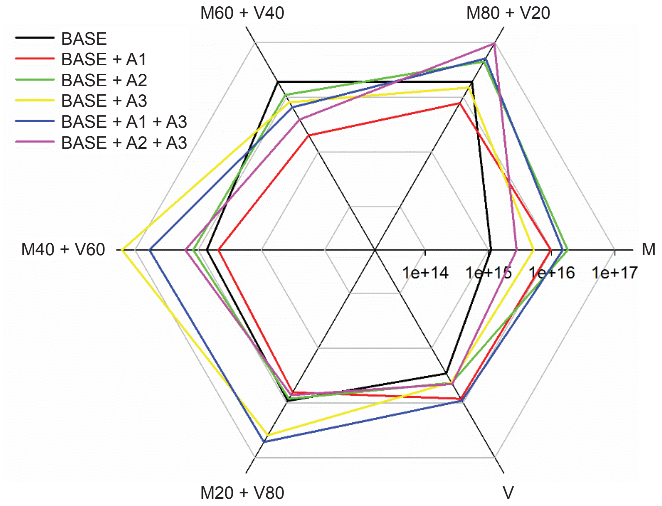

Figure 3. The dissipative coefficient U_w *of lubrications under the AW test for lubricant samples at different base oil compositions and additives formulations.*

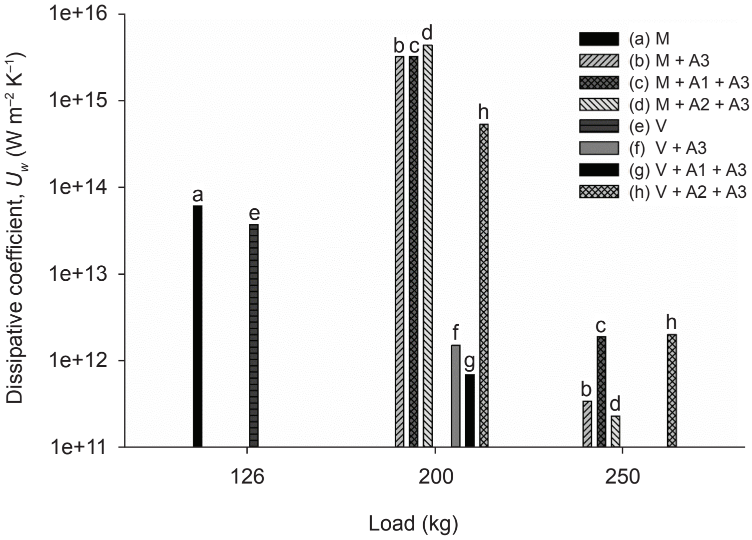

Figure 4. The dissipative coefficient U_w *of lubrications under the EP test for lubricant samples at different base oils and additives formulations.*

The Model Implication and Applications

In practice, the conventional tribological data such as friction coefficient and wear scar diameter can only be used in comparing the relative performance of two or more lubricants. If the tribological data obtained from the comparison is similar or not significantly different, no conclusive remark can be drawn. To overcome this problem, the modelling approach introduced in this work helps the lubrication assessment from the thermodynamic perspective. This modelling approach utilises U_w parameter to gauge the effectiveness of the lubrication in minimising wear and to sustain the activity of the tribosystem. One can select the best lubricant or lubricant composition based on the magnitude of U_w, in which the larger the magnitude of U_w, the better the lubrication and its mechanism to minimise wear at the designated operating condition. Despite the fact that U_w is not a physical parameter that can be measured experimentally, it is useful in the assessment and interpretation of lubrication performance. In terms of reliability, the friction-wear relationship and the performance region bounded by the specific ranges of U_w (*Table 4*) are very consistent and reliable across different lubricant samples, such as M and V in this work and also other synthetic oils in the previous work (Chan et al., 2020).

TABLE 5. RANKING OF THE LUBRICANT FORMULATIONS IN THE AW AND EP TESTS

Item	Traditional approach[a]	Modelling approach[b]
Anti-wear test	M40 + V60 + A1 + A3 (0.34 mm)	M40 + V60 + A3 (1.6E+17 W m^{-2} K^{-1})
	M40 + V60 + A3 (0.39 mm)	M80 + V20 + A2 + A3 (9.6E+16 W m^{-2} K^{-1})
	M20 + V80 + A1 + A3 (0.39 mm)	M40 + V60 + A1 + A3 (5.9E+16 W m^{-2} K^{-1})
	M20 + V80 + A2 + A3 (0.39 mm)	M20 + V80 + A1 + A3 (5.2E+16 W m^{-2} K^{-1})
	M80 + V20 + A2 + A3 (0.40 mm)	M80 + V20 + A1 + A3 (5.1E+16 W m^{-2} K^{-1})
Extreme pressure test	M + A3[c] (250 kg)	V + A2 + A3 (2.0E+12 W m^{-2} K^{-1})
	M + A1 + A3[c] (250 kg)	M + A1 + A3 (1.9E+12 W m^{-2} K^{-1})
	M + A2 + A3[c] (250 kg)	M + A3 (3.4E+11 W m^{-2} K^{-1})
	V + A2 + A3[c] (250 kg)	M + A2 + A3 (2.3E+11 W m^{-2} K^{-1})

Note: [a] - The value in parentheses is wear scar diameter (AW test) and weld point (EP test); [b] - The value in parentheses is the dissipative coefficient U_w; [c] - Unable to determine the ranking of lubricant formulation.

In this study, the top lubricant formulations in the AW test and also the EP test are selected and listed in *Table 5*. The selection is based on the traditional approach (i.e., tribological data) and the modelling approach (i.e., dissipative coefficient U_w). As can be seen in the table, the ranking of lubricant formulations based on the modelling approach is different from the traditional approach. In view of the AW test, the best-performing lubricants gave wear scar diameter ranged from 0.34–0.40 mm and there are three lubricants which scored similar results (0.39 mm). In this case, it is not possible to rank the lubrication performance according to wear scar diameter. However, ranking of the lubrication performance is possible based on their dissipative coefficients. On the other hand, a similar issue was found in the EP test as well. The best lubricant formulation is difficult to determine based on weld point alone since all scored 250 kg. With the use of the dissipative coefficient, the ranking of lubrication performance can be conducted. In this study, the modelling approach concluded that M40 + V60 + A3 lubricant is the best formulation for the anti-wear test, while V + A2 + A3 is the best in the extreme-pressure test.

CONCLUSION

This study has pioneered a comprehensive modelling approach designed to elevate the assessment of lubrication effectiveness within the realms of the ASTM D2783 EP and ASTM D4172 AW tests. The modelling approach serves to determine the optimal high-performance lubrication and synergistic additive combination, even in scenarios where the evaluated lubricants yield similar outcomes in tribological assessments. This is possible through the determination of the dissipative coefficient U_w of the lubrication. U_w operates as a reliable assessment parameter, offering insight into the lubrication capability to minimise wear and sustain tribological operations effectively. From the thermodynamic viewpoint, a lubricant boasting a higher U_w value demonstrates heightened efficiency in quelling the accumulation of frictional entropy inherent to a lubricated tribological system. As a result, this attribute reduces the tendency and intensity of wear within the system. Expanding beyond the realms of lubrication assessment, the dissipative coefficient U_w also finds use in constructing the friction-wear relationship for lubrication. This mapping of lubrication performance proves invaluable for the design of lubricant formulations. It serves as a tool to investigate the synergistic interactions between base oils and additives, to gauge the lubrication efficacy across diverse sliding conditions and to facilitate comparative analyses between two distinct lubricants. The prowess of this modelling approach finds substantial validation within a comprehensive case study involving 36 lubricant samples, comprising M, V and their various blends, supplemented by an array of tribological additives.

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GC/Q-TOF-MS-BASED METABOLOMICS: UNVEILING THE TEMPORAL METABOLIC PATHWAYS IN *Ganoderma boninense* USING PATHWAY ANALYSIS TOOLS

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ABSTRACT

Metabolomics research aims to uncover the complex biochemical pathways involved in biological processes, but the interpretation of metabolites, which play diverse roles within biological systems, remains a significant challenge. In this study, we utilised pathway analysis modules, such as Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper and MetaboAnalyst to facilitate the visualisation and interpretation of Ganoderma boninense *metabolomics data, derived from the gas chromatography/quadrupole-time-of-flight (GC/Q-TOF) mass spectrometry-based experiments. Our analysis revealed a time-dependent classification of 39 identified extracellular metabolites from the methanolic extract of* G. boninense, *where several metabolic pathways, i.e. starch and sucrose metabolism, galactose metabolism, valine, leucine and isoleucine degradation and citrate (TCA) cycles were found significantly enriched in* G. boninense. *The integration of pathway analysis tools enabled enhanced biological interpretation, contributing to a deeper understanding of temporal primary metabolic pathways linked to the* G. boninense *developmental process, energy production and cellular functions over time. These findings underscore the importance of the pathway analysis tools in metabolomics, helping to reveal the biological insights which are hidden within the complex metabolite profiles and thus advancing our understanding of the* G. boninense *developmental process* in vitro.

Keywords: functional analysis, *Ganoderma boninense*, GC/Q-TOF, pathway.

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INTRODUCTION

A comprehensive study of metabolites has become an essential approach that advances the understanding of the complex molecular relationships found in biological systems (Chen et al., 2022). The multifunctional roles of metabolites are being analysed for their consistent change in biological processes that underlie the profiles, as shown in metabolomics experiments (Oh et al., 2023; Qiu et al., 2023). In the context of metabolomics research, pathway analysis links the detected metabolites to known metabolic pathways, thus enhancing our understanding of their roles in various physiological and pathological conditions (Kanehisa & Sato, 2020; Xia & Wishart, 2011). In metabolomics, pathway analysis increasingly relies on advanced methods such as over-representation analysis (ORA) and topology-based approaches to interpret complex datasets. These approaches map the metabolites onto established biological pathways and evaluate their significance under various conditions (Wieder et al., 2021, 2022).

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Understanding the microbial metabolism and evaluating its biological importance to various environmental signals, requires recent advances in measuring extracellular metabolites (Pinu & Villas-Boas, 2017). With the various analytical equipment and high throughput methodologies available, the extracellular metabolite analysis has become a significant technique for monitoring microorganism growth parameters (Liu et al., 2022; Qiao et al., 2020). One of the promising analytical platforms in metabolite analysis is gas chromatography-mass spectrometry (GC-MS), which provides sensitive, reproducible and robust techniques for measuring volatile metabolites. Furthermore, GC-MS has an advantage over other analytical techniques, as it allows for faster and more reliable identification of compounds based on their mass spectra, using highly established and widely used public spectral databases (Fiehn, 2016).

Recent research highlights the importance of pathway analysis in metabolomics for data visualisation, as it enables researchers to interpret complex datasets by mapping metabolites to known biochemical pathways. Pathway tools like MetaboAnalyst and Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper are commonly used for this purpose, offering statistical workflows and visualisation features that assist in identifying significant metabolic changes under different conditions, including disease states or environmental changes (Kanehisa et al., 2022; Li et al., 2023; Pang et al., 2024). For example, in fungal research, KEGG enrichment analysis has identified 47 significant metabolic pathways which are associated with *Metarhizium anisopliae* sporulation, thus highlighting the role of amino acid metabolism, particularly glutamate, aspartate, serine, glycine, arginine and leucine in the sporulation process (Yang et al., 2023). Using the pathway tools, several metabolisms associated with *G. boninense* growth and development, virulence and pathogenicity have also been identified (Santiago et al., 2024).

This current study focuses on *G. boninense*, a wood-decaying basidiomycete fungus responsible for oil palm basal stem rot (BSR). *G. boninense* is of interest because it is the causal agent of BSR disease in oil palm, particularly in Malaysia and Indonesia. *G. boninense* presents a major threat to the oil palm industry, with projections suggesting it could infect up to 860,610 ha of mature oil palms by 2040, thus posing significant economic risks (Olaniyi & Szulczyk, 2020). Within just six months, an infection can lead to a 43% economic loss in affected plantations (Khoo & Chong, 2023). In terms of combating the immediate threat of *Ganoderma*, breakthroughs have been made in using molecular techniques to diagnose infections at the early stages (Murphy, 2014). For example, GC-MS-based metabolomics has been increasingly applied to study the interaction between oil palm and *G. boninense*, particularly for identifying bioactive compounds and understanding metabolic responses under various conditions (Abdullah et al., 2021; Hailini et al., 2020; Isha et al., 2020; Rozali et al., 2017; Rupaedah et al., 2024).

Despite extensive research on *G. boninense*, most studies have only primarily focused on the metabolites produced by its oil palm host, thus leaving gaps in our understanding of the metabolites generated by the fungus itself. Moreover, interpreting the functions of metabolites, which often have diverse roles within the biological systems, remains a significant challenge. To address this, our study aimed to investigate the metabolic pathways underlying the complex *G. boninense* metabolite profiles, through pathway analysis tools in metabolomics. This approach enhances biological interpretations, thus offering deeper insights into the intricate metabolic profiles of *G. boninense* and contributing to a better understanding of the dynamic metabolic processes of *G. boninense*.

MATERIALS AND METHODS

Preparation of Fungal Culture

Ganoderma boninense isolate PER71 was provided by the Plant Pathology and Biosecurity Unit, Malaysian Palm Oil Board (MPOB), Malaysia. *G. boninense* was cultivated using a liquid culture protocol (Nurazah et al., 2021b; Wahab, 2016). The culture was initially grown on six Difco™ PDA (Becton, Dickinson and Company, USA) plates and incubated at 27 ± 1ºC for eight days, as biological replicates. One plug of 9.55 mm from each of the six individual PDA plates was inoculated into 50 mL of MEB (Becton, Dickinson and Company, USA) in a 175 cm^3 Nunclon™ cell culture flask (ThermoFisher Scientific, USA). Three technical replicates were prepared. A total of six mycelial plugs were grown in the cell culture flask at 27 ± 1°C for six days. After six days, the mycelial plugs were collected on sterile filter paper, washed with sterile distilled water (3x) and placed into 50 mL of basal medium supplemented with 5 g of glucose (carbon source) and 3.9 g of 2-N-morpholinoethanesulphonic acid (MES) (nitrogen source) at pH 5.5, in a 175 cm^3 Nunclon™ cell culture flask (ThermoFisher Scientific, USA). A negative control with only growth medium was prepared and labelled as day 0. The culture fluids containing *G. boninense* extracellular metabolites from each flask were collected at different time points (day 2, 4, 6 and 8) to study the dynamic changes in *G. boninense* metabolite profiles over time (Nurazah et al., 2021b).

Three replicate samples were obtained from each time point, for 15 samples (including an additional three samples from day 0).

Metabolite Extraction

Metabolite extraction was conducted according to Nurazah et al. (2021b). The culture fluids containing *G. boninense* extracellular metabolites were freeze-dried using a freeze dryer for two days using a FreeZone® Freeze Drier System (Labconco, USA). About 0.1 g of the powdered culture media was dissolved in 3 mL of methanol (Merck, Germany), vortexed for 1 min and sonicated for 30 min. The mixture was then filtered through a 0.2 µm cellulose acetate Minisart syringe filter (Merck, Germany). A volume of 150 µL of individual methanolic extract was placed into a 250 µL micro-insert and dried under nitrogen for at least an hour. Next, a total of 15 dried samples were subjected to gas chromatography/quadrupole-time-of-flight (GC/Q-TOF) derivatisation procedure according to Rozali et al. (2021). The dried samples were derivatised in 80 µL of 20 mg mL^{-1} methoxyamine hydrochloride in pyridine and incubated at 37ºC for 90 min, followed by derivatisation with 80 µL N-methyl-N-trimethylsilyltrifluoroacetamide (MSTFA) for 30 min. The derivatised samples were allowed to rest for 60 min before injection. All the derivatisation procedures were conducted using an automated GERSTEL multipurpose sampler (MPS) XT 4.2.0 (GERSTEL GmbH & Co. KG, Germany).

Gas Chromatography/Quadrupole-Time-of-Flight Mass Spectrometry

The derivatised samples of 1 µL each were injected into the GC/Q- mass spectrometer (MS) system comprising a Gerstel Autosampler, a 7890B Agilent gas chromatography, and a 7200B Agilent Q-TOF MS (Agilent Technologies, USA). The GC was operated with helium as the carrier gas (1 mL min^{-1}) with VF-5ms (10 m guard column) column (30 m long x 0.25 mm inner diameter x 0.25 µm film thickness). The injection temperature was set at 260ºC with the following temperature program; injection at 80ºC, held for 2 min, followed by a 10ºC oven temp, ramp to 325ºC held for 9 min with a run time of 46 min. Raw data were deconvoluted using the Unknown Analysis tool from MassHunter Quantitative Analysis Software Version B.07.01 (Agilent Technologies, USA) and the identification of compounds was performed by comparison of the spectra to the G1676AA Agilent Fiehn 2013 GC/MS Metabolomics Retention Time Locking (RTL) Library with 70% mass spectrum similarity.

Data Processing and Multivariate Data Analysis

The generated bucket data was uploaded to the MetaboAnalyst ver. 6.0 online platform (Xia et al., 2009) for principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA)). The uploaded data were normalised by sum, log-transformed and Pareto scaled. Finally, the model was validated as described by Nurazah et al. (2021a), where the prediction accuracy was based on 5-fold CV (cross-validation), R^2 (predictive quality), and Q^2 (goodness of fit) values. The R^2 values demonstrate model fitness by explaining the variation inside the model and the Q^2 values denote model predictability, illustrating the model's capacity to predict a new data set. The highest R^2 values indicate a robust fit of the data, ranging from 0.0–1.0. R^2 levels approaching 1.0 signify an excellent model description, whereas Q^2 values over 0.5 denote proficient prediction, and values above 0.9 imply excellent prediction (Rozali et al., 2023; Eriksson et al., 2005). Permutation tests measured the significance ($p < 0.01$) of class discrimination: i) Prediction accuracy during training and ii) separation distance based on the ratio between group sum of squares (B/W ratio). The *p*-values less than 0.05 signify a statistically significant model (Khan et al., 2022). Variable importance was also determined using the variable importance in projection (VIP) analysis to detect metabolites that are responsible for the separation in the PLS-DA score scatter plot. Metabolites exhibiting a VIP larger than one (VIP > 1) were selected for further investigation (Liu et al., 2020).

Metabolic Pathway Analysis

Metabolite features with VIP values exceeding 1.0 was selected from the PLS-DA (Yang et al., 2021). It is provided as input for the KEGG pathway mapping tools, giving an overview of their positions within metabolic pathways using KEGG Mapper ver. 5.0 (https://www.kegg.jp/kegg/mapper) based on organism-specific, *Saccharomyces cerevisiae* (budding yeast) (Kanehisa et al., 2022). Pathway analysis was conducted by integrating pathway enrichment and topology analyses using MetaboAnalyst version 6.0 (Xia & Wishart, 2010, 2011) to identify the pathways most significantly affected by the selected metabolites. The KEGG database ID was used to map the names of the compounds with the following parameters: (1) Enrichment analysis was performed using the over-representation analysis hypergeometric test to identify differentially expressed metabolites within functionally related groups, (2) topology analysis was measured using out-degree centrality to assess the compound significance in a specific metabolic network and (3) pathway library code *sce* (*S. cerevisiae*) in the KEGG database were used as reference metabolic pathways. The multiple testing-Holm-Bonferroni method and false discovery rate (FDR) were then applied to the statistically significant *p*-values obtained from the pathway enrichment analysis. The metabolic pathways with significant *p*-values ($p < 0.05$) were selected.

RESULTS AND DISCUSSION

Visualisation of *G. boninense* Extracellular Metabolite Profiles

Using different days for the extraction of metabolites is essential for capturing the dynamic changes in the metabolic profile of *G. boninense* throughout its growth stages. Each time point corresponds to a specific stage of growth, allowing us to observe the varying production of extracellular metabolites, as the organism progresses from one stage to another. Studies by Ellstrom et al. (2015) and Nagappan et al. (2024) highlight that these different growth stages of fungi exhibit distinct metabolic activities, which can influence the production of metabolites. By assessing the metabolic profiles at various time points, we gain a comprehensive understanding of the metabolic responses of an organism and potential adaptations during its lifecycle. This approach ultimately provides insights into the ecological and functional roles of the metabolites in relation to the growth phases of *G. boninense*.

PCA allows the visualisation of underlying patterns in GC/Q-TOF data, highlighting the intergroup variability of *G. boninense* metabolites associated with cultivation time. The PCA scores plot indicated that the principal component (PC)1 (30.0%) and PC2 (25.5%) contributed to 55.5% of the total variation in the dataset. Sample groupings were observed along PC1 and PC2 according to the days of cultivation (*Figure 1a*). A close sample grouping was observed for day 6 and 8, separated from days 2 and 4 by PC1, while the sample grouping for day 2 was separated from day 4 by PC2. Further analysis of the observed sample groupings was conducted using PLS-DA. As depicted in the scores plot, individual groupings of *G. boninense* samples cultivated at day 0, 2, 4, 6 and 8 were also observed with 42.8% of the total variation in the dataset from PC1 and PC2 (*Figure 1b*). The metabolite profiles were distributed along PC1, suggesting the time-dependent metabolic changes in *G. boninense*. The assessment of the classification significance was done by the 5-fold cross-validation (CV) that showed excellent fitting (R^2) and predictive (Q^2) values of 0.9951 and 0.9605, respectively. The model also showed significance at $p < 0.01$ according to the permutation tests. The variable importance in projection (VIP > 1.0) identified from the loadings plot was sorted by importance, and has identified 39 discriminant metabolites ($p < 0.05$) with greater contributions to the observed groupings, and also identified the changes in *G. boninense* according to cultivation time (*Figure 2*).

Pathway Analysis by KEGG Mapper and MetaboAnalyst

Metabolic perturbations examined using GC-Q/TOF showed distinct changes in metabolic pathways in *G. boninense* which may explain

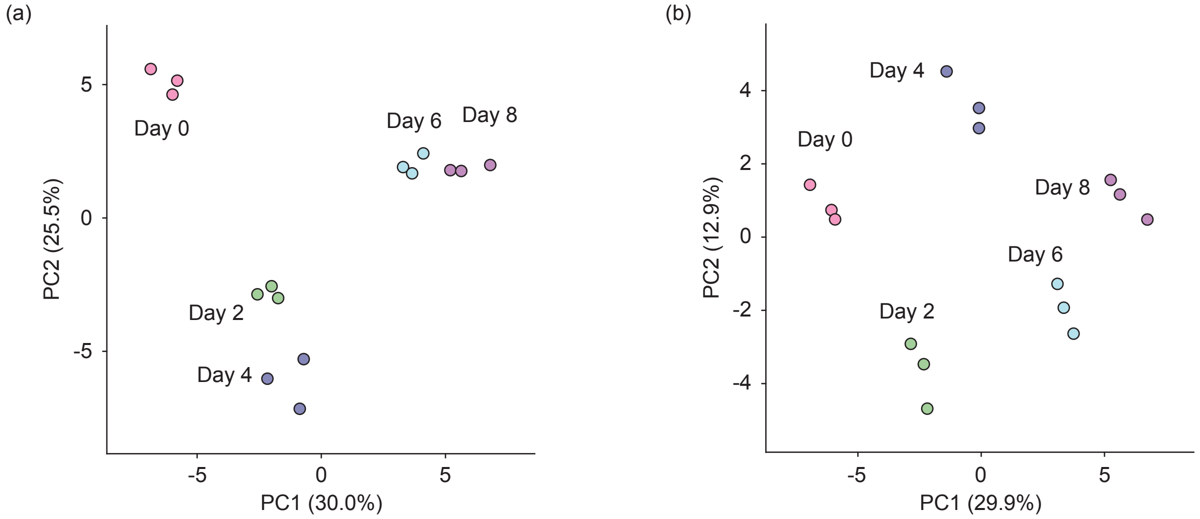

Figure 1. The scores plot of (a) principal component analysis (PCA) and (b) partial least squares-discriminant analysis (PLS-DA) for Ganoderma boninense *metabolites at day 0, 2, 4, 6 and 8 of cultivation.*

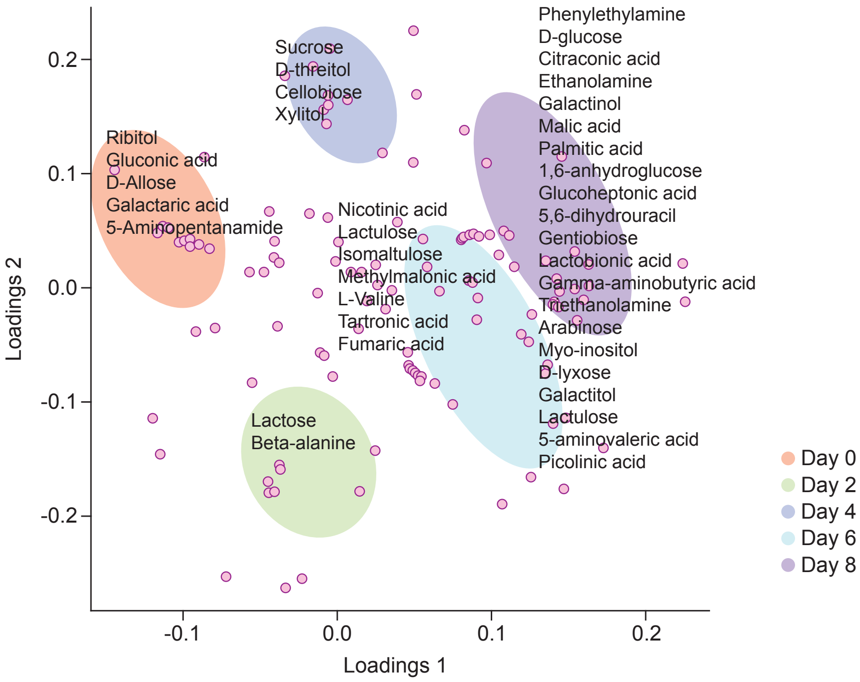

Figure 2. The loadings plot of partial least squares-discriminant analysis (PLS-DA) for Ganoderma boninense *metabolites at day 0, 2, 4, 6 and 8 of cultivation.*

the continuous metabolic perturbation across the cultivation time. By scrutinising the data through the lenses of PCA and PLS-DA, we gained an overview of metabolite patterns and identified key metabolites associated with *G. boninense* cultivation time. Beginning with the list of discriminant metabolites, the pathway analysis served as the next step that allowed us to distinguish the biological pathways that enlighten the collective behaviour of metabolites, in response to the experimental conditions (Karnovsky & Li, 2020).

Global visualisation of the differential metabolites according to the cultivation time of *G. boninense* in their specific biological pathways was viewed using KEGG Mapper (Kanehisa & Sato, 2020). KEGG Mapper comprises a set of mapping tools, designed to link molecular (such as genes, proteins, metabolites and glycans) with higher-level objects (such as pathways, modules, hierarchical structures, taxonomy and diseases) (Kanehisa et al., 2017). Based on the identified metabolites from the multivariate analysis, KEGG Mapper revealed 51 PATHWAYS and 31 MODULES. The specific metabolic pathways respective to the differential metabolites are highlighted (*Figure 3*). Details of the KEGG metabolic pathways according to the mapped metabolites are shown in *Table 1*. The result shows the involvement of fungal primary metabolism, with possible pathways altered in *G. boninense*, which include glycan metabolism, biosynthesis of terpenoids and polyketides, lipid metabolism, carbohydrate metabolism, energy metabolism, amino acid metabolism, nucleotide metabolism and the metabolism of cofactors and vitamins. The KEGG pathway classification shows changes in carbohydrate metabolism across the cultivation time, which could potentially have significant roles during the growth of *G. boninense* (*Figure 4*) (Zhou et al., 2021).

KEGG Mapper is particularly useful for exploring individual pathways and understanding the roles of specific metabolites within those pathways. However, it may not include some of the more advanced statistical analyses. Thus, implementing Pathway Analysis in MetaboAnalyst provides a more comprehensive and systematic approach that incorporates various statistical methods, namely enrichment and pathway topology analysis (Xia & Wishart, 2010). To better understand the dynamics of the observed alterations, we depicted 17 altered metabolic pathways in the pathway analysis plot, using a criterion of pathway impact values exceeding 0.01 (Wang et al., 2022) (*Figure 5*). Among these, four metabolic pathways were found to be significant in the enrichment analysis (highlighted as dark red circles at the top of the pathway analysis plot). These pathways include starch and sucrose metabolism, galactose metabolism, valine, leucine and isoleucine degradation and citrate (TCA) cycle (*Table 2*). These findings highlighted the potential pathways in primary metabolism, underlying the time-dependent metabolic alterations that occur during the growth, reproduction and survival of *G. boninense*.

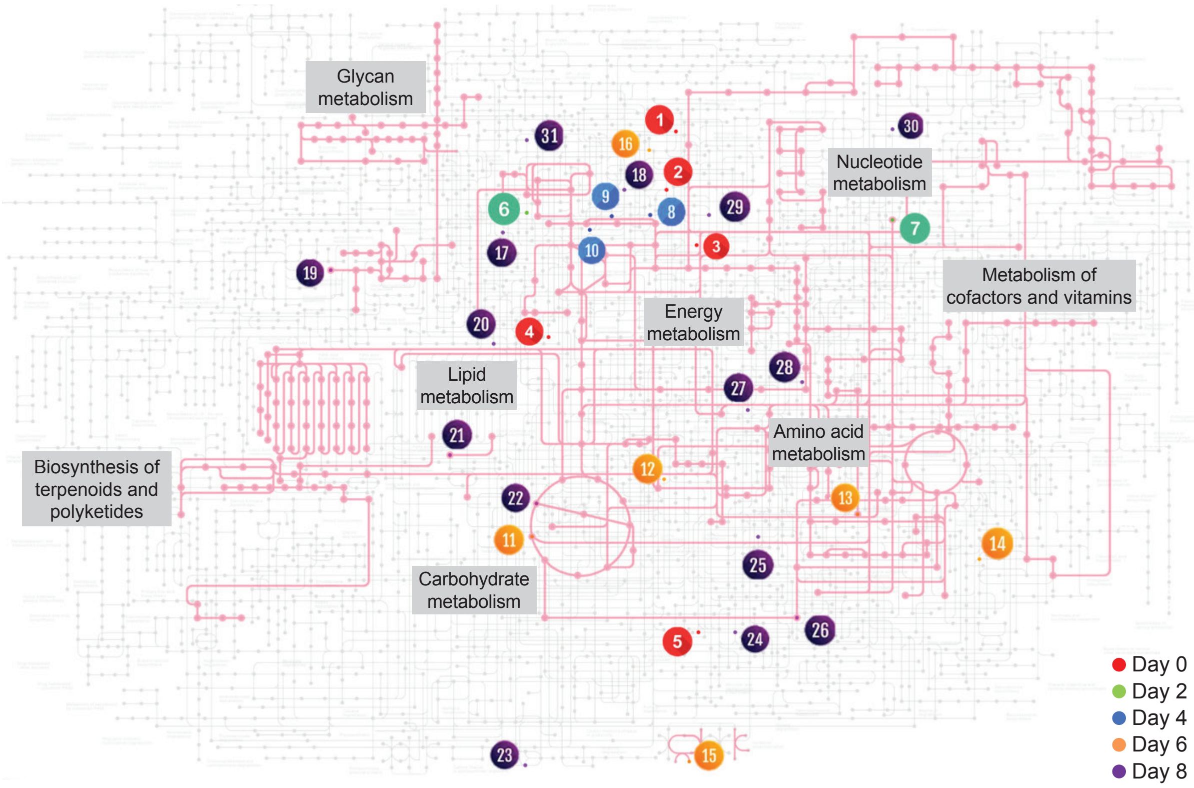

Figure 3. The KEGG metabolic map of Ganoderma boninense *metabolites associated with specific metabolic pathways. The coloured numbers represent the differential metabolites at day 0, 2, 4, 6 and 8 of cultivation. The nodes represent metabolites and the edge links two nodes if they are involved in a reaction as substrate and product.*

TABLE 1. THE KEGG METABOLIC PATHWAYS ASSOCIATED WITH *Ganoderma boninense* METABOLITES

Time points (day)	Putative metabolite (KEGG ID)	Metabolic pathway
0	1. Galactaric acid (C00879) 2. Gluconic acid (C00257) 3. Ribitol (C00474) 4. D-Allose (C01487) 5. 5-Aminopentanamide (C00990)	• Carbohydrate metabolism • Metabolism of cofactors and vitamins • Amino acid metabolism
2	6. Lactose (C00243) 7. Beta-alanine (C00099)	• Carbohydrate metabolism • Nucleotide metabolism
4	8. Xylitol (C00379) 9. Cellobiose (C00185) 10. Sucrose (C00089)	• Carbohydrate metabolism
6	11. Fumaric acid (C00122) 12. Methylmalonic acid (C02170) 13. L-Valine (C00183) 14. Nicotinic acid (C00253) 15. Fumaric acid (C00122) 16. Isomaltulose (C00252)	• Carbohydrate metabolism • Energy metabolism • Amino acid metabolism • Nucleotide metabolism • Metabolism of other amino acids • Metabolism of cofactors and vitamins
8	17. Galactitol (C01697) 18. D-Glucose (C00031) 19. Ethanolamine (C00189) 20. Inositol (C00137) 21. Palmitic acid (C00249) 22. Malic acid (C00149) 23. Malic acid (C00149) 24. 5-aminovaleric acid (C00431) 25. Phenylethylamine (C05332) 26. γ -aminobutyric acid (C00334) 27. Picolinic acid (C10164) 28. Citraconic acid (C02226) 29. Arabinose (C00259) 30. 5,6-dihydrouracil (C00429) 31. Galactinol (C01235)	• Carbohydrate metabolism • Lipid metabolism • Amino acid metabolism • Nucleotide metabolism

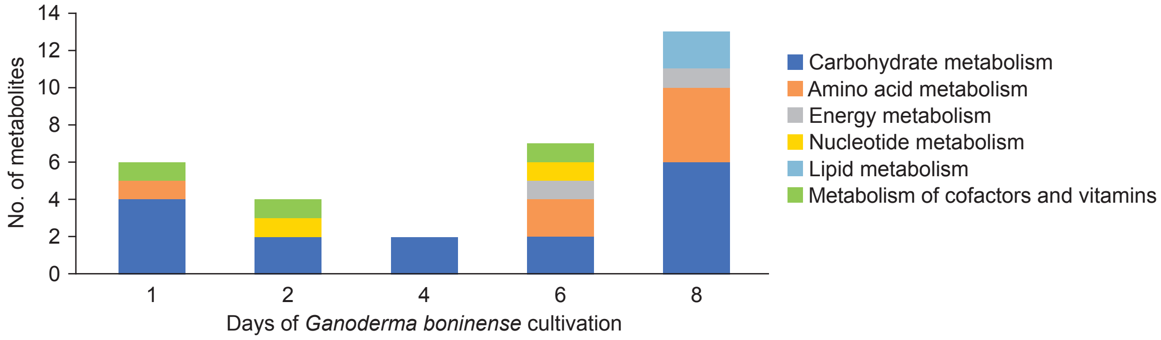

Figure 4. The specific KEGG pathways associated with the metabolites expressed during Ganoderma boninense *cultivation.*

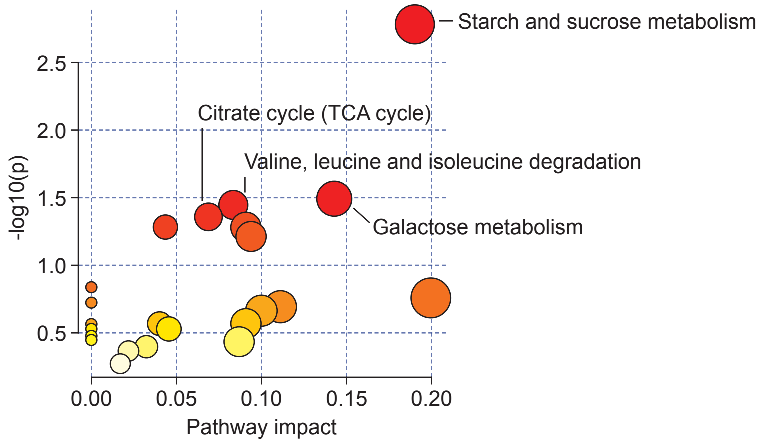

Figure 5. Pathway analysis plot that represents the pathway impact values (x-axis) and p-*value from the enrichment analysis (y-axis). The x-axis represents the pathway impact values computed from the pathway topological analysis, and the y-axis is the -log of the* p-*value obtained from the pathway enrichment analysis. Larger circles represent nodes with greater pathway enrichment and darker colors represent more significance.*

TABLE 2. OVERVIEW OF KEY METABOLIC PATHWAYS IN *Ganoderma boninense*

No.	Metabolic pathway	Putative matched metabolite	*p*-value	FDR	Pathway impact
1.	Starch and sucrose metabolism	Sucrose (C00089[a]) D-Glucose (C00031) Isomaltose (C00252)	0.0016	0.1203	0.1905
2.	Galactose metabolism	Sucrose (C00089) D-Glucose (C00031)	0.0322	0.6340	0.1429
3.	Valine, leucine and isoleucine degradation	L-Valine (C00183) Methylmalonic acid (C02170)	0.0360	0.6340	0.0833
4.	Citrate cycle (TCA cycle)	Malic acid (C00149) Fumaric acid (C00122)	0.0437	0.6340	0.0690

Note: [a] Letters in parentheses indicate the KEGG ID.

Primary metabolism, i.e. carbohydrate and amino acid metabolisms in fungi plays a vital role in biochemical processes required for growth, energy production, and cellular maintenance. Carbohydrate metabolism in *G. boninense*, including starch and sucrose metabolism, galactose metabolism, and citrate cycle (TCA cycle) exhibit enriched metabolic activities. This observation highlights the response of *G. boninense* to the availability of carbohydrates as a source of nutrients, supplying the energy and essential coenzymes needed to sustain growth and reproduction (Wisecaver et al., 2014; Zhou et al., 2021). Furthermore, the presence of key metabolites, i.e., sucrose, glucose, malic acid and fumaric acid in these metabolic activities, play important roles in energy production and maintenance of the cellular metabolism of *G. boninense* (Chroumpi et al., 2020). As reported by Li et al. (2024), a monosaccharide namely, glucose is consumed by microbes during the early and middle stages of growth. The upregulation of the carbohydrate metabolism pathway was also observed in a previous study by

Nagappan et al. (2024), highlighting *G. boninense*'s ability to degrade polysaccharides found in the oil palm cell walls. Being a hemibiotrophic fungus, this finding suggests the key importance of carbohydrate metabolism in *G. boninense*'s survival and pathogenicity, which may support its early biotrophic lifestyle in accessing nutrients (Chong et al., 2017).

G. boninense also exhibits enriched metabolic activities in the amino acid metabolism pathway, i.e. valine, leucine and isoleucine degradation. The alteration in this metabolism emphasises the crucial role of L-valine and methylmalonic acid in this pathway, facilitating nutrient absorption and balance, producing the building blocks of cells, and providing the energy necessary for life-sustaining functions (Borin & Oliveira, 2022). Moreover, this pathway linked to the TCA cycle, serves as an alternative route for energy generation, especially when primary carbon sources are limited (Berger et al., 2007). This observation suggests that as the nutrient levels decline, *G. boninense* may enter the growth stage where its primary metabolism shifts towards sustaining cell survival.

CONCLUSION

Although the biological interpretation of metabolites, which are involved in multiple roles within a biological system, remains challenging, our findings demonstrated utilising the pathway analysis tools, i.e. KEGG Mapper and pathway analysis in MetaboAnalyst, which enhance the visualisation and interpretation of metabolomics data in relation to biological processes. As in the context of *G. boninense*, several metabolic pathways, i.e. starch and sucrose metabolism, galactose metabolism, valine, leucine and isoleucine degradation and TCA cycle were found significantly enriched during cultivation. The pathway analysis tools enhanced biological interpretation, offering deeper insights into the dynamics of *in vitro* metabolite production, and temporal primary metabolic pathways associated with *G. boninense* development, energy production and cellular functions over time. These findings highlight the crucial role of pathway analysis in metabolomics workflow, revealing hidden biological insights within the complex metabolite profiles and advancing our understanding of *G. boninense* developmental processes.

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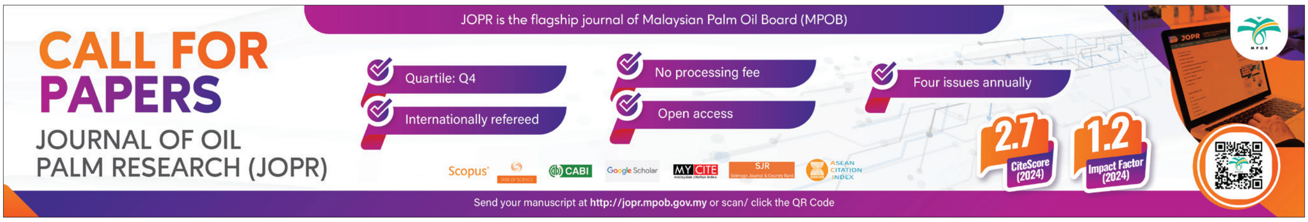

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