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Mechanising Oil Palm Loose Fruits Collection – A Review

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MECHANISING OIL PALM LOOSE FRUITS COLLECTION – A REVIEW

MOHD RAMDHAN KHALID*; ABD RAHIM SHUIB* and NORMAN KAMARUDIN*

ABSTRACT

The presence of oil palm loose fruits (LF) on the ground is one of the indicators that the fresh fruit bunch (FFB) is ready to be harvested. LF are also present when the bunch falls to the ground during the cutting operation, and these fruits need to be collected together to maximise the oil content during processing. Even after a century since this crop was first planted commercially in Malaysia, no major changes have been made in terms of how LF are being collected in the plantation. The collection is done manually by hand picking or by using a raking device and the LF are eventually placed into a bag or directly into a container or trailer. This activity involves frequent bending movement which causes backache to the worker. To minimise this problem and to increase the collection productivity, various tools and machines, from using a simple mechanical picking mechanism to vacuum-type collecting machines were developed. Recently, the focus was concentrated towards the unmanned collection concept. This article reviews most of the developing technologies related to mechanised oil palm LF collection and their technical limitations. Design, working system and cost considerations for the future development of LF collecting machine are also described.

Keywords: oil palm, loose fruits, manual collection, mechanised collection, cost consideration.

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INTRODUCTION

Oil palm planted area in Malaysia has reached 5.85 million hectares since it was first planted as a commercial crop in 1917 (Kushairi *et al.*, 2019). The RM 67.74 billion industry is still relying extensively on foreign labour especially in the plantation sector. Mechanising field operation seems to be the best approach to minimise foreign labour dependency by providing greater output per hectare. However, the acceptance and the implementation of mechanisation in the industry can still be considered very low. Azman *et al.* (2017) rated the level of mechanisation in the oil palm plantation at 1.9% for the harvesting and 16.2% for the in-field collection operations. Workers remain using either the manual or semi-mechanised tools. To overcome these issues, improvement on the human-machine connection is required during product design and product manufacturers need to play a role to disseminate information and to provide training to the workers (Nur Syazwani *et al.*, 2015).

Two areas needing major changes in terms of technology adoption are harvesting and loose fruits (LF) collection. The introduction of a motorised cutter (Cantas) can be considered as a breakthrough in mechanising harvesting operation (Abdul Razak *et al.*, 2008). However, it is limited by height (less than 5 m palm height). Numerous on-going studies are being conducted by the Malaysian Palm Oil Board (MPOB) to increase the reachable height and its performance. Nevertheless, for LF collection, the industry is still counting on manual method although several technologies were introduced by research institutes and universities, together with trials conducted by the industry itself.

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LOOSE FRUITS

LF generally have the highest oil content than most of the attached fruits in the bunch. The presence of LF in the field are from two sources: first, those naturally detached when the fresh fruit bunch (FFB) is ripe and ready to be harvested, and second, those that are scattered on the ground during the harvesting activity. It is a common practice that when there are 1-10 LF on the ground, the cutting activity can be performed immediately as the bunch is already ripe. A study by Azali *et al.* (2015) revealed that during harvesting, the majority of LF are scattered in a radius of 1.5-2.5 m from the FFB impact point.

Depending on their variety and palm age, the weight of the FFB ranges between 10-40 kg and the fruitlet ranges from 6-20 g (Razali *et al.*, 2012). The average length, width and thickness of fruitlets vary from 30-35 mm, 20-27 mm and 17-24 mm, respectively (Owolarafe *et al.*, 2006; Mohd Hafiz and Abdul Rashid, 2011; Renel *et al.*, 2013). They also stated that oil palm fruit has about 70%-80% of sphericity which indicates that the fruitlet has a spherical shape.

Years ago, LF were neglected by plantations, being left in the field due to the perception of their small size, hence, not worth to be collected. Nowadays, thanks to intensive research works, uncollected LF is considered as losses to the oil palm industry due to its maximum level of oil content. The majority of the detached LF are from the outer diameter of the bunch. The fruits are huge and contain the highest oil. Studies showed that fruits from the outer diameter of a bunch contribute up to 40% of palm oil compared to the middle and inner regions of spikelet, *i.e.* 35% and 29% (Henson, 2012). Hence, there will be a significant reduction in oil extraction rate (OER) if LF are not collected and processed. A study by Gan *et al.* (1993) indicated that if 20 LF per bunch are not collected, there will be a reduction on OER by 0.92%, 0.46% and 0.37% for palm aged 1-5 years, 6-15 years and above 15 years (years after the first harvesting), respectively.

Many plantations are now realising this fact and efforts are geared towards ensuring that all LF are collected from the field to maximise their profits. Sime Darby Plantation Sdn Bhd reported that the company would make an additional RM 30 million in net profit if estate workers collect six LF per palm (Seedlink, 2008). Unfortunately, studies by Afroza *et al.* (2015) and Nurul Fadilah *et al.* (2019) revealed that there are still between 10%-30% of LF which are not being collected by the independent smallholders. The uncollected LF will eventually grow and turn into weeds or volunteer oil palm seedlings (VOPS) (Maizatul-Suriza and Idris, 2012). There are also possibilities that the VOPS will be taken by irresponsible individuals, to be sold as planting materials. It is, therefore, vital that LF are fully collected. The typical LF collection system can be summarised as in *Figure 1*.

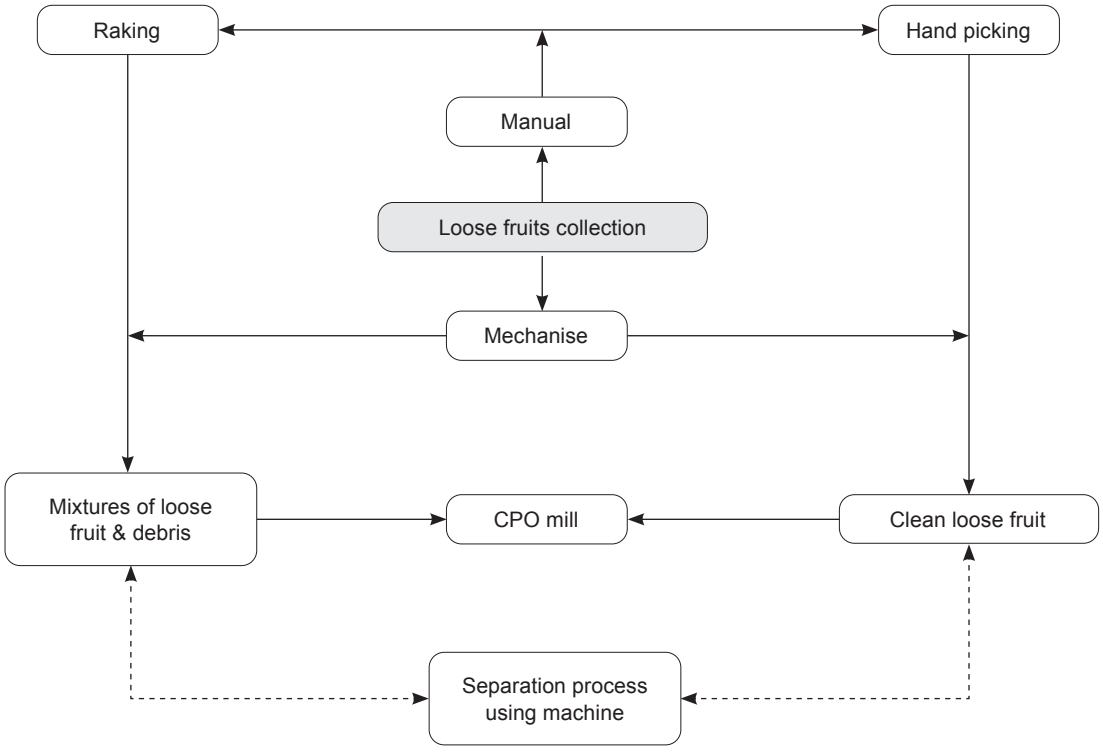


Figure 1. Flow chart of loose fruit collection system.

Manual Collection of Loose Fruit

LF must be collected and gathered with other bunches. Currently, conventional methods are being widely practised in plantations with the productivity ranging from 200-250 kg per worker per day (Abd Rahim *et al.*, 2018). LF are normally picked up by hand and collected in a bucket or bag. Besides that, there are also different options like using rakes, planks and scoops (*Figure 2*). It is estimated that the time taken to collect LF is about 30%-60% of the total FFB handling time (Mohd Zohadie, 1992; Henson, 2012; Nizaroyani Saibani *et al.*, 2015).

Depending on the estate's standard practice, the collection of LF is done either by the harvesters themselves or by a separate group of female workers (Martin *et al.*, 2016). To accomplish the job, the worker has to frequently bend down to collect the scattered LF and move from palm to palm. Although this activity is performed within a short period, it is however being repeated daily, which indirectly promotes an incorrect body posture (Ng *et al.*, 2013; Ezrin *et al.*, 2016). The worker is prone to fatigue and back pain which may lead to poor productivity (Nur Syazwani *et al.*, 2016).

The presence of debris that accumulate together with LF during collection is one of major problems. A study by Darius and Fairulnizam (2014) revealed that a manual method of using sack or wheelbarrow, about 27%-38% debris was accumulated within the collected LF. These trashes are potential to contribute for lower OER as the trash will absorb oil during processing. Hence, there is a need to remove the debris before the LF are sent for processing. Several potential cleaning or segregating machines are available for the industry (Ahmad Zamri and Mohd Zulfahmi, 2017; Gemka, 2017; Mohd Ramdhan and Abd Rahim, 2017).

Mechanised Methods

A practical and cost-effective mechanised LF collection system is still one of the primary targets of the oil palm industry. There is a need to increase the efficiency of the LF collection but at the same time, the cost is kept at a minimum. Efforts to lighten and improve the method of collecting LF were initiated in the late 1980s as conducted by Muhammad Salih and Razak (1988). Abd Rahim *et al.* (2011) reported that several designs to collect LF have been invented, but most have never been commercialised due to the various technical limitations and constraints.

Two approaches were tested to assist LF collection activity, *i.e.* mechanical and suction systems. Both of these approaches have their advantages and disadvantages, which will be discussed.

Mechanical type. Among the earliest works on mechanical LF collecting devices were invented by students from the Universiti Putra Malaysia (UPM), Selangor, Malaysia which employed the sweeping or brushing mechanism and discs as the collecting device (Rimfiel and Abadanjumi, 2007). They further concluded that the development of a chain and rake type oil palm LF collector was found satisfactory in picking LF on uneven ground and grass area.

Later, realising the need to come up with different approaches, other researchers have initiated student's projects related to the mechanical type collectors such as studies by Muhammad Azam (2011) and Muhamad Khairul (2013) by using tiny spike or nail type collector (*Figure 3*). Even though these devices can collect LF, however they are less preferred by the industry as they tend to bruise the fruits. High level of bruised fruits will lead to an increase of free fatty acid (FFA) during storage (Fatin *et al.*, 2014; Hadi *et al.*, 2009).

Figure 2. A typical body posture of worker using basket and scoop for collecting loose fruits.

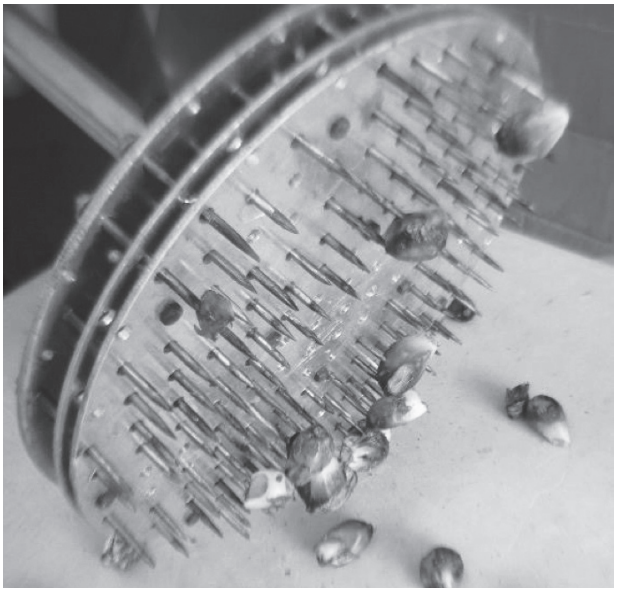


Figure 3. Nail type collector.

A utility innovation by Zainuddin (2014) revealed that instead of using nails to punch into the LF, the invention used flexible 'fingers' to entrap the LF between the 'fingers'. The entrapped LF were then ejected using a spring mechanism attached to a handle. A similar concept of a mechanical LF collector is the segmented multiple wheels, with each wheel having many synthetic rubber tines that can pick the oil palm fruit during rolling motion as developed by Ahmad Zamri *et al.* (2016). The device can be manually operated or motorised and with this type of mechanism, it does not bruise the fruits (*Figure 4)*. A comparable concept was developed earlier by Mohd Amir (2009) that utilised the deflecting 'fingers' concept to collect LF in rolling motion. Again, the ability to be operated on uneven terrain and how much productivity gained still need to be further studied in the real oil palm environment.

Another method is an adaptation of the nut collector principle for oil palm LF collection, comprising of a roller-type, cage mechanism to collect LF. The oval-shaped cage is made of several flexible rods that will split open once being pushed towards the LF. Once the LF is entrapped in the cage, the rods will return to their normal position, hence, leaving only clean LF inside. It was claimed that around 30-60 kg hr^{-1} of LF can be collected using this device (Mohd Solah *et al.*, 2009). However, based on observations, the roller picker was found not effective for use in soft and wet soils as the LF will tend to further sink into the soil rather than going into the cage.

Some limitations on the mechanical collectors in oil palm plantations are the terrain condition, the vegetation and the various residues at palm circle (Rimfiel and Abadanjumi, 2007). Hence, for effective LF collection, the field must be well prepared to accommodate any machines or equipment.

Although the mechanical type collectors offer a simple and low-cost solution, the total productivity is still uncertain. Furthermore, at one collection point, a longer time is required to collect the scattered LF, with limited amount of LF collected during a single roll. An effective device should be able to overcome all these conditions. If successful, smallholders and small-medium plantations would probably be the main users for this type of collection. Some commonly available mechanical type LF collectors are summarised in *Table 1*.

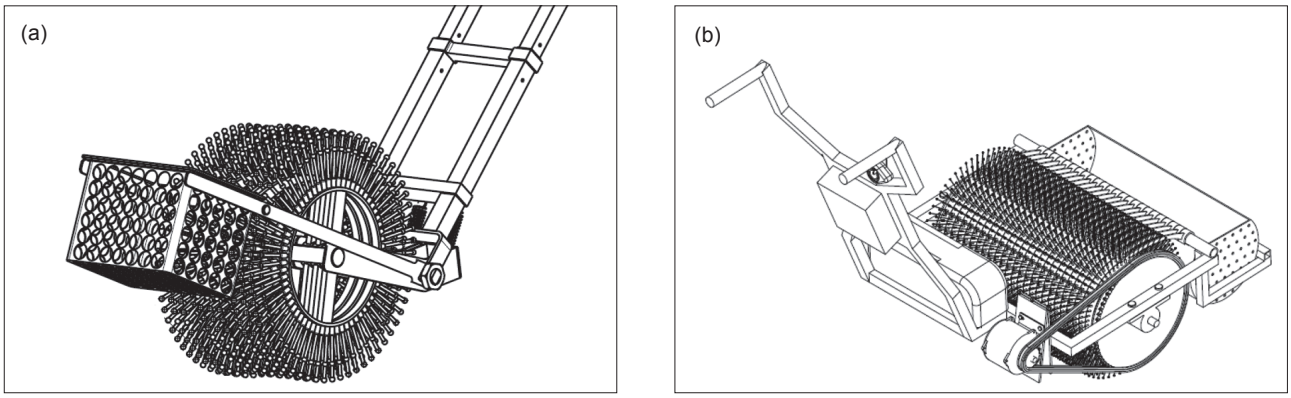


Figure 4. Roller type with flexible 'finger' collector: (a) manual and (b) motorised.

TABLE 1. SUMMARY OF AVAILABLE MECHANICAL TYPE LOOSE FRUITS (LF) COLLECTING CONCEPTS

Source	Collecting concept/technique	Operation methods	Productivity (kg min^{-1})	Cost indicator*
Rimfiel and Abadanjumi (2007)	Chain and rake	Forward/backward push	n.a.	Medium
Mohd Solah *et al.* (2009)	Rotating cage	Roll	1 - 6	Low
Zainuddin (2014)	Nail	Downward push	2	Low
Muhammad Azam (2011)	Nail	Downward push	n.a.	Low
Muhamad Khairul (2013)	Nail	Downward push	n.a.	Low
Mohd Amir (2009)	Segmented wheels with tines	Forward/backward push	n.a.	Medium
Ahmad Zamri *et al.* (2016)	Segmented wheels with tines	Forward/backward push	n.a.	Medium
Mohd Izmer (2018)	Rotating brushes and screw type conveyor	Tractor mounted	5-6	High

Note: *Low <RM 10 000; RM 10 000 <Medium <RM 50 000; High >RM 50 000 (including prime mover).
n.a - not available.

One of the promising machines that is currently available in the market is the tractor-mounted LF collecting machine known as Scavenger 3 which employs a rotating brush at the rear of a mini tractor to sweep the LF into its container (Mohd Izmer, 2018). It also has a cleaning mechanism where the collected LF will be conveyed into a series of parallel rods that will filter the debris. With a properly working system, it was claimed that the machine is able to collect LF up to 5 million tonnes per day with debris content of less than 5%.

Suction type. A typical LF collecting machine uses an air blower to create a partial vacuum to 'suck up' LF from the ground. Similar to a vacuum cleaner, the machine utilises the same principle where a vacuum suction is created by making pressure differential between the inside and outside of the machine. The LF entering the hose are being pushed into the machine by the outer atmospheric pressure when the blower reduces the pressure inside the barrel. There are two types of vacuum system which are the venturi (open system) and the closed vacuum system (*Figure 5*). Both systems were tried by numerous researchers to collect the LF and *Table 2* shows the comparison between the two systems.

Among the earliest LF collection machines is the one developed by Ahmad *et al.* (1995) in a joint collaboration between MPOB and UPM. The machine uses a wheelbarrow frame to place two containers; one for LF, while the other is for trash. Using petrol engine as a power source, LF are sucked up through a flexible nozzle. The LF together with trashes go through a separation box, where the heavier LF go into the LF container while the lighter trash goes into the trash container. This machine, on average is capable of collecting 300-400 kg per day of clean LF, with less than 10% trash. However, the need to be pushed from palm to palm and limited storage capacity are the weaknesses of this machine.

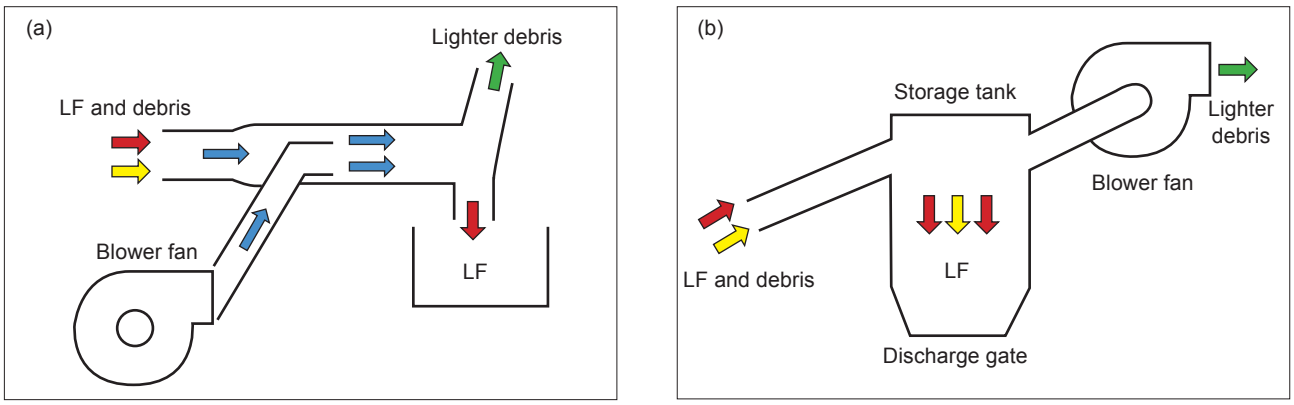
TABLE 2. VENTURI TYPE *vs.* CLOSED VACUUM

Suction system	Advantages	Disadvantages
Venturi	• Loose fruits (LF) can be directly transferred into a bin or container.	• The kinetic energy per volume of air is low, hence, less efficient.
	• No injuries to the LF as no direct contact with the fan.	• Higher blower rate is needed thus increasing power consumption.
Closed vacuum	• Higher suction can be generated.	• Maintenance issue – *e.g.* need access to the suction tank. • Mud could easily coat in the suction tank and hoses.

Improvements were made later by Ahmad Zamri and Ahmad (1999) where the suction mechanism was mounted to a three-wheeled vehicle. It was able to collect and carry LF from palm to palm, suitable for uneven and terraced ground, easy to operate and can be handled by just one person. The use of this machine minimised damage to the LF, but the percentage of damage was not stated. This ride-on type machine was capable of collecting LF between 40-60 kg hr^{-1} with a single operator. The only drawbacks were the dirt's clogging issues especially during wet condition and limited storage capacity.

Patent owned by Anthuan (1996) claimed that his invention uses the suction power of a vacuum mechanism to pick up loose fruitlets with ease. It is powered by the power take-off (PTO) from the tractor that also tows the entire assembly. It is also equipped with a rotating separator drum to separate the debris from the LF.

Several attempts have also been conducted by students at UPM where they have developed and tested different suction methods to collect LF (Rimfiel and Abadanjumi, 2007).



Note: LF - loose fruits.

Figure 5. A typical concept of venturi suction (a) and closed vacuum system (b).

An upgrade version of LF collecting machine that utilises vacuum cyclone concept was developed by Abd Rahim *et al.* (2012). Using the advantages of cyclone in the barrel, the machine is able to separate debris, hence producing clean LF at the bottom of the barrel. With the capability to collect LF around 4.2-5.1 kg min^{-1}, this machine is capable of collecting 1200-1500 kg of clean LF in a day with less than 15% debris. Abd Rahim *et al.* (2018) later made an improvement by integrating the elevated discharge mechanism to enable the collected LF to be discharged directly into a bin or lorry.

An integrated LF collecting machine was developed by Mohd Zulfahmi *et al.* (2020) with a combination of vacuum, double layer rotating drums and a tipping bucket. Mounted to a three-wheel transporter, it was found that the output was around 600-1000 kg clean LF in a day with a total operation cost of between RM 73.90-RM 113 per day. Additionally, it was claimed that the debris content was around 5%-10% by weight and with the tipping ability, the clean LF was able to be transferred into a 3 t bin once the bucket was full.

Based on the above inputs and *Table 3*, it is clear that the suction method is the best option for the plantation industry, because of its greater output. A study by Mohd Ramdhan *et al.* (2019) found that the minimum air velocity that is required to lift up a single loose fruit is approximately 22.4 m s^{-1} or airflow of 0.21 m^3 s^{-1}. However, to collect groups of LF effectively, airflow of between 0.28-0.33 m^3 s^{-1} (air velocity of 30-35 m s^{-1}) is required. Hence, more works need to be done to further improve the technology so that it can be commercially used by the industry.

Cost Considerations

Before introducing a machine to the market, the inventors or machine manufacturers need to understand the situation and expectations from the industry. One of the important factors that need to be emphasised is the working system. It is agreed that collecting individual LF scattered around the palm base is inadequate to improve collection productivity. Therefore, there is a need to improve the system by using a separate gang consisting of three persons (one driver, one collector and one helper to heap the LF). The LF scattered on the ground are first gathered into piles by one dedicated person before the machine performs its task. In this way, however, more workers are needed, making it less appealing to estate management. Therefore, the productivity of the total system must be greater than the manual method to make it economically viable.

Apart from the selling price, the output of the machine is also crucial to be cost-effective. Hence, an estimation of cost per tonne with varying machine costs and productivity was developed as shown in *Table 4*.

Manual labour cost with assumptions of:
Average productivity: 20 bags per day (20 kg per bag)
Labour cost : RM 2.50 per bag
Labour cost : RM 50 per man per day or RM 150 per day for three workers

To calculate the operational cost of machine, straight line depreciation is used with assumptions of:

Machine's economic life: 5 years
25 working days per month
Labour cost : RM 50 per man per day or RM 150 per day for three workers
Fuel cost : RM 15 per litre per day @RM 2.20 per litre
: RM 33 per day
Repair and maintenance cost: RM 30 per day

Hence, to introduce LF collecting machine in the oil palm plantation, the machine must be able to produce at least 600 kg LF per man per day or 1.8 t per day for three workers to be economical (below RM 150 per day). This means that productivity must be at least three folds than the manual method.

TABLE 3. SUMMARY OF AVAILABLE VACUUM TYPE LOOSE FRUITS (LF) COLLECTING CONCEPTS

Source	Collecting concept/technique	Prime mover	Productivity (kg min^{-1})	Cost indicator*
Ahmad *et al.* (1995)	Direct suction	Wheelbarrow	1-1.2	Medium
Anthuan (1996)	Direct suction with rotating separator	Tractor	n.a	High
Ahmad Zamri and Ahmad (1999)	Direct suction	3-wheeler	1.13	Medium
Abd Rahim *et al.* (2012)	Direct suction with cyclone	Tractor or 3-wheeler	4.2 – 5.1	High
Mohd Zulfahmi *et al.* (2020)	Direct suction with rotating separator	3-wheeler	1.3 – 2.1	Medium

Note: *Low <RM 10 000; RM 10 000<Medium<RM 50 000; High >RM 50 000 (including prime mover).

TABLE 4. ESTIMATED TOTAL OPERATION COST (R t^{-1*}) AT VARYING MACHINE COST PER UNIT AND PRODUCTIVITY

Machine cost (RM)	Estimated productivity (kg per man per day)			
	200	400	600	800
20 000	377.22	188.61	125.74	94.31
30 000	388.33	194.17	129.44	97.08
40 000	399.44	199.72	133.15	99.86
50 000	410.56	205.28	136.85	102.64
60 000	421.67	210.83	140.56	105.42
70 000	432.78	216.39	144.26	108.19

Note: * Using three workers.

FUTURE DESIGN CONSIDERATIONS

The design criteria of suction type LF collectors, the machine must have, among others:

a) The ability to collect at least 600 kg per man per day as a new benchmark.
b) Maximum of three persons to run the system but preferably a single operator. The operator does not need to move from his seat to collect the LF.
c) Having adequate temporary storage capacity (at least able to cover four rows of palm before the compartment is fully loaded with LF).
d) Operated with a minimum cost of materials and maintenance and has a high commercial value.
e) Suited to various types of ground conditions.
f) Ergonomically designed and easy to move along the collection areas in the field.
g) Does not physically injure or damage the collected LF.
h) Having the capability of isolating trash and foreign materials is an advantage to the system.
i) User-friendly, where the operator does not need special skills to operate the machine.

A study indicates that by using the harvesting machine, the amount of detached LF produced during harvesting was reduced by 45% compared to manual harvesting as the bunch does not hit the ground (Mohd Ramdhan and Rahim, 2014). Perhaps, alternatively, inventors and users should now consider eliminating the task of LF collection by preventing the bunch from falling and hit the ground. Such work has already begun with the development of bunch catcher machine by Azali *et al.* (2015) where the harvested FFB and LF will drop into the catchment head and then later dropped down into a bucket. Harvesting machine that incorporates the catching mechanism such as grapple to hold the bunch after cutting should be the way forward. This concept of cut and catch has been developed by Abd Rahim *et al.* (2005; 2010) to minimise the amount of LF during the harvesting process.

Works on exploring the potential of robotic technology to be integrated with LF collecting machine have also been initiated. The possibility of having day and night operation with fewer workers is an interesting opportunity. The presence of open source with affordable cost microcontroller in today's markets has paved the way for an innovative and cost-effective breakthrough.

Most of agricultural autonomous robotics research has been performed in controlled environments such as for fruits picking (Reid *et al.*, 2001; Van Henten *et al.*, 2003; Scarfe *et al.*, 2009). These robots were designed using either vision, global positioning system (GPS), laser and sensor-based navigation control system (Jayantha *et al.*, 2005) and integrated with the manipulators to perform their functions (Christoph *et al.*, 2014). Hence, to suit the concept of autonomous robotic in oil palm plantation environment, more studies need to be conducted.

Siti Nur Amalina *et al.* (2018) has explored the concept of having incorporated automation features, which involved the elastic cage auto feeding and on a tracking system to track the motion of the loose fruit collector. Nevertheless, this work is still in the early stage.

A robot was developed by Muzakkir and Afandi (2016), with the combination of an embedded platform (Arduino Mega) with robotic mechanism, which revealed convincing results, in terms of its movement, quantity and the quality of the collections as well as stability. Although these works can be considered as still in the infancy stage, the efforts need to be supported. However, we are of the opinion that the highest priority right now is to design an effective collecting mechanism first. Once achieved and proven to work well, this mechanism can be further integrated with available robotic technologies to further enhance its capability.

Another area that is worth exploring is the self-propelled harvester for collecting (harvest) shell fruits such as hazelnuts, chestnuts, almonds, walnuts, olives, coffee, *etc.* Few machines were successfully developed and marketed by foreign companies. As for example, Facma Srl, an Italian company, has produced a vacuum collecting machine with rotating sweepers (*Figure 6*). These sweepers could collect and gather the fruits before being sucked by vacuum into the rear container. With some modifications, this type of machine can be used to collect LF with proper field preparation (Mohd Ramdhan, 2018).

Additionally, various types of unmanned tracked prime mover are available in the overseas market. These transporters have the intelligent system installed where the machine can be remotely operated (*Figure 7*). Having navigation and camera system, this labour-saving technology has the potential to be utilised in oil palm plantation for various operations from collecting LF to field maintenance activities.

Figure 6. The self-propelled harvester for collecting (harvest) shell fruits.

Figure 7. The remote-controlled tool carrier is ideal for slope areas that present the risk of overturning the vehicle with a man on board, or in areas impenetrable by other means.

CONCLUSION

Conventionally, the technique for collecting LF is by the manual method or hand picking whereby the collected LF are transferred into a bucket or bag. This technique is not only labour demanding and time-consuming but is also tedious. A cost-effective loose fruit collection system remains the main requirement of oil palm estates. Currently, there are several types of LF collectors such as roller picker, mechanical LF collector and disc plate collector. However, several design concepts that can be improved in terms of the surface condition during operation, minimum damage towards the fruits, quantity of the LF and cost for the tool. In practice, the method of collections should be suitable to operate in various field conditions. In addition, the operator should be comfortable when operating the implement without incurring back pain. Therefore, the criteria of the new design of the collector should work well in various types of field condition, ergonomic for the operator, leading to higher productivity.

Apart from machine performance, other factors such as working system, payment scheme, machinery incentive and workers and management acceptance are among the key components that need to be carefully structured for successful implementation of the mechanised operation.

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REFERENCES

Abd Rahim, S; Abdul Razak, J; Salmah, J; Mohd Solah, D; Mohd Ramdhan, K; Ahmad, H; Yusof, B and Mohd Basri, W (2005). Development of a machine for harvesting tall palms. *MPOB Information Series No. 224*. palmolis.mpob.gov.my/publication/TOT/TT-217.pdf

Abd Rahim, S; Mohd Ramdhan, K; Mohd Azwan, M B; Mohd Solah, D and Norman, K (2018). Development of oil palm loose fruit collecting machine with elevated discharge mechanism (Mark iii). *Int. J. Engineering Research and Technology (IJERT), Vol. 7 Issue 10*: 225-234.

Abd Rahim, S; Mohd Ramdhan, K; Wan Ishak, W I and Norazman, A (2010). Oil palm mechanical harvester - A technical perspective. *MPOB Technology No. 32*: 1-25.

Abd Rahim, S; Mohd Ramdhan, K and Mohd Solah, D (2011). Chapter 17: Innovation and technologies

for oil palm mechanization. *Further Advances in Oil Palm Research (2000-2010)* (Mohd Basri, W; Choo, Y M and Chan, K W eds.). Vol. 1. MPOB, Bangi. p. 569-597.

Abd Rahim, S; Mohd Ramdhan, K; Mohd Solah, D and Aminulrashid, M (2012). Oil palm loose fruits collecting machine (MK III). *MPOB Information Series No. 592*. palmolis.mpob.gov.my/ publication/TOT/TT-505.pdf

Abdul Razak, J; Ahmad, H; Johari, J; Malik, N; Yosri, G and Omar, A (2008). *Cantas*™ – A tool for the efficient harvesting of oil palm fresh fruit bunches. *J. Oil Palm Res. Vol. 20*: 548-558.

Afroza, K L; Fatimah, M and Rohaya, M H (2015). Determination of the harvesting factors of independent smallholder oil palm estate. *J. Advanced and Applied Sciences (JAAS) 3(4)*: 136-144.

Ahmad, H; Ahmad Zamri, Y and Mohd Salih, J (1995). Loose fruit collector. *PORIM Information Series No. 19*. palmoilis.mpob.gov.my/publications/TOT/TT-19.pdf

Ahmad Zamri, Y and Ahmad, H (1999). Mechanical loose fruit collector (MKII). *PORIM Information Series No. 57*. palmoilis.mpob.gov.my/publications/TOT/TT-57.pdf

Ahmad Zamri, Y and Mohd Zulfahmi, Y (2017). Loose oil palm fruit segregating machine and method of segregating loose oil palm fruits using the same. World Intellectual Property Organisation (WIPO) WO 2017010862 A1.

Ahmad Zamri, Y; Mohd Zulfahmi, Y; Ahmad, J and Shawaluddin T (2016). A motorised device for picking and collecting loose fruits. World Intellectual Property Organisations (WIPO) WO 2016072829A1 and PCT/MY2015/000058.

Anthuan, R (1996). Oil palm loose fruitlets picking machine. Malaysian patent No. MY-96004243.

Azali, A; Syed Salim, S A; Abdul Halim, Z A and Azahah, A (2015). Conceptual of mobile oil palm fresh fruit bunch catcher. *Int. J. Agriculture Innovations, Vol. 4 Issue 6*: 1159-1163.

Azman, I; Siti Mashani, A; Norhidayu, A; Abd Razak, J and Abd Rahim, S (2017). An economic study on mechanisation in oil palm plantations in Malaysia. Palm Oil Economic Review and Outlook Seminar 2017. Pullman Kuala Lumpur City Centre, 17 January 2017.

Christoph Schütz; Julian Pfaff; Jörg Baur; Thomas Buschmann and Heinz Ulbrich (2014). A modular robot system for agricultural applications. *Proc. of the International Conference of Agricultural Engineering, Zurich*. 6-10 July 2014. p. 1-8.

Darius, E P and Muhammad Fairulnizam, H (2014). Effects of collecting systems and plantation environment on debris accumulation in a collected oil palm loose fruits. *Proc. of lnt. Conf. Plt. Phy. 2014*. p. 147-151.

Ezrin Hani Sukadarin; Baba Md Deros; Nur Syazwani Mohd Nawi; Jaharah A Ghani; Ahmad Rasdan Ismail and Junaidah Zakaria (2016). Back pain and the observed factors among oil palm workers. *Int. J. Engineering Technology and Sciences Vol. 5 Issue 1*: 70-78. DOI: http://dx.doi.org/10.15282/ijets.5.2016.1.9.1048

Fatin, S A; Rosnah, S and Yunus, R (2014). Effect of chopping oil palm fruit spikelets on the free fatty acid content release rate and its mechanical properties. *Int. J. Research in Engineering and Technology Vol. 3 Issue 1*: 511-516.

Gan, L T; Ho, C Y; Chiew, J S and Lam, K S (1993). Optimum harvesting standards to maximize labour productivity and oil recovery. *1993 PORIM International Palm Oil Congress - Update and Vision (Agriculture)*. PORIM, Bangi. p. 195-211.

Gemka Sdn Bhd (2017). Loose fruit cleaner. https://www.gemka.com.my/loose-fruit-cleaner, accessed on 24 March 2017.

Hadi, S; Ahmad, D and Akande, F K (2009). Determination of the bruise indexes of oil palm fruits. *J. Food Engineering, 95*: 322-326.

Henson, I E (2012). Ripening, harvesting and transport of oil palm bunches. *Palm Oil*. p. 137-162. DOI: 10.1016/b978-0-9818936-9-3.50008-3.

Jayantha, K; Ray, E; Anthony, C and Craig, M (2005). Automation of an agricultural tractor for fruit picking. *Proc. of the 2005 IEEE International Conference on Robotics and Automation*. Spain. April 2005. p. 3201-3206.

Kushairi, A; Ong-Abdullah, M; Nambiappan, B; Hishamuddin, A; Bidin, M N I Z; Ghazali, R; Subramaniam, V; Sundram, S and Parveez, G K A (2019). Oil palm economic performance in Malaysia and R&D progress in 2018. *J. Oil Palm Res. Vol. 31(2)*: 165-194. DOI: https://doi.org/10.21894/jopr.2020.0026.

Martin, K; Robert, E and Dublin, S (2016). Harvesting and crop recovery: SALCRA's experience. *12th ISP National Seminar 2016*. Kuching, Sarawak. p. 135-147.

Maizatul-Suriza Mohamed and Abu Seman Idris (2012). Occurrence of common weeds in immature plantings of oil palm plantations in Malaysia. *The Planters Vol. 88 No. 1037*: 537-547.

Mohd Amir (2009). An apparatus for picking loose fruits such as oil palm or the likes lying on the ground. Malaysian patent No. MY13777l-A.

Mohd Hafiz, M H and Abdul Rashid, M S (2011). Oil palm physical and optical characteristics from two different planting materials. *Research J. Applied Sciences, Engineering and Technology, 3(9)*: 953-962.

Mohd Izmer, A (2018). Tractor mounted loose fruit collecting implement (Scavenger 3). Paper presented at the Labour and Mechanisation Seminar (LAMP 2018). Hotel Mudzaffar, Melaka. 17 October 2018.

Mohd Ramdhan, K (2018). Report on Visit to the International Agricultural and Gardening Machinery Exhibition (EIMA 2018). 7-11 November 2018. Bologna, Italy. *MPOB Report OP 455*. p. 3-32.

Mohd Ramdhan, K and Abd Rahim, S (2017). Performance of oil palm loose fruits separating machine. *J. Oil Palm Res. Vol. 29(3)*: 358-365.

Mohd Ramdhan, K and Abd Rahim, S (2014). Field evaluation of oil palm mechanical harvesting machines for tall oil palms. *J. Oil Palm Res. Vol. 26(2)*: 125-132.

Mohd Ramdhan, K; Abd Rahim, S and Norman, K (2019). Determination of minimum suction level for collecting oil palm loose fruits. *Proc. of the National Convention of Agricultural and Food Engineering 2019.* Wisma Tani, Putrajaya. 21 March 2019. p. 198-201.

Mohd Solah, D; Abd Rahim, S and Mohd Saleeh, J (2009). Roller-type oil palm loose fruit picker. *MPOB Information Series No. 419*. palmoilis.mpob.gov.my/publications/TOT/TT-419.pdf

Mohd Zohadie, B (1992). Harvesting and transportation of oil palm fresh fruit bunches. *Proc. of the National IRPA Seminar (Agriculture Sector).* Vol. 1. p. 839-840.

Mohd Zulfahmi Mohd Yusoff; Zamri, A; Abd Kadir, M Z A; Wan Hassan, W Z and Azis, N (2020). Development of integrated loose fruit collector machine for oil palm plantations. *Bulletin of Electrical Engineering and Informatics Vol. 9 No. 2*: 500-506.

Muhamad Khairul, S (2013). Design and *Fabrication of Handy Disc Loose Coconut Palm Collector*. Final year student project report. Universiti Malaysia Pahang.

Muhammad Azam, I (2011). *Design and Development of Coconut Palm Collector*. Final Year Student Project Report. Universiti Malaysia Pahang.

Muhammad Salih, J and Razak, J (1988). *Kajian awal terhadap alat memungut buah kelapa sawit terlerai. Seminar Penyelidikan Kejuruteraan Pertanian*. 16-17 November. Serdang: Fakulti Kejuruteraan, UPM.

Muzakkir, M N and Afandi, A (2016). Roller picker robot (ropicot 1.0) for loose fruit collection system. *J. Engineering and Applied Sciences Vol. 11 No. 14*: 8983-8986.

Ng, Y G; Shamsul Bahri, M T; Irwan Syah, M Y; Mori, I and Hashim, Z (2013). Ergonomics observation: Harvesting tasks at oil palm plantation. *J. Occup. Health, 55(5)*: 405-414.

Nizaroyani Saibani; Ariff Azly Muhamed; Mohd Faizal Maliami and Rasyidah Ahmad (2015). Time and motion studies of manual harvesting methods for oil palm fruit bunches: A Malaysian case study. *Jurnal Teknologi (Sciences and Engineering), 74(3)*: 77-83.

Nur Syazwani Mohd Nawi; Baba Md Deros; Mohd Nizam Ab Rahman; Ezrin Hani Sukadarin and Norani Nordin (2016). Malaysian oil palm workers are in pain: Hazards identification and ergonomics related problems. *Malaysian J. Public Health Medicine 2016, Vol. (Suppl. 1)*: 50-57.

Nur Syazwani Mohd Nawi; Baba Md Deros; Mohd Nizam Ab Rahman; Norani Nordin and Ezrin Hani Sukadarin (2015). Mechanized tools and technovation machinery in palm oil plantations: Utilization or rejection? Paper presented at the International Symposium on Advancement in Ergonomics and Safety 2015. Universiti Malaysia Perlis, Perlis. 30 November 2015.

Nurul Fadilah, M N; Er, A C; Rewati, K; Yaakob, H I and Amran, A (2019). Good agricultural practices amongst oil palm smallholders: A case study in Sabah. *Revista de Ciencias Sociales y Humanidades Vol. 4 No. 17*: 347-355.

Owolarafe, K; Olabige, M T and Faborode, M O (2006). Physical and mechanical properties of two varieties of fresh oil palm fruit. *J. Food Engineering, 78(2007)*: 1228-1232.

Razali, H; Halim, A S M A and Roslan, S (2012). A review of crop plant production and ripeness forecasting. *Int. J. Agriculture and Crop Sciences Vol. 4 No. 2*: 54-63.

Renel, M A; Pepito, M B; Delfin, S S and Engelbert, K P (2013). Determination of some physical and mechanical properties of oil palm fruit. *USM R&D J. Vol. 21 No. 2*: 61-70.

Rimfiel, J and Abadanjumi, E (2007). Performance evaluation of a terrain accommodating oil palm loose fruit collector. *Pertanika J. Science and Technology Vol. 15 No. 1*: 15-23.

Reid, J N; Miles, S J; Butler, J; Baldwin, M and Noble, R (2001). Automatic mushroom harvesting development. *J. Agricultural Engineering Research, 78*: 15-23.

Scarfe, A J; Flemmer, R C; Bakker, H H and Flemmer, C L (2009). Development of an autonomous kiwifruit picking robot. *Proc. of the 4th International Conference on Autonomous Robots and Agents*. 10-12 February, New Zealand. p. 380-384.

Seedlink (2008). Loose fruit *vs.* lost income. *Seedlink Vol. 2/6:* 8. A bimonthly publication by Sime Darby Plantation Sdn Bhd, March 2008. Subang Jaya.

Siti Nur Amalina, M S; Muhammad Razif, M; Zulhakim, W; Muhamad Aznan, M and Nur Hanani, Z (2018). Integrating automation in an oil palm loose fruits collector. Paper presented at the 2018 MSAE Conference. Serdang, Selangor, Malaysia. 7-8 February 2018. Paper No: MSAE2018-AMA014. p. 279-283.

Van Henten, E J; Van Tuijl, B A J; Hemming, J; Kornet, J G; Bontsema, J and Van Os, E A (2003). Field test of an autonomous cucumber picking robot. *Biosystems Engineering, 86(3)*: 305-313.

Zainuddin, I (2014). Loose fruit picker. Malaysian patent No. MY152054.

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A RAPID AND EFFICIENT DNA EXTRACTION PROTOCOL FOR *Ganoderma zonatum*, A BASAL AND UPPER STEM ROT PATHOGEN OF OIL PALM IN MALAYSIA

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ABSTRACT

Ganoderma zonatum *is associated with both the basal and upper stem rot diseases in oil palm. Despite the severity of these diseases, there is only limited information on the molecular characteristics of this oil palm pathogen. Most of the studies on* G. zonatum *related to oil palm are focused on the epidemiology and genetic diversity of the organism. In other palm species,* G. zonatum *has also been identified as the causal agent of bud rot disease. To further characterise the organism using molecular techniques, the ability to isolate good quality DNA samples is important. In this study, seven DNA extraction protocols were evaluated and the best protocol, Boehm protocol, had the highest yield of good quality DNA. The protocol was able to yield 208.95 ± 4.52 µg DNA per gram of sample with purities above 1.80 for* $A_{260/280}$ *and 2.0 for* $A_{260/230}$*. This extraction protocol is a rapid and efficient protocol that employs cetyl trimethylammonium bromide (CTAB), sodium dodecyl sulphate (SDS), β-mercaptoethanol and proteinase K in the lysis buffer. The Boehm protocol was further tested on three other* Ganoderma *species found in the oil palm plantations and a medicinal fungus,* G. lucidum*. It was noted that the protocol was efficient, with high yields for* G. zonatum *when compared to the other four species. This is probably due to the fact that extraction protocols for each organism requires specific optimisation to obtain optimal yield and purity. In conclusion, the Boehm protocol was best suited for genomic DNA extraction of* G. zonatum *and found suitable for downstream applications such as PacBio sequencing.*

Keywords: basal stem rot, DNA extraction, *Ganoderma zonatum,* mycelium, upper stem rot.

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INTRODUCTION

Ganoderma zonatum is a fungus that belongs to the phylum Basidiomycota, class Agaricomycetes, order Polyporales and family Ganodermataceae (Steyart, 1967). *Ganoderma zonatum* has been associated with basal stem rot (BSR) disease of oil palm in South-east Asia, a disease that has threatened the Malaysian oil palm industry with an export revenue of RM 65.12 billion (Kushairi *et al*., 2019). It is a well-known fact that BSR in Malaysia, Indonesia and Papua New Guinea (PNG) is caused predominantly by the pathogen *Ganoderma boninense*. Another *Ganoderma*

species that was reported to cause BSR was *G. miniatocinctum,* while *G. tornatum* is a saprophyte that is found in oil palm plantations (Idris, 1999).

Idris (1999) reported the presence of both *G. boninense* and *Ganoderma miniatocinctum* was associated with the presence of *Ganoderma zonatum* in the oil palm plantations. *Ganoderma zonatum* was also reported to cause other diseases such as upper stem rot (USR) disease in Malaysia (Hassan *et al*., 2005; Rakib *et al*., 2014; 2017), Indonesia (Rees *et al*., 2012) and PNG (Pilotti, 2005). Rakib *et al*. (2015) found that *G. zonatum* from USR-infected palms tend to be more aggressive than *G. zonatum* from BSR-infected palms in Malaysia. On the contrary, *G. boninense* from BSR-infected palms were more aggressive than *G. boninense* from USR-infected palms. Here the different degree of aggressiveness was evaluated based on criteria such as area under disease progress curve (AUDPC), epidemic rate, severity of foliar symptoms, disease severity index (DSI), stem bole and primary root necrosis. As an example, the mean DSI recorded for *G. zonatum* of USR-infected palms (6.84%) was significantly higher compared to BSR-infected palms (2.39%) at 12 weeks after infection. In the USA, *G. zonatum* is also a lethal pathogen that causes butt rot in over 60 palm species (Elliott *et al*., 2018) and this is of great concern in Florida. The only way to curb the spread of butt rot is through prevention, as commonly practised in the USA (Elliott and Broschat, 2000).

Recently, Elliott *et al*. (2018) examined the genetic variability of 25 *G. zonatum* isolates infecting 12 different palm species in Florida and an additional 17 more isolates from eight different countries. The DNA were extracted using the Gentra® Puregen® Cell Kit (Qiagen) (Elliott *et al*., 2018). As for *G. zonatum* in BSR (Rakib *et al*., 2017; Wong *et al*., 2012) and USR (Rakib *et al*., 2017) studies in oil palm, the DNA were extracted using DNeasy Mini Plant Kit (Qiagen). Numerous studies have highlighted that cell lysis efficiency, DNA yield and quality depend largely on sample type and protocols used (Gul *et al*., 2017; Nagi *et al*., 2013; Natarajan *et al*., 2016; Perry *et al*., 2014; Umesha *et al*., 2016). Most of the fungi studied to date require modifications to existing extraction protocols to produce good quality and high yield of nucleic acids (Motkova and Vytrasova, 2011; Nagappan *et al*., 2018; Verant *et al*., 2016). It is pertinent to establish the best extraction protocol for each fungus that is studied in depth. Moreover, it would be an added advantage if one DNA extraction protocol is feasible to be used across most fungi species or on closely related species.

It would be beneficial to be able to go a step further to study any organism at the nucleotide level (Choi and Kim, 2017; Li *et al*., 2018). In order for any sequencing project to proceed, such as PacBio sequencing, large amount of good purity DNA is required to meet this requirement. Usage of extraction kit is not suitable as it yields low amount of DNA and is not cost-effective (Pipan *et al*., 2018; Psifidi *et al*., 2015; Umesha *et al*., 2016). Thus, the current study explored and recommend a time efficient and cost-effective DNA extraction protocol of *G. zonatum* found in the oil palm plantations in Malaysia. Here, we presented a rapid and suitable DNA extraction protocol with minimal amount of tissues required to produce high yields of good purity DNA.

MATERIALS AND METHODS

Biological Materials

Ganoderma zonatum, *G. boninense*, *G. miniatocinctum* and *G. tornatum* stocks were obtained from the GanoDROP Unit, Biology Division, Malaysian Palm Oil Board (MPOB), Malaysia. The fruiting body of *G. lucidum* was purchased from the Cepaul Mushroom Nursery, Kajang, Selangor, Malaysia. Actively growing eight day old mycelia were transferred into 250 ml of Potato Dextrose Broth and incubated at 27±1°C for 14 days. The cultures were harvest and washed with sterilised distilled water and frozen in liquid nitrogen prior to DNA extraction.

Genomic DNA Isolation of *Ganoderma* Species

A total of seven protocols were tested for genomic DNA isolation of *G. zonatum*. The first and second protocol used cetyl trimethylammonium bromide (CTAB) (Voigt *et al*., 1999) and sodium dodecyl sulphate (SDS) (Moslem *et al*., 2010) extraction buffers, respectively. The third protocol tested, as described by Kim *et al*. (1997) used polyvinylpyrrolidone (PVP) and SDS, whereby, the subsequent protocol also applied SDS as one of the components in the extraction buffer (Weiland, 1997). The fifth protocol was an online protocol by Vilgalys (2018), whereby he applied a 2 X CTAB extraction buffer. The second last extraction protocol investigated, employed Triton X-100 and SDS as the lysis buffer (Extraction method 1: Van Burik *et al*., 1998). The final protocol employed a combination of CTAB, SDS, β-mercaptoethanol and proteinase K (Boehm, 2004). RNAse was added in all the protocols evaluated. All the protocols explored were described using the respective author's name for easy identification. The quantity and quality of extracted nucleic acids were measured using Nanodrop™ 1000 Spectrophotometer.

Analysis of DNA by Agarose Gel Electrophoresis

The quality and size of DNA was determined on 0.8% (w/v) agarose gel in 1 x TAE buffer (40 mM Tris, 20 mM acetic acid, and 1mM EDTA), which was

precast with 3 µl of EtB 'Out' nucleic acid staining solution (Yeastern Biotech Co., Ltd). Two microlitres (50 ng μl^{-1}) of sample were loaded into each lane. Electrophoresis was performed at 80 V for 40 min and the agarose gel was post-stained with the same staining solution for 10 min. The agarose gel was visualised using the G:BOX Chemi XX9 (Syngene).

Statistical Analysis

All extractions were performed in triplicates to account for variability. One way analysis of variance (ANOVA) was used to determine statistical differences of DNA yield between protocols using a significance level of $P<0.05$. A T-test was used to compare mean DNA yield between two protocols. Data are shown as the mean ± SD.

Polymerase Chain Reaction (PCR) Amplification

To verify the quality of the extracted DNA, the DNA was used as a template in a PCR experiment to amplify a microsatellite region, as described by Merciere *et al*. (2015). The size of DNA was determined on 4.0% (w/v) Super Fine Resolution (SFR) agarose gel in 1 x TAE buffer (40 mM Tris, 20 mM acetic acid, and 1mM EDTA), which was precast with 12.5 µl of EtB 'Out' nucleic acid staining solution (Yeastern Biotech Co., Ltd). Six microlitres of PCR product were loaded into each lane. Electrophoresis was performed at 100 V for 3 hr and the SFR agarose gel was post-stained with the same staining solution for 10 min. The agarose gel was visualised using the G:BOX Chemi XX9 (Syngene).

RESULTS AND DISCUSSION

In the process of identifying the best DNA extraction protocol for *G. zonatum*, seven protocols were evaluated on this pathogen that is associated with BSR, USR and butt rot. Six protocols that were evaluated have been used on fungi from different phylum such as Basidiomycota, Ascomycota, Zygomycota, Deuteromycota and Oomycota. The seventh protocol (Kim protocol) is a simple and rapid protocol used to extract DNA from fruit trees and conifers that have high levels of polyphenols. This protocol was selected as high level of polyphenols have been reported in *Ganoderma lucidum* (Dong *et al*., 2019) and was thought to be suitable for *G. zonatum*. We also included a commonly used protocol, CTAB as a positive control in this study.

The starting mycelial weight used in these seven protocols was 0.1 g and the amount of DNA recovered ranged from ~13 - ~260 µg DNA g^{-1} of sample (*Table 1*). The integrities of the extracted DNA were separated and observed in 0.8% agarose gel (*Figure 1)*. All the seven protocols were able to extract intact high molecular weight genomic DNA. The bands from the Moslem and Kim protocols in lanes 2 and 3, respectively appeared to be brighter with a slight smear, indicating some levels of DNA contamination has occurred. The protocol that produced the highest yield recorded from this study was with the Van Burik protocol that used a combination of two components in the lysis buffer. In this protocol, the application of Triton X-100 and SDS were capable of disrupting the fungal cell wall and membrane wall producing a yield of 259.77 ± 6.67 µg DNA g^{-1} of sample, which is significantly higher ($P<0.05$) compared to the other six protocols. However, the average purity for $A_{260/230}$ was way below the standard absorbance ratio of 2.0 and above. The average absorbance ratio of $A_{260/230}$ measured by Nanodrop was 0.89 ± 0.05, which indicates high levels of polysaccharides detected in the extracted DNA.

The protocols by Kim, Vilgayls, Weiland and Moslem also had high levels of polysaccharide contamination, with low absorbance ratios between 0.26 ± 0.01 to 1.14 ± 0.04 for $A_{260/230}$. These are indications of the presence of contaminant biomolecules. These protocols also had exceptionally low yields, between 12.67 ± 0.03 to 65.17 ± 2.00 µg DNA g^{-1} of sample. The results demonstrated that the lysis buffers employed were not sufficient in breaking down the cell and membrane walls of *G. zonatum*. On the contrary, the Boehm protocol with a mixture of several components such as CTAB, SDS, β-mercaptoethanol and proteinase K in the buffer was able to remove contaminated biomolecules such as proteins, carbohydrates, lipids or other nucleic acids, producing absorbance ratios of 2.14 and 2.22 for $A_{260/280}$ and $A_{260/230}$ respectively. The expected ratios of $A_{260/280}$ and $A_{260/230}$ for 'pure' DNA are commonly within the range of 1.8 - 2.0 and 2.0 - 2.2 (Seo *et al*., 2011; Lucena-Aguilar *et al*., 2016). It was notable that this protocol produced a yield of 208.95 ± 4.52 µg DNA g^{-1} of sample, which was only a reduced yield of ~50 µg DNA g^{-1} of sample when compared to the van Burik protocol. The combination of components in this buffer were extremely efficient in the lysis of cell wall and cell membrane of *G. zonatum*. Due to the anionic and cationic conditions of SDS and CTAB, these buffers work well with each other in solubilising proteins and lipids (Tripathy *et al*., 2017). Generally, *Ganoderma* species contains β-glucans as the major active polysaccharides (Obodai *et al*., 2017) and are rich in phenolic compounds from fruiting bodies and mycelia (Mishra *et al*., 2018). The inclusion of β-mercaptoethanol with SDS and CTAB aided in the breaking of disulfide bonds between the cysteine residue present in the crude extract (Wingfield, 2001; Winther and Thorpe, 2014). Furthermore, β-mercaptoethanol is an antioxidant that is commonly used to address issues related to

phenolics (Calderon-Cortes *et al*., 2010; Sahu *et al*., 2012). Meanwhile, proteinase K which works better in the presence of SDS, inactivates DNases and leaves the DNA complete and intact. This suggests that the DNA extracted by the Boehm protocol was able to produce good quality DNA for *G. zonatum*. The Boehm protocol was reported by Gracia *et al*. (2014), where it was applied as a modification to the existing Moller protocol to produce good quality DNA from two fungal pathogens of alfalfa, *Phoma medicaginis* and *Colletotrichum trifolii* that was suitable for internal transcribe spacer sequencing.

The commonly used Voigt (CTAB) protocol also produced good quality DNA in terms of absorbance ratio of $A_{260/280}$ and $A_{260/230}$ (*Table 1*). Nevertheless, the yield was 2.4x lower with 87.32 ± 5.21 µg DNA g^{-1} of sample when compared to the Boehm protocol. Albeit, the Voigt protocol is still viable but the drawback to this protocol is that additional material is required to achieve higher yields of DNA and it is labour intensive. The duration required for the Voight protocol is two days, in contrast to the Boehm protocol which takes approximately 4 hr from the beginning of the process until DNA is ready for use. Time is of the essence especially when a large number of samples are needed to be tested or sequenced.

To further assess the reliability of the Boehm extraction protocol and attest on the viability of one protocol used across closely related fungi species, the three *Ganoderma* species that were found in oil palm plantations and one reference species, *G. lucidum* were evaluated. It was noted that *G. boninense*, *G. miniatocinctum*, *G. tornatum* and *G. lucidum* produced rather low yields compared to *G. zonatum*, ranging from 33.40 ± 1.25 to 89.83 ± 1.26 µg DNA g^{-1} of sample (*Table 2*). Nevertheless, the *G. miniatocinctum* DNA was of good quality, with absorbance ratios of 2.04 ± 0.02 and 2.00 ± 0.02 for $A_{260/280}$ and $A_{260/230}$, suggesting that the sample is free from proteins and polysaccharide compounds. In *Figure 2*, *G. boninense* and *G. lucidum* are shown to have bands with higher intensity compared to the other *Ganoderma* species. This could be due to the presence of carbohydrates, lipids, salts or phenol that absorb strongly at 230 nm (Lucena-Aguilar *et al*., 2016). The results correlate well with the $A_{260/230}$ absorbance ratio as presented in *Table 2*. Kuhad *et al*. (2004) concluded that the selection of extraction buffers such as CTAB or SDS is fungus-specific when tested with six species of basidiomycetes. Hence, with further optimisation, the Boehm protocol could be suitable for use as a standard extraction protocol for closely related species though the yield produced is low.

TABLE 1. GENOMIC DNA YIELD AND QUALITY OF *G. zonatum* EXTRACTED FOLLOWING SEVEN PROTOCOLS

Protocols	Duration	Parameters	Replicate 1	Replicate 2	Replicate 3	Average
Voigt	~2 days	A260/280	1.90	1.92	1.91	1.91 ± 0.01
		A260/230	1.93	1.77	1.85	1.85 ± 0.08
		µg DNA g^{-1} sample	81.50	91.55	88.90	87.32 ± 5.21[a]
Moslem	~2 hr	A260/280	1.75	1.71	1.76	1.74 ± 0.03
		A260/230	1.18	1.10	1.15	1.14 ± 0.04
		µg DNA g^{-1} sample	49.59	53.28	50.49	51.12 ± 1.92[b]
Kim	~4 hr	A260/280	1.11	1.09	1.12	1.11 ± 0.02
		A260/230	0.27	0.26	0.25	0.26 ± 0.01
		µg DNA g^{-1} sample	12.69	12.63	12.69	12.67 ± 0.03[c]
Weiland	~3 hr	A260/280	1.65	1.64	1.65	1.65 ± 0.01
		A260/230	1.14	1.13	1.12	1.13 ± 0.01
		µg DNA g^{-1} sample	58.55	58.40	59.20	58.72 ± 0.43[d]
Vilgalys	~2 days	A260/280	1.99	1.98	1.97	1.98 ± 0.01
		A260/230	1.28	1.17	1.15	1.20 ± 0.07
		µg DNA g^{-1} sample	63.15	65.20	67.15	65.17 ± 2.00[d]
Van Burik	~4 hr	A260/280	1.83	1.78	1.80	1.80 ± 0.03
		A260/230	0.94	0.86	0.86	0.89 ± 0.05
		µg DNA g^{-1} sample	267.30	254.60	257.40	259.77 ± 6.67[e]
Boehm	~4 hr	A260/280	2.14	2.14	2.14	2.14 ± 0.00
		A260/230	2.26	2.17	2.22	2.22 ± 0.05
		µg DNA g^{-1} sample	204.70	213.70	208.45	208.95 ± 4.52[f]

Note: Means ± standard deviation (S.D.) in a column between protocols with different superscripts differ significantly ($P < 0.05$).
Data are presented as mean ± S.D. of three biological replicates.

Next, we also assessed the integrity of the extracted DNA of *G. zonatum* across the seven protocols and also on the selected Boehm protocol on the five *Ganoderma* species. This was performed with PCR using Simple Sequence Repeat (SSR) primer pair that has an amplicon size of 150 bp. Firstly, we conducted experiments that showed PCR amplification of the extracted DNA from all the seven protocols (*Figure 3*). Despite disparity between the purity and concentrations of DNA, PCR amplification was successful from the seven protocols evaluated (Pipan *et al*., 2018). Apart from DNA quality and concentration, there are many factors to consider when assessment is based on PCR, such as suitability of primers, quality of reagents or even the thermal cycler used. Nevertheless, it is important to note that the Boehm protocol was able to produce the highest yield of highly pure DNA, making it suitable for most molecular biology applications. DNA from the five *Ganoderma* species extracted using the Boehm protocol was also suitable for PCR amplification (*Figure 4*). Nevertheless, the SSR band from the *G. lucidum* DNA was less intense compared to the amplicons from the other species. This is probably due to the lower quality of DNA produced from *G. lucidum* (*Table 2*).

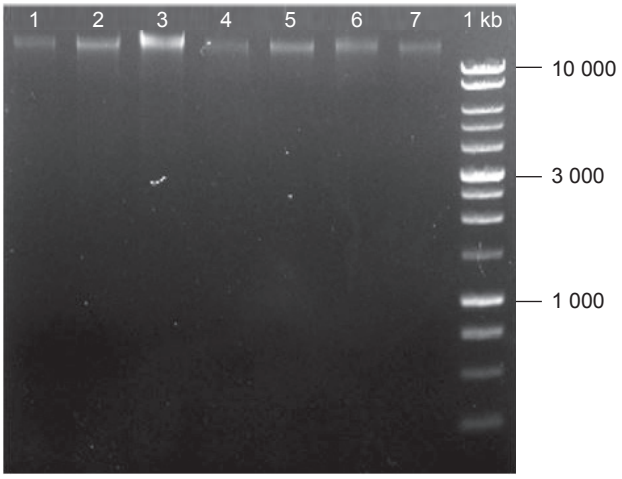

Figure 1. Agarose gel electrophoresis of extracted genomic DNA. Two microlitres of sample (50 ng ul^{-1}) were loaded into each lanes. Ganoderma zonatum *extracted with seven published protocols: 1) Voigt, 2) Moslem, 3) Kim, 4) Weiland, 5) Vilgalys, 6) Van Burik and 7) Boehm.*

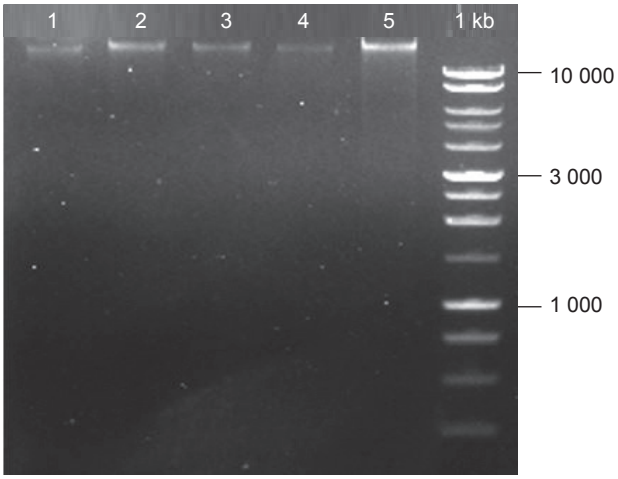

Figure 2. Agarose gel electrophoresis of extracted genomic DNA. Two microlitres of sample (50 ng ul^{-1}) were loaded into each lanes. Ganoderma *species tested with the Boehm protocols were: 1)* Ganoderma zonatum, *2)* G. boninense, *3)* G. miniatocinctum, *4)* G. tornatum *and 5)* G. lucidum.

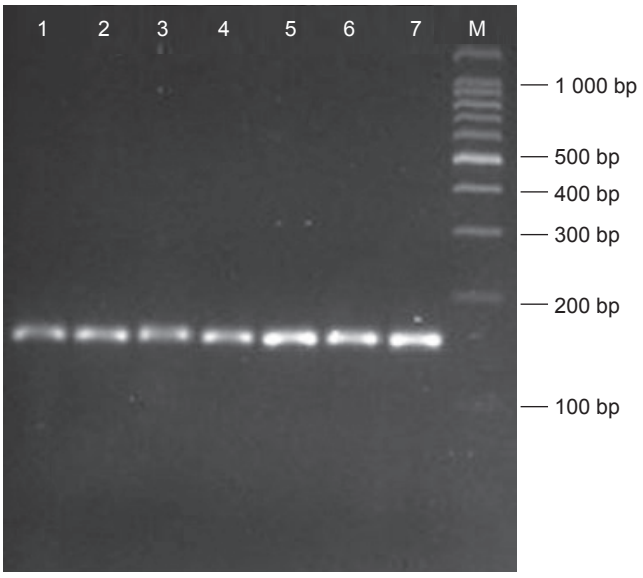

Figure 3. Agarose gel electrophoresis of genomic DNA. Polymerase chain reaction products of amplified microsatellite regions of Ganoderma zonatum *extracted with seven published protocols: 1) Voigt, 2) Moslem, 3) Kim, 4) Weiland, 5) Vilgalys, 6) Van Burik, 7) Boehm and M-100 bp DNA ladder.*

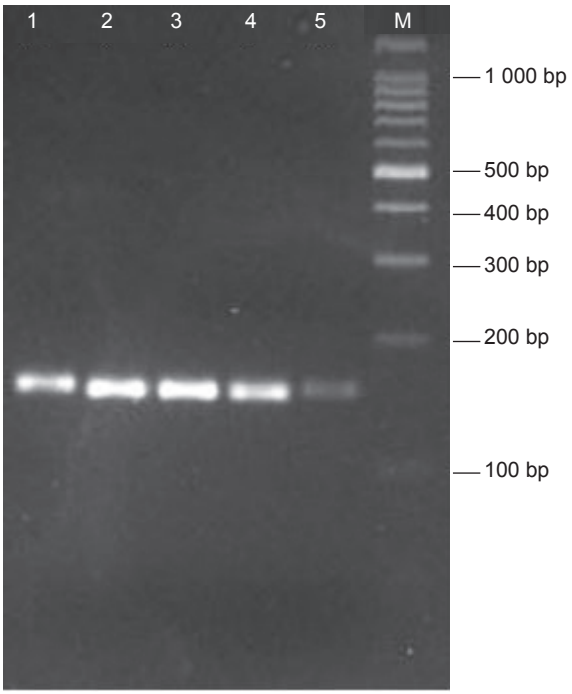

Figure 4. Agarose gel electrophoresis of genomic DNA. Polymerase chain reaction products of amplified microsatellite regions of Ganoderma *species extracted via the Boehm protocol. Lane 1-5:* Ganoderma zonatum, G. boninense, G. miniatocinctum, G. tornatum, G. lucidum *and M-100 bp DNA ladder.*

TABLE 2. GENOMIC DNA YIELD AND QUALITY OF FIVE *Ganoderma* SPECIES EXTRACTED VIA BOEHM (2004) PROTOCOL

Species	Parameters	Replicate 1	Replicate 2	Replicate 3	Average
G. zonatum	$A_{260/280}$	2.14	2.14	2.14	2.14 ± 0.00
	$A_{260/230}$	2.26	2.22	2.17	2.22 ± 0.05
	µg DNA g^{-1} sample	204.70	208.45	213.70	208.95 ± 4.52
G. boninense	$A_{260/280}$	1.99	1.97	2.09	2.02 ± 0.06
	$A_{260/230}$	1.69	1.60	1.55	1.61 ± 0.07
	µg DNA g^{-1} sample	41.35	41.40	41.70	41.48 ± 0.19
G. miniatocinctum	$A_{260/280}$	2.02	2.05	2.05	2.04 ± 0.02
	$A_{260/230}$	2.02	2.00	1.98	2.00 ± 0.02
	µg DNA g^{-1} sample	65.00	65.00	66.05	65.35 ± 0.61
G. tornatum	$A_{260/280}$	2.03	2.01	2.03	2.02 ± 0.01
	$A_{260/230}$	1.80	1.73	1.69	1.74 ± 0.06
	µg DNA g^{-1} sample	88.65	89.70	91.15	89.83 ± 1.26
G. lucidum	$A_{260/280}$	1.90	1.83	1.81	1.85 ± 0.05
	$A_{260/230}$	1.46	1.35	1.21	1.34 ± 0.13
	µg DNA g^{-1} sample	34.40	32.00	33.80	33.40 ± 1.25

Note: Data are presented as mean ± S.D. of three biological replicates.

CONCLUSION

The Boehm protocol was the most efficient, simple and reliable DNA extraction protocol tested on the mycelium of *G. zonatum*. This protocol eliminates the use of phenol and requires very low amount of tissue to produce high yield and good quality DNA. It takes about 4 hr to complete the procedure compared to the commonly used CTAB protocol which takes approximately two days. The evaluation of protocols was aimed at obtaining good quality DNA for downstream applications, such as PacBio sequencing. Therefore, for approaches such as PacBio or Southern blot analysis which normally require large quantity and good quality DNA (Nagappan *et al*., 2018), the short duration of the Boehm protocol was able to achieve that.

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REFERENCES

Boehm, E W (2004). Fungal genomic DNA extraction. http://www.protocol-online.org/prot/Protocols/Fungal-Genomic-DNA-Extraction-1200.html, accessed on 1 March 2019.

Calderon-Cortes, N; Quesada, M; Cano-Camacho, H and Zavala-Paramo, G (2010). A simple and rapid method for DNA isolation from xylophagous insects. *Int. J. Mol. Sci*., *11(12)*: 5056-5064.

Choi, J J and Kim, S-H (2017). A genome tree of life for the fungi kingdom. *PNAS*, *114(35)*: 9391-9396.

Dong, Q; He, D; Ni, X; Zhou, H and Yang, H (2019). Comparative study on phenolic compounds, triterpenoids and antioxidant activity of *Ganoderma lucidum* affected by different drying methods. *J. Food Meas. Charact*., *13(4)*: 3198-3205.

Elliott, M L and Broschat, T K (2000). *Ganoderma* butt rot of palms. https://edis.ifas.ufl.edu/pdffiles/PP/PP10000.pdf, accessed on 1 November 2018.

Elloitt, M L; Des Jardin, E A; Ortiz, J V and Macias, T (2018). Genetic variability of *Ganoderma zonatum* infecting palms in Florida. *Mycologia*, *110(2)*: 339-346.

Gracia, A N; Ayub, N D; Fox, A R; Gomez, M C; Dieguez, M J; Pagano, E M; Berini, C A; Muschietti, J P and Soto, G (2014). Alfalfa snakin-1 prevents fungal colonization and probably coevolved with rhizobia. *BMC Plant Biology*, *14(1)*: 248-260.

Gul, F; Hussain, A; Jan, G and Hamayun, M (2017). Genomic DNA extraction for molecular identification of endophytic fungi: An easy and efficient protocol. *Biosci. Biotech. Res. Asia*, *14(2)*: 667-671.

Hassan, Y; Foster, H L and Flood, J (2005). Investigations on the causes of upper stem rot (USR) on standing mature oil palms. *Mycopathologia*, *159(1)*: 109-112.

Idris, A S (1999). *Basal Stem Rot (BSR) of Oil Palm* (Elaeis guineensis *Jacq.*) *in Malaysia: Factors Associated with Variation in Disease Severity*. Ph.D thesis. Wye College, United Kingdom.

Kim, C S; Lee, C H; Shin, J S; Chung, Y S and Hyung, N I (1997). A simple and rapid method for isolation of high quality genomic DNA from fruit trees and conifers using PVP. *Nucleic Acids Res*., *25(5)*: 1085-1086.

Kuhad, R C; Kapoor, R K and Lal, R (2004). Improving the yield and quality of DNA isolated from white-rot fungi. *Folia Microbiol*., *49(2)*: 112-116.

Kushairi, A; Ong-Abdullah, M; Nambiappan, B; Hishamuddin, E; Bidin, M N I Z; Ghazali, R; Subramaniam, V; Sundram, S and Parveez, G K A (2019). Oil palm economic performance in Malaysia and R&D progress in 2018. *J. Oil Palm Res*. *Vol. 31(2)*: 165-194.

Li, H; Wu, S; Ma, X; Chen, W; Zhang, J; Duan, S; Gao, Y; Kui, L; Huang, W; Wu, P; Shi, R; Li, Y; Wang, Y; Li, J; Gua, X; Luo, X; Li, Q; Xiong, C; Liu, H; Gui, M; Sheng, J and Dong, Y (2018). The genome sequence of 90 mushrooms. *Sci. Rep*., *8(1)*: 9982-9986.

Lucena-Aguilar, G; Sanchez-Lopez, A M; Barberan-Aceituno, C; Carrillo-Avial, J A; Lopez-Guerrero, J A and Aguilar-Quesada, R (2016). DNA source selection for downstream applications based on DNA quality indicators analysis. *Biopreserv. Biobank*., *14(4)*: 264-270.

Merciere, M; Laybats, A; Carasco-Lacombe, C; Tan, J S; Klopp, C; Durand-Gasselin, T; Alwee, S S R S; Camus-Kulandaivelu, L and Breton, F (2015). Identification and development of new polymorphic microsatellite markers using genome assembly for *Ganoderma boninense*, causal agent of oil palm basal stem rot disease. *Mycol. Prog*., *14(11)*: 103-113.

Mishra, J; Joshi, A; Rajput, R; Singh, K; Bansal, A and Misra, K (2018). Phenolic rich fractions from mycelium and fruiting body of *Ganoderma lucidum* inhibit bacterial pathogens mediated by generation of reactive oxygen species and protein leakage and modulate hypoxic stress in HEK 293 cell line. *Advances in Pharmacological Sciences*, *2018:* 6285615.

Moslem, M A; Bahkali, A H; Abd-Elsalam, K A and Wit, P J G M (2010). An efficient method for DNA extraction from Cladosporioid fungi. *Genet. Mol. Res*., *9(4)*: 2283-2291.

Motkova, P and Vytrasova, J (2011). Comparison methods for isolating fungal DNA. *Czech J. Food Sci., 29(Special Issue)*: S76-S85.

Nagappan, J; Chin, C F; Angel, L P L; Cooper, R M; May, S T and Low, E T L (2018). Improved nucleic acid extraction protocols for *Ganoderma boninense*, *G. miniatocinctum* and *G. tornatum*. *Biotechnol. Lett., 40(2)*: 1541-1550.

Nagi, M A; Abd-Elsalam, K A; Bahkali, A H and Mongy, A A A (2013). Improved method for DNA isolation from different types of soil infested with three fungal genera. *Rom. Agric. Res*., *30*: 229-333.

Natarajan, V P; Zhang, X; Morono, Y; Inagaki, F and Wang, F (2016). A modified SDS-based DNA extraction method for high quality environmental DNA from seafloor environments. *Front. Microbiol*., *7(986)*: 1-13.

Obodai, M; Mensah, D L N; Fernandes, A; Kortei, N K; Dzomeku, M; Teegarden, M; Schwartz, S J; Barros, L; Prempeh, J; Takli, R K and Ferreira, I C F R (2017). Chemical characterization and antioxidant potential of wild *Ganoderma* species from Ghana. *Molecules*, *22(2)*: 196-213.

Perry, M D; White, P L and Barnes, R A (2014). Comparison of four automated nucleic acid extraction platforms from the recovery of DNA from *Aspergillus fumigatus*. *J. Med. Microbiol*., *63(9)*: 1160-1166.

Pilotti, C A (2005). Stem rots of oil palm caused by *Ganoderma boninense*: Pathogen, biology and epidemiology. *Mycopathologia*, *159(1)*: 129-137.

Pipan, B; Zupancic, M; Blatnik, E; Dolnicar, P and Meglic, V (2018). Comparison of six genomic DNA extraction methods for molecular downstream applications of apple tree (*Malus X domestica*). *Cogent Food and Agriculture, 4(1)*: 1540094.

Psifidi, A; Dovas, C I; Bramis, G; Lazou, T; Russel, C L; Arsenos, G and Banos, G (2015). Comparison of eleven methods for genomic DNA extraction suitable for large-scale whole-genome genotyping and long-term DNA banking using bold samples. *PLoS ONE, 10(1)*: e0115960.

Rakib, M R M; Bong, C F J; Khairulmazmi, A and Idris, A S (2014). Genetic and morphological diversity of *Ganoderma* species isolated from infected oil palms (*Elaeis guineensis*). *Int. J. Agric. Biol*., *16(4)*: 691-699.

Rakib, M R M; Bong, C F J; Khairulmazmi, A and Idris, A S (2015). Aggressiveness of *Ganoderma boninense* and *G. zonatum* isolated from upper and basal stem rot of oil palm (*Elaeis guineensis*) in Malaysia. *J. Oil Palm Res. Vol. 27(3)*: 229-240.

Rakib, M R M; Bong, C F J; Khairulmazmi, A; Idris, A S; Jalloh, M B and Wahida, N H (2017). *Ganoderma* species of basal and upper stem rots in oil palm (*Elaeis guineensis*) in Sarawak, Malaysia. *J. Academia UiTM*, *5*: 27-35.

Rees, R W; Flood, J; Hasan, Y; Wills, M A and Cooper, R M (2012). *Ganoderma boninense* basidiospores in oil palm plantations: Evaluation of the possible role in stem rots of *Elaeis guineensis*. *Plant Pathol*., *61(3)*: 567-578.

Sahu, S K; Thangaraj, M and Kathiresan, K (2012). DNA extraction protocol for plants with high levels of secondary metabolites and polysaccharides without using liquid nitrogen and phenol. *ISRN Mol. Biol*., *2012*: 205049.

Seo, A N; Kim, J-H; Lee, D; Jeong, J Y and Park, J-Y (2011). Comparison of the DNA preservation in neutral-buffered formalin fixed paraffin-embedded tissue and in non-buffered formalin fixed paraffin-embedded tissue. *Korean J. Pathol., 45(6)*: 549-556.

Steyaert, R L (1967). Les *Ganoderma* palmicoles. *Bull. Jar. Bot. Nat. Belg., 37(4)*: 465-492.

Tripathy, S K; Maharana, M; Ithape, D M; Lenka, D; Mishra, D; Prusti, A; Swain, D; Mohanty, M R and Raj, K R R (2017). Exploring rapid and efficient protocol for isolation of fungal DNA. *Int. J. Curr. Microbiol. App. Sci*., *6(3)*: 951-960.

Umesha, S; Manukumar, H M and Raghava, S (2016). A rapid protocol for isolation of genomic DNA from food-borne fungal pathogens. *3 Biotech*., *6(2)*: 123-131.

Van Burik, J A H; Schreckhise, R W; White, T C and Bowden, R A (1998). Comparison of six extraction techniques for isolation of DNA from filamentous fungi. *Med. Mycol*., *36(5)*: 299-303.

Verant, M L; Bohuski, E A; Lorch, J M and Blehert, D S (2016). Optimization methods for total nucleic acid extraction and quantification of the bat white-nose syndrome fungus, *Pseqdogymnoascus destructans*, from swab and environmental samples. *J. Vet. Diagn. Invest*., *28(2)*: 110-118.

Vilgalys Lab (2018). http://www.umich.edu/~mycology/protocols_assets/DNAminipreps.doc, accessed on 14 February 2018.

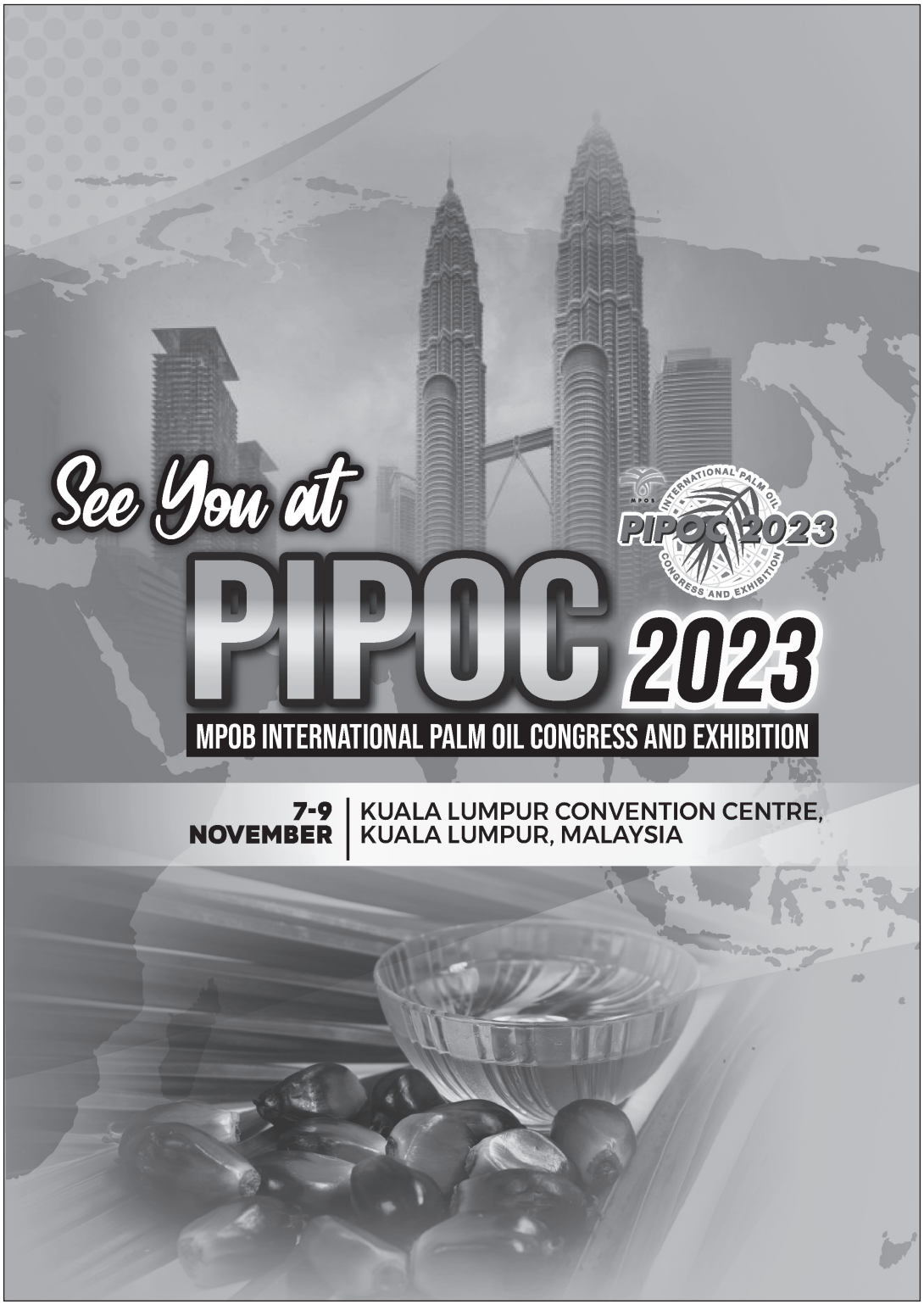
Voigt, K; Cigelnik, E and O'Donnell, K (1999). Phylogeny and PCR identification of clinically important Zygomycetes based on nuclear ribosomal-DNA sequence data. *J. Clin. Microbiol*., *37(12)*: 3957-3964.

Weiland, J J (1997). Rapid procedure for the extraction of DNA from fungal spores and mycelia. *Fungal Genet. Rep., 44(22)*: 1-7.

Wingfield, P T (2001). Use of protein folding reagents. *Curr. Protoc. Protein Sci., 00(1)*: A.3A.1-A.3A.4.

Winther, J R and Thorpe, C (2014). Quantification of thiols and disulfides. *Biochim. Biophys. Acta, 1840(2)*: 383-346.

Wong, L C; Bong, C F J and Idris, A S (2012). *Ganoderma* species associated with basal stem rot disease of oil palm. *Am. J. Appl. Sci., 9(6)*: 879-885.



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POPULATION DENSITY OF *Elaeidobius kamerunicus* FAUST IN DIFFERENT SPIKELET POSITION AT ANTHESISING MALE INFLORESCENCE OF *Elaeis guineensis* Jacq. IN SABAH AND SARAWAK, MALAYSIA

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ABSTRACT

The oil palm male inflorescences provide food source and breeding sites for the pollinating weevil, Elaeidobius kamerunicus (E. kamerunicus). *The present study examined the difference in the formation of the oil palm male inflorescences on different soil types, and how the differences affect the population density of* E. kamerunicus. *Samplings of adult* E. kamerunicus *and the spikelets of fully-anthesising oil palm male inflorescences were conducted monthly for a period of 18 months at four oil palm plantations on peat and mineral soils in East Malaysia.* Elaeidobius kamerunicus *population density in peat soil areas was found to be lower than those in mineral soils (P<0.05). It was also found that the male inflorescences spikelet length in the study sites on peat soil areas were less uniform; spikelets on the upper part of the inflorescence were significantly shorter (= 12.80 cm) compared to the spikelets sampled from the bottom part of the inflorescence (= 14.91 cm, P< 0.05, P= 0.000-0.031). In addition, compared to the lower part of the inflorescence, significantly fewer weevils were found lodged on the spikelets sampled from the upper part of the inflorescence (Site 1^{Top} = 8 adult weevils per spikelet; Site 2^{Top} = 12 adult weevils per spikelet; Site 1^{Bottom} = 18 adult weevils per spikelet; Site 2^{Bottom}= 17 adult weevils per spikelet). However, no significant correlation was found between the length of the spikelet and the weevil density on each spikelet (r= 0.021-0.181). The result from this study demonstrates the influence of the soil types on the formation of the inflorescence, which requires further investigation.*

Keywords: *Elaeis guineensis, Elaeidobius kamerunicus*, different soil types, male inflorescence.

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INTRODUCTION

Oil palm (*Elaeis guineensis*) produces separate male and female flowers on the same plant in an alternating cycle, which is governed by varieties of factors, *e.g.* genetic, age and environmental conditions (Corley, 1976). The male inflorescence consists of a 40 cm stout peduncle bearing around 100-300 spikelets, which individually measures 10-30 cm long. Each of the spikelets usually holds 400-1500 male flowers. The female inflorescence has a shorter and stouter stalk, which holds 150 spikelets that contain 30 flowers each (Janick and Paull, 2008).

The oil palm pollinating weevil, *Elaeidobius kamerunicus* feeds on pollens and the soft part of male flowers, which is also the location for egg-laying (Corley and Tinker, 2015). Both male and female inflorescences emit characteristic aniseed smell to attract adult *E. kamerunicus* and permit

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pollen transfers from male to female inflorescences (Muhamad Fahmi *et al*., 2016; Janick and Paull, 2008; Aisagbonhi *et al*., 2004; Hussein *et al*., 1991). However, due to lesser incentives offered (*i.e*. breeding sites, food), the number of weevils visiting the female inflorescences is lesser than the number of weevils visiting the male inflorescences (Chiu, 1984; Syed, 1980).

Sufficient pollination is important in the process of oil palm fruit development and yield production. Incomplete pollination can cause bunch failure, low bunch production, and low fruit set (Kamaruddin *et al*, 2018; Haniff and Roslan, 2002). Due to the ineffectiveness of locally-available oil palm pollinators, *E. kamerunicus* was introduced to Malaysia from Cameroon in 1981 to improve fruit set formation (Syed *et al*., 1982).

Subsequently, monitoring of the population establishment of the introduced pollinator was conducted to determine its population level in the oil palm ecosystem (Nurul Fatihah *et al*., 2018; Rahardjo *et al*., 2018; Syarifah Nadiah and Idris, 2016; Basri *et al*., 1987). The monitoring of the pollinator has to be done regularly to ensure its sustainability as well as to monitor changes that might positively or negatively affect the species. Ming and Bong (2017) and Chiu *et al*. (1984) reported that sampling of the spikelets of the anthesising male inflorescences as more reliable and able to give a better estimation to the pollinating weevil densities of the area. In order to get an estimation of *E. kamerunicus* population per unit area, adults *E. kamerunicus* should be sampled from anthesising male inflorescences, indicated by florets opening. Three spikelets should be sampled from each region of the inflorescence, namely top, middle and bottom parts to get a more reliable estimate (Nurul Fatihah *et al*., 2018; Basri *et al*., 1987).

The population of *E. kamerunicus* is affected by the sex ratio of oil palm inflorescence. The development of high yielding planting materials produced a greater number of female inflorescences and a lower number of male inflorescences (Prasetyo *et al*., 2014). In addition, environmental stimuli also influence the sex ratio of the inflorescence (Corley and Tinker, 2015). *Elaeidobius kamerunicus* depends on male inflorescences for its food source and breeding sites, so the scarcity of male inflorescences in a particular area may negatively affect the population levels of *E. kamerunicus*, which will also influence the pollination of oil palm. The plant growth is influenced by the soil type on which the palm is grown (Yahya *et al*., 2012; Chan *et al*., 2005; Hamza and Anderson, 2005). Additionally, the amount and composition of volatile organic compounds emitted by the inflorescences are also influenced by the soil type (Muhamad Fahmi *et al*., 2016). It is acknowledged that *E. kamerunicus* spends most of its entire lifetime on the male inflorescence (Ming and Bong, 2017; Chiu, 1984; Syed, 1980). However, to date, no detailed observation on the effects of the male inflorescence morphology on the population levels of the pollinating weevil was conducted. The influence of the soil types on the male inflorescence morphological development was also not studied previously.

Therefore, this study was conducted to investigate the population density of *E. kamerunicus* lodged at each region of the male inflorescences. This information can be used to assess the differences between the formations of male inflorescences, in terms of uniformity of spikelet length, in different areas of sampling. In addition, this study was also conducted to compare the population level of *E. kamerunicus* on different types of soil.

MATERIALS AND METHODS

Location of the Study

The study was conducted in four localities of oil palm plantations with commercial DxP planting materials, with three sites located in Sarawak and one site is in Sabah. Site 1 was a 5-year old oil palm (planted in 2010) planting on undulating compacted peat area in the Roban region, Sarawak (N1°53'58" E111°12'19"). Site 2 was a 4-year old planting (planted in 2011) on peat in Pusa region, Sarawak (N1°40'15" E111°13'13"), Site 3 was a 3-year old oil palm area (planted in 2012) on mineral soil in the Segangan area, Sabah (N5°06'26.0" E118°26'50.3") and Site 4 was a 5-year old planting on mineral soil in Miri, Sarawak (N4°7'52" E113°58'28").

Samples Collection

The samplings of male inflorescences and adults *E. kamerunicus* at the experimental sites were conducted monthly from January 2015 until June 2016. At each site, an area of 10 ha was selected for the study. A total of 150 healthy, bunch-producing palms were then systematically selected, by marking every tenth palm within the study area. The samples of spikelets from male inflorescences were then collected at random from the marked-palms. At Site 1, Site 2, and Site 4, three spikelets each from the top, middle, and bottom portion (*Figure 1*) of 10 fully anthesising male inflorescences were sampled each month, giving a total of 90 spikelets monthly. At Site 3, due to lack of fully anthesising male inflorescences during sampling, three spikelets each from the top, middle, and bottom portion of four fully anthesising male inflorescences were sampled, giving a total of 36 spikelets monthly. The anthesis of male inflorescence starts with the opening of florets at the base which then opens gradually upwards in about three days (Syed, 1980). Fully anthesising spikelet is usually obtained on the third day of

anthesis, when all florets opening up from the distal to the apical part of the floret, bearing the highest amount of pollen and emitting the highest amount of weevil-attracting volatile organic compounds.

The spikelets were carefully cut from each region of the inflorescences and stored in individual plastic bags. Samples were then brought to the laboratory for further processing. The total number of adult *E. kamerunicus* was then summed up and categorised according to the positions of the spikelets on the inflorescences. The length of each spikelet was also measured and compared.

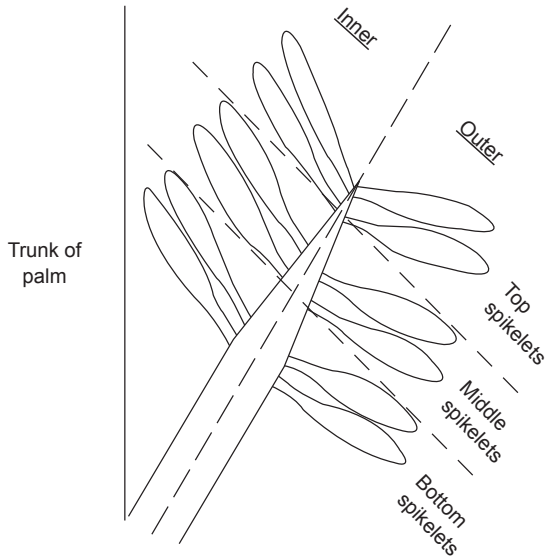

Figure 1. The six sections of the male inflorescence used in stratified sampling suggested by Chiu et al. *(1984).*

Statistical Analysis

All measured parameters were subjected to analysis of variance by using Minitab 17 software. Where applicable, means are then separated using Tukey's procedure (P< 0.05).

RESULTS

Population Density of *E. kamerunicus* in Different Soil Types

A comparison between the number of adults *E. kamerunicus* lodged at the spikelets of the male inflorescences was conducted (*Table 1*). Based on statistical analysis, except for a few months (*i.e.* May 2015, July-October 2015), it was observed that the mean population density of *E. kamerunicus* per spikelet in mineral areas was significantly greater than that in peat areas (P<0.05, P=0.000).

In addition, a comparison between each study site was also made and illustrated in *Figure 2*. The number of adult weevils per spikelet at both mineral soils at Site 3 and Site 4 were greater than those at peat soils (Site 1 and Site 2). The number of adult weevils per spikelet at Site 3 (= 31.51 weevils per spikelet) was significantly greater than the number of weevils at Site 4 (= 25.98 weevils per spikelet), Site 1 (= 21.20 weevils per spikelet) and Site 2 (= 15.11 weevils per spikelet) (P<0.05, P=0.000).

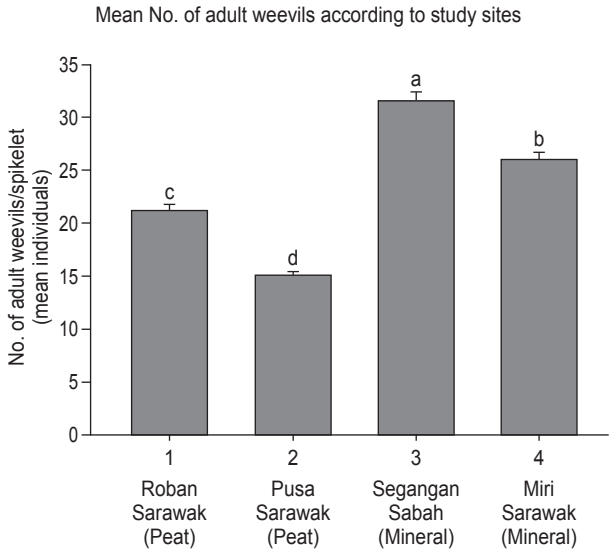

Figure 2: The mean number of adult weevils per spikelet in fully anthesising male inflorescences at the study locations (means that do not share a letter are significantly different).

Male Inflorescence Spikelet Lengths from Different Portion of Inflorescence

The length of spikelets from different parts of the male inflorescences from samples collected in the peat soil areas in Site 1, Sarawak, and Site 2, Sarawak were significantly different (P< 0.05, P= 0.000-0.021) during the period of the study (*Table 2*, *Figures 3* and *4*). Meanwhile, in mineral soil areas in Site 3, Sabah, only samples collected in May 2015, June 2015, October 2015, January 2016 and March 2016 showed significant differences (P<0.05 P= 0.000-0.031) in terms of the length of the spikelets (*Table 2*). No significant differences in the length of the spikelets from different positions of male inflorescences were observed from the samples collected in another mineral soil site at Site 4, Sarawak (P>0.05, P=0.406-0.979) (*Table 2*).

From samples collected at Site 1 and Site 2, it was observed that the length of spikelets sampled from the top part of the inflorescences was generally shorter (Site 1^{Top} = 11.91 cm; Site 2^{Top} = 12.55 cm) compared to samples collected from other parts of the inflorescences (*Tables 3* and *4*). On the other hand, the length of the spikelets from the bottom part (Site 1^{Bottom} = 14.68 cm; Site 2^{Bottom} = 15.13 cm) was significantly longer than the spikelets collected from the top part, although the length of spikelets from those two parts with the spikelets from

the middle part was not significantly different (Site 1^{Middle} = 14.04 cm; Site 2^{Middle} = 14.93 cm) (P>0.05).

Meanwhile, at Site 3, samplings conducted in May 2015, June 2015, September 2015, October 2015, January 2016, March 2016, May 2016, and June 2016 showed that the length of spikelets from the bottom part of the inflorescences (Site 3^{Bottom} = 14.79 cm) was significantly longer than those from the top part (Site 3^{Top} = 12.97 cm) (*Table 5*). Whereas, in other months of samplings, no significant differences were found between the length of spikelets from different portions of male inflorescence (P>0.05, P=0.088-0.657) (*Table 2*, *Figure 5*).

Samples collected from Site 4 showed no significant differences in terms of the length of spikelets from a different portion of the male inflorescences (P>0.05, P=0.406-0.979) (Site 4^{Top} =14.07cm; Site 4^{Middle} =14.16 cm; Site 4^{Bottom} =14.18 cm) (*Tables 2* and *6*, *Figure 6*).

Number of Adult Weevils in Spikelets of Male Inflorescences

Generally, there was a significant difference between the number of adult weevils found in all three different spikelet positions of the male inflorescence during the study period on samples collected in peat soil areas at Site 1 and Site 2, Sarawak (P<0.05, P= 0.000-0.0047) (*Table 2*, *Figures 7* and *8*).

From samples collected in Site 1, only those collected in January 2016 showed no significant differences between the number of adult weevils lodged at different positions of spikelet (P>0.05, P = 0.129) (*Table 2*). Whereas, in Site 2, samples collected in February, March, April, May, August, September, November 2015 and May 2016 showed no significant differences in terms of the number of adult weevils at the spikelets sampled from three different positions on the male inflorescences (P>0.05, P= 0.061-0.555) (*Table 2*).

In Site 1, the number of adults *E. kamerunicus* sampled from the top part of the inflorescence was significantly lesser compared to those collected from the bottom part (P<0.05, P= 0.000-0.047, Site 1^{Top} = 13.04 weevils per spikelet; Site 1^{Bottom} = 26.83 weevils per spikelet). However, there were no significant differences in the number of adult weevils collected from the middle and bottom parts of the inflorescence (Site 1^{Middle} = 23.74 weevils per spikelet, P>0.05).

In Site 2, the adult weevils found lodged at the top spikelets at the inflorescences were significantly lesser than those at the bottom spikelets (P<0.05, P= 0.000-0.041, Site 2^{Top} = 11.78 weevils per spikelet; Site 2^{Bottom} = 16.77 weevils per spikelet).

Throughout the study period, the analysed data based on the monthly census conducted at the sites located on the mineral soils showed no significant differences between the number of adult weevils sampled from different portions of male inflorescences (*Tables 2*, *5* and *6*, P> 0.05, P= 0.078-0.981, Site 3^{Top} = 28.51 weevils per spikelet,Site 3^{Middle} = 32.43 weevils per spikelet, Site 3^{Bottom} = 33.60 weevils per spikelet, Site 4^{Top} = 26.67 weevils per spikelet, Site 4^{Middle} = 26.33 weevils per spikelet, Site 4^{Bottom} = 27.22 weevils per spikelet). The graph illustrating this is shown in *Figure 9* (Site 3) and *Figure 10* (Site 4).

TABLE 1. MEAN ADULT *Elaeidobius kamerunicus* POPULATION DENSITY IN OIL PALM MALE INFLORESCENCES FROM DIFFERENT SOIL TYPES

Sampling month	Mean population density of weevils/ spikelet				F value	P value
	Peat	±SE	Mineral	±SE		
Jan 15	13.8222b	0.74	24.4537a	1.58	47.16	0.000*
Feb 15	8.9333b	0.48	28.9524a	1.66	178.36	0.000*
Mar 15	15.2278b	0.78	29.8056a	2.17	62.73	0.000*
Apr 15	21.0222b	1.53	34.3889a	2.27	25.69	0.000*
May 15	22.5778b	1.57	27.1905a	2.39	2.36	0.126
Jun 15	16.5772b	1.02	26.6270a	2.26	20.07	0.000*
Jul 15	30.55a	1.98	30.5139a	1.83	0.00	0.991
Aug 15	19.3889a	1.35	20.0238a	1.42	0.10	0.751
Sep 15	34.0778a	2.49	31.0694a	2.76	0.49	0.485
Oct 15	18.5500a	1.05	16.5556a	1.19	1.55	0.214
Nov 15	19.1722b	1.03	31.5694a	2.25	32.79	0.000*
Dec 15	16.1556b	1.45	34.7857a	1.98	60.14	0.000*
Jan 16	13.7278b	0.59	41.5000a	4.50	135.35	0.000*
Feb 16	13.8056b	1.04	40.0556a	4.18	77.42	0.000*
Mar 16	12.7889b	0.6	31.2500a	3.54	79.71	0.000*
Apr 16	18.6556b	1.05	39.5833a	4.26	47.91	0.000*
May 16	16.3278b	1.03	34.8611a	3.90	41.45	0.000*
Jun 16	15.6889b	0.99	34.6389a	4.35	41.38	0.000*

Note: Means that do not share a letter are significantly different.
SE - standard error.
*P<0.05.

TABLE 2. RESULT OF ANOVA FOR THE COMPARISON OF THE NUMBER OF ADULT *Elaeidobius kamerunicus* AND THE SPIKELET LENGTH SAMPLED FROM DIFFERENT POSITION AT THE ANTHESISING MALE INFLORESCENCES (α = 0.05)

Sampling month	Comparisons of No. of adult weevils/spikelet between different positions of spikelet sites								Comparisons of spikelet length from different positions sites							
	Roban		Pusa		Segangan		Miri		Roban		Pusa		Segangan		Miri	
	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value	F value	P value
Jan 15	9.11	0.000*	3.36	0.039*	1.65	0.207	0.93	0.399	30.11	0.000*	20.89	0.000*	0.43	0.657	0.20	0.815
Feb 15	11.67	0.000*	0.59	0.555	0.87	0.428	0.03	0.970	19.11	0.000*	4.06	0.021*	1.64	0.209	0.02	0.978
Mar 15	6.44	0.002*	2.89	0.061	0.39	0.683	0.03	0.966	5.32	0.007*	17.74	0.000*	2.93	0.067	0.15	0.864
Apr 15	4.29	0.017*	0.27	0.767	1.01	0.375	0.21	0.806	19.29	0.000*	6.71	0.002*	1.97	0.155	0.05	0.952
May 15	5.90	0.004*	1.59	0.210	2.63	0.087	0.02	0.981	5.58	0.005*	20.22	0.000*	3.89	0.030*	0.46	0.638
Jun 15	4.65	0.012*	9.22	0.000*	0.92	0.407	0.03	0.966	13.47	0.000*	7.89	0.001*	6.74	0.004*	0.03	0.966
Jul 15	6.44	0.002*	6.62	0.002*	0.53	0.594	0.17	0.843	42.07	0.000*	41.36	0.000*	2.61	0.088	0.93	0.406
Aug 15	5.27	0.007*	1.48	0.233	0.32	0.726	0.35	0.703	13.14	0.000*	21.35	0.000*	1.09	0.348	0.27	0.768
Sep 15	8.60	0.000*	1.13	0.328	0.21	0.810	0.05	0.955	12.92	0.000*	37.59	0.000*	3.23	0.042*	0.46	0.638
Oct 15	6.85	0.002*	5.66	0.005*	2.75	0.078	0.18	0.835	4.17	0.019*	7.39	0.001*	3.86	0.031*	0.04	0.960
Nov 15	13.82	0.000*	1.32	0.273	0.37	0.696	0.05	0.948	27.98	0.000*	13.23	0.000*	1.28	0.291	0.07	0.931
Dec 15	3.018	0.047*	4.61	0.012*	1.12	0.337	0.39	0. 679	5.78	0.004*	25.66	0.000*	2.21	0.125	0.22	0.803
Jan 16	2.09	0.129	4.88	0.010*	0.02	0.979	0.20	0.815	21.88	0.000*	21.24	0.000*	10.43	0.000*	0.35	0.855
Feb 16	5.45	0.006*	3.33	0.041*	0.04	0.959	0.02	0.978	25.61	0.000*	20.24	0.000*	0.79	0.464	0.05	0.979
Mar 16	5.46	0.006*	5.10	0.008*	0.37	0.691	0.15	0.864	9.38	0.000*	53.33	0.000*	16.46	0.000*	0.15	0.689
Apr 16	4.29	0.017*	4.34	0.016*	0.01	0.992	0.05	0.952	30.42	0.000*	12.79	0.000*	1.53	0.232	0.15	0.955
May 16	5.59	0.005*	1.34	0.268	0.06	0.938	0.46	0.638	12.28	0.000*	39.78	0.000*	9.78	0.000*	0.49	0.679
Jun 16	6.94	0.002*	7.96	0.001*	0.20	0.820	0.03	0.966	9.88	0.000	25.88	0.000	5.16	0.011*	0.09	0.968

Note: *P<0.05.
ANOVA - analysis of variance.

TABLE 3. MONTHLY MEAN SPIKELET LENGTH AND NUMBER OF ADULT *Elaeidobius kamerunicus* IN THE SPIKELETS OF OIL PALM MALE INFLORESCENCES FROM DIFFERENT POSITIONS OF SPIKELETS – ROBAN SAMPLES

Sampling month	Roban											
	Number of adult weevils (individuals per spikelet)						Length of spikelet (cm)					
	Top	SE	Middle	SE	Bottom	SE	Top	SE	Middle	SE	Bottom	SE
01/15	8.37b	1.07	13.7ab	1.68	18.3a	2.04	12.11c	0.18	13.51b	0.2	14.31a	0.22
02/15	5.07b	0.35	11.8a	1.3	11.4a	1.37	10.76b	0.38	13.55a	0.3	13.89a	0.47
03/15	12.7b	1.41	24.17a	2.72	18.93ab	2.44	11.21b	0.29	12.88a	0.54	13.36a	0.58
04/15	16.83b	2.08	31.47a	4.49	31.07a	4.91	12.39b	0.44	14.81a	0.35	15.35a	0.27
05/15	17.43b	2.31	35.23a	4.97	31.9a	3.94	13.21b	0.55	15.55a	0.58	15.28a	0.49
06/15	15.53b	2.34	27.37a	3.3	25.13ab	3.02	13.54b	0.4	16.27a	0.42	16.07a	0.42
07/15	18.83b	2.46	30.8ab	4.17	42.87a	6.62	12.81c	0.23	14.3b	0.23	15.74a	0.22
08/15	14.63b	1.8	25.63ab	3.55	33.13a	5.79	11.84b	0.44	14.77a	0.43	14.64a	0.50
09/15	23.77b	3.17	52.87a	8.62	64.77a	8.33	11.28b	0.41	13.22a	0.44	14.34a	0.44
10/15	11.77b	1.45	21.73a	2.28	22.53a	2.9	12.63b	0.42	13.92ab	0.36	14.11a	0.40
11/15	13.97b	0.98	27.63a	2.53	33.4a	3.78	11.25b	0.35	14.04a	0.36	15.3a	0.46
12/15	12.93b	2.38	22.07ab	4.38	29.6a	6.4	10.35b	0.5	11.62ab	0.46	12.46a	0.36
01/16	10.03a	0.67	13.03a	1.38	12.57a	1.17	11.34b	0.41	14.55a	0.47	14.83a	0.36
02/16	8.47b	1.07	15.17ab	2.19	18.33a	2.84	11.1c	0.29	13.07b	0.35	14.52a	0.37
03/16	7.67b	0.91	13.3ab	1.71	15.57a	2.31	12.31b	0.41	13.92a	0.46	14.92a	0.42
04/16	16.43b	2.26	25.8ab	3.4	28.23a	3.24	11.3b	0.35	14.28a	0.35	15.09a	0.39
05/16	10.07b	1.43	21.23a	3.35	23.9a	3.95	12.95b	0.34	14.55a	0.34	15.34a	0.36
06/16	10.13b	1.27	14.37ab	1.73	21.37a	3.05	11.99b	0.44	13.82a	0.41	14.65a	0.44

Note: Means that do not share a letter are significantly different.
SE - standard error.

TABLE 4. MONTHLY MEAN SPIKELET LENGTH AND NUMBER OF ADULT *Elaeidobius kamerunicus* IN THE SPIKELETS OF OIL PALM MALE INFLORESCENCES FROM DIFFERENT POSITIONS OF SPIKELETS – PUSA SAMPLES

Sampling month	Pusa											
	Number of adult weevils (individuals per spikelet)						Length of spikelet (cm)					
	Top	SE	Middle	SE	Bottom	SE	Top	SE	Middle	SE	Bottom	SE
01/15	10.70b	1.23	14.67ab	1.70	17.20a	2.28	13.25b	0.23	16.27a	0.48	15.90a	0.32
02/15	8.40a	1.32	9.33a	1.25	7.60a	0.70	11.03b	0.55	12.98a	0.60	12.92a	0.49
03/15	10.47b	0.77	13.47a	0.87	11.63ab	1.01	10.79b	0.37	13.65a	0.36	13.68a	0.45
04/15	16.17a	3.96	16.77a	2.61	13.83a	2.13	12.63b	0.44	14.84a	0.55	14.71a	0.44
05/15	12.27a	1.61	19.20a	3.98	19.43a	3.59	11.63b	0.45	15.00a	0.40	14.79a	0.41
06/15	7.80b	0.64	12.73a	0.94	10.87a	0.85	12.37b	0.41	13.92a	0.42	14.77a	0.46
07/15	17.40b	1.92	34.57a	5.34	38.83a	5.11	11.46c	0.25	13.34b	0.27	14.71a	0.24
08/15	12.60a	1.16	14.23a	1.25	16.10a	1.82	12.19b	0.26	14.72a	0.34	14.72a	0.34
09/15	19.80a	1.48	23.17a	1.74	20.10a	2.00	12.80b	0.36	16.36a	0.29	16.00a	0.31
10/15	11.37b	1.16	21.37a	2.24	22.53a	3.69	13.08b	0.48	15.54a	0.49	14.99a	0.45
11/15	12.23a	0.96	14.77a	1.36	13.03a	1.03	13.64b	0.40	15.75a	0.37	16.27a	0.38
12/15	9.30b	0.69	12.37a	0.71	10.67ab	0.74	13.45b	0.34	15.87a	0.24	15.96a	0.25
01/16	11.70b	0.95	16.80ab	1.73	18.23a	1.84	12.73b	0.21	14.97a	0.35	15.03a	0.28
02/16	8.07b	1.32	14.90ab	2.75	17.90a	3.69	12.61c	0.33	14.64b	0.42	16.12a	0.42
03/16	11.57b	0.72	15.80a	1.08	12.83ab	1.05	12.64b	0.19	14.96a	0.17	15.21a	0.22
04/16	10.90b	0.80	16.73a	1.74	13.83ab	1.49	13.44b	0.38	15.70a	0.32	15.43a	0.33
05/16	12.47a	1.28	15.37a	1.28	14.93a	1.49	13.93b	0.17	15.87a	0.19	15.90a	0.18
06/16	8.90b	1.02	17.03a	2.33	22.33a	3.29	12.24b	0.28	14.28a	0.32	15.20a	0.30

Note: Means that do not share a letter are significantly different.
SE - standard error.

TABLE 5. MONTHLY MEAN SPIKELET LENGTH AND NUMBER OF ADULT *Elaeidobius kamerunicus* IN THE SPIKELETS OF OIL PALM MALE INFLORESCENCES FROM DIFFERENT POSITIONS OF SPIKELETS – SEGANGAN SAMPLES

Sampling month	Segangan											
	Number of adult weevils						Length of spikelet (cm)					
	Top	SE	Middle	SE	Bottom	SE	Top	SE	Middle	SE	Bottom	SE
01/15	18.83a	3.45	24.08a	4.69	31.67a	6.46	13.73a	1.00	14.94a	0.83	14.58a	1.03
02/15	23.75a	3.66	28.25a	3.78	31.08a	4.40	13.32a	0.67	15.01a	0.77	13.63a	0.66
03/15	26.50a	4.70	32.75a	5.75	30.33a	4.69	10.73a	0.69	12.07a	0.47	12.34a	0.27
04/15	22.25a	5.83	32.42a	8.06	36.58a	7.89	14.31a	0.42	15.78a	0.63	14.32a	0.72
05/15	17.42a	3.59	33.83a	6.57	37.00a	8.36	12.35b	0.42	13.39ab	0.34	13.60a	0.23
06/15	22.83a	4.79	34.67a	7.73	34.50a	8.18	11.33b	0.31	12.00ab	0.32	12.83a	0.22
07/15	34.42a	4.21	32.92a	4.76	28.50a	3.64	11.65a	0.53	13.29a	0.49	12.23a	0.52
08/15	22.75a	3.90	27.58a	5.42	27.42a	5.03	13.61a	0.42	14.85a	0.69	14.35a	0.65
09/15	40.42a	7.57	40.92a	5.79	46.17a	7.25	11.06b	0.41	11.74ab	0.45	12.53a	0.37
10/15	10.67a	2.29	9.25a	1.40	17.42a	3.68	17.15b	0.69	19.02ab	0.52	19.67a	0.76
11/15	32.92a	5.29	38.08a	6.86	30.92a	6.07	13.91a	0.98	15.49a	1.02	15.90a	0.77
12/15	19.92a	3.14	26.08a	5.07	31.00a	6.84	12.81a	1.35	15.45a	1.17	15.99a	0.86
01/16	40.67a	7.98	41.00a	8.04	42.83a	8.06	11.58b	0.50	14.11a	0.64	15.27a	0.61
02/16	38.33a	7.57	40.58a	8.13	41.25a	6.55	12.85a	0.55	13.38a	0.87	14.08a	0.61
03/16	34.58a	7.41	32.08a	5.93	27.08a	5.19	10.76b	0.45	12.78a	0.45	14.14a	0.34
04/16	39.58a	8.17	40.25a	6.89	38.92a	7.69	15.01a	0.59	16.22a	0.51	16.53a	0.81
05/16	36.67a	6.85	33.17a	6.87	34.75a	7.09	10.63b	0.54	12.80ab	0.66	14.96a	0.84
06/16	30.75a	6.75	35.83a	7.85	37.33a	8.47	11.49b	0.82	14.45ab	0.84	15.31a	0.97

Note: Means that do not share a letter are significantly different.
SE - standard error.

TABLE 6. MONTHLY MEAN SPIKELET LENGTH AND NUMBER OF ADULT *Elaeidobius kamerunicus* IN THE SPIKELETS OF OIL PALM MALE INFLORESCENCES FROM DIFFERENT POSITIONS OF SPIKELETS – MIRI SAMPLES

Sampling month	Miri											
	Number of adult weevils						Length of spikelet (cm)					
	Top	SE	Middle	SE	Bottom	SE	Top	SE	Middle	SE	Bottom	SE
01/15	23.96a	3.15	21.29a	2.89	27.50a	3.60	12.23a	0.52	12.21a	0.54	12.66a	0.61
02/15	28.70a	3.99	29.90a	3.82	29.77a	3.39	14.50a	0.51	14.39a	0.46	14.52a	0.43
03/15	29.58a	6.11	30.92a	5.92	28.75a	5.49	13.53a	0.68	13.70a	0.69	14.06a	0.72
04/15	36.73a	4.52	33.53a	4.46	37.67a	5.08	12.98a	0.42	12.80a	0.42	12.85a	0.44
05/15	24.56a	4.08	24.44a	3.57	23.67a	2.73	12.74a	0.61	13.78a	0.93	13.64a	0.91
06/15	25.87a	5.18	24.10a	4.54	25.07a	4.44	16.47a	0.64	16.62a	0.61	16.70a	0.66
07/15	27.67a	4.59	31.42a	5.54	28.17a	4.53	14.42a	0.80	13.95a	0.51	13.01a	0.88
08/15	16.33a	1.92	17.13a	3.30	19.53a	3.00	14.92a	0.49	14.49a	0.46	14.53a	0.43
09/15	18.67a	4.81	19.42a	5.12	20.83a	5.34	14.23a	0.91	15.23a	0.77	15.26a	0.92
10/15	16.90a	2.54	18.80a	2.47	18.90a	2.91	14.23a	0.70	14.42a	0.66	14.16a	0.64
11/15	29.58a	5.35	27.83a	4.97	30.08a	5.04	14.42a	0.60	14.71a	0.70	14.78a	0.85
12/15	41.43a	4.52	37.20a	3.48	36.67a	4.48	14.13a	0.52	13.65a	0.52	14.02a	0.56

Note: Means that do not share a letter are significantly different.
SE - standard error.

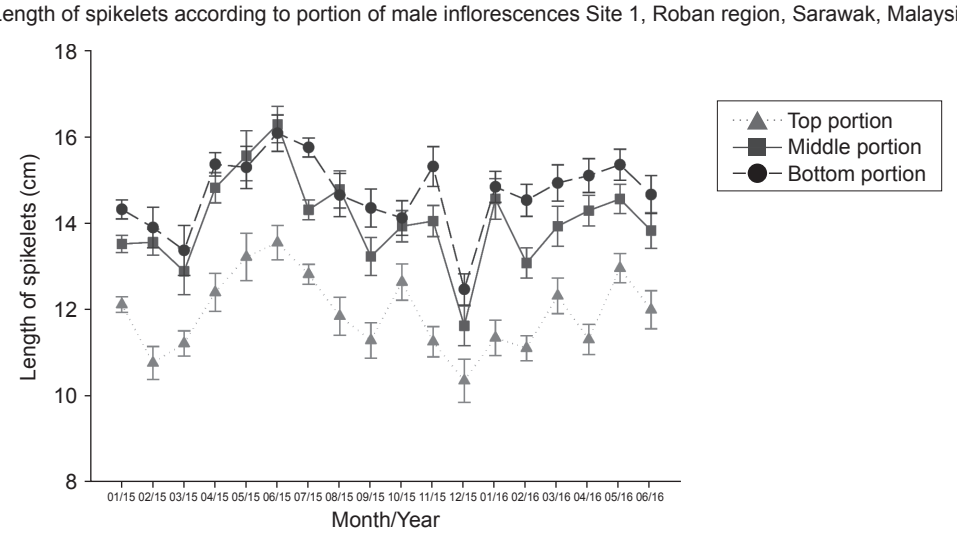

Figure 3. The length of spikelets (cm) in fully anthesising male inflorescence vs. *the position of spikelets at Site 1, Roban, Sarawak, Malaysia.*

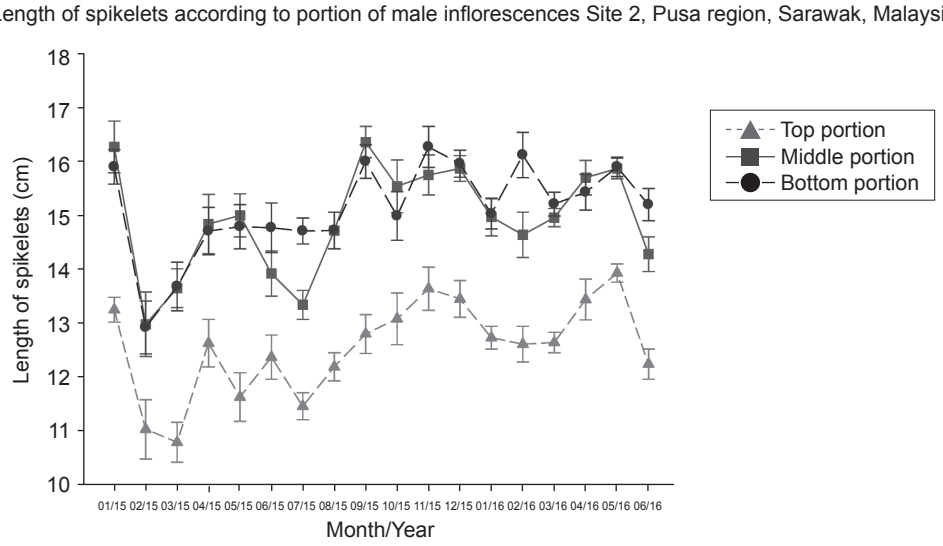

Figure 4. The length of spikelets (cm) in fully anthesising male inflorescence vs. *the position of spikelets at Site 2, Pusa, Sarawak, Malaysia.*

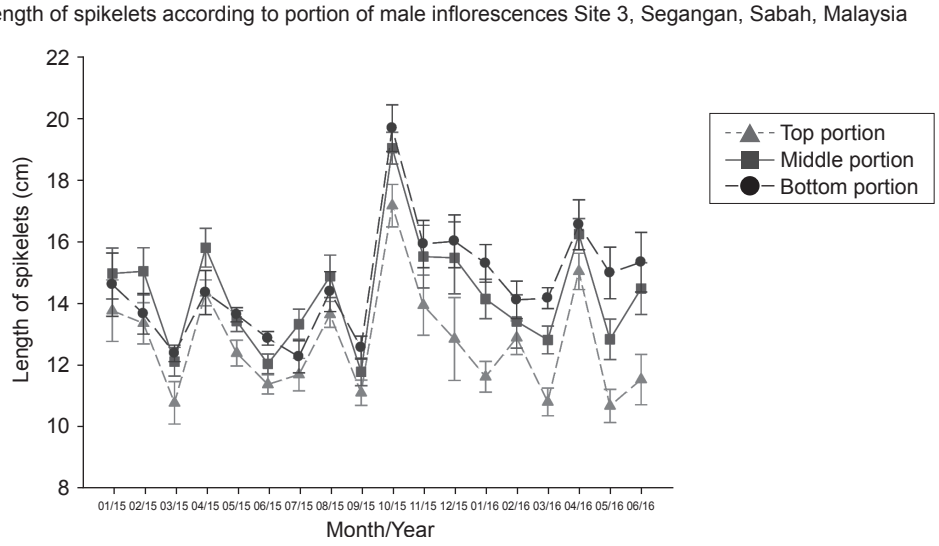

Figure 5. The length of spikelets (cm) in fully anthesising male inflorescence vs. *the position of spikelets at Site 3, Segangan, Sabah, Malaysia.*

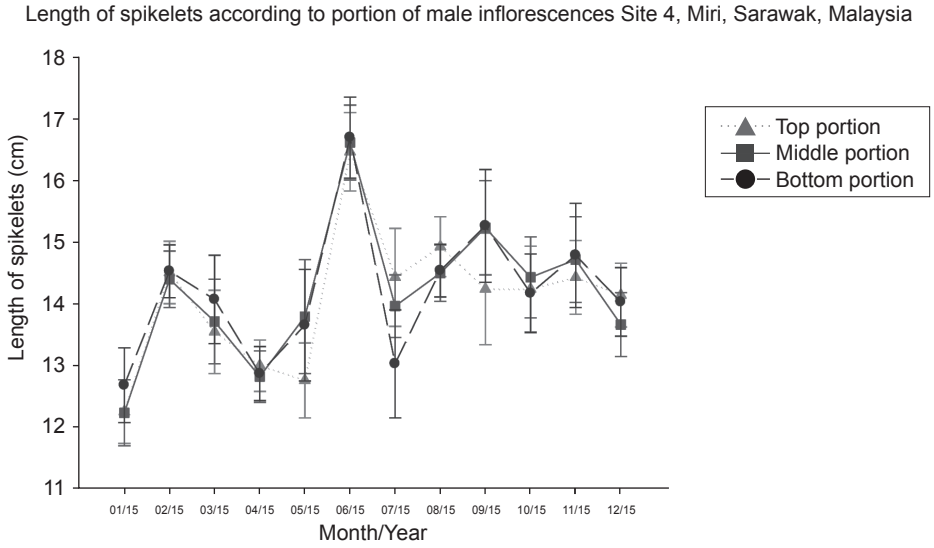

Figure 6. The length of spikelets (cm) in fully anthesising male inflorescence vs. *the position of spikelets at Site 4, Miri, Sarawak, Malaysia.*

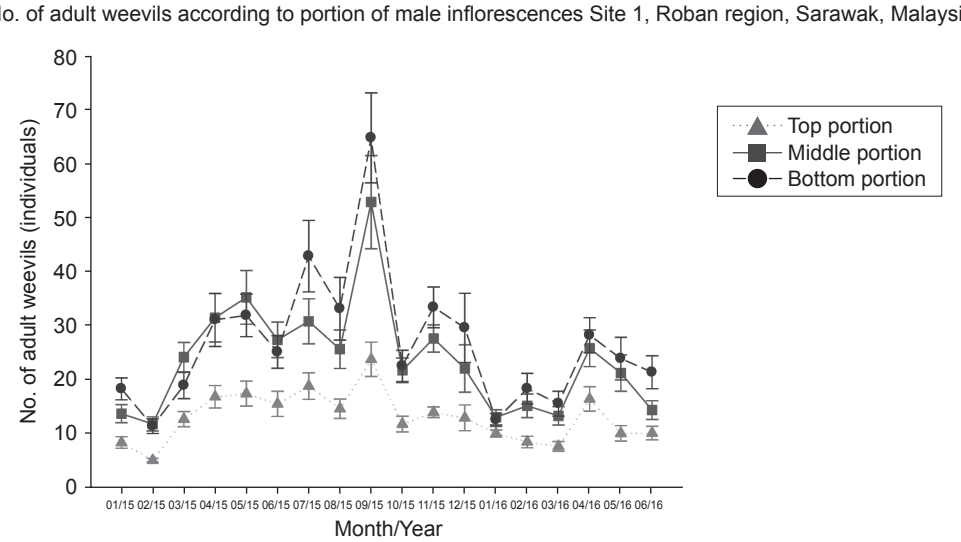

Figure 7. The number of adult Elaeidobius kamerunicus *in fully anthesising male inflorescence* vs. *the position of spikelets at Site 1, Roban, Sarawak, Malaysia.*

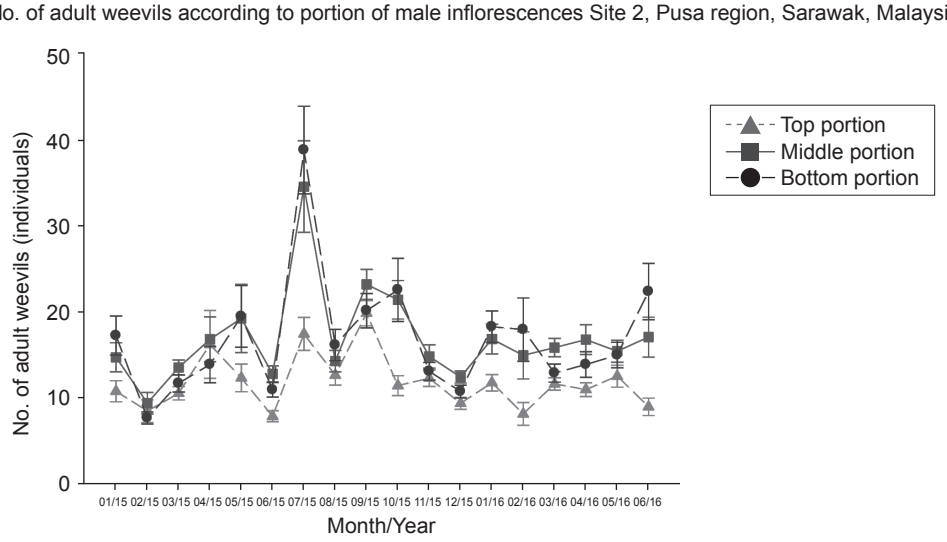

Figure 8. The number of adult Elaeidobius kamerunicus *in fully anthesising male inflorescence* vs. *the position of spikelets at Site 2, Pusa, Sarawak, Malaysia.*

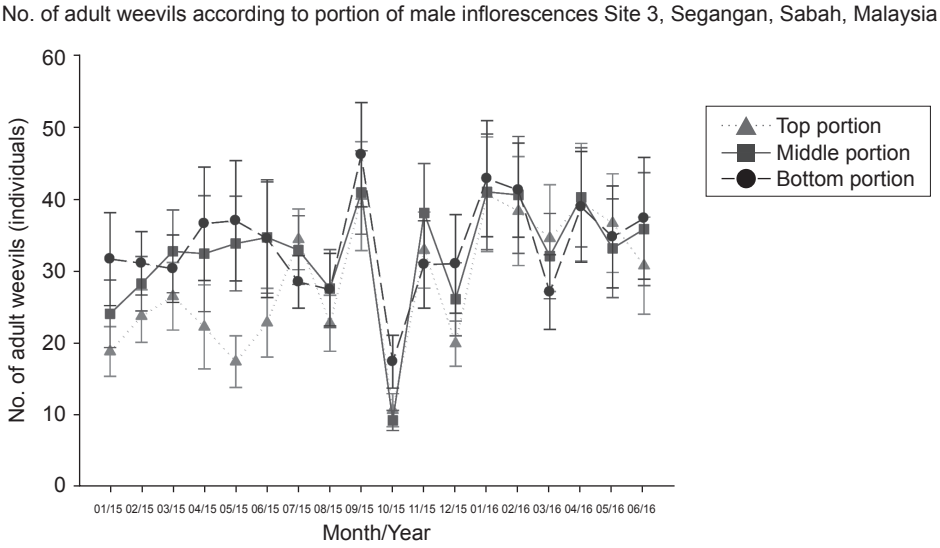

Figure 9. The number of adult Elaeidobius kamerunicus *in fully anthesising male inflorescence* vs. *the position of spikelets at Site 3, Segangan, Sabah, Malaysia.*

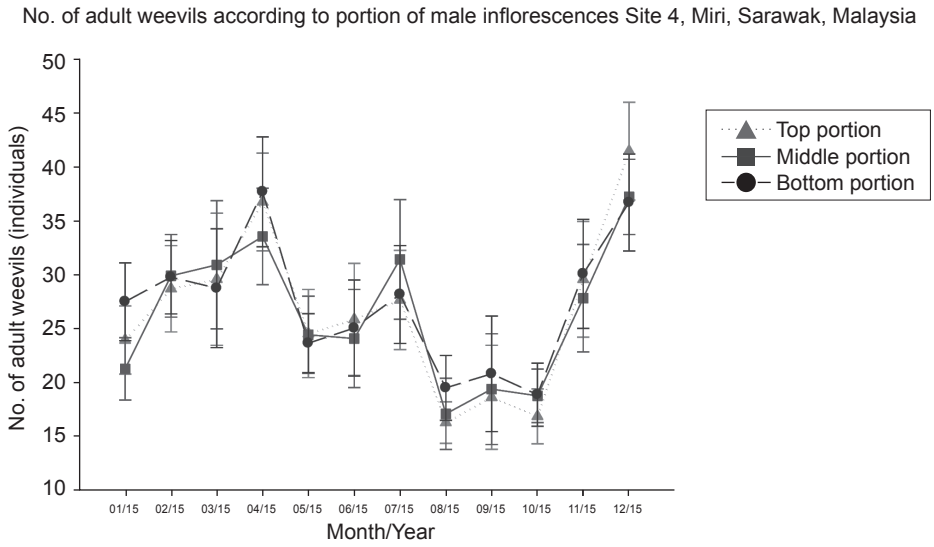

Figure 10. The number of adult Elaeidobius kamerunicus *in fully anthesising male inflorescence* vs. *the position of spikelets at Site 4, Miri, Sarawak, Malaysia.*

Correlation between the Number of Adult Weevils and the Spikelet Length

Weak positive correlations were found between the number of adults *E. kamerunicus* and the length of fully anthesising spikelets from the male inflorescences at Site 1, Sarawak (*Table 7*). The correlation coefficient at the site was at r = 0.154 (top), r = 0.181 (middle) and r = 0.155 (bottom). Similarly, at Site 2, Sarawak, a positive correlation was found between the number of adults *E. kamerunicus* and the length of fully anthesising spikelets from the bottom part of the inflorescence. However, at r = 0.161, it was considered as weak and unreliable. Additionally, a weak positive correlation was also found for samples from the top part (r = 0.021) and the bottom part (r = 0.054) of the inflorescence (*Table 7*).

There were no significant correlations found between the number of adults *E. kamerunicus* and the lengths of fully anthesising spikelets from the top (r = -0.090), middle (r = -0.076), and bottom portion (r = -0.059) of male inflorescences at Site 3, Sabah (*Table 7*). Similarly, there were also no correlations found between the number of adult weevils and the length of male inflorescences spikelet at Site 3, Sarawak (*Table 7*, r= 0.041-0.072).

TABLE 7. CORRELATION COEFFICIENTS BETWEEN THE NUMBER OF ADULT *Elaeidobius kamerunicus* AND THE LENGTH OF SPIKELET IN FULLY ANTHESISING MALE INFLORESCENCES IN FOUR LOCALITIES IN SABAH AND SARAWAK, MALAYSIA

Experimental site	Spikelet position	Mean numbers of adult weevil	SD	Mean length of spikelet (cm)	SD	Correlation coefficients
Site 1, Roban, Sarawak	Top	13.04	10.69	11.91	2.27	0.154**
	Middle	23.74	21.97	14.04	2.40	0.181**
	Bottom	26.83	25.83	14.68	2.36	0.155**
Site 2, Pusa, Sarawak	Top	11.70	8.43	12.55	2.09	0.021 n.s.
	Middle	16.92	13.32	14.93	2.28	0.054 n.s.
	Bottom	16.71	14.75	15.13	2.11	0.161**
Site 3, Segangan, Sabah	Top	28.51	20.94	12.68	2.83	-0.090 n.s.
	Middle	32.43	22.33	14.26	2.89	-0.076 n.s.
	Bottom	33.60	22.93	14.57	2.83	-0.059 n.s.
Site 4, Miri, Sarawak	Top	26.98	21.46	14.19	3.06	0.041 n.s.
	Middle	26.31	20.17	14.17	2.97	0.045 n.s.
	Bottom	27.57	21.00	14.23	3.09	0.072 n.s.

Note: **Correlation is significant at 1% level.
n.s. - not significant
SD - standard deviation.

DISCUSSION

Since its introduction into Malaysia in the early 1980s, the early phase of the pollinating weevil population study was conducted subsequently to determine the species establishment in the country. The study conducted by Basri *et al.* (1987) confirmed the establishment of the weevil population. In the study, it was reported that the stable population levels in Sabah and Sarawak were at 31.5 weevils per spikelet. Afterward, except for a study by Cik Mohd Rizuan *et al.* (2013), no recent study to monitor the population of the pollinating weevil in East Malaysia was conducted. Compared to the findings by Basri *et al.* (1987), the results from this study revealed that the average weevil population at Site 1, Site 2, and Site 4, which are located in Sarawak, is lower. Only the average weevil population at Site 3 (Sabah) remains at 31.51 weevils per spikelet.

Throughout the period of this study, the weevil population density per spikelet fluctuated, from as high as more than 35 weevils per spikelet to less than 10 weevils per spikelet. Apparently, this trend was also reported in previous studies (Nurul Fatihah *et al*., 2018; Lubis *et al.*, 2017; Syarifah Nadiah and Idris, 2016; Cik Mohd Rizuan *et al.*, 2013). As highlighted by Khaliq *et al.* (2014), the insect population dynamics can be influenced by both abiotic and biotic factors. In *E. kamerunicus* case, especially in South-east Asia, the fluctuation in the population can be due to varieties of factors, including high rainfall (Prasetyo *et al*., 2010), excessive and improper insecticide usage especially on male inflorescences (Ming and Bong, 2017; Najib *et al*., 2012; Purba *et al*., 2012; Hutauruk *et al*., 1985), predation by rats, ants, spiders, birds, mites and nematodes (Muhammad Luqman *et al*., 2017; Bettycopa *et al*., 2015; Puan *et al*., 2011; Krantz and Poinar, 2004; Aisagbonhi *et al*., 2004; Poinar *et al*., 2002; Sipayung *et al*., 1987; Syed, 1979) and reduction in the number of male inflorescences arising from the planting of high yielding materials, which focus on the production of the greater amount of female inflorescences (Prasetyo *et al*., 2012; Purba *et al*., 2009). Some of the factors arose due to the management practices itself and are avoidable. The extreme and sustained decline in the pollinating weevil population may have deleterious effects on the oil palm fruit formation. However, in this study, the effects of the temporary reduction of *E. kamerunicus* populations on the pollination efficiency were not studied, and perhaps can be considered for future studies.

Compared to the study locations on the peat soil areas, higher weevil population density per spikelet was observed in sites located on mineral soil areas. Similar results were also reported by Lubis *et al.* (2017). As the age profile and the planting materials of the palms in all study locations were more or less the same, the differences in terms of the weevil population could be attributed to the different types of soil that the oil palms were planted on.

Different soil types have significant influences on oil palm yield (Veloo *et al.*, 2015). They reported that the different peat characteristic (*i.e.* peat maturity and stage of composition) has the most significant effect on oil palm yield. Generally, oil palm planted on sapric soils has a higher yield compared to hemic soils. Mineral soils (*i.e.* sandy spodosol) also performed better than hemic soils. This was possible because oil palm roots are in

contact with highly decomposed sapric materials, which is a better rooting and growth medium, compared to semi-decomposed hemic materials. In addition, hemic peat with a higher level of porosity may not have good nutrient retention properties, especially in areas with high rainfall. In a review by Woittiez *et al.* (2017), biochemically constrained soils such as peat soils were identified as one of the oil palm yield-limiting factors. This perhaps, explains the difference in oil palm inflorescence development between the sites located in peat soils and mineral soils.

In addition to climatic factors, Prasetyo *et al*. (2010) reported that the lower pollinating weevil population in peat soil areas was also due to a lack of anthesising male inflorescences, which is predominant in young mature areas. Montes *et al.* (2018) also concluded that the number of male inflorescences directly affecting the pollinator populations. The quantification of male inflorescence density was not conducted in this current study but it was found that the formation of male inflorescence, especially in terms of spikelet development in peat soil, was less uniform compared to those in mineral soil areas. The length of spikelets sampled from sites on mineral soils was similar throughout all different positions of the spikelets on the male inflorescences. However, in samples from peat soil areas, in most of the time, the top spikelets were significantly shorter than the bottom spikelets. To a certain extent, this has also influenced the number of weevils lodged at each spikelet. On peat soil, the number of adult weevils per spikelet from the top portion (which has the shortest length) of the inflorescences, was significantly less than those at the bottom portion (longest spikelets). This finding suggests that more weevils can be found on longer spikelets, although, in this study, no strong positive correlation was found between the length of spikelets and the number of weevils. However, in contrast, a study by Ponnamma *et al.* (2006) found a strong positive correlation between the number of weevils that emerged under laboratory conditions and the length of spikelets (r=0.4315). Ponnamma *et al.* (2006) suggested that longer spikelets provide more incentives (*e.g.* food sources and breeding sites) to the pollinating weevils compared to the shorter ones, and thus, more attractive. At Site 3 and Site 4, where the length of the spikelets was more uniform, no significant differences were found for both number of weevils for each part of the inflorescence. Similar length of spikelets in each portion of the male inflorescences meant that a similar amount of food sources and breeding sites were available to the weevils, regardless of the spikelets position. Different length of spikelets in male inflorescences on peat soil areas may also influence the population levels of the pollinator, and affected the pollination process.

CONCLUSION

The population of the oil palm pollinating weevils, *E. kamerunicus* at sites located on peat soil areas was lower than those on mineral soil areas. One of the factors that may contribute to this fact was the difference in the formation of male inflorescences spikelets, which serve to provide food sources and breeding sites for the weevil. It has been observed that the formation of the spikelets on peat areas tend to be less uniform than the spikelets formation on mineral soil areas. The spikelets from the upper part of the inflorescences were shorter compared to the spikelets from other parts of the inflorescences, which means a lower amount of food and breeding sources were available in these spikelets. This was further substantiated by a significantly lower number of weevils lodged at the spikelets sampled from the top portion of the male inflorescences. However, future studies can be done to study the effects of lower pollinator populations on the oil palm fruit formation. Additional studies on the cause of the differences in the inflorescence's formation and physical properties on peat soil and mineral soil areas are also recommended. It is also useful to know how the differences in the soil physical properties and nutrient availability influence the sexual determination of the inflorescences, causing the lack of male inflorescences availability in the field.

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REFERENCES

Aisagbonhi, C I; Kamarudin, N; Okwuagwu, C O; Wahid, M B; Jackson, T A and Adaigbe, V (2004). Preliminary observations on a field population of the oil palm pollinating weevil *Elaeidobius kamerunicus* in Benin City, Nigeria. *Int. J. Tropical Insect Science, 24(3)*: 255-259.

Basri, M W; Zulkefli, M; Abdul Halim, H and Tayeb, D M (1987). The population census and the pollinating efficiency of the weevil, *Elaeidobius kamerunicus* in Malaysia - A status report, 1983-1986. *Proc. of the 1987 International Oil Palm/Palm Oil Conferences. Progress and Prospect*. PORIM, Bangi. p. 535-549.

Bettycopa, A; Andrew, A T; Khalid, H; Mohd Haniff, H and Kamarudin, N (2015). The diet of yellow-vented bulbul (*Pycnonotus goiavier*) in oil palm agro-ecosystems. *J. Oil Palm Res. Vol. 27(4)*: 417-424.

Chan, K Y; Oates, A; Swan, A D; Hayes, R C; Dear, B S and Peoples, M B (2005). Agronomic consequences of tractor wheel compaction on a clay soil. *Soil and Tillage Research, 89(1)*: 13-21.

Chiu, S B (1984). *Some Aspects of the Ecology of* Elaeidobius kamerunicus *Faust, the Pollinating Weevil of Oil Palm, with Emphasis on Developing Sampling Techniques*. Master thesis. Universiti Pertanian Malaysia, Selangor, Malaysia. http://psasir.upm.edu.my/10210/1/FP_1984_1_A.pdf

Chiu, S B; Khoo, K C and Hussein, M Y (1984). A method of estimating the natural population of the pollinating weevil, *Elaeidobius kamerunicus* Faust, of oil palm. *Proc. of the First Regional Symposium on Biological Control*. 4-6 September 1985, Universiti Pertanian Malaysia.

Cik Mohd Rizuan, Z A; Noor Hisham, H and Samsudin, A (2013). Role of pollinating weevil (*Elaeidobius kamerunicus*) seasonal effect and its relation to fruit set in oil palm area of FELDA. *Proc. of the PIPOC 2013 International Palm Oil Congress*. MPOB, Bangi. p. 19-21.

Corley, R H V (1976). Germination and seedling growth. *Developments in Crop Science* (Corley, R H V; Hardon, J J and Wood, B J E eds.). Vol. 1. Elsevier, Amsterdam, Netherlands. p. 37-54.

Corley, R H V and Tinker, P B (2015). *The Oil Palm*. 5th Edition. John Wiley and Sons. West Sussex, United Kingdom. p. 37-42.

Hamza, M A and Anderson, W K (2005). Soil compaction in cropping systems. A review of the nature, causes and possible solutions. *Soil and Tillage Research, 89(2)*: 121-145.

Haniff, M H and Roslan, M M N (2002). Fruit set and oil palm bunch components. *J. Oil Palm Res. Vol. 14(2)*: 24-33.

Hussein, M Y; Lajis, N H and Ali, J H (1991). Biological and chemical factors associated with the successful introduction of *Elaeidobius kamerunicus* Faust, the oil palm pollinator in Malaysia. *Acta Horticulturae, 288(1)*: 81-87.

Hutauruk, C H; Sudharto, P S; Siman-Gunsong, G and Sipayung, A (1985). *Menjelang dua tahun serangga penyerbuk kelapa sawit* Elaeidobius kamerunicus *di Indonesia*. *Simposium Kelapa Sawit*. 27-28 March 1985. Medan, Indonesia. p. 183-225.

Janick, J and Paull, R E (2008). *The Encyclopedia of Fruit and Nuts*. CABI Publishing, Oxfordshire, United Kingdom. 954 pp.

Kamaruddin, N; Moslim, R; Mohamad, S A and Sulaiman, M R (2018). Fruit set and weevil pollination issues in oil palm. *The Planters*: 565-581.

Khaliq, A; Javed, M; Sohail, M and Sagheer, M (2014). Environmental effects on insects and their population dynamic. *J. Entomology and Zoology Studies, 2(2)*: 1-7.

Krantz, G W and Poinar, G O (2004). Mites, nematodes and the multimillion-dollar weevil. *J. Natural History, 38(2)*: 135-141.

Lubis, F I; Sudarjat and Dono, D (2017). *Populasi serangga penyerbuk kelapa sawit* Elaeidobius kamerunicus *Faust dan pengaruhnya terhadap nilai fruit set pada tanah berliat, berpasir dan gambut di Kalimantan Tengah, Indonesia. Jurnal Agrikultura, 28(1)*: 39-46.

Ming, S C and Bong, C F (2017). Effect of different insecticides on the survival of the oil palm pollinator, *Elaeidobius kamerunicus* (Coleoptera: Curculionidae). *The Planter, 93(1100)*: 777-788.

Montes, B L G; Sanchez, L A; Prada, F; Daza, E S; Bustillo, A E and Romero, H M (2018). Relationships between inflorescences and pollinators and their effects on bunch components in *Elaeis guineensis* in Colombia. *J. Entomological Science, 53(4)*: 554-568.

Muhamad Fahmi, M H; Ahmad Bukhary, A K; Norma, H and Idris, A B (2016). Analysis of volatile organic compound from *Elaeis guineensis* inflorescences planted on different soil types in Malaysia. The 2016 UKM FST Postgraduate Colloquium. *AIP Conf. Proc., 1784(1)*. DOI: https://doi.org/10.1063/1.4966858.

Muhammad Luqman, H A; Dzulhelmi, M N; Idris, A B and Izfa Riza, H (2017). The potential natural predators of *Elaeidobius kamerunicus* Faust, 1878 (Coleoptera: Curculionidae) in Malaysia. *Serangga, 22(2)*: 239-252.

Najib, M A; Siti Ramlah, A A; Mohamed Mazmira, M M and Basri, M W (2012). Effect of Bt products, LEPCON-1, BAFOG-1 (S) and ECOBAC-1 (EC), against the oil palm pollinating weevil, *Elaeidobius kamerunicus* and beneficial insects associated with *Cassia cobanensis*. *J. Oil Palm Res. Vol. 24*: 1442-1447.

Nurul Fatihah, A L; Muhamad Fahmi, M H; Luqman, H A; Al-Talafha, H; Noor Nasuha, A A; Che Mohd Rizuan, Z A and Idris, A B (2018). Effect of oil palm planting materials, rainfall, number of male inflorescence and spikelet on the population abundance of oil palm pollinator, *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae). *Serangga, 23*(*1*): 35-45.

Poinar, G O; Jackson, T A; Bell, N L and Wahid, M B (2002). *Elaeolenchus parthenonema* n. g., n. sp. (Nematoda: Sphaerularioidea: Anandranematidae n. fam.) parasitic in the palm-pollinating weevil *Elaeidobius kamerunicus* Faust, with a phylogenetic synopsis of the Sphaerularioidea Lubbock, 1861. *Systematic Parasitology*, *52(3)*: 219-225.

Ponnamma, K N; Asha, V and Sajeebkhan, A (2006). Progeny emergence in *Elaeidobius kamerunicus*. *Planter, 82(962)*: 333-336.

Prasetyo, A E; Arif, M and Hidayat, T C (2012). Buah landak pada tanaman muda kelapa sawit. *Warta Pusat Penelitian Kelapa Sawit, 17(1):* 13-20.

Prasetyo, A E; Purba, W P and Susanto, A (2014). *Elaeidobius kamerunicus*: Application of hatch and carry technique for increasing oil palm fruit set. *J. Oil Palm Res. Vol. 26*: 195-202.

Prasetyo, A E; Supriyanto, E; Susanto, A and Purba, A R (2010). Population dynamics of *Elaeidobius kamerunicus* Faust, a case study on upland oil palm plantation. *Proc. of the PIPOC 2011 International Oil Palm Congress.* MPOB, Bangi. 668 pp.

Puan, C L; Goldizen, A W; Zakaria M; Hafidzi, M N and Baxter, G S (2011). Relationships among rat numbers, abundance of oil palm fruit and damage levels to fruit in an oil palm plantation. *Integr. Zool., 6(2)*: 130-139.

Purba, A R; Supriyanto, E; Supena, N and Arif, M (2009). *Peningkatan produktivitas kelapa sawit dengan menggunakan bahan tanaman unggul*. *Prosiding Pertemuan Teknis Kelapa Sawit (PTKS)*. 28-30 May 2009. Jakarta, Indonesia. p. 1-14.

Purba, R Y; Rozziansha, T A P and Pangaribuan, Y (2012). Strategies to improve effectiveness of pollination and productivity on early mature oil palm. *Proc. of the Fourth IOPRI-MPOB International Seminar. Existing and Emerging of Oil Palm Pests and Diseases – Advance in Research and Management*. 13-14 December 2012. Bandung, Indonesia. 409 pp.

Rahardjo, B T; Rizali, A; Utami, I P; Karindah S, Puspitarini, R D and Sahari, B (2018). Populasi *Elaeidobius kamerunicus* Faust (Coleoptera: Curculionidae) pada beberapa umur tanaman kelapa sawit. *J. Entomologi Indonesia, 15(1)*: 31-39.

Sipayung, A; Duryadi, D and Lubis, A U (1987). *Preferensi tikus terhadap jenis makanan dalam ekosistem perkebunan kelapa sawit*. Laporan akhir kerjasama penelitian Pusat Penelitian Marihat- Biotrop SIMEO Bogor, Indonesia.

Syarifah Nadiah, S M D and Idris, A G (2016). Population density of oil palm pollinator weevil *Elaeidobius kamerunicus* based on seasonal effect and age of oil palm. The 2016 UKM FST Postgraduate Colloquium. *AIP Conf. Proc. 1784(1)*. 5pp. DOI: https://doi.org/10.1063/1.4966889

Syed, R A (1979). Studies on oil palm pollination by insects. *Bull. Entomol. Res., 69 (Dec 1979)*: 213-224.

Syed, R A (1980). *Pollinating Insects of Oil Palm*. Commonwealth Institute of Biological Control (CIBC) Report.

Syed, R A; Law, I H and Corvey, R H V (1982). Insect pollination of oil palm: Introduction, establishment and pollinating efficiency of *Elaeidobius kamerunicus* in Malaysia. *The Planter, 58*: 504-561.

Veloo, R; Ranst, E V and Selliah, P (2015). Peat characteristics and its impact on oil palm yield. *NJAS-Wageningen J. Life Sciences, 72-73*: 33-40.

Woittiez, L S; Wijk, M T V; Slingerland, M; Noordwijk, M V and Giller, K E (2017). Yield gaps in oil palm: A quantitative review of contributing factors. *Euro. J. Agronomy, 83*: 57-77.

Yahya, Z; Mohammed A T; Harun, M H and Shuib, A R (2012). Oil palm adaptation to compacted alluvial soil (Typic Endoaquepts) in Malaysia. *J. Oil Palm Res. Vol. 24*: 1533-1541.

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ECONOMIC INJURY LEVEL OF OIL PALM BUNCH MOTH, *Tirathaba mundella* WALKER FOR PEST MANAGEMENT RECOMMENDATIONS IN OIL PALM PRODUCTION

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ABSTRACT

Oil palm bunch moth, Tirathaba mundella *Walker is a notorious bunch feeding pest typically among oil palm aged 3-7 years old planted on peat. In order to manage the pest, an economic injury level (EIL) for the pest needs to be determined which could assist in decision-making if a control tactic is justified. In order to determine the EIL, the percentage of fertile oil palm fruitlets and oil to bunch content were determined for fruit bunches with different pest infestation severity. The severity was characterised based on the mean larvae present in fruit bunches and male inflorescences. The study found that the mean larvae count was positively correlated with the economic losses and number of parthenocarpic fruitlets. The overall oil extraction rate (OER) of moderate and severely infested fruit bunches was significantly reduced as compared to clean fruit bunches. Based on average crude palm oil (CPO) market price and production per hectare, an EIL for* T. mundella *was able to be estimated. This study suggested the EIL at 10% of oil palms per hectare moderately or severely infested. The finding of this study would benefit future pest management practice in oil palm plantation established on peatland.*

Keywords: economic injury level, oil palm bunch moth, fruit set, oil to bunch, *Tirathaba mundella*.

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INTRODUCTION

Today, Indonesia and Malaysia are the world's biggest palm oil producers contributing 85% of the global palm oil supply which accounted for 34% of world vegetable oils consumption in 2018 (Kushairi *et al*., 2019). Compared with other oil bearing crops, oil palm is a highly efficient producer of vegetable oil. It needs less land, only 0.26 ha to produce 1 t of oil compared with 2.2, 2.0 and 1.5 ha for soyabean, sunflower and rapeseed respectively (Wahid *et al*., 2011). On average, the oil palm industry contributes 5%-7% of Malaysia's gross domestic product (GDP) with export revenue for the last five years averaging at RM 64.24 billion annually (Balu *et al*., 2018). However, the oil palm industry is facing multifaceted challenges; among them are severe pest infestation that adversely affected the oil palm yield.

Tirathaba mundella Walker, a menacing oil palm bunch moth that feeds on oil palm inflorescences as well as oil palm fruit bunches, are reported to severely negative impacted on palms especially those planted on peat and approaching maturity (5-7 years old) (Su, 2016; Lim, 2012; Lim *et al*., 2012). In severe infestation cases, abortion of male inflorescences and fruit bunches were reported (Su, 2016). This will cause a substantial loss in yield. However, pest control measures are often costly. Therefore, it is important to recognise at which point will the pest population begins to cause sufficient

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damage to justify the time and expense of control measures. To a great extent, the answer depends on two fundamental pieces of economic information: (1) how much financial loss is the pest causing? and (2) how much will it cost to control the pest? Therefore, in this study, the economic injury level (EIL) of *Tirathaba mundella* (*T. mundella*) for an oil palm plantation was investigated to estimate the economic losses and determine the break out point where a pest control treatment must be carried out (Stejskal, 2003). The estimation of the EIL of *T. mundella* in oil palm plantation will enable the oil palm operators to fully appreciate the data of their pest field census as the capacity to make appropriate management decisions. A severity index is proposed in this study as a standard for field census and the EIL could be used as effective pest management strategy tool.

MATERIALS AND METHODS

Assessment of Pest Infestation Severity in Mineral and Peat Estates

Two young mature (7-year old) oil palm estates were selected as study sites. Estate A was established at peatland (N 4° 02´ 57.660 E 114° 13´ 10.380) and Estate B was established on mineral soil (N 4° 07´ 43.248 E 113° 58´ 49.188), both under Miri district, Sarawak, Malaysia. The severity of the oil palm bunch moth, *T. mundella*'s infestation was assessed, which covered an area of 5 ha per estate, with each replicate covered an area size of 1 ha. In both study sites, there were only one round of pesticide treatment applied in previous year and no treatment during the study period (Pin, 2018, Pers. Comm.). All fruit bunches and male inflorescences were collected from each assessment area and examined then categorised into three groups based on their infestation severity stages, namely light to clean, moderate and severe. Light to clean category was determined as fruit bunches or male inflorescences that did not show any obvious sign of infestation, shown in *Figures 1a* and *1b* or had less than 25% percentage of the surface covered with pest frass. Moderate infestation referred to a condition where 25%-50% of the surface of either a bunch or a male inflorescence was covered with pest frass (*Figures 2a* and *2b*) and the severe infestation was referred to a condition where more than 50% of the surface was covered by the frass (*Figures 3a* and *3b*).

Larvae Count for Oil Palm Male Inflorescences and Fruit Bunches Based on Severity Categories

Thirty oil palm male inflorescences and 30 fruit bunches in each category (light to clean, moderate and severe) were further assessed to evaluate the

Figure 1a. Light to clean oil palm fruit bunch.

Figure 1b. Light to clean oil palm male inflorescence.

Figure 2a. Ripe oil palm fruit bunch with moderate oil palm bunch moth infestation.

Figure 2b. Post-anthesis oil palm male inflorescence with moderate oil palm bunch moth infestation.

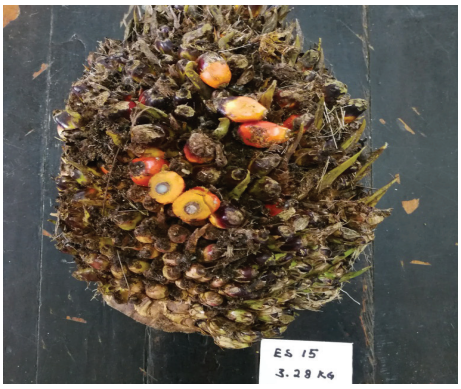

Figure 3a. Ripe oil palm fruit bunch with severe bunch moth infestation.

Figure 4. Spikelets of fruitlets after dissection.

Figure 3b. Post-anthesis oil palm male inflorescence with severe bunch moth infestation.

Figure 5a. Fertilised fruitlets: fully formed fruitlets with kernel and nut.

Figure 5b. Parthenocarpic fruitlets: fully formed fruitlets without kernel and nut.

average number of larvae present in each category. All fruit bunches and male inflorescences were dissected and the larvae residing in the sample were extracted. After each dissection, the sample was soaked in clean water for 5 min to capture the remaining larvae. Total number of larvae and their instar stages were recorded. The larvae count data were subjected to logarithmic transformation to normalise and after transformation, the larvae count data responded were subjected to analysis of variance using statistical analysis system (SAS) version 8.2.

Percentage of Fruit Set for Oil Palm Fruit Bunches in Each Severity Categories

Forty oil palm fruit bunches at their full ripening stages from each severity category, namely, light to clean, moderate and severe infestation were randomly sampled. The sampled bunches were weighed, dissected, and the bunch stalks were weighed. Then all the spikelets of fruitlets (*Figure 4*) were counted for their fertilised (*Figure 5a*) and parthenocarpic fruitlets (*Figure 5b*) ratio. Percentage of fruit set, which is the percentage of the total number of fertilised fruitlets to the total number of fertile plus parthenocarpic fruitlets in the sampled bunch (Sugih *et al*., 1996; Lawton, 1981; Harun and Roslan, 2002) was calculated based on the following formula:

$$\text{Percentage of fruit set} = (\text{total fertilised fruitlets} / \text{total fruitlets}) \times 100\%$$

The percentage of fruit sets were subjected to ANOVA using SAS version 8.2 and the mean percentage of fruit sets for each category were tested for their significance difference using the Duncan New Multiple Range Test at a significant level of $p<0.05$.

Bunch Weight and Oil Extraction Rate for Each Severity Category of Oil Palm Fruit Bunches

The weight of fresh fruit bunches (FFB) from each severity category was recorded immediately after harvesting. The bunches were then dissected with an axe to separate the spikelets from the stalk. All the spikelets and loose fruits were weighed to get spikelets per bunch data. All the fruitlets were stripped from the spikelet and weighed to obtain the fruit to spikelet ratio. The fruitlets from each sample were then depericarped to separate the mesocarp fibre and the kernel nut. All the mesocarp from each sample was weighed before drying at 80°C overnight. The dried mesocarp was weighed after cooling to obtain the dry mesocarp to fruitlet ratio. The dried mesocarp was then ground using an electric grinder. The ground mesocarp fibre was then dried at 80°C for another 4 hr to remove any remaining moisture. Oil was then extracted in 200 cc Soxhlets for 10 hr aggregate. After extraction, the samples were air-dried overnight followed by oven dry at 80°C for another 2 hr the next day. After drying, the samples were cooled in a desiccator before final weighing (Blaak *et al*., 1963). The oil in oil palm fruit mesocarp was calculated by the difference before and after drying. The formula for oil is shown below:

$$\text{Oil to bunch} = (\text{spikelet/bunch}) \times (\text{fruitlet/spikelet}) \times (\text{dried mesocarp/fruitlet}) \times (\text{oil/dry mesocarp}) \times 100\%$$

The oil to bunch data obtained from each category of bunch was then compiled and subjected to ANOVA using SAS version 8.2 and the mean percentage of oil to bunch for each category was tested for their significance difference using the Duncan New Multiple Range Test at a significant level of $p<0.05$.

RESULTS AND DISCUSSION

Comparison of *Tirathaba mundella* Infestation Percentage between Mineral and Peat Oil Palm Estates

Field assessments were carried out in two 7-year old palm estates. The severity of the infestation of *T. mundella* in peat estate was compared to mineral estate, as shown in *Figure 6*. Peat estate had significantly higher percentage of fruit bunches in moderate to severe categories than mineral estate. The mineral estate, on the other hand, had 100% of the collected fruit bunches in light to clean category.

This finding is expected as many studies have reported oil palm bunch moth is a major pest in oil palm plantations established on peat and sandy soils whereas mineral estates suffered in a lesser extent (Prasetyo, 2018). *Tirathaba mundella* feasts on male and female oil palm inflorescences in many peat estate (Lim, 2012; Wood and Ng, 1974; Su and Bong, 2017). It was reported that the first three months of oil palm inflorescences development were the most susceptible stage (Su, 2016). The larvae of *T. mundella* gain their entry through small openings of the female inflorescences when the sheaths had burst (Ng, 1977). The larvae build and move along in the tubes of silk which are attached with the granular faeces and other detritus. They feed on the ovules of flowers and plant tissues leaving behind all their faeces (Wood and Ng, 1974; Ng, 1977). When the damage was fresh, the faeces were in reddish colour (*Figure 7a*) and turned brownly black when aged (*Figure 7b*).

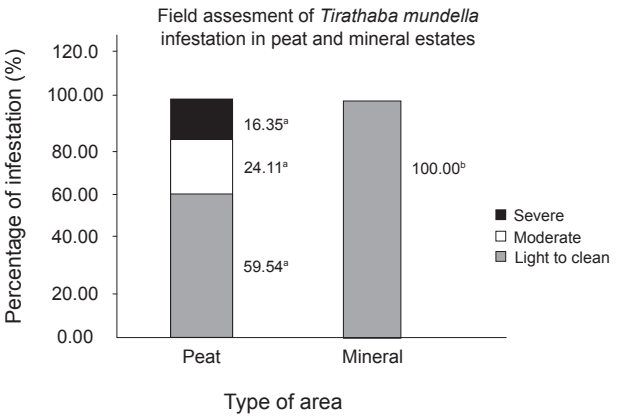

Figure 6. Difference of mean percentages of light to clean, moderate and severe infestation of oil palm fruit bunches between mineral and peat estates. Mean percentage with different superscripts were significantly different using T-test.

Tirathaba mundella Infestation Severity Index

The severity of *T. mundella* infestation was classed into three categories and the number of pest larvae found in the inflorescences as well as the fruit bunches based on each category were determined. The mean number of larvae found per male inflorescence in severe category was 24 (rounded up figure), 11 larvae in moderate category and five larvae in light to clean category (*Table 1*). The mean number of larvae found

Figure 7a. Oil palm fruit bunches with severe new infestation covered by reddish faeces (red circle).

Figure 7b. Oil palm fruit bunches with severe old infestation covered by brownish black faeces (red circles).

in oil palm fruit bunches were 22 larvae in the severe category, 10 larvae in moderate category and two in light category (*Table 1*).

Table 1 could serve as a standard severity index that is practical and easy to be adopted by oil palm operator in their field census to estimate the pest population density. They only need to make visual estimation on the percentage of frass coverage on the fruit bunches as well as inflorescences (*Figures 1* to *3*) to determine the severity category and then estimate the larvae present in the field based on *Table 1*. A field census on pest density would soundly inform the management if a control measure should be taken. It is crucial as the pest management is usually costly.

An interesting observation was noted that the majority of the infested male inflorescence and fruit bunches were less than four months old where most of the inner fruitlets were still under development stage. This suggests that *T. mundella* preferred to infest on young fruit bunches (1-3 months old) than those more than four months old bunches where most of the fruitlets were fully formed.

The young larvae of *T. mundella* tunnelled through the soft tissue of young fruitlets and feed on the juicy embryo kernel and which later developed into fruitlets with a hollow centre due to prior destruction of the kernel (Su, 2016). Larvae of the bigger instar fed on 1-3 months old fruitlets, often resulting to scarring and pitting of the fruit surface (Ng, 1977).

For oil palm male inflorescences that were infested with *T. mundella*, a depressions on the spikelets can be obeserved. Depending on the degree of damage, each spikelet would still develop until full anthesis stage where it would attract oil palm pollinators, *Elaeidobius kamerunicus* to visit and complete the pollination process (Su and Bong, 2017).

For oil palm fruit bunches in moderate severity category, scarring and pitting were frequently observed on the fruitlets at the outer layer of the bunch while smaller fruitlets in the inner layer could become aborted due to destruction of the kernel. The fruit bunches in this severity category could continue to develop into maturity. The ripened bunches were with portions of undeveloped fruitlets and corky appearance as the outcome of mesocarp damage by the pest. For severely infested fruit bunches, the damage could lead to bunch abortion (*Figure 8*), and put the bunch development to a halt.

Figure 8. Aborted and rotten oil palm fruit bunch associated to severe Tirathaba mundella *infestation.*

Male inflorescence with severe infestation were noted to have most of their spikelets damaged and unable to develop into the anthesis stage. When less spikelets developed into the anthesis stage, it would attract less weevils to visit and indirectly negatively adversed the pollination process and resulted in poor fruit set. In some very severe cases when most of the spikelets were attacked, the male inflorescence would just abort and never reached anthesis stage (Su, 2016).

Reduced pollination due to the damages caused by *T. mundella* on the inflorescences would result in poor fruit set formation and subsequently affecting the oil palm yield. In addition to that, pest damage on the fruit bunches would directly impinge the palm oil yield quantity. Lim (2012) estimated that in a field with an outbreak of the pest and where most of the bunches were severely damaged, the yield losses could be as high as 50%.

In order to assist in pest management decision making, an EIL was determined in this study. EIL which is one of the important component of integrated pest management (IPM) can be defined as the lowest population density that will cause economic damage (Stern *et al*., 1959).

The mean percentages of fertilised fully formed fruitlets, malformed small fruitlets and parthenocarpic fruitlets were compared among the three pest infestation severity categories as shown in *Figure 9*. Fruit bunches with fertilised fully formed fruitlets in moderate and severe categories were significantly lower as compared to light to clean category (*Figure 9*) and they also had more malformed small fruitlets and parthenocarpic

TABLE 1. MEAN NUMBER OF *Tirathaba mundella* LARVAE FOUND ON BOTH OIL PALM FRUIT BUNCHES AND MALE INFLORESCENCES BASED ON DIFFERENT CATEGORIES OF INFESTATION SEVERITY

Tirathaba mundella infestation severity category	Percentage of pest faeces covered on surface (%)		Mean number of larvae	
	Fruit bunch	Male inflorescence	Fruit bunch	Male inflorescence
Light to clean	0 - 25	0 - 25	1.67±0.60^{c}	4.86±1.64^{b}
Moderate	25 - 50	25 - 50	9.93±1.95^{b}	10.86±3.08^{b}
Severe	>50	>50	21.13±3.50^{a}	23.71±6.96^{a}

Note: Mean with different superscripts were significantly different at p<0.05 by Duncan Multiple Range Test.

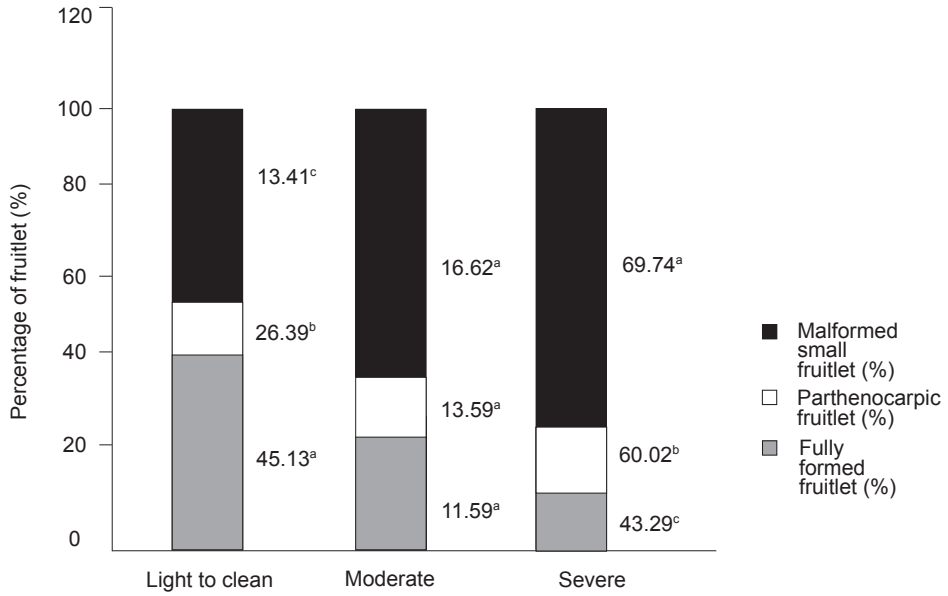

Figure 9. Percentage of fully formed, malformed and parthenocarpic fruitlets found on light to clean, moderate and severe infested fruit bunches. Mean percentage with different superscripts were significantly different at p<0.05 by Duncan Multiple Range Test.

fruitlets. This established the association of pest infestation severity with poor fruit set. This association was also noted in a study conducted in Indonesia by Prasetyo *et al*. (2018). Although poor fruit set formation has multifacted factors such as pollinators density, inflorescences sex ratio (Rao and Law, 1998; Syed and Saleh, 1987), the pest feeding on inflorescences is a undoubtedly the cause. Therefore controlling the pest population is crucial to maintain healthy fruit set formation.

Fruit bunch weight is used as one of parameters to measure oil palm plantation yield. The bunch weight for each severity categories were compared as shown in *Table 2*. The light to clean fruit bunches carried significantly heavier bunch weight than those in moderate and severe categories. Lower bunch weight means lower yield performance of an oil palm estate (Rao and Law, 1998).

Based on Rao and Law (1998), total yield is calculated based on the following formula:

Total yield = Total number of bunches x average weight per bunch

Therefore, with the data of total number of bunches harvested in a light to clean, moderate or severely infested oil palm estate, the yield of that estate can be estimated based on average weight per bunch in *Table 2*. The total number of bunches collected is usually available and accurately recorded as harvesting payment is made based on the number of bunches harvested.

Table 2 shows the fruit bunches in severe category carried 50% less weight than light to clean category. The loss of bunch weight is anticipated as there were significantly higher percentage of the malformed fruitlets in a bunch (*Figure 9*). The size of malformed fruitlets was significantly smaller than the normal fully-formed fruitlets. Loss in bunch weight has seriously impinged on both the yield per hectare and overall economy for the oil palm plantation.

The oil production per unit of a planted area is estimated by weight of FFB produced and its oil extraction rates (OER) in a mill (Donough *et al*., 2015; Julia *et al*., 2017). Therefore, another measurement to be taken into account to estimate the economic loss associated with pest infestation is the OER.

TABLE 2. MEAN BUNCH WEIGHT FOR BUNCHES IN LIGHT TO CLEAN, MODERATE AND SEVERE INFESTATION CATEGORIES

T. mundella infestation severity category	Percentage of pest faeces covered on fruit surface (%)	Mean bunch weight
Light to clean	0 - 25	6.56±0.33[a]
Moderate	25 - 50	4.49±0.19[b]
Severe	>50	3.79±0.19[b]

Note: Mean with different superscripts were significantly different at p<0.05 by Duncan Multiple Range Test.

The OER is a useful management tool to assess the quantity of crude palm oil produced per area of planted oil palm and from there loss or gain of the oil palm enterprise could be estimated (Chang *et al*., 2003). Oil to bunch analysis on the other hand, could be done by any laboratory using very basic facilities. It would provide a basic data on intrinsic oil in the bunch which could be used to estimate the realised oil extraction rate commonly used by the industry to calculate the revenue. *Table 3* shows the mean percentage of oil content in all three pest infestation severity categories. Both moderately and severely infested fruit bunches had significantly lower oil content than light to clean fruit bunches. This is related to the significantly higher percentage of malformed fruitlets found among fruit bunches in moderate and severe categories (*Figure 9*). Mostly malformed fruitlets carry very minimal or negligible oil content.

TABLE 3. THE OIL TO BUNCH PERCENTAGE FOR BUNCHES IN LIGHT TO CLEAN, MODERATE AND SEVERE INFESTATION CATEGORIES

Tirathaba mundella infestation severity category	Percentage of pest faeces covered on fruit surface (%)	Oil to bunch
Light to clean	0 - 25	23.63±0.80[a]
Moderate	25 - 50	14.29±0.75[b]
Severe	>50	11.93±1.03[b]

Note: Mean with different superscripts were significantly different at p<0.05 by Duncan Multiple Range Test.

According to Chan (1981), the oil loss from mill processing averages about 8% of the oil recovered. Oil losses could incur due to many factors that include poor operation management such as uncollected loose fruits during routine harvesting intervals. Pertaining to this, a factor of 0.855 is suggested by the Institut de Recherche pour les Huile et Oleagineux (IRHO) to account for field and mill losses when converting laboratory oil to bunch figures to realised mill OER. The oil content of different categories of infested bunch was analysed and converted OER as shown in *Table 4*.

There was a significant reduction in OER for moderately and severely infested fruit bunches than light to clean fruit bunches. Significant reduction in oil extraction rate would result in monetary losses for a company (Farahida *et al*., 2017). In this study, the OER loss was estimated at 8% for moderately infested fruit bunches and 10% for severely infested bunches.

The average production of CPO per hectare is 4.01 t whereas the production of palm kernel oil is 0.5 t ha^{-1} (Rao and Law, 1998; Donough *et al*., 1996). Based on 2019 average market price of CPO at RM 2100 t^{-1}, and RM 2600 t^{-1} for crude palm kernel oil (MPOC, 2019), a 1% reduction in OER is equivalent to a loss of RM 97 ha^{-1} (*Table 5*).

The average cost of a standard control measure using *Bt*. based insecticides is estimated as RM 77 ha^{-1} (RM 35 for pesticides and RM 42 for labour cost). Therefore, a 0.8% drop of OER should alarm an intervention to be taken as the economic loss is at par with the control measure cost. Based on the finding of this study, if 10% of a hectare of an oil palm estate is moderately or severely infested by *T. mundella*, it will cause an average 0.8%-1% drop in OER, valued at RM 77.60-RM 97. Therefore, the EIL for *T. mundella* in oil palm estate is set at 10% of a hectare of an oil palm estate is moderately or severely infested by *T. mundella*. This is lower than the recommended EIL for *T. rufivena* proposed in 1991 by IRHO, which had set the EIL for at 30% of palms with at least one bunch more than 50% attacked (young plantings) and 60% (older/mature planting). The difference may due to the variance in genus of insect pest. The proposed EIL can serve as a guideline for the management of *T. mundella*.

TABLE 4. POTENTIAL OIL EXTRACTION RATE (OER) REDUCTION FOR MODERATE AND SEVERE PEST INFESTATION BASED ON BUNCH ANALYSIS AND CALCULATED OER

Infestation category	Oil content (%) (bunch analysis)	Calculated OER (%)	Potential OER reduction (%)
Light to clean	23.63	20.20	-
Moderate	14.29	12.21	-7.99
Severe	11.93	10.20	-10.00

TABLE 5. AVERAGE OIL PRODUCTION PER HECTAR AND MONETARY LOSS BASED ON 1% OIL REDUCTION

Oil type	Average production per hectare (t)	Reduction of 1% in oil extracted (t)	Loss in RM (rounded up)
Crude palm oil	4.01	0.0401	84
Crude palm kernel oil	0.5	0.005	13
Total			97

CONCLUSION

Tirathaba mundella infestation is more profound in 7-year old oil palm peat estate than mineral estate of the same age. Using an easy to use *T. mundella* infestation severity index produced in this study, a field survey based on visual estimation of the percentage of faeces covered area on male inflorescences or fruit bunches can categorise an area into one of the three severity categories. Based on the category, the pest density can be roughly estimated as the mean number of larvae found corresponded to the severity categories. The bunch weight, the mean percentage of fruit set and oil to bunch were used to determine the overall oil extraction yield which translated into monetary value to estimate EIL. Based on this study, the EIL for *T. mundella* in oil palm estate is suggested at 10% of a hectare of an oil palm estate under moderate or severe *T. mundella* infestation. Further research is recommended to investigate ways to sustainably reduce the pest population without harming the beneficial insects.

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REFERENCES

Balu, N; Azman, I; Norfadilah, H; Nazlin, I; Dayang, N S; Nik, A N I; Noraida, O; Kamalrudin, M S; Hassan, N A M and Kushairi, A (2018). Malaysia: 100 years of resilient palm oil economic performance. *J. Oil Palm Res. Vol. 30(1)*: 13-25.

Blaak, G; Sparnaaij, L D and Menendez, T (1963). Breeding and inheritance in the oil palm (*Elaeis guineensis* Jacq.) Part II. Methods of bunch quality analysis. *J. West African Institute of Oil Palm Research*, *4*: 146-155.

Chan, C O (1973). Some notes on the oil palm bunch moth (*Tirathaba mundella* Walk.) and its control. *Advances in Oil Palm Cultivation* (Wastie, R L and Earp, D A eds.). Incorporated Society of Planters, Kuala Lumpur. p. 396-401.

Chan, K S (1981). The estimation of mill oil losses. *The Selangor Planters' Association Journal*.

Chang, L C; Abdullah Sani, A R and Basran, Z (2003). An economic perspective of oil extraction rate in the oil palm industry of Malaysia. *Oil Palm Industry Economic J. Vol. 3(1)*: 25-31.

Corley, R H V and Tinker, P B (2003). *The Oil Palm*. 4th edition. 562 pp.

Donough, C R; Chew, K W and Law, I H (1996). Effect of fruit set on OER and KER: Results from studies at Pamol Estates (Sabah) Sdn Bhd. *The Planter*, *72(841)*: 203-219.

Donough, C R; Cock, J; Oberthur, T; Indrasuara, K; Rahmadsyah; Gatot, A R and Dolong, T (2015). Estimating oil content of commercially harvested oil palm fresh fruit bunches - A step towards increasing palm oil yields. *Oil Palm Bulletin No. 70*: 8-12.

Farahida, Z; Nasuddin, O; Syahrizan, S; Zaini, M R and Muhammad, A B (2017). Fresh fruit bunch quality and oil losses in milling processes as factors that affect the extraction rate of palm oil. *Int. J. Agriculture, Forestry and Plantation, 5:* 99-103.

Harun, M H and Noor, M R M (2002). Fruit set and oil palm bunch components. *J. Oil Palm Res. Vol. 14(2)*: 24-33.

Julia, A; Alawi, S; Mokhtar, M N; Baharuddin, A S and Daud, N M (2017). Development of palm oil extraction performance index (EPI) based on oil extraction rate (OER) and oil loss (OL). *Pertanika J. Sci. and Technol*., *25(S)*: 335-344.

Kushairi, A; Meilina, O A; Balu, N; Elina, H; Mohd Noor, I Z B; Razmah, G; Vijaya, S; Shamala, S and Ghulam, K A P (2019). Oil palm economic performance in Malaysia and R&D progress in 2018. *J. Oil Palm Res. Vol. 31(2)*: 165-194.

Lawton, D M (1981). Pollination and fruit set in the oil palm (*Elaeis guineensis* Jacq.). *Oil Palm in the Eighties* (*Malaysian Oil Palm Conference*). p. 241.

Lim, K H (2012). Integrated pest management of *Tirathaba* bunch moth on oil palm planted on peat. *The Planter*, *88(1031)*: 97-104.

Lim, K H; Lim, S S; Parish, F and Suharto, R (2012). *RSPO Manual on Best Management Practices (BMPs) for Existing Oil Palm Cultivation on Peat*. RSPO, Kuala Lumpur.

Malaysian Palm Oil Council (MPOC) (2019). Monthly palm oil trade statistic. http://www.mpoc.org.my/monthly-palm-oil-trade-statistics-2019.

Ng, K Y (1977). *Bionomics of* Tirathaba mundella, *A Pest of Oil Palm*. Master of Agricultural Science thesis, Universiti Malaya, Kuala Lumpur.

Ng, S K; Helmut, V U and Hardter, R (2003). Botanical aspects of the oil palm relevant to crop management. *Oil Palm Management for Large and Sustainable Yields*. p. 13-24.

Pin, O K (2018). Personal communication. Sarawak Oil Palms Berhad, Miri, Sarawak.

Prasetyo, A E; Lopez, J A; Eldridge, J R; Zommick, D H and Susanto, A (2018). Long-term study of *Bacillus thuringiensis* application to control *Tirathaba rufivena*, along with the impact to *Elaeidobius kamerunicus*, insect biodiversity and oil palm productivity. *J. Oil Palm Res. Vol. 30(1)*: 71-82.

Rao, V and Law, I H (1998). The problem of poor fruit set in parts of East Malaysia. *The Planter*, *74(870)*: 463-483.

Stern, V M; Smith, R F; Van Den Bosch, R and Hagen, K S (1959). The integration of chemical and biological control of the spotted alfalfa aphid: The integrated control concept. *Hilgardia, 29(2)*: 81-101. DOI:10.3733/hilg.v29n02p081.

Stejskal, V (2003). Economic injury level and preventive pest control. Research Institute of Crop Production. *J. Pest Science, 76*: 170-172.

Su, C M (2016). *Management of Oil Palm Bunch Moth* (Tirathaba mundella *Walker*) *in Young Mature Oil Palm Plantation on Peat Soil in Sarawak, Malaysia*. M. Sc. thesis, Universiti Putra Malaysia.

Su, C M and Bong, C F (2017). Effect of different insecticides on the survival of the oil palm pollinator, *Elaeidobius kamerunicus* (Coleoptera: Curculionodae). *The Planter*, *93(1100):* 777-788.

Sugih, W; Heru, S; Ahmad, F and Thiagarajan, S (1996). Influence of rainfall, palm age and assisted pollination on oil palm fruit set in Riao, Indonesia. *Proc. of the 1996 PORIM International Palm Oil Congress (Agriculture).* PORIM, Bangi. p. 207-220.

Syed, R and Saleh, A (1987). Population of *Elaeidobius kamerunicus* Faust. in relation to fruit set. *Proc. of the 1987 International Oil Palm /Palm Oil Conferences: Progress and Prospects – Module I Agriculture*. p. 528-534.

Wahid, M B; May, C Y and Weng, C K (2011). Strategic direction in oil palm research. *Further Advances in Oil Palm Research*. MPOB, Bangi. p. 1-27.

Wood, B J (1968). *Pests of Oil Palms in Malaysia and their Control*. The Incorporated Society of Planters, Kuala Lumpur. p. 111-113.

Wood, B J and Ng, K Y (1974). Studies on the biology and control of oil palm bunch moth, *Tirathaba mundella* Walk. (Lepidoptera: Pyralidae). *Malay. Agric. J., 49(3)*: 310-331.

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ESTIMATING THE YIELD LOSS OF OIL PALM DUE TO *Ganoderma* BASAL STEM ROT DISEASE BY USING BAYESIAN MODEL AVERAGING

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ABSTRACT

It is very crucial to planters to estimate the yield loss due to Ganoderma *basal stem rot (BSR) disease in oil palm. However, currently there is a limited mathematical model available that can be used for that purpose. Therefore, this empirical study was conducted to build a mathematical model which can be used for yield loss estimation due to the disease. Three commercial oil palm plots with different production phases (*i.e. *steep ascent phase, plateau phase and declining phase) were selected as the study sites. The yield and disease severity of the selected palms in the three study sites were recorded for the duration of 12 months. Model averaging approach using Bayes theorem was used to build the model. This is also known as Bayesian Model Averaging (BMA). The BMA model revealed that planting preparation technique was the most important predictor of oil palm yield loss, followed by disease progress (measured using area under the disease-progress curve, AUDPC), disease severity, number of infected neighbouring palms, and two interaction terms. By using the developed BMA model, it was estimated that the economic loss can be up to 68% compared to the attainable yields of all the infected palms.*

Keywords: yield loss, oil palm, *Ganoderma* basal stem rot, total bunch weight, Bayesian model averaging.

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INTRODUCTION

Ganoderma basal stem rot (BSR) disease is the most widely studied oil palm disease in Malaysia (Idris, 2012). The disease is caused by the white rot fungi, *Ganoderma* species (Flood *et al.*, 2000; Mercière *et al.*, 2017). *Ganoderma boninense* was identified as the main species that causes the disease (Ho and Nawawi, 1985; Wong *et al.*, 2012; Siang *et al.*, 2013). In the region of South-east Asia, especially in Malaysia and Indonesia, it is considered as the most devastating disease. Cases of the disease were also reported in several oil palm producing countries such as Angola, Cameroon, Ghana, Nigeria, Zambia, San Tome and Principe, Tanzania, Zimbabwe, and the Republic of Congo in Africa, Honduras in the region of Central America, and Papua New Guinea in the Oceania region (Ariffin *et al.*, 2000). The disease can reduce the yield of the infected palms either from total yield loss by killing the infected palms (called direct loss) or by reducing the weight or the number of fresh fruit bunches (FFB) of the infected palms but still living palms (or indirect loss) (Roslan and Idris, 2012).

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Despite the fact that the disease has been present for many years, information on the relationship between disease severity and yield loss is still very limited (Assis *et al*., 2016). There is no specific study that has been conducted to model the relationship between the *Ganoderma* BSR disease development (*i.e*. disease incidence and disease severity) and oil palm yield loss by taking into account the growth of oil palm. This means that the information on the relationship between the disease progress and oil palm yield is still lacking. A study conducted by Roslan and Idris (2012) is the latest effort to estimate the yield loss due to *Ganoderma* BSR disease, but the study did not take into account the severity of the disease. Furthermore, there is a limited mathematical model available to estimate the loss. The economic loss assessment was only based on the infected dead palms. In other words, the economic loss was underestimated since the loss in yield had already occurred even before the infected palms collapsed. The same goes with the study conducted by Singh (1991) where the yield loss estimation was only based on the disease incidence without considering the disease severity and no mathematical model was developed to estimate the loss. Both of these studies had estimated the yield loss but the estimations were not based on the individual palm level which can give more accurate loss estimation.

This relationship must be quantified, developed, modelled and validated based on empirical data which are collected through observation or experiment, as opposed to relying solely on theory. By having this mathematical disease-yield loss relationship, it will be very helpful especially to the management team of oil palm producers in determining the effectiveness of their practical disease managements and also to researchers and by extension to workers in evaluating their experiments (Savary *et al*., 2006). The oil palm producers can accurately determine their yield loss and therefore economics of applying any treatments. The predicted loss will then play a very important role in guiding the management to formulate their future programme, such as proposed low-input and environmental-friendly strategies to the epidemic development of the disease in order to decrease the yield losses and reduce their consequences.

MATERIALS AND METHODS

Study Sites

The data were collected based on the statistical approach where the level of disease occurred naturally in the fields of study without any intervention. FAO (1983) calls this approach as an individual plant comparison approach. The advantages of this statistical approach are the yield loss-disease relationship is based on the natural disease epidemic and there will also be no side effects of treatments on the yield which possibly can affect the yield loss-disease relationship. Based on this design or approach, all the study sites must have a certain level of disease incidence.

A single-plant method was used in this study. In this method, each plant was considered as a single datum point for regression analysis (Gaunt, 1990), *i.e.* the sampling unit was the individual palm. The individual palms studied include infected palms and uninfected palms (*i.e*. healthy palm). All the infected palms in the studied sites were sampled and monitored for the duration of 12 months starting from the first disease census. For the uninfected palms, only the selected palms were monitored. A simple random sampling using a uniform distribution was therefore used to select certain number of palms as a control. This is due to the fact that the spatial distribution of the disease is random (Assis *et al*., 2015). This random sampling was conducted based on the identification number which has been assigned for every single palm planted in the study sites.

There were three study sites selected as shown in *Table 1*. These sites were selected from three different estates but owned by the same company located in Tawau division, Sabah, Malaysia. The main criterion used in selecting the study sites was the growth phases of oil palm. These three study sites covered three production phases of oil palm; which are steep ascent phase (3-10 yr after planting) where the yield is in increasing phase, plateau phase (10-15 yr after planting) where the yield is in flat or maximum phase, and declining phase (older than 15 yr after planting) where the yield is in declining phase (Amstrong, 1999). The age of oil palm is among the most important confounding factor of *Ganoderma* BSR disease in affecting the yield (Ariffin *et al*., 2000). Besides, any disease assessment data must be qualified by the growth stage of the crop at the time of assessment (Brown and Keane, 1997). Other criterion of selecting these study sites was the *Ganoderma* BSR incidence must be considerably significant to study the relationship between yield loss and disease (Cooke, 2006). All the study sites were managed under the same plantation company. This was done to ensure similarity in terms of the effect of agronomic practices that could also affect the yield loss-disease relationship (Virdiana *et al*., 2010; Chung, 2011). The study sites used in this study is the same with Assis *et al*. (2016) but different in terms of disease census data (disease census at month 6 *vs*. month 12), time frame (6 months *vs*. 12 months), and also modelling approach (single model *vs*. model averaging approach).

TABLE 1. DESCRIPTIONS OF THE STUDY SITES

Description	Study site 1	Study site 2	Study site 3
ID	MBE0702	SKE0224	MDE8717
Location	Latitude 4° 25′ 53.76″ N; Longitude 117° 45′ 8.64″ E	Latitude 4° 19′ 24.96″ N; Longitude 118° 05′ 26.88″ E	Latitude 4° 46′ 19.35″ N; Longitude 118° 8′ 18.67″ E
Production phase	Ascent phase	Plateau phase	Declining phase
Year of planting (age)	2007 (8 yr after planting)	2002 (13 yr after planting)	1987 (28 yr after planting)
Total area	13.47 ha	10.75 ha	6.8 ha
Number of palms	2042	1462	999
Soil type	Lumisir	Lumisir	Bulanat/Lating
Previous crop	Oil palm	Oil palm	Forest
Generation	2nd generation	2nd generation	1st generation
Planting technique	Zero burning with evenly spread chip	Zero burning with evenly spread chip	Jungle to oil palm clearing

Source: Sawit Kinabalu Sdn Bhd (2013).

Variables of Study

The predict and or dependent variable in this study was yield loss in total bunch weight (TBW) of oil palm due to *Ganoderma* BSR disease. The predictors were disease severity, area under the disease progress curve (AUDPC), number of infected neighbouring palms, age of palm, previous crop, soil type and planting preparation technique. Even though nutrient availability in the soil is also one of the predisposing factors of the disease (Ariffin *et al.*, 2000), this factor was not considered in the yield loss model building. This factor was assumed to be constant since all the study sites were using the same agronomic practices (*i.e.* all the study sites were under the same plantation company).

By following the standard measurement of yield loss used by FAO (1983), the yield loss (YL) in TBW for each individual palm studied was calculated by using Equation (1) (Savary and Willocquet, 2014).

$$YLTBW_i = Y_a - Y_i \qquad \text{Equation (1)}$$

where Y_a denotes the average attainable TBW in 12 months for the uninfected palms and Y_i denotes the actual TBW in 12 months for *i*th palm (*i* is the sampling unit). The Y_a was calculated using Equation (2) (Teng, 1990).

$$Y_a = \frac{\sum_{i=1}^{n} Y_{i_{\text{uninfected}}}}{n} \qquad \text{Equation (2)}$$

where $\sum_{i=1}^{n} Y_{i_{\text{uninfected}}}$ refers to the total actual TBW of the selected uninfected palms in the three study sites and n denoted the number of selected uninfected palms. The normality of the $Y_{i_{\text{uninfected}}}$ data distribution was first checked by using statistical as well as graphical methods before calculating the Y_a. This was done to ensure that the Y_a is an unbiased estimate for representing the average yield of uninfected palms.

Disease severity was measured by using visual method instead of non-visual method which is time-consuming and very costly. This visual method was conducted by following the standard procedures used by the Malaysian Palm Oil Board (MPOB) in conducting *Ganoderma* BSR disease census (Idris *et al.*, 2016). A standard procedure of measurement must be used to ensure consistency in terms of measurement between observers and simplicity for speed of operation (Cooke, 2006). The disease severity was labelled as R1 (uninfected), R2 (mild infection), R3 (moderate infection), R4 (severe infection), and R5 (dead). This variable was considered as categorical data, thus dummy transformation was performed. The R1 was chosen as the reference category since it represented uninfected palms. The disease census was conducted quarterly to monitor the disease development progress.

AUDPC was used to measure the disease progress. AUDPC has been found as one of the important predictors that can predict the yield loss of crops due to diseases or pests (Wolf and Verreet, 2009; Lal *et al.*, 2014). The AUDPC for each palm was calculated by using Equation (3) (Brown and Keane, 1997).

$$AUDPC = \sum_{i=1}^{n} \frac{DS_i + DS_{i+1}}{2} + (t_{i+1} - t_i) \qquad \text{Equation (3)}$$

where DS_i is the disease assessment at time i, n is the number of disease assessments, and $(t_{i+1} - t_i)$ is the interval between two consecutive assessments. The AUDPC takes into consideration of the amount of disease (*i.e.* $\frac{DS_i + DS_{i+1}}{2}$) as well as the duration of the disease (*i.e.* $t_{i+1} - t_i$).

Root to root contact has been found to be the main spread mode of *Ganoderma* BSR disease (Sanderson, 2005; Cooper *et al*., 2011; Naher *et al*., 2013). This means that there is a possibility that infection occurs due to the infected neighbouring palms. Therefore, this study considered the number of infected neighbouring palms as one of the possible predictors to estimate the yield loss due to the disease. In this study, the neighbouring palms were limited to the eight nearest palms to the studied palms. The minimum and maximum numbers of infected neighbouring palms for each of the studied palm were therefore 0 (if no infected neighbouring palms) and 8 (if all the neighbouring palms are infected), respectively. The calculation of the number of infected neighbouring palms was based on the first disease census which was during the first month of monitoring.

Age of palm is one of the predisposing factors of *Ganoderma* BSR disease (Idris *et al*., 2011). Older palms are more susceptible to be infected by the disease (Idris *et al*., 2010). Due to the limited resources, there were only three different palm ages covered in this study (*Table 1*). However, it covered all the three production phases of oil palm (*i.e*. ascent phase, plateau phase, and declining phase). Additionally, there is also a difference of FFB weight according to the age of oil palm. There is positive correlation between the weight of FFB and age of palm (Breure and Menendez, 1990; Corley and Tinker, 2016).

Type of previous crop has been confirmed to be one of the predisposing factors of *Ganoderma* BSR disease (Singh, 1991). The previous crop for MBE0702, SKE0224, and MDE8717 were oil palm, oil palm, and forest respectively (Sawit Kinabalu Sdn Bhd, 2013). Since this variable was a categorical variable, hence, dummy transformation was also performed.

Another predisposing factor of the disease is type of soil. Previous studies have found that the disease is most serious in coastal areas as compared to inland areas (Lim *et al*., 1992; Suriya Rao *et al*., 2003). The disease incidence is also high especially in areas with low water levels (*e.g*. more than 75 cm from the peat surface). On peat areas, therefore it is important to maintain a water level of 50-75 cm from the peat surface to minimise the *Ganoderma* BSR disease infections and spread of this deadly disease on oil palms planted on peat (Roundtable of Sustainable Palm Oil, 2012; Supriyanto *et al*., 2020). In this study, there were two types of soil involved, which are Lumisir and Bulanat/Lating. The major particle for Lumisir is sand. However, there was no clear picture of the major particle for Bulanat/Lating (The Malaysian Society of Soil Science, 1977). Therefore, there was no clear guideline whether the effect of soil type on YLTBW is positive or negative.

Availability of inoculum source is one of the important factors of *Ganoderma* BSR disease distribution (Chung, 2011). Planting preparation technique can potentially determine the availability of inoculums. Burning crop residues including diseased materials is an effective way of sanitation during replanting especially in areas where the BSR incidence is increasing in second and third generation oil palm planting (Chung, 2011). However, permission from the authorities is required for this replanting technique. Currently, zero burning with evenly spread chip is the standard practice by big oil palm companies in land preparation. Zero burning is considered as one of the best management practices in oil palm (Roundtable of Sustainable Palm Oil, 2012). In this study, there were two planting preparation techniques involved, namely zero burning with evenly spread chip (*i.e*. for MBE0702 and SKE0224 study sites) and jungle clearing (*i.e*. for MDE8717 study site). Zero burning with evenly spread chip can reduce the inoculum source of *Ganoderma* BSR disease, thus this category of planting technique was set as the reference category in this study.

Large number of potential predictors causes large number of possible models. One of the solutions to reduce the number of potential predictors is by removing predictors that demonstrate multicollinearity (Bush, 2012). Besides, one of the assumptions of multiple linear regression model estimated by ordinary least square (OLS) is not an exact linear relationship (*i.e.* multicollinearity) among the predictors. There were two methods used in identifying the main source of multicollinearity, namely the correlation-based and the variance-based method. This screening, however, involved only the main effects because multicollinearity is not a serious issue when involving interaction effects (Gujarati, 2003). It is clear that there will be high collinearity between the main effects and the interaction effects. The main effects include disease severity of R2 (labelled as R2), disease severity of R3 (labelled as R3), disease severity of R4 (labelled as R4), number of infected neighbouring palms (labelled as N), age of palm (labelled as AGE), type of previous crop (labelled as PREVIOUSCROP), soil type (labelled as SOILTYPE), and planting technique (labelled as PT), while the interaction effects include AUDPC, AUDPC*N, AUDPC*AGE, AUDPC*PREVIOUS, AUDPC*SOILTYPE, and AUDPC*PT. The AUDPC was considered as an interaction effect (*i.e*. integral variable) since it was calculated based on the disease severity (*i.e*. R1, R2, R3, R4, and R5) [Equation (3)]. The disease severity is one of the main effects in this study.

Bayesian Model Averaging

Bayesian Model Averaging (BMA) is an alternative to estimation-post-selection approach. The basic idea of this approach is that there may be

more than one possible model can fit into the data well and give accurate predictions of the quantity of interest. Combining these possible models by averaging the parameters of the selected predictors can give higher accuracy of prediction as compared to a single 'best' model. Besides incorporating model selection uncertainty, this approach also incorporates other forms of uncertainty such as predictor selection, transformations, outliers and model form (Clyde, 2003). Furthermore, model averaging approach also integrates two main problems in model selection; which are model search and model selection criterion by averaging or combining the information from all the possible models or from a subset of the possible models during the estimation, inference, or prediction (Hoeting, 2002). In many cases, models developed by BMA have better predictive performances as compared to any single model (Wang *et al.*, 2004; Prost *et al.*, 2008; Genell *et al.*, 2010; Hayden *et al.*, 2010; Zou *et al.*, 2013; Morozova *et al.*, 2015).

In this study, each model considered in BMA was a linear regression model. The main principles of BMA are explained as follows (Montgomery and Nyhan, 2010). Let Y be the dependent variable, β_0 denotes the constant term, β_j denotes the coefficients of k predictors (or also called as limiting factors in yield gap studies), X_j, and μ denotes the error term with normal distribution, $\mu \sim N(0,\sigma^2)$, then

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \mu = X\beta + \mu \qquad \text{Equation (4)}$$

BMA estimates this model by taking into consideration all the possible combinations of $\{X\}$. The problem arises when there is a large number of possible predictors, k, to consider. If there is k possible variables, then there will be 2^k possible models. The aim of BMA is to compute the posterior distribution of β. Let β in Equation (4) is estimated by $\hat{\beta}$, then

$$P(\hat{\beta}|D) = \sum_{i=1}^{n=2^k} P(\hat{\beta}|D,M_1)\, P(M_1|D) \qquad \text{Equation (5)}$$

where M_1, M_2,..., M_n is the set of possible models, D denotes the data set, and $P(.|.)$ denotes a conditional density probability function. The $P(\hat{\beta}|D)$ is the sum of the posterior distributions [or posterior model probability (PMP)], $P(M_i|D)$, under each of the models, weighted by their PMP. The $P(\hat{\beta}|D)$ is also called the posterior inclusion probabilities (PIP). PIP is the probabilities that each variable belongs to the final model. In this study, Bayesian information criterion (BIC) approximation was used to obtain approximate posterior model probabilities. The approximate posterior model probabilities using BIC was calculated as

$$P(M_i|D) = \frac{P(M_i)\exp[-.5BIC(M_i)]}{\sum_{i=1}^{n} P(M_j)\exp[-.5BIC(M_j)]} \qquad \text{Equation (6)}$$

where $BIC\ (M_i)$ is

$$BIC\ (M_i) = -2\log(\text{maximum likelihood}\,|M_i) + q_k \log(N) \qquad \text{Equation (7)}$$

The q_k is the dimension of model M_i and N is the number of cases. The estimated posterior means and standard deviations of $\hat{\beta} = \hat{\beta}_1, \hat{\beta}_2,..., \hat{\beta}_k$, were then constructed as Equation (8) and Equation (9) respectively.

$$E[\hat{\beta}|D] = \sum_{j=1}^{n=2^k} \hat{\beta} P(M_n|D) \qquad \text{Equation (8)}$$

$$V[\hat{\beta}|D] = \sum_{j=1}^{n=2^k} (Var\,[\hat{\beta}|D, M_n] + \hat{\beta}^2)\, P(M_n|D) - E[\beta|D]^2 \qquad \text{Equation (9)}$$

The R package or specifically 'library(BMA)' was used to develop model using BMA approach. The function of 'bicreg' of the BMA library was used to compute the posterior parameter means using the simple BIC approximation to the posterior model probabilities (Raftery *et al.*, 2015). It implements Occam's window algorithm for linear regression (Raftery, 1995).

RESULTS AND DISCUSSION

Yield Loss Model

Table 2 shows the summary of the BMA model showing 10 selected best models. It was clear that the first model which is labelled as model 1 is the best model among all the 256 possible models since it has the lowest BIC and the largest PMP (*i.e.* of being the correct model). This model includes R2, AUDPC and PT, which is the same with the best model selected under best-subset selection. But the estimation of yield loss in BMA was not only based on this single model, but it considered all the 10 selected best models.

Based on the PIP, PT was the most important predictor with PIP value of 100. This means that this predictor was 100% included in all 10 models selected. The second important predictor is AUDPC (with the PIP value of 72.1), followed by R2 (with the PIP value of 69.2), R4 (with the PIP value of 34.8), R3 (with the PIP value of 33.6), N (with the PIP value of 15.5), AUDPC_N (with the PIP value of 8), and AUDPC_PT (with the PIP value of 2.8). But both of R2 and AUDPC_PT had negative effects on the oil palm yield loss. But all of these eight predictors were included in the final model of BMA regardless of

the sign of effect and contribution of the predictors. This means that there was no subset selection as in backward stepwise subset selection and also in best-subset selection. Based on the posterior distribution mean for each coefficient, the BMA model can be written as

$$YLTBW = -24.632 - 18.307(R2) + 13.456(R3) + 21.531(R4) + 2.346(AUDPC) + 0.551(N) + 35.11(PT) + 0.014(AUDPC\_N) - 0.011(AUDPC\_PT)$$

The identification of the main sources of multicollinearity was performed based on correlation-based analysis (Pearson product-moment correlation coefficient) and also variance-based analysis (variance inflation factor, VIF). The results of these two analyses show that three variables; age of palm, type of previous crop, and soil type caused multicollinearity problems. Hence, these variables were removed from the model. Residual analysis on the BMA model was performed to check whether this model violated the assumptions of zero mean of errors, normality, homoscedasticity, and no outliers. The mean and standard deviation of the standardised residual of the model were zero and close to 1 (*i.e.* 0.997 ≈ 1) respectively. The result of Kolmogorov-Smirnov test shows that the distribution of the errors was also normal (statistic value = 0.045, df = 378, p = 0.061) (Dhamu and Ramamoorthy, 2012) and the scatterplot also reveals that no extreme values or outliers were detected in the errors of the model where all the standardised residual fall within the range of ±3 (Field, 2009).

The constant value in the model represents the average yield difference of healthy palms which were not surrounded by any infected palm and also planted in the area with the planting preparation technique of zero burning with evenly spread chip. The negative value means that any healthy palm that fulfilled all the criteria mentioned above will produce higher TBW per year as compared to the average TBW of reference palms (*i.e.* R1) with the difference is approximately 24.632 kg yr^{-1}.

If a palm is infected with disease severity of R3 (moderate) or R4 (severe), there will be a reduction in TBW of about 13.455 kg or 21.531 kg respectively within one year as compared to the average yield of the reference palms' (*i.e.* R1) TBW by assuming other predictors are constant. However, the model also suggested that if a palm is infected with disease severity of R2 (mild), there will be no reduction in TBW. Instead, the TBW will be higher than the average yield of the reference palms' (*i.e.* R1) by 18.307 kg yr^{-1}.

One additional neighbouring palm infected will cause the central palm a loss of approximately 0.551 kg within one year. If there is a positive increase in disease progress (AUDPC) by one unit, it will cause a loss of 2.346 kg of FFB in 12 months' time if other predictors are still unchanged. For planting preparation technique, zero burning with evenly spread chip will reduce the loss by 35.113 kg of FFB one year after the disease census as compared to the jungle clearing planting technique. Planting preparation technique was the most influencing predictor in the model. The coefficient of this predictor was significantly different from zero in all the selected 10 best models (*Table 2*).

For the two interaction effects, the disease severity with the number of infected neighbouring palms (AUDPC_N) and the disease severity with the planting preparation technique (AUDPC_PT), their effects on the YL in TBW were only 0.014 kg and 0.011 kg yr^{-1} respectively. Every unit increase in the interaction between disease severity and number of infected neighbouring palms will cause the YL in TBW to increase by 0.014 kg yr^{-1}. However, this interactions term will have no effect on the YL if a palm is healthy or not surrounded by any infected neighbouring palms. For the interaction between the disease severity and the planting preparation technique, every unit increase in this interaction term will cause the YL in TBW to decrease by only 0.011 kg yr^{-1}. And again, this interactions term will also have no effect on the YL if a palm is healthy or planted in the area with the planting preparation technique of zero burning with evenly spread chip.

Economic Loss Estimation

Table 3 shows how the economic loss was estimated. Based on the first disease census conducted, there were 461 infected palms from the three study sites. Specifically, a total of 78 palms, 98 palms, 71 palms, 214 palms were rated as R2, R3, R4, and R5, respectively. Based on the attainable yield (*i.e.* 118 kg of TBW yr^{-1}) estimated in this study, all the 461 infected palms attainably can produce in total 54 398 kg of FFB yr^{-1} [*i.e.* (78 x 118 kg = 9204) + (98 x 118 kg = 11 564) + (71 x 118 kg = 8378) + (214 x 118 kg = 25 252) = 54 398]. Unfortunately, due to *Ganoderma* BSR disease, there were some reductions in TBW. The YL model developed in this study was used to estimate the reductions (or losses). Based on the BMA model, the total YL due the infected palms with disease severity of R2, R3, R4, and R5 were approximately 590.68 kg, 5811.73 kg, 5734.52 kg, and 25 252 kg respectively. In total, the yield loss was 37 388.93 kg. When converted into monetary value with the exchange rate of RM 1 = USD 0.2576 (*i.e.* the average exchange rate in 2015), the total economic loss was USD 4112.78 yr^{-1} (*i.e.* 37 388.93 kg x USD 0.11 = USD 4112.78). The price per kilogram was the average monthly FFB price (mill gate) for Sabah region in 2015 (MPOB, 2015). This economic loss is equivalent to 68.73% of the attainable yield of 461 palms if not infected by the disease.

TABLE 2. SUMMARY OF THE BEST 10 MODELS IN BAYESIAN MODEL AVERAGING (BMA)

Predictor	PIP	EV	SD	Model 1	Model 2	Model 3	Model 4	Model 5	Model 6	Model 7	Model 8	Model 9	Model 10	Cumulative
Intercept	100.0	-24.632	11.226	-30.836	-8.421	-34.024	-30.389	-11.331	-28.592	-16.319	-31.139	-32.997	-9.536	-
R2	69.2	-18.309	13.260	-26.536	-	-26.476	-26.301	-	-24.246	-27.869	-27.869	-27.221	-	-
R3	33.6	13.455	20.893	-	45.405	-	-	45.013	-	-4.663	-4.663	-	42.728	-
R4	34.8	21.531	31.921	-	70.387	-	-	70.471	9.673	-	-	-	67.029	-
AUDPC	72.1	2.346	1.545	3.372	-	3.373	3.188	-	3.071	3.472	3.472	3.562	-	-
N	15.5	0.551	1.513	-	-	3.617	-	3.380	-	-	-	-	-	-
PT	100.0	35.113	5.643	36.491	32.852	34.400	34.903	30.953	36.368	36.841	36.841	42.353	31.387	-
AUDPC_N	8.0	0.014	0.060	-	-	-	0.171	-	-	-	-	-	0.178	-
AUDPC_PT	2.8	-0.011	0.125	-	-	-	-	-	-	-	-	-0.415	-	-
No. of predictors	-	-	-	3	3	4	4	4	4	4	4	4	4	-
R^2	-	-	-	0.395	0.392	0.400	0.397	0.397	0.397	0.396	0.395	0.395	0.395	-
BIC	-	-	-	-171.932	-170.468	-169.227	-167.706	-167.336	-167.173	-166.560	-166.484	-166.428	0.166.416	-
Posterior probability	-	-	-	0.432	0.208	0.112	0.052	0.043	0.040	0.029	0.028	0.028	0.027	1.000

Note: The column 'PIP' indicates the probability that the coefficient for a given predictor is not zero, among the 10 models returned. The column 'EV' displays the BMA posterior distribution mean for each coefficient and the column 'SD' displays the BMA posterior distribution standard deviation for each coefficient.
R2 - mild infection, R3 - moderate infection, R4 - severe infection, AUDPC - age under the disease progress curve, N - number of infected neighbour palms, PT - planting technique, AUDPC_N - disease severity with the number of internal neighbour palms, AUDPC_PT - disease severity with the planting preparation technique, BIC - Bayesian information criterion.

TABLE 3. YIELD LOSS DUE TO REDUCTION IN TOTAL BUNCH WEIGHT (TBW)

Disease severity (after 12 months)	Field data				Modelling results			
	8 years after planting	13 years after planting	28 years after planting	Total infected palms	Attainable yield (kg yr^{-1})*	Attainable economic yield (USD yr^{-1})**	Estimated yield loss kg yr^{-1})***	Estimated economic loss (USD yr^{-1})*
Mild infection	9	36	33	78	9 204.00	1 012.44	590.68	64.97
Moderate infection	14	22	62	98	11 564.00	1 272.04	5 811.73	639.29
Severe infection	9	13	49	71	8 378.00	921.58	5 734.52	630.80
Dead	169	32	13	214	25 252.00	2 777.72	25 252.00	2 777.72
Total loss	-	-	-	461	54 398.00	5 983.78	37 388.93	4 112.78
Percentage of loss as compared to the attainable level	-	-	-	-	-	-	68.73%****	68.73%*****

Note: *Calculated based on the average attainable yield of healthy palms (*i.e.* R1) which is 118 kg $palm^{-1}$ yr^{-1}
**Estimated by using the model developed which is Bayesian model averaging (BMA) model.
***Estimated by using the fresh fruit bunch (FFB) price of USD0.11 kg^{-1} (MPOB, 2015).
**** (37 388.93 kg / 54 398 kg) x 100.
***** (USD 4112.78 / USD 5983.78) x 100.

This is considered as a huge loss to the planter since it represents 68.73% of the attainable yield per year which is higher compared to the estimated loss by Assis *et al*. (2016), 43.32% per six months. But these two studies are not perfectly comparable since the approach used (*i.e*. single model approach versus model averaging approach) to develop the YL model as well as the time frame used (*i.e*. 6-month *vs*. 12-month) are different. The loss estimation using this BMA model is also more detailed as compared to the loss estimation done by Roslan and Idris (2012) and Singh (1991). The loss estimation in this present study considered dead palms as well as infected but still productive palms.

CONCLUSION

By using the YL model developed, it was estimated that the economic loss due to the disease was equivalent to 68.73% of the attainable yield of all the infected palms (*i.e*. 461 palms) after 12 months observation. This model has the potential to be used by oil palm planters including estate and smallholders in helping them to estimate the potential YL as well as economic loss due to *Ganoderma* BSR disease. However, the model developed still needs to be validated in different setting, such as different plantation companies, areas, *etc*. Once the model is validated, it can potentially be used to estimate the potential loss as a baseline data in deciding the right time to carry out replanting especially in the hot spot areas of *Ganoderma* BSR disease. Additionally, the potential loss estimated from the model can also be used to compare the effectiveness of any preventive or control measures taken to reduce the economic loss due to the disease.

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REFERENCES

Amstrong, D L (1999). Oil palm nutrition management. *Better Crops International, 13(1)*: 3-6.

Ariffin, D; Idris, A S and Singh, G (2000). Status of *Ganoderma* in oil palm. Ganoderma *Diseases of Perennial Crops* (Flood, J; Bridge, P D and Holderness, M eds.). United Kingdom: Cabi Publishing. p. 49-68.

Assis, K; Chong, K P; Idris, A S; Hoong, H W and Ho, C M (2015). Spatial statistical analysis of basal stem root disease under natural field epidemic of oil palm. *AIP Conference Proceedings*. p. 13-18. DOI: 10.1063/1.4907420.

Assis, K; Chong, K P; Idris, A S and Ho, C M (2016). Economic loss due to *Ganoderma* disease in oil palm. *Int. J. Social, Behavioral, Educational, Economic, Business and Industrial Engineering, 10(2)*: 604-608.

Breure, C J and Menendez, T (1990). The determination of bunch yield components in the development of inflorescences in oil palm (*Elaeis guineensis*). *Experimental Agriculture, 26(1)*: 99-115. DOI: 10.1017/S0014479700015441.

Brown, J and Keane, P (1997). Chapter 20: Assessment of disease and effects on yield. *Plant Pathogens and Plant Diseases* (Brown, J F and Ogle, H J eds.). Armidale: Rockvale Publications. p. 315-329.

Bush, H M (2012). *Biostatistics: An Applied Introduction for the Public Health Practitioner*. New York: Delmar Cengage Learning. 368 pp.

Chung, G F (2011). Management of *Ganoderma* diseases in oil palm plantations. *The Planter, 87(1022)*: 325-339.

Clyde, M (2003). *Subjective and Objective Bayesian Statistics: Principles, Models and Applications*. New Jersey: Wiley-Interscience. 600 pp.

Cooke, B M (2006). Chapter 2 disease assessment and yield loss. *The Epidemiology of Plant Diseases* (Cooke, B M; Gareth Jones, D and Kaye, B eds.). Second edition. Springer. p. 43-80.

Cooper, R M; Flood, J and Rees, R W (2011). *Ganoderma boninense* in oil palm plantations: Current thinking on epidemiology, resistance and pathology. *The Planter, 87(1024)*: 515-526.

Corley, R H V and Tinker, P B H (2016). *The Oil Palm*. 5th edition. United Kingdom: Wiley-Blackwell. 614 pp.

Dhamu, K P and Ramamoorthy, K (2012). *Statistical Methods*. Jodhpur: Agrobios (India). 360 pp.

FAO (1983). *Assessment and Collection of Data on Pre-harvest Food Grain Losses*. FAO Econom. Rome: Food and Agriculture Organization (FAO). 134 pp.

Field, A (2009). *Discovering Statistics Using SPSS*. London: Sage Publication Inc. 856 pp.

Flood, J; Hasan, Y; Turner, P D and O'Grady, E (2000). The spread of *Ganoderma* from its infective

sources in the field and its implications for management of the disease in oil palm. Ganoderma *Diseases of Perennial Crops* (Flood, J; Bridge, P D and Holderness, M eds.). United Kingdom: Cabi Publishing. p. 101-112.

Gaunt, R E (1990). Practical methods of quantifying diseases and pathogen populations. *Crop Loss Assessment in Rice* (Teng, P S ed.). Manila: International Rice Research Institute (IRRI). p. 67-74.

Genell, A; Nemes, S; Steineck, G and Dickman, P W (2010). Model selection in medical research: A simulation study comparing Bayesian Model Averaging and stepwise regression, BMC medical research methodology. *BioMed Central Ltd, 10(1)*: 108. DOI: 10.1186/1471-2288-10-108.

Gujarati, D N (2003). *Basic Econometrics*. 4th edition. New York: McGraw-Hill. DOI: 10.1126/science.1186874. 1027 pp.

Hayden, E; Stomper, A; Sloan, M I T and Westerkamp, A (2010). Selection *vs*. averaging of logistic credit risk models. *Social Science Research Network*. p. 1-8. DOI: 10.2139/ssrn.1724124.

Ho, Y W and Nawawi, A (1985). *Ganoderma boninense* Pat. from basal stem rot of oil palm (*Elaeis guineensis*) in Peninsular Malaysia. *Pertanika, 8(3)*: 425-428.

Hoeting, J A (2002). Methodology for Bayesian Model Averaging: An update. *International Biometric Conference*. p. 231-240.

Idris, A S; Nur Rashyeda, R; Mohd Hefni, R; Shamala, S and Norman, K (2016). *Standard Operating Procedures (SOP) Guidelines for Managing* Ganoderma *Disease in Oil Palm*. MPOB, Bangi. p. 41.

Idris, A S (2012). *Ganoderma* disease of oil palm in Malaysia: Latest technologies on detection, control and management. *Proc. of the 17th International Conference on Oil Palm and Expopalma.* Cartagena De Indias.

Idris, A S; Mior, M H A Z; Maizatul, S M and Ahmad Kushairi, D (2011). Survey on status of *Ganoderma* disease of oil palm in Malaysia 2009-2010. *Proc. of the PIPOC 2011 International Palm Oil Congress*. MPOB, Bangi. p. 235-238.

Idris, A S; Mior, M H A Z; Wahid, O and Ahmad Kushairi, D (2010). Geostatistics fo monitoring *Ganoderma* outbreak in oil palm plantations. *MPOB Information Series No. 74*: 2 pp.

Keane, P and Kerr, A (1997). Factors affecting disease development. *Plant Pathogens and Plant Diseases* (Cooke, B M; Gareth Jones, D and Kaye, B eds.). Second edition. Dordrecht: Springer. p. 287-298.

Lal, M; Sharma, S; Ahmad, I and Yadav, S (2014). Development of yield loss assessment model for potato late blight disease in indo-gangetic plains. *Potato J., 41(2)*: 130-136.

Lim, T K; Chung, G F and Ko, W H (1992). Basal stem rot of oil palm caused by *Ganoderma boninense*. *Plant Pathology Bulletin, 1*: 147-152.

MPOB (2015). Monthly FFB yield (tonnes/ hectare). http://bepi.mpob.gov.my/images/Yield/Yield-2015//FFB Yield (July-December) 2015.pdf

Mercière, M; Boulord, R; Carasco-Lacombe, C; Klopp, C; Lee, Y P; Tan, J S; Syed Alwee, S S R; Zaremski, A; De Franqueville, H; Breton, F and Camus-Kulandaivelu, L (2017) About *Ganoderma boninense* in oil palm plantations of Sumatra and Peninsular Malaysia: Ancient population expansion, extensive gene flow and large scale dispersion ability. *Fungal Biology, 121(6-7)*: 529-540. DOI: 10.1016/j.funbio.2017.01.001.

Montgomery, J and Nyhan, B (2010). Bayesian model averaging: Theoretical developments and practical applications. *Political Analysis, 18(2)*: 245-270. DOI: 10.1093/pan/mpq001.

Morozova, O; Levina, O; Uusküla, A and Heimer, R (2015). Comparison of subset selection methods in linear regression in the context of health-related quality of life and substance abuse in Russia. *BMC Medical Research Methodology, 15(1)*: 71. DOI: 10.1186/s12874-015-0066-2.

Naher, L; Umi Kalsom, Y; Ismail, A; Tan, S G and Mondal, M M A (2013). Ecological status of *Ganoderma* and basal stem rot disease of oil palms (*Elaeis guineensis* Jacq.). *Australian J. Crop Science, 7(11)*: 1723-1727.

Prost, L; Makowski, D and Jeuffroy, M H (2008). Comparison of stepwise selection and Bayesian model averaging for yield gap analysis. *Ecological Modelling, 219(1-2)*: 66-76. DOI: 10.1016/j.ecolmodel.2008.07.026.

Raftery, A E (1995). Bayesian model selection in social research. *Sociological Methodology, 25*: 111-163. DOI: 10.2307/271063.

Raftery, A E; Hoeting, J A; Volinsky, C T; Painter, I S; Yeung, K Y and Sevcikova, H (2015). *Package 'BMA'*. 47 pp.

Roslan, A and Idris, A S (2012). Economic impact of *Ganoderma* incidence on Malaysian oil palm plantation – A case study in Johor. *Oil Palm Industry Economic J. Vol. 12(1)*: 24-30.

Roundtable of Sustainable Palm Oil (2012). *RSPO Manual on Best Management Practices (BMPs) for Existing Oil Palm Cultivation on Peat* (Lim, K H; Lim, S S; Faizal, P and Suharto, R eds.). Kuala Lumpur. 79 pp.

Sanderson, F R (2005). An insight into spore dispersal of *Ganoderma boninense* on oil palm. *Mycopathologia, 159(1)*: 139-141. DOI: 10.1007/s11046-004-4436-2.

Savary, S; Teng, P S; Willocquet, L and Nutter, F W (2006). Quantification and modeling of crop losses: A review of purposes. *Annual Review of Phytopathology, 44*: 89-112. DOI: 10.1146/annurev.phyto.44.070505.143342.

Savary, S and Willocquet, L (2014). Simulation modeling in botanical epidemiology and crop loss analysis. *The Plant Health Instructor*. DOI: 10.1094/PHI-A-2014-0314-01.

Sawit Kinabalu Sdn Bhd (2013). Background information of the study sites. Tawau.

Siang, M K; Yit, K G; Hun, J T; Kah, J G; Wei, C W and You, K G (2013). *In vitro* growth of *Ganoderma boninense* isolates on novel palm extract medium and virulence on oil palm (*Elaeis guineensis*) seedlings. *Malaysian J. Microbiology, 9(1)*: 33-42.

Singh, G (1991). *Ganoderma* - The scourge of oil palms in the coastal areas. *The Planter, 67*: 421-444.

Supriyanto; Purwanto; Poromarto, S H and Supyani (2020). The relationship of some characteristics of peat with oil palm basal stem rot (BSR) caused by *Ganoderma* in peatlands. *IOP Conf. Series: Earth and Environmental Science*. IOP Publishing. p. 1-7. DOI: 10.1088/1755-1315/423/1/012064.

Suriya Rao, A V; Lim, C C; Chia, C C and Teo, K W (2003). Studies on *Ganoderma* spread and control. *The Planter, 79*: 67-83.

Teng, P S (1990). Crop loss assessment: A review of representative approaches and current technology. *Crop Loss Assessment in Rice* (Teng, P S ed.). International Rice Research Institute (IRRI). p. 19-37.

The Malaysian Society of Soil Science (1977). Characteristics of some soils in Sabah and Sarawak. *The Malaysian Society of Soil Science*. p. 95.

Virdiana, I; Hasan, Y; Aditya, R and Flood, J (2010). Testing the effects of oil palm replanting practices (windrowing, fallowing and poisoning) on incidence of *Ganoderma*. *4th International Oil Pollution Compensation (IOPC)*. Oil Palm Research Institute (IOPRI), Yogjakarta: Indonesian. 7 pp.

Wang, D; Zhang, W and Bakhai, A (2004). Comparison of Bayesian Model Averaging and stepwise methods for model selection in logistic regression. *Statistics in Medicine, 23(22)*: 3451-3467. DOI: 10.1002/sim.1930.

Wolf, P F J and Verreet, J A (2009). Empirical-deterministic prediction of disease and losses caused by Cercospora leaf spots in sugar beets Originalarbeit. *J. Fur Kulturflanzen, 61(5)*: 168-177.

Wong, L C; Bong, C F and Idris, A S (2012). *Ganoderma* species associated with basal stem rot disease of oil palm. *Amer. J. Applied Sciences, 9(6)*: 879-885. DOI: 10.3844/ajassp.2012.879.885.

Zou, Y; Lord, D; Zhang, Y and Peng, Y (2013). Application of the Bayesian Model Averaging in predicting motor vehicle crashes. *J. Transportation and Statistics, 10(1)*: 49-60.

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HERBICIDE EFFECTS ON *Ganoderma boninense* INFECTION IN OIL PALM SEEDLINGS

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ABSTRACT

The use of herbicides for weed management is a common practice in oil palm plantation. However, previous studies have shown that herbicide can reduce plant susceptibility and could also cause physiological injury to plant parts such as roots which will ultimately provide entry points for pathogens. This study aimed to investigate the effect of commonly used herbicides in the oil palm plantation as a predisposing factor to Ganoderma *disease development.* In vitro *study on 11 commercial herbicides with four different dosages have shown that the highest percentage inhibition of radial growth (PIRG) of* Ganoderma boninense *was recorded by paraquat dichloride treatment (100%) followed by diuron (87%) and monosodium methyl arsenate (MSMA) (79%) at concentration of 100 µg ml^{-1}. Based on nursery trial, oil palm seedlings inoculated with* G. boninense *and treated with diuron showed the highest* Ganoderma *disease progression at 92.73% followed by seedlings treated with metsulfuron-methyl and glyphosate monoammonium (83.27%) and by glyphosate glyphosate isopropylammonium with (73.81%). These findings will help the oil palm industry in Malaysia to choose the best herbicides in order to mitigate the development of* Ganoderma *disease incidence.*

Keywords: basal stem rot, *Ganoderma boninense*, herbicide, phytotoxicity.

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INTRODUCTION

The oil palm is an economically important crop and considered as one of the world's major sources of edible oil and a significant precursor of biodiesel fuel (Mohd Ali *et al.*, 2012; Hameed *et al.*, 2009). Unfortunately, it faces the threat of a devastating disease. Basal stem rot (BSR) disease caused by the fungus *Ganoderma boninense* is considered as the greatest threat to oil palm production in South-east Asia including Malaysia (Ariffin *et al.*, 2000; Assis *et al.*, 2016). The disease incidence increases progressively, although slowly, but certainly erodes the profitability of this major industry. It has been reported that the economic loss due to this disease in our country is between RM 225 million to RM 1.5 billion (up to USD 500 million) a year (Arif *et al.*, 2011). Several control methods have been introduced and practised to control BSR disease including cultural practices, chemical and biological controls. Cultural practices such as surgery, soil mounding, sanitation by removing and destroying infected old palm stumps, root masses and ploughing along the new planting row at the replanting area have been applied to manage BSR. Thorough studies have been conducted on managing the disease with chemical fungicides such as hexaconazole (Idris *et al.*, 2004a) and biological control agents such as endophytic microorganisms (Sapak *et al.*, 2008), *Trichoderma* (Mohd and Faridah, 2008; Nusaibah *et al.*, 2017) and mycorrhizal fungi (Sundram *et al.*, 2015). The research findings showed

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the potential of these approaches in delaying the disease and prolonging the economic life-span of the infected oil palms. Apart from these control approaches, herbicides commonly used to manage weeds in oil palm plantation could be explored for their effects towards *G. boninense*. The side effects of herbicides on plant pathogens and hosts either promoting or supressing the disease development have been discussed extensively by researchers (Hess, 2018; Johal and Huber, 2009; Manju *et al.*, 2015; Sanogo *et al.*, 2000). The interaction of herbidices with *G. boninense* and oil palm roots, however, have not yet fully investigated and tested. Therefore, this study aimed to assess the side effects of several herbicides commonly used in oil palm plantation on *G. boninense* development in oil palm seedlings.

MATERIALS AND METHODS

Herbicides and Source of BSR Pathogen

Eleven herbicides commonly used in oil palm plantations were selected in these experimental studies (*Table 1*). A pure culture of fungal pathogen *G. boninense* (PER 71) was obtained from the Malaysian Palm Oil Board (MPOB), Bangi, Selangor. The fungus was grown on potato dextrose agar (PDA) for eight days and incubated at room temperature (28°C±2°C).

TABLE 1. SELECTION OF HERBICIDES WITH DIFFERENT ACTIVE INGREDIENTS AND MODES OF ACTION

Active ingredient of herbicides	Mode of action	Product rate (litre ha^{-1})
Metsulfuron-methyl	Systemic	17
Triclopyr butoxy ethyl ester	Systemic	33
Fluroxypyr-1-methyl heptyl ester	Systemic	22
Glyphosate isopropylammonium	Systemic	60
Glyphosate monoammonium	Systemic	34
2, 4-D dimethylamine	Systemic	49
Diuron	Systemic	45
Sodium chlorate	Contact	180
Glufosinate ammonium	Contact	330
Paraquat dichloride	Contact	235
Monosodium methyl arsenate	Contact	125

Direct Effect of Eleven Herbicides on *G. boninense* Radial Growth

Inhibitions of radial growth of *G. boninense* by 11 herbicides were assessed using a poison agar technique as previously reported by Marzuki *et al.* (2015). A mycelial disc, 5 mm diameter, was obtained from the actively growing *G. boninense* margins and was placed on the middle of a poison agar plate (9 cm in diameter). The poison agar was prepared by mixing PDA with herbicide at four different concentrations which were 1, 10, 100 and 1000 µg ml^{-1}. For each herbicide concentration, five replicates of the plate agar were used to inoculate with the mycelial disc of the pathogen. All inoculated poison agar plates were incubated at room temperature (28°C±2°C) for eight days. A mycelial disc plated on PDA without any herbicides served as a control treatment. All of the treatments were distributed in a completely randomised design. After the incubation period, the radial growth of *G. boninense* on the poison agar plates was measured in percentage inhibition of radial growth (PIRG) by using Equation (1) by Skidmore and Dikson (1976).

$$PIRG = r_1 - r_2 / r_1 \times 100 \qquad \text{Equation (1)}$$

where r_1 is the radial growth of *G. boninense* in a control plate and r_2 is the radial growth of *G. boninense* in the poison agar plate.

Effects of Selected Herbicides in Suppressing *G. boninense* on Oil Palm Seedlings

The experiment was conducted in an open nursery area at Bandar Baru Bangi, Selangor, Malaysia. Oil palm seedlings were supplied by the Federal Land Development Authority (Felda), Malaysia. Twelve months old *dura* x *pisifera* crosses oil palm seedlings were grown in large polybag (38.1 cm x 50.8 cm). Seven commonly used herbicides namely metsulfron-methyl, triclopyr butoxy ethyl ester, paraquat dichloride, glyphosate isopropylammonium, glyphosate monoammonium, diuron and monosodium methyl arsenate based on the results from *in vitro* experiment were used in this study (selected from *Table 1*). A stock solution of each herbicide was made based on the manufactures recommendation for field application. *Ganoderma boninense* rubber wood block (RWB) inoculums (3 cm x 3 cm x 6 cm) were prepared as described by Sapak *et al.* (2008). The seedlings were artificially inoculated with *G. boninense* RWB via root inoculation technique (Idris *et al.*, 2004b). The seedlings were treated with selected herbicides that previously prepared before inoculation with the pathogen. Each herbicide was drenched carefully around the oil palm seedling collar without contact with the stem, fronds and stalk as suggested by Tan and Chan (1994). The experiment was laid down in a completely randomised design. Each herbicide treatment was replicated three times consisted of 10 seedlings per treatment. The seedlings without pathogen treated with herbicides were used as a

negative control and the seedlings with pathogen but without herbicide served as a positive control. The effects of herbicides on BSR disease development were assessed monthly based on disease incidence (DI) and disease severity (DS). The experiment was conducted for 15 months.

The assessment of DI was performed according to the method proposed by Sapak *et al.* (2008). In this method, oil palm seedlings were visually assessed as infected seedlings once the seedlings displayed disease symptoms of chlorosis and necrosis of leaves, with or without production of white mycelium or fruiting bodies of *G. boninense*. Percentage of DI was then calculated based on Equation (2) by Campbell and Maiden (1990).

$$\text{DI}\ (\%) = \frac{\text{Total number of seedlings infected by BSR}}{\text{Total number of seedlings assessed}} \times 100 \qquad \text{Equation (2)}$$

A reduction of disease incidence due to herbicide effects was then measured as the area under the disease progress curve (AUDPC) and disease reduction (DR). The value of AUDPC was calculated based on an Equation (3) by Campbell and Madden (1990).

$$AUDPC\ (Unit^2) = \sum_{i-1}^{n-1} \left[\frac{y_{i+}\, y_{i+1}}{2}\right] \times (t_{i+1} - t_1) \qquad \text{Equation (3)}$$

where y_i is an assessment of a DI at the ith observation, t_i is time at the ith observation, and n is the total number of observations. Meanwhile, *DR* value was calculated based on an Equation (4) of Sivan and Chet (1986).

$$DR = (1 - DT \mid DC) \times 100 \qquad \text{Equation (4)}$$

where *DC* is a percentage of DI in the positive control treatment and *DT* is an AUDPC of the herbicide treatment.

DS

DS was measured based on disease severity foliar index (DSFI) and disease severity internal symptoms (DSIS) of the infected tissues. Development of external foliar symptoms on oil palm seedlings was recorded at a sequence of 1, 3, 6, 9, 12 and 15 months after treatment. The oil palm seedlings with BSR disease foliar symptoms were scored based on five class indexes which are 0 = asymptomatic seedling without appearance of white fungal mass (mycelium) fruiting body and without any foliar symptoms such as chlorosis and necrotic of leaves, 1 = appearance of white fungal mass and/or fruiting body but without any foliar symptoms, 2 = appearance of white fungal mass and/or fruiting body with evidence of less than 25% of leaf areas showing foliar symptoms, 3 = appearance of white fungal mass and/or fruiting body with evidence of more than 26% of leaves with foliar symptoms, and 4 = appearance of white fungal mass and/or fruiting body with evidence of more than 75% of leaves with foliar symptoms. The DSFI was then calculated based on an Equation (5) as proposed by Mohd and Faridah (2008).

$$\text{DSFI} = \frac{\sum(\text{A}\times\text{B})\times 100)}{\sum \text{n}\times 4} \qquad \text{Equation (5)}$$

where *A* is the disease scale ranged from 0 to 4, *B* is a total number of plants showing that disease class per treatment, *n* is the total number of replicate with 4 indicating the highest level of disease index assessment. Meanwhile, the DSIS was assessed based on internal symptoms of BSR disease severity index on root (DSIR) and disease severity index on bole (DSIB) tissues of oil palm seedlings. This assessment was conducted during the final stage of experiment by using a destructive sampling method. Prior to the DSIS assessment, *Ganoderma* selective media were used to confirm the presence of *G. boninense* as the infected root and bole tissues would produce a halo zone on the media (Ariffin and Idris, 1991). The infected seedlings were then split longitudinally at the inoculated root point to examine the extension of disease symptoms to the bole tissue by using a grid method with size of 1 cm^2 per grid (Sapak *et al.*, 2008). The disease symptom of rotten tissues caused by *G. boninense* was rated based on a disease scale as suggested by Nur Sabrina *et al.* (2012) as 0 = asymptomatic root and bole tissues without any internal symptom of rot, 1 = root and bole tissues displaying <20% symptom of rot, 2 = root and bole tissues displaying 20%-50% symptom of rot, 3 = root and bole tissues displaying >50%-80% symptom of rot and 4 = root and bole tissues displaying >80% symptom of rot. Equation (6) was applied to calculate the internal severity of the infected seedlings (Liu *et al.*, 1995).

$$\text{DSIS}\ (\%) = \frac{\text{Number of seedlings in the scoring} \times \text{scoring scale}}{\text{Total number of seedlings assessed} \times \text{the score}} \times 100 \qquad \text{Equation (6)}$$

Data Analysis

All collected data were subjected to the analysis of variance (ANOVA) and Turkey's Test ($P \leq 0.05$)

by using SAS® statistical packages version 9.0 (SAS Institute Carey, North Carolina, USA).

RESULTS AND DISCUSSION

Effects of Herbicides on Radial Growth of *G. boninense in vitro*

Results of *in vitro* study revealed that PIRG of *G. boninense* upon direct exposure to 11 herbicides at different concentrations are varied as presented in *Table 2*. The highest PIRG of *G. boninense* was recorded in the paraquat dichloride treatment at both concentrations of 100 and 1000 μg ml^{-1} which gave 100.0% of PIRG or zero growth of *G. boninense*. This result indicated that paraquat at 100 μg ml^{-1} would be an adequate concentration to totally inhibit the growth of *G. boninense*. The ability of paraquat to inhibit plant pathogen has been discussed by Toubia-Rhame *et al.* (1995) and Duke *et al.* (2007a) where both studies have reported that paraquat was able to suppress the growth of *Dreschlera teres* in barley via direct toxic to the pathogen. Previous study by Smith and Lyon (2006) also showed that paraquat could inhibit other fungal species such as *Mucor* sp., *Penicillium* sp. and *Aspergillus* sp. up to 70.0% of PIRG. Nevertheless, low concentrations of paraquat dichloride at 1 μg ml^{-1} and 10 μg ml^{-1} did not inhibit the growth of *G. boninense* as evidenced by the pathogen growing on the poison agar plates. The second highest PIRG value at the concentration of 100 μg ml^{-1} was recorded by diuron at 87.2%. Monosodium methyl arsenate and glufosinate ammonium recorded PIRG values of 79.5% and 72.2%, respectively. Furthermore, at the highest concentration of 1000 μg ml^{-1}, herbicides of diuron, glufosinate ammonium and monosodium methyl arsenate gave similar effect on the pathogen growth with PIRG values between 93.0%-94.0%. In contrast, the less effective herbicides on suppression of radial growth of pathogen at the highest concentration (1000 μg ml^{-1}) were observed in the treatment of sodium chlorate with only 35.5%, followed by 2-4-D dimethylamine, glyphosate isopropylammonium and metsulfuron-methyl with PIRG values of 62.2%, 63.3% and 65%, respectively.

Phytotoxicity Effect of Herbicides on *Ganoderma* Disease Incidence

Disease progression. The external symptoms of *Ganoderma* infection were observed during the course of the experiments. These symptoms included the progressive yellowing of lower leaves, the subsequent desiccation from the oldest to the younger leaves, and the rapid development of sphorophore, like small white button and fruiting bodies. Assessment of the exposure data presented here indicated that the above-mentioned herbicides used in oil palm plantation would have the potential to inhibit and encourage the aggressiveness of fungal *G. boninense* populations upon direct exposure *in vitro*. However, due to natural processes in the field, the inhibitory effect on the growth of *G. boninense* was different depending on the mode of actions and active ingredient of the herbicides. Herbicides treated in *in vitro* studies caused significant reduction to growth and development of *G. boninense*. Johnston *et al.* (1980) reported that fungal pathogens

TABLE 2. EFFECT OF DIFFERENT HERBICIDES AT DIFFERENT CONCENTRATIONS ON RADIAL GROWTH OF *G. boninense* ON POISON AGAR (cm)

Active ingredient	Concentration (μg ml^{-1})			
	1	10	100	1 000
Paraquat dichloride	9.44±4.65^{c}	34.46±3.73^{b}	100.00±0.00^{a}	100.00±0.00^{a}
Diuron	6.68±1.52^{c}	8.88±2.32^{c}	87.22±1.52^{b}	93.86±1.24^{a}
Monosodium methyl arsenate	18.88±3.62^{d}	45.54±3.17^{c}	79.46±2.48^{b}	94.14±1.52^{a}
Glufosinate ammonium	21.08±1.52^{d}	35.54±1.24^{c}	72.20±1.96^{b}	93.86±1.24^{a}
Glyphosate monoammonium	4.48±1.52^{d}	8.88±2.32^{c}	31.68±1.52^{b}	74.44±2.32^{a}
Fluroxypyr-1-methyl heptyl ester	3.36±1.24^{c}	34.42±1.52^{b}	35.54±2.32^{b}	71.10±2.48^{a}
Triclopyr butoxy ethyl ester	3.36±1.24^{c}	33.88±2.32^{b}	35.54±2.32^{b}	71.10±2.48^{a}
Metsulfuron-methyl	8.88±2.32^{d}	22.20±2.78^{c}	36.66±2.32^{b}	65.00±3.73^{a}
Glyphosate isopropylammonium	4.46±2.48^{d}	15.58±1.52^{c}	25.56±3.04^{b}	63.34±4.56^{a}
2, 4-D dimethylamine	10.00±3.17^{d}	23.88±4.21^{c}	35.54±2.32^{b}	62.22±3.17^{a}
Sodium chlorate	5.58±3.40^{b}	11.68±4.97^{b}	12.20±5.41^{b}	35.54±1.81^{a}

Note: Means ± standard error. Values sharing same letters between columns differ non-significantly ($P \leq 0.05$).

may also differ in their response to the herbicides in field conditions. Anderson and Kolmer (2005) also reported that the results from greenhouse and field study confirmed that application of glyphosate can reduce or eliminate plant diseases caused by *Puccinia triticina* and also *Puccinia graminis*.

Oil palm seedlings treated with herbicides and inoculated with *G. boninense* showed zero incidences in all treatments for the first two months of observation. Formation of sporophore or small white button of *G. boninense* was then observed in all treatments except for triclopyr butoxy ethyl ester at three months after treatment (*Table 3*). Based on the assessment after 15 months, seedlings treated with diuron showed the highest DI value at 92.73%. Recent study by Adejoro *et al.* (2019) recorded that *Corchorus olitorius* plants were treated with diuron displayed the disease symptoms of dieback and stunted growth. Their study also recorded a reduction in soil microbial population due to diuron application. Furthermore, Lima *et al.* (2017) also reported that the application of diuron could cause anatomical and physiological injuries to *Bauhina variegate*. Based on these studies, it is higly possible that the application of diuron directly (into the polybag) could cause injury to the root system thus provided an opening for the pathogen to infect the plant. The second highest value of DI was recorded in the treatments of metsulfuron-methyl and glyphosate monoammonium with the same value at 83.27% followed by glyphosate isopropyl ammonium at 73.81%. On the other hand, the seedlings treated with triclopyr butoxy ethyl ester recorded the lowest DI value of 56.62%. In the case of auxin like herbicide such as triclopyr butoxy ethyl ester, volatility and drift could cause injury to non-target plant (Sciumbato *et al.*, 2004), thus it is consistent with the results obtained that even though triclopyr butoxy ethyl ester showed the lowest DI value, the DI was still significantly high.

The disease development was also evaluated using the AUDPC. The AUDPC is a quantitative summary of the disease intensity over time and can be used for best management strategies. The percentage of DR and AUDPC are shown in *Table 4*. AUDPC was calculated based on the DI. In this study, treatment with the lowest AUDPC values indicated low severity of disease in the treated seedlings by the selected herbicides. The seedlings treated with triclopyr butoxy ethyl ester showed

TABLE 3. DISEASE INCIDENCE (%) OBSERVED ON OIL PALM SEEDLINGS 1 TO 15 MONTHS AFTER TREATMENT WITH HERBICIDES AND INOCULATION WITH *G. boninense*

Active ingredient	Month after treatment					
	1	3	6	9	12	15
Metsulfuron-methyl	0	34.15^{c}	56.07^{a}	75.54^{b}	83.27^{a}	83.27^{b}
Triclopyr butoxy ethyl ester	0	0.00e	34.15^{c}	41.88^{e}	48.88^{d}	56.62^{e}
Paraquat dichloride	0	27.14^{d}	48.88^{b}	56.62^{d}	64.35^{c}	64.35^{d}
Glyphosate isopropylammonium	0	27.14^{d}	48.88^{b}	56.62^{d}	73.81^{b}	73.81^{c}
Glyphosate monoammonium	0	27.14^{d}	41.15^{bc}	83.27^{a}	83.27^{a}	83.27^{b}
Diuron	0	56.62^{a}	56.62^{a}	73.81^{b}	73.81^{b}	92.73^{a}
Monosodium methyl arsenate	0	49.61^{b}	49.61^{b}	64.35^{c}	64.35^{c}	83.27^{b}
Control	0	56.62^{a}	56.62^{a}	73.81^{b}	73.81^{b}	92.73^{a}

Note: Means ± standard error. Values sharing same letters between columns differ non-significantly ($P \leq 0.05$).

TABLE 4. EFFECTIVENESS OF DIFFERENT HERBICIDES IN CONTROLLING BASAL STEM ROT DISEASE CAUSED BY *G. boninense* ON OIL PALM SEEDLINGS MEASURED AS DISEASE INCIDENCE BASED ON AREA UNDER DISEASE PROGRESS CURVE (AUDPC) AND DISEASE REDUCTION (DR)

Treatment	AUDPC (unit^{2})	DR (%)
Metsulfuron-methyl	854.92	4.30
Triclopyr butoxy ethyl ester	459.66	48.55
Paraquat dichloride	673.93	24.56
Glyphosate isopropylammonium	716.50	19.80
Glyphosate monoammonium	815.83	8.68
Diuron	893.37	0.00
Monosodium methyl arsenate	783.86	12.34

the lowest AUDPC of 459.66 unit^2, followed by the paraquat dichloride (673.93 unit^2) and glyphosate isopropylammonium (716.50 unit^2). Thus, triclopyr butoxy ethyl ester had the most effect on *Ganoderma* disease development with DR at 48.55% followed by paraquat dichloride (24.56%) and glyphosate isopropylammonium (19.80%). The lowest DR was recorded by diuron at 0% unit indicating no reduction in suppression the BSR and followed by metsulfuron-methyl 4.30%, glyphosate monoammonium 8.68% and monosodium methyl arsenate 12.34%.

The lower percentage of DI, AUDPC and higher value of DR in the seedlings treated with the selected herbicides suggested that the herbicides have a potential to inhibit the growth of *G. boninense*.

The external symptoms of DSFI at 15 months also showed that seedlings treated with diuron recorded the highest DSFI value at 85% whilst the metsulfron-methyl shown the lowest DSFI at 28.33%. The second lowest were recorded by paraquat dichloride and monosodium methyl arsenate with DSFI value of 56.67%, followed by the glyphosate monoammonium at 50% (*Table 5*).

At the end of the experiment, the destructive sampling was carried out at 15 months to assess the extent of root rot and bole decay. The seedlings were sampled and their roots washed under running water to assess the extent of the root rot. The bole was cut longitudinally for assessment of percentage infection of bole tissues and roots. Severe root rot was seen in seedlings under positive control treatment which suffered foliar desiccation. The highest level of root rot decay on DSIR, with extensive colonisation of fungal masses on the root surface, was observed in seedlings treated by diuron in which 41.11% of root showed brown discolouration whilst the lowest DSIR value was shown by seedlings treated with metsulfuron-methyl at 21.67% (*Table 6*). In relation to DSIR, seedlings treated by diuron also showed the highest DSIB at at 40.00% whereas the lowest DSIB was recorded by seedlings treated by metsulfuron-methyl and paraquat dichloride with the same value at 21.67%.

TABLE 5. EFFECTIVENESS OF DIFFERENT HERBICIDES ON CONTROLLING BASAL STEM ROT DISEASE CAUSED BY *G. boninense* ON OIL PALM SEEDLINGS BASED ON DISEASE SEVERITY FOLIAR INDEX (%)

Herbicide (active ingredient)	Observation month					
	1	3	6	9	12	15
Metsulfuron-methyl	$0.00^{a}\pm0.00$	$6.67^{abc}\pm0.48$	$15.00^{ab}\pm0.55$	$16.67^{cd}\pm0.33$	$30.00^{c}\pm0.59$	$28.33^{d}\pm1.28$
Triclopyr butoxy ethyl ester	$5.00^{a}\pm0.33$	$11.67^{abc}\pm0.51$	$11.67^{ab}\pm0.33$	$26.67^{bcd}\pm0.48$	$31.67^{c}\pm0.94$	$63.00^{bc}\pm1.69$
Paraquat dichloride	$3.33^{a}\pm0.18$	$8.33^{abc}\pm0.51$	$13.33^{ab}\pm0.30$	$18.33^{cd}\pm0.95$	$45.00^{abc}\pm1.13$	$56.67^{bc}\pm0.73$
Glyphosate isopropylammonium	$3.33^{a}\pm0.18$	$21.67^{ab}\pm0.74$	$23.33^{ab}\pm0.55$	$38.33^{b}\pm0.96$	$41.67^{bc}\pm1.34$	$70.0^{ab}\pm1.03$
Glyphosate monoammonium	$0.00^{a}\pm0.00$	$3.33^{bc}\pm0.33$	$5.00^{b}\pm0.45$	$13.33^{d}\pm0.33$	$30.00^{c}\pm0.45$	$50.00^{d}\pm1.02$
Diuron	$6.67^{a}\pm0.45$	$23.33^{a}\pm0.66$	$28.33^{a}\pm0.15$	$60.00^{a}\pm1.39$	$60.0^{a}\pm0.89$	$85.00^{a}\pm1.05$
Monosodium methyl arsenate	$11.67^{a}\pm0.63$	$21.67^{ab}\pm0.66$	$21.67^{ab}\pm0.70$	$30.00^{bc}\pm0.89$	$45.00^{abc}\pm0.96$	$56.67^{bc}\pm1.10$
Control	$0.00^{a}\pm0.00$	$1.67^{c}\pm0.51$	$5.00^{b}\pm0.51$	$16.70^{cd}\pm0.64$	$38.33^{c}\pm1.20$	$63.0^{bc}\pm1.10$

Note: Mean ± standard deviation. Values sharing same letters between columns differ non-significantly ($P \leq 0.05$).

TABLE 6. DISEASE SEVERITY OBSERVED IN ROOT (DSIR) AND DISEASE SEVERITY IN BOLE (DSIB) TISSUES OF OIL PALM SEEDLINGS AT 15 MONTHS AFTER TREATED WITH HERBICIDES AND *G. boninense* INOCULATION

Treatment	DSIR±SD	DSIB±SD
Metsulfuron-methyl	21.67 ± 1.26^{a}	21.67 ± 1.32^{a}
Triclopyr butoxy ethyl ester	31.67 ± 1.28^{a}	25.00 ± 1.21^{a}
Paraquat dichloride	28.89 ± 1.47^{a}	21.70 ± 1.37^{a}
Glyphosate isopropylammonium	30.00 ± 1.31^{a}	25.00 ± 1.34^{a}
Glyphosate monoammonium	40.00 ± 1.46^{a}	28.33 ± 1.34^{a}
Diuron	41.11 ± 1.61^{a}	40.00 ± 1.33^{a}
Monosodium methyl arsenate	33.33 ± 1.63^{a}	28.33 ± 1.19^{a}
Control	55.00 ± 1.28^{a}	46.70 ± 1.51^{a}

Note: Mean ± standard deviation (SD). Values sharing same letters differ non-significantly ($P \leq 0.05$).

CONCLUSION

The *in vitro* study has shown that certain concentration of herbicides could inhibit *G. boninense*. The effect of herbicides on plant pathogen have been reported by many previous studies (Duke *et al*., 2007b). Nevertheless, most studies found that herbicides generally do not tend to move in significant quantities below the top 15 cm of soil. Recent findings found that *G. boninense* in oil palm roots and trunk were more persistence at 60 cm below soil (Sundram, 2019). Thus, although certain herbicides are toxic to *G. boninense* but due to the movement and distribution of the herbicide in the field, the herbicide will have little effect on *G. boninense*. Nevertheless, it is worth to note that herbicides with different mechanisms of action can also stimulate anti-microbial compound such as phytoalexins and therefore can have an effect on plant disease resistance. Nevertheless, there are limited studies on the effect of tested herbicides on plant disease resistance.

Based on the nursery study, it was evident that direct application of herbicides into root will have an adverse affect to the oil palm seedlings. Herbicides such as diuron, metsulfuron-methyl and glyphosate monoammonium that have been previously reported to cause injury to plant roots had the highest DI compared to other herbicide treatments. This information is important especially in the context of suitable application of these herbicides in the field. It is recommend that no application of herbicides should be made directly to the root or bold of oil palm seedlings.

In conclusion, this study suggests that herbicides which potentially can cause injury to oil palm seedlings should be reassessed for their application in oil palm nursery to avoid damage to oil palm which would provide entry routes for the pathogen. Neverthless, the findings in this study only provide a basic understanding on the effect of herbicides that are commonly used to control weeds to young oil palm and *G. boninense* based on observable symptoms. Therefore, further intensive study at cellular and molecular levels to get better understanding on the interaction between herbicides, oil palm and pathogen need to be carried out in the future.

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REFERENCES

Adejoro, S A; Ajoke, C A and Rex, D A (2019). Effects of diuron residues on the growth performance of jute (*Corchorus olitorius*) plant and its rhizosphere soil microbial population. *J. Biology and Life Science, 10*: 35-45.

Anderson, J A and Kolmer, J A (2005). Rust control in glyphosate tolerant wheat following application of the herbicide glyphosate. *Plant Disease, 89*: 1136-1142.

Arif, M S; Roslan, A; Idris, A S and Ramle, M (2011). Economics of oil palm pests and *Ganoderma* disease and yield losses. *Proc. of the Third MPOB-IOPRI International Seminar: Integrated Oil Palm Pests and Diseases Management*. Kuala Lumpur Convention Centre, Kuala Lumpur.

Ariffin, D and Idris, A S (1991). A selective medium for the isolation of *Ganoderma* from diseased tissues. *Proc. of the International Palm Oil Conference, Progress, Prospect and Challenges toward the 21st Century (Modul 1, Agriculture)* (Yusuf *et al.* eds.). PORIM, Bangi. p. 517-519.

Ariffin, D; Idris, A S and Singh, G (2000). Status of *Ganoderma* in oil palm. Ganoderma *Diseases of Perennial Crops* (Flood, J; Bridge, P and Holderness, M eds.). Wallingford, United Kingdom. CABI Publishing. p. 49-68.

Assis, K; Chong, K P; Idris, A S and Ho, C M (2016). Economic loss due to *Ganoderma* disease in oil palm. *World Academy of Science, Engineering and Technology, International Journal of Social, Behavioral, Educational, Economic, Business and Industrial Engineering, 10(2)*: 631-635.

Campbell, C L and Madden, L V (1990). *Introduction to Plant Disease Epidemiology*. New York, USA: Wiley. 532 pp.

Duke, S; Wedge, D E; Cerdeira, A L and Matallo, M B (2007a). Herbicide effects on plant disease. *Outlooks on Pest Management, 8*: 36-40.

Duke, S O; Wedge, D E; Cerdeira, A L and Matallo, M B (2007b). Interactions of synthetic herbicides with plant disease and microbial herbicides. *Novel Biotechnologies for Biocontrol Agent Enhancement and Management* (Maurizio Vurro and Jonathan Gressel eds.). Dordrecht: Springer. p. 277-296.

Hameed, B H; Lai, L F and Chin, L H (2009). Production of biodiesel from palm oil (*Elaeis guineensis*) using heterogeneous catalyst: An optimized process. *Fuel Processing Technology, 90*: 606-610.

Hess, F D (2018). Herbicide effects on plant structure, physiology, and biochemistry. *Pesticide Interactions in Crop Production* (Altman, J ed.). New York: CRC Press. p. 13-34.

Idris; A S; Ismail, S and Ariffin, D (2004a). Prolonging the productive life of *Ganoderma*-infected palms with hexaconazole. *MPOB Information Series No. 214*: 4 pp.

Idris, A S; Kushairi, A; Ismail, S and Ariffin, D (2004b). Selection for partial resistance in oil palm progenies to *Ganoderma* basal stem rot. *J. Oil Palm Res. Vol. 16*: 12-18.

Johal, G S and Huber, D M (2009). Glyphosate effects on diseases of plants. *European J. Agronomy, 31*: 144-152.

Johnston, H W; Ivany, J A and Cutcliffe, J A (1980). Effect of herbicides applied to soil on *Fusarium* root rot of processing peas. *Plant Disease, 64*: 942-943.

Lima, D A; Muller, C; Costa, A C; Batista, P F; Dalvi, V C and Domingos, M (2017). Morphoanatomical and physiological changes in *Bauhinia variegate* as indicators of herbicide diuron reaction. *J. Ecotoxicology and Environmental Safety, 141*: 242-250.

Liu, L; Kloepper, J W and Tuzun, S (1995). Induction of systemic resistance in cucumber against bacterial angular leaf spot by plant growth-promoting rhizobacteria. *Phytopathology, 85*: 843-847.

Manju, M J; Sabu, P I; Shankarappa, T H; Jacob, C K; Prem, E and Jacob, M (2015). Effect of herbicide and fungicide application to curb *Corynespora cassiicola* inoculum in rubber plantation. *Environment and Ecology, 33*: 1293-1298.

Marzuki, N F; Goh, Y K; Tung, H J; Goh, Y K and Goh, K J (2015). Evaluation of the cultural characteristics and antagonistic activities of *Cladobotryum semicirculare* against *Ganoderma boninense in vitro*. *J. Oil Palm Res. Vol. 27*: 326-338.

Mohd Ali, A A; Mohd Zainudin, M H; Idris, A; Baharuddin, A S; Sulaiman, A; Matsui, T; Osaka, N; Oshibe, H; Hassan, M A and Shirai, Y (2012). Enhanced biogas production from palm oil mill effluent supplemented with untreated oil palm empty fruit bunch biomass with a change in the microbial community. *Japan J. Food Engineering, 13(3)*: 37-41.

Mohd, Z and Faridah, A (2008). Disease suppression in *Ganoderma*-infected oil palm seedlings treated with *Trichoderma harzianum*. *Plant Protection Science, 44*: 101-107.

Nur Sabrina, A A; Sariah, M and Zaharah, A R (2012). Suppression of basal stem rot disease progress in oil palm (*Elaeis guineensis*) after copper and calcium supplementation. *Pertanika J. Tropical Agricultural Science, 35*: 13-24.

Nusaibah, S A; Saad, G and Hun, T G (2017). Antagonistic efficacy of *Trichoderma harzianum* and *Bacillus cereus* against *Ganoderma* disease of oil palm via dip, place and drench (DPD) artificial inoculation technique. *Int. J. Agriculture and Biology, 19*: 299-306.

Sanogo, S; Yang, X B and Scherm, H (2000). Effects of herbicides on *Fusarium solani* f. sp. glycines and development of sudden death syndrome in glyphosate-tolerant soybean. *Phytopathology, 90*: 57-66.

Sapak, Z; Meon, S and Ahmad, Z A M (2008). Effect of endophytic bacteria on growth and suppression of *Ganoderma* infection in oil palm. *Int. J. Agriculture and Biology, 10*: 127-132.

Sciumbato, A S; Chandler, J M; Senseman, S A; Bovey, R W and Smith, K L (2004). Determining exposure to auxin-like herbicides. I. Quantifying injury to cotton and soybean. *Weed Technology, 18*: 1125-1134.

Sivan, A and Chet, I (1986). Biological control of *Fusarium* spp. in cotton, wheat and muskmelon by *Trichoderma harzianum*. *J. Phytopathology, 116*: 39-47.

Skidmore, A M and Dickinson, C H (1976). Colony interactions and hyphal interference between *Septoria nodorum* and phylloplane fungi. *Transactions of the British Mycological Society, 66*: 57-64.

Smith, S and Lyon, A (2006). The uptake of paraquat by soil fungi. *New Phytologist, (76)*: 479-484.

Sundram, S; Meon, S; Seman, I A and Othman, R (2015). Application of arbuscular mycorrhizal fungi with *Pseudomonas aeruginosa* UPMP3 reduces the development of *Ganoderma* basal stem rot disease in oil palm seedlings. *Mycorrhiza, 25*: 387-397.

Sundram, S (2019). *Ganoderma* biology, etiology and epidemiology. *Proc. of International Seminar on Breeeding for* Ganoderma *Tolerance in Oil Palm*. Kuala Lumpur Convention Centre Kuala Lumpur.

Tan, C H and Chan, C L (1994). Injury symptoms on young oil palms induced by topical and basal herbicides. *The Planter, 70*: 341-358.

Toubia-Rahme, H; Ali-Haimoud, D E; Barrault, G and Albertini, L (1995). Inhibition of *Drechslera teres* sclerotioid formation in barley straw by application of glyphosate or paraquat. *Plant Disease, 79*: 595-598.

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PERFORMANCE EVALUATION OF A MOTORISED PALM OIL EXTRACTOR WITH QUALITY ASSESSMENT OF THE PALM OIL EXTRACTED IN COMPARISON WITH A MANUAL VERTICAL PRESS

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ABSTRACT

Traditional methods of palm oil extraction from palm fruits (Elaeis guineensis) *produce low quality and quantity of oil. This study sought to design, construct and test a motorised palm oil extractor with evaluation of the oil extracted in comparison with a manual vertical press. The performance parameters tested were oil extraction ratio (OER), oil extraction efficiency (OEE), machine discharge efficiency (MDE) and oil extraction losses (OEL) while the tested physio-chemical parameters were free fatty acids (FFA), iodine value (IV), saponification value (SV) and peroxide value (PV). There were significant differences ($p \leq 0.05$) in OER (16.20% and 13.53%), OEE (77.13% and 64.44%) and OEL (18.30% and 24.76%) while the MDE (81.70% and 73.13%) were not significantly different ($p > 0.05$) for the motorised and vertical press, respectively. No significant differences were observed for IV and SV while FFA showed significant differences ($p \leq 0.05$). The PV was not detected for both methods. A motorised palm oil extractor produced oil of higher quality and had higher performance efficiencies as compared to the manual vertical press. The novelty of this work was in producing an efficient equipment that is affordable to a smallholder farmer which extracts palm oil of high quality.*

Keywords: palm oil, quality parameters, screw press, efficiency, extraction.

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INTRODUCTION

Oil palm (*Elaeis guineensis*) originated from the tropical rain forest region of West Africa (Azodo *et al.*, 2013; Poku, 2002). Oil palm grows mainly in tropical climates (Razali *et al.*, 2012) and due to its economic importance as a high yielding source of edible oil, it is grown as a plantation crop in most countries with tropical climates (Poku, 2002). In 2003, the government of Uganda with BIDCO Uganda Limited, signed an agreement to undertake the oil palm project at Bugala Islands Kalangala district. All plantation related activities and the extraction of crude oil from the fresh fruit bunches was managed by the Oil Palm Uganda limited (OPUL). IFAD (2010) reported that there were 10 088 ha of oil plantation with 1600 ha under smallholders.

Oil palm produces two types of oils; palm oil and palm kernel oil (Azodo *et al.*, 2013). Most oils are recovered from oil bearing crops by cooking, grinding, expelling, pressing by chemical methods or solvent extraction of the raw materials (Anebi *et al.*, 2014). Small scale processors mainly in

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developing countries extract their oils traditionally by use of a constructed vertical press that is operated by about three to four workers (*Figure 1*). The commonest method of extracting oil from oilseeds is the mechanical pressing method which may be hydraulic press or screw press principle (Moses, 2014). Presses that have been developed over the years include manual vertical screw press, motor-jack press, spindle press, hydraulic press, and the combined screw/hydraulic press (*Figure 2*). The screw press principle is more reliable, has a higher efficiency and usually more adaptable for small and medium scale producers (Olaniyan *et al.*, 2012) than the hydraulic press which is otherwise more expensive, need more maintenance, requires more labour and involves risking contamination of the oil with poisonous hydraulic fluid (Aremu and Ogunlade, 2013; Olaniyan *et al.*, 2012).

The oil extracted from oil palm is commonly referred to as crude palm oil (CPO) because it is not refined. In most developing countries, most of the CPO produced by small scale processors does not meet the quality standards for industrial use mainly due to presence of high levels of free fatty acids (Osei-Amponsah *et al.*, 2012). In this regard, non-industrial CPO must fulfill the requirements of quality applicable to all oils and fats as their consumption can be harmful to human beings due to presence of components that can trigger reactions that lead to degradation of these oils (Ngando *et al.*, 2011). The quality of vegetable oils varies with the quality of the fruits, the method of processing, handling and storage used (Ohimain *et al.*, 2013; Okogeri and Otika, 2014) and according to Ngando *et al.* (2011) and Kukeera *et al.* (2015), the mostly examined quality parameters include moisture content, iodine value (IV), peroxide value (PV), saponification value (SV) and free fatty acids (FFA). The presence of FFA indicates the initiation of acidification and quality deterioration of an oil sample (Ngando *et al.*, 2013) as well as indicating its degree of hydrolysis (Tarmizi *et al.*, 2016). The IV is an index for the adulteration of oils as it measures the unsaturation levels of the oil while the PV is the measure of the degree of oil oxidation giving an indication of the levels of primary lipid oxidation, quality and stability of fats and oils (Agbaire, 2012). SV indicates the molecular weights of triglycerides and it is inversely proportional to the length of fatty acids (Agbaire, 2012).

Source: Photos courtesy of Jjagwe, J.

Figure 1. Traditional method of oil extraction used in Buwama-Uganda: (a) manual vertical press with digested oil palm fruits ready for extraction and (b) workers extracting oil manually.

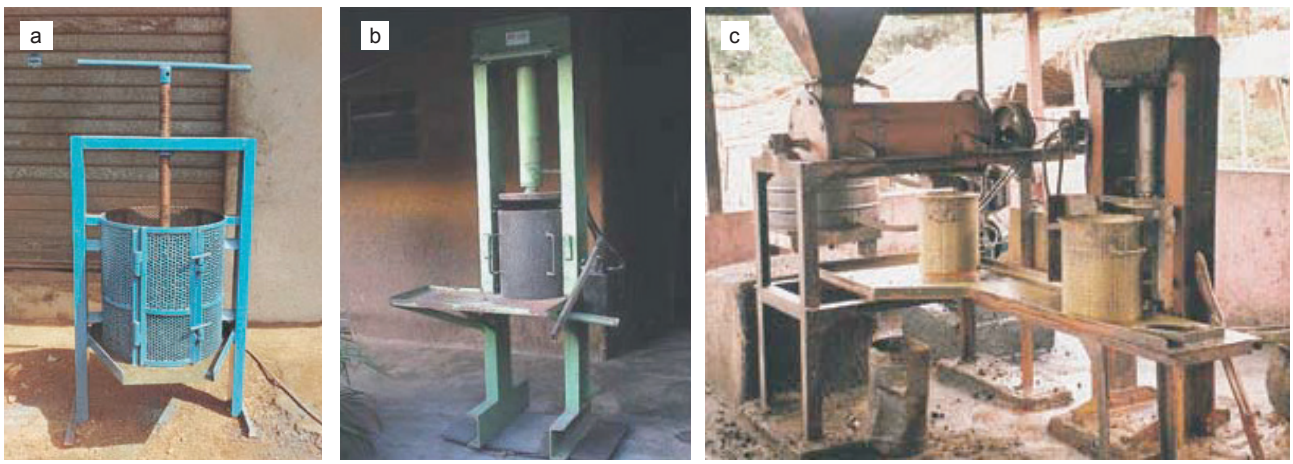


Figure 2. Commonly used presses for oil extraction: (a) spindle press, (b) hydraulic press and (c) combined screw and hydraulic press (Poku, 2002).

There is a need to increase the supply of palm oil for meeting the food security needs of the world (Mahmud *et al*., 2010) since the demand for palm oil is not only growing in the food sector but also in industries where it is used for product development like cosmetics and soaps (Nsiah *et al*., 2012). In Sub-Saharan Africa, advances in agricultural productivity have been hindered by low technology and therefore the development of new technologies will work towards changing the optimal size of production in favour of large holdings (Aremu and Ogunlade, 2013). The novelty of this study is justified by the fact that most of the knowledge related to the design and development of screw press equipment belongs to the holders of large manufacturers in industrial scale production systems (Olaniyan *et al*., 2012). Besides, these screw presses are not available on the Ugandan market and hence are just imported by the large-scale oil extraction companies. Therefore with an increased number of small scale holders of oil palm farms in Uganda, there is a need to develop technology that will be affordable to the small producers to meet the demand of palm oil that is projected to increase to 60 million tonnes per year by 2020 from the current 46 million tonnes per year (World Growth, 2011) as well as increasing incomes to the small scale farmers. The objective of this work was to design and construct a low scale motorised extractor to effectively extract palm oil at an affordable price while maintaining quality, thus the screw press principle was adopted in designing and constructing a palm oil extractor to effectively extract palm oil from the digested palm fruits.

MATERIALS AND METHODS

Design Considerations

In the design of the machine, key considerations made included high oil yield, high oil extraction efficiency and ratio, low extraction loss, availability and cost of construction materials. Other considerations included the simplicity in design and ease to fabricate the machine, usability of the machine without previous technical training, a strong frame to ensure structural stability and strong support for the machine, and ability to easily dismantle the machine for cleaning purposes.

Design Concept

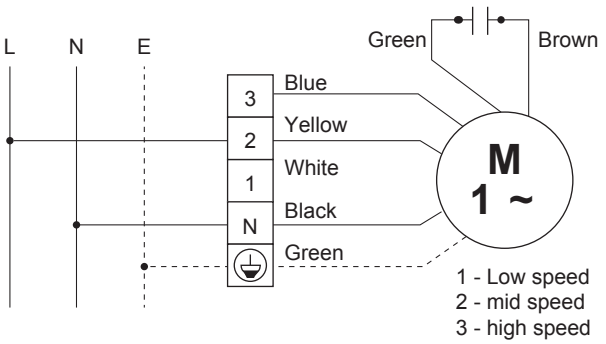
The machine consisted of four major components; the feeding component, extraction component, power component and the frame. The feeding component consisted of the hopper; the extraction component consisted of the worm screw shaft, the perforated frustum barrel (squeezing chamber) and both the oil and residual outlets; the power component consisted of the prime mover (geared motor) and the chain while the frame served as a stand for the machine on which all other components were mounted. The worm screw shaft rotates in the perforated barrel and conveys the digested fruits from the feeding section towards the discharge section where there are outlets for both the cake and the extracted oil. All the components in contact with the fruits were made out of stainless steel because it is the recommended standard material for edible food materials. Pressure was achieved in the machine by the operation of the tapered worm shaft which was designed as a step-down volume from the feed end towards the discharge end, thus, reducing the area available for the fruits and increasing pressure to efficiently extract the oil.

Design and Selection of the Shaft

The worm shaft is the main component of the screw press and is acted upon by weights of material being processed (sprocket and screw threads). In operation, the worm shaft with the aid of screw threads conveys, presses and squeezes the material (digested palm fruits) for oil extraction. Therefore, in order to safeguard against bending and tensional stresses, the diameter of the shaft was determined from Equation (1) as given by Mrema and McNulty (1985).

$$d_S = \sqrt[3]{\frac{P}{N}} \times 112 \qquad \text{Equation (1)}$$

where; d_s - diameter of the shaft in mm, P - power rating of the motor in kW, N - rotational speed of the shaft in rpm. Working with what was available in the workshop, a geared motor (*Figure 3*) of 0.75 kW was available and in order to effectively squeeze the fruits, a speed of 120 rpm was taken, therefore substituting for P= 0.75 kW and N= 120 rpm into Equation (1) d_s= 20.6 mm, therefore a stainless steel rod of 20 mm was selected for the worm shaft.



Source: https://www.fantech.com.au/images/PDF/Catalogue/WiringDiag.pdf

Figure 3. Wiring schematic of a single phase motor.

Design of the Screw Threads

The worm shaft is essentially a tapered screw conveyor with the volumetric displacement being decreased from the feed end of the barrel to the discharge end. The screw threading system was designed with aid of stainless steel rods of 6 mm thickness welded onto the shaft with a decrease in the pitch and depth towards the discharge end using Equation (2) as given by Shigley and Mischeke (2001).

$$U_n = a + (n-1)d \qquad \text{Equation (2)}$$

where; U_n - screw depth at the discharge end in mm, a - screw depth at the feed end in mm, d - common difference between the next successive screw depths (decrease in pitch) and n - number of turns. Depending on the mass of the fruits that was desired to be processed per given time within the working chamber of the designed volume, a = 110 mm and depending on the mean diameter of the kernel, U_n = 35 mm a decrease in pitch (d) of 10 mm was taken hence designing a screw thread with 8 turns.

Design of the Working Chamber

The working chamber (squeezing section) was designed based on the configuration of the screw thread that was to rotate within it. This working chamber was designed as a frustum from stainless steel sieve of 3 mm thickness and with perforations of 2 mm and its volume was determined from Equation (3).

$$V = \frac{\pi h}{3}(R^3 + Rr + r^3) \qquad \text{Equation (3)}$$

where; V - volume of the frustum in m^3, h - height in m, r - radius at the lower end (discharge end) in m, R - radius at the upper base (feed in end) in m. Taking a clearance of 5 mm between the screw threads and the working chamber, h = 0.44 m, R = 0.125 m, r = 0.05 m, therefore a frustum of volume 3.8373×10^{-3} m^3 was designed as the working chamber.

Design of the Load Lifted by the Screw

The load that can be lifted by the screw was determined from Equations (4), (5) and (6) as given by Hall *et al.* (1961).

$$W_e = T\frac{(1-\mu tan\theta cos\alpha)}{\frac{D_m}{2}(tan\theta + \frac{\mu}{cos\alpha})} \qquad \text{Equation (4)}$$

$$T = \frac{P}{2\pi N} \qquad \text{Equation (5)}$$

$$\alpha = \tan^{-1}(\tan\theta_n \cos\theta) \qquad \text{Equation (6)}$$

where; W_e - load lifted by the screw in N, T - torque transmitted by the screw shaft in Nm, D_m - mean thread diameter at the feed in end (a) in m, μ - coefficient of friction, θ_n - thread angle in degrees and θ - tapering angle in degrees. Substituting N = 2 rev/s, P = 750 W, T = 59.68 Nm, D_m = 110 mm, $\theta = 5^0$, $\theta_n = 15^0$, $\mu = 0.15$ hence, $\alpha = 14.98^0$ W_e = 60 N hence, 6 kg of digested fruits can be processed per unit time.

Design of the Pressure to be Developed by the Screw Thread

The pressing area and the pressure to be developed by the screw thread were determined by Equations (7) and (8) as given by Hall *et al.* (1961).

$$A_P = \pi D_m n U_n \qquad \text{Equation (7)}$$

$$P_r = \frac{W_e}{A_p} \qquad \text{Equation (8)}$$

where; P_r - pressure developed by the screw thread, A_p - pressing area.

Substituting π = 3.142, D_m = 110 mm, n = 8, U_n = 35 mm hence, A_p = 96 774 mm^2 and $P_r = 3.1\times10^{-3}$ Nmm^{-2}. Therefore, a pressure of 0.0031 MPa would be available for pressing and squeezing oil from the digested fruits during operation.

Design for the Pressure of the Working Chamber

The pressure that can be withstood by the pressing chamber was determined by Equation (9) (Khurmi and Gupta, 2005).

$$P_b = \frac{2t\delta_a}{D_i} \qquad \text{Equation (9)}$$

where; P_b - pressure to be withstood by the chamber in Pa, t - thickness of the chamber in mm, δ_a - allowed stress - 0.27 yield stress in Nmm^{-2} and D_i - inside diameter of the chamber at the feed in end in mm. The yield stress of stainless steel is given as 241 MPa by Khurmi and Gupta (2005). Substituting t = 3 mm, δ_a = 65. 07 MPa, D_i = 260 mm hence P_b = 1.5 Nmm^{-2} or 1.5 MPa. This means that the pressure the working chamber can withstand (1.5 MPa) is greater than the pressure developed by the screw press (0.0031 MPa). Therefore, the chamber will withstand the extraction pressure without bursting.

Design for the Capacity of the Screw Press

The theoretical capacity of the expeller was determined using Equation (10) (Onwuala *et al.*, 2006).

$$Q_e = \frac{\pi}{4}(D_S^2 - d_S^2)P\,N_S\varphi\ell \qquad \text{Equation (10)}$$

where; Q_e - theoretical capacity of the expeller in kgh^{-1}, D_s - diameter of the screw thread in m, d_s - base diameter of the screw shaft in m, P_s - screw pitch at the feeding end in m, N_s - rotational speed of the screw shaft in rpm, φ - filling factor and ℓ - bulk density of palm fruit in kgm^{-3}. Substituting $D_s = 130$ mm, $d_s = 20$ mm, $P_s = 90$ mm, $N_s = 120$ rpm, $\varphi = 0.8$ and $\ell = 913$ kg m^{-3} into Equation (10) hence, $Q_e = 102$ kgh^{-1}.

Testing the Machine Extraction Performance

Palm fruits for the testing of the machine were obtained from BAK Ecological Farm located in Buwama Mpigi district (N0.02898, E32.06071), which close to the equator with consistent weather variables over the years. The annual rainfall of the area is about 1264 mm and average temperature is between 16°C-29°C (Wortmann and Sones, 2017). The soils are mainly Vertisols and Gleyic Arenosols which are often acidic with moderate levels of organic matter (Wortmann and Sones, 2017). Harvesting of palm fruits at the farm is done throughout the year after observing that fruit bunches are ripe and ready for harvesting. For experimental purposes, sampling of fruits was done in the months of January, March and May in 2016. On each sampling, fruits were grab sampled five times and then mixed to form a single composite sample that was used for oil extraction. The fruits were digested to rupture the oil containing cells and ease the process of oil extraction. Digestion was achieved by boiling thoroughly cleaned fruits from a stainless-steel saucepan on a cook stove using charcoal as the fuel at temperature ranges of 130°C-150°C for 2 hr. The machine was started and 6 kg of the digested fruits were continuously fed into the machine through the hopper every minute. Thus, the feeding rate of the machine was 360 kg hr^{-1} which was greater than the batch feeding rate of 200 kg per extraction time which varied between 1 hr and 1.5 hr depending on the extraction efficiency of the workers. The screw press conveyed, squeezed and pressed the fed in fruits in order to extract the oil. The digested fruits fed in, the residual cake and the amount of oil extracted were collected and weighed separately and this was done in triplicates. The same parameters were taken in triplicates for the traditional extraction method that was being used in Buwama so that a quantitative performance analysis could be done with the two methods of oil extraction. The values obtained were used to calculate the oil yield (oil extraction ratio), oil extraction efficiency, machine discharge efficiency and oil extraction losses as by Equations (11), (12), (13) and (14) (Owalarafe *et al.*, 2007).

$$OER = \frac{M_{OE}}{M_{FF}} \times 100 \qquad \text{Equation (11)}$$

$$OEE = \frac{OER}{AEO} \times 100 \qquad \text{Equation (12)}$$

$$MDE = \frac{M_{OE} + M_{RC}}{M_{FF}} \times 100 \qquad \text{Equation (13)}$$

$$OEL = \frac{M_{FF} - (M_{OE} + M_{RC})}{M_{FF}} \times 100 \qquad \text{Equation (14)}$$

where; *OER* - oil extraction ratio (%), *OEE* - oil extraction efficiency (%), *MDE* - material discharge efficiency (%), *OEL* - oil extraction loss (%), M_{OE} - mass of oil extracted (kg), M_{FF} - mass of fruits fed into the machine (kg), MRC - mass of residue cake (kg), AEO - amount of oil expected (kg) which is 21% of M_{FF} according to Razali *et al.* (2012).

Physio-chemical Quality Analysis of the Extracted Oil Samples

Oil samples as extracted using the two methods (motorised and traditional methods) were taken to the Food Analysis Laboratory of the Department of Food Science, Technology and Bio-engineering of Makerere University. The physio-chemical parameters that were analysed included FFA, PV, IV and SV. All the parameters were analysed in triplicates for each sample as extracted by each method.

Determination of FFA

FFA concentration was determined by first mixing 25 ml of diethylether, 25 ml alcohol, 1 ml of phenolphthalein and neutralising this mixture with 0.1 M sodium hydroxide and then adding 2 g of the oil sample to form an alcoholic solution of the oil (Pearson, 1976). The alcoholic oil solution was then titrated with aqueous 0.1 M of sodium hydroxide using 1 ml of phenolphthalein as the indicator with constant shaking until a pink persistent colour was obtained. This procedure was done for both the oil samples in triplicates and FFA was determined using Equation (15).

$$FFA = \frac{titration\ (ml) \times 5.61}{wt} \qquad \text{Equation (15)}$$

where; *FFA* - free fatty acids (% of oleic acid), *wt* - weight of the oil sample (g).

Determination of PV

The PV was determined by weighing accurately 3 g of the oil sample into a dry 250 ml conical flask

and then adding 10 ml of chloroform, followed by 15 ml of glacial acetic acid and 1 ml of aqueous potassium iodide solution (Pearson, 1976). The flask was shaken for 1 min and after 75 ml of water was added and the mixture (V ml) titrated with 0.01 M sodium thiosulphate solution using soluble starch as the indicator. A reagent blank (V_o ml) oil sample determination was also carried out using the same procedure. The PV was determined using Equation (16).

$$PV = \frac{(V - V_o) \times 1000}{wt} \qquad \text{Equation (16)}$$

where; *PV* - peroxide value (meq kg^{-1}), *V* - sample titer (ml), V_o - blank titer (ml), *T* - molarity of sodium thiosulphate, *wt* - weight of the oil sample (g).

Determination of IV

The IV was determined by weighing 2 g of the oil sample into a 250 ml conical flask and then adding 10 ml of carbon tetrachloride followed by 20 ml of Wijs' solution and then allowing the mixture to stand in the dark for 30 min (Pearson, 1976). To the solution, 15 ml of potassium iodide solution and 100 ml of water were added and the mixture titrated with 0.1 M thiosulphate solution using starch as the indicator just before the end point (a ml). A blank test of the oil sample was also carried out using the same procedure (b ml). The IV was determined from Equation (17).

$$IV = \frac{(b - a) \times 1.269}{wt} \qquad \text{Equation (17)}$$

where *IV* - iodine value, *b* - blank titer (ml), *a* - sample titer (ml), *wt* - weight of the oil sample (g).

Determination of SV

The SV was determined by weighing 2 g of the oil sample into a conical flask and adding 25 ml of alcoholic potassium hydroxide solution. The flask was then heated in boiling water for 1 hr with frequent shaking (Pearson, 1976). To the hot solution, 1 ml of phenolphthalein solution was added and then titrated the hot excess alkali with 0.5 M hydrochloric acid (a ml). A blank titration was also carried out at the same time (b ml). The SV was determined from Equation (18).

$$SV = \frac{(b - a) \times 28.05}{wt} \qquad \text{Equation (18)}$$

where; *SV* - saponification value, *a* - sample titer (ml), *b* - blank titer (ml), *wt* - weight of the oil sample (g).

Statistical Analysis

Both the performance and oil quality parameters for each extraction method were determined in triplicates and the results were analysed with SPSS 16.0 to compare means with the independent samples T-test.

RESULTS AND DISCUSSION

Production of the Machine Parts

The stand was constructed of stainless steel angle lines of 40 mm x 40 mm with a thickness of 3 mm. Four pieces of 1122 mm were cut and bent at an angle of 30° at a distance of 142 mm from the base to increase on the stability. These were welded together to form the stand. From the bent end, two pieces of angle lines of 459 mm were welded to form the motor seat. The hopper was made from a stainless steel sheet of 3 mm thickness and 300 mm x 200 mm was bent to form the round hopper. A stainless-steel pipe of 35 mm diameter was welded on the machine to act as the oil outlet (*Figure 4*). The screw press was made by welding stainless round bars of 6 mm thickness onto the shaft, these were made at a depth of 220 mm, 200 mm, 180 mm, 160 mm, 140 mm, 120 mm, 100 mm

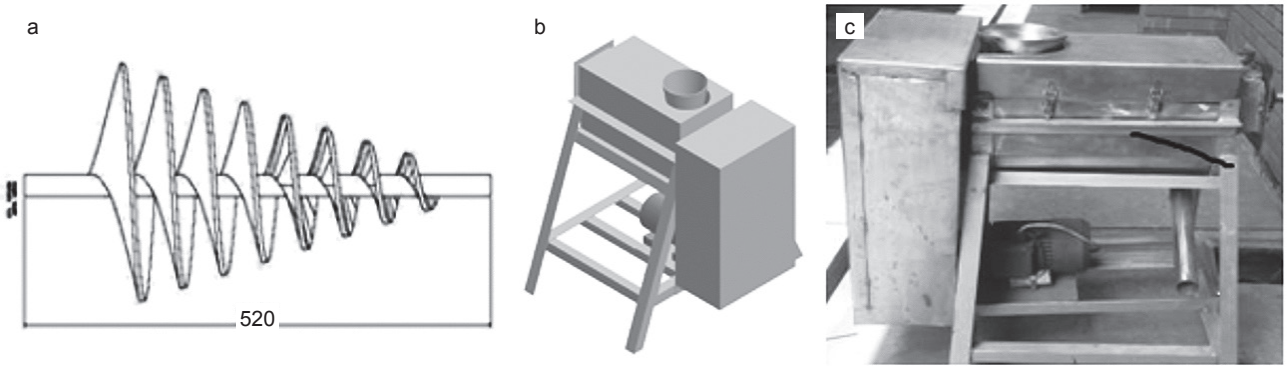


Figure 4. Machine components: (a) screw press, (b) computer aided designed machine and (c) fabricated machine.

and 80 mm respectively to form the tapering screw thread with the feed in pitch at 90 mm and decreasing by 10 mm up to the discharge end. The pressing chamber was made from a stainless steel sieve of 3 mm thickness with uniform perforations of 2 mm. This was bent using a bending machine to form a frustum of length 440 mm, upper diameter (feeding end) of 260 mm and lower diameter (at discharge end) of 100 mm. Production process included; marking out, cutting, drilling, machining, joining, fitting and fabrication. All the material components were made from stainless steel and welding done by stainless welding rods. The total production cost of the machine was USD 1142. The production drawings, part and materials list of the machine components are shown in *Figures 5* and *6*.

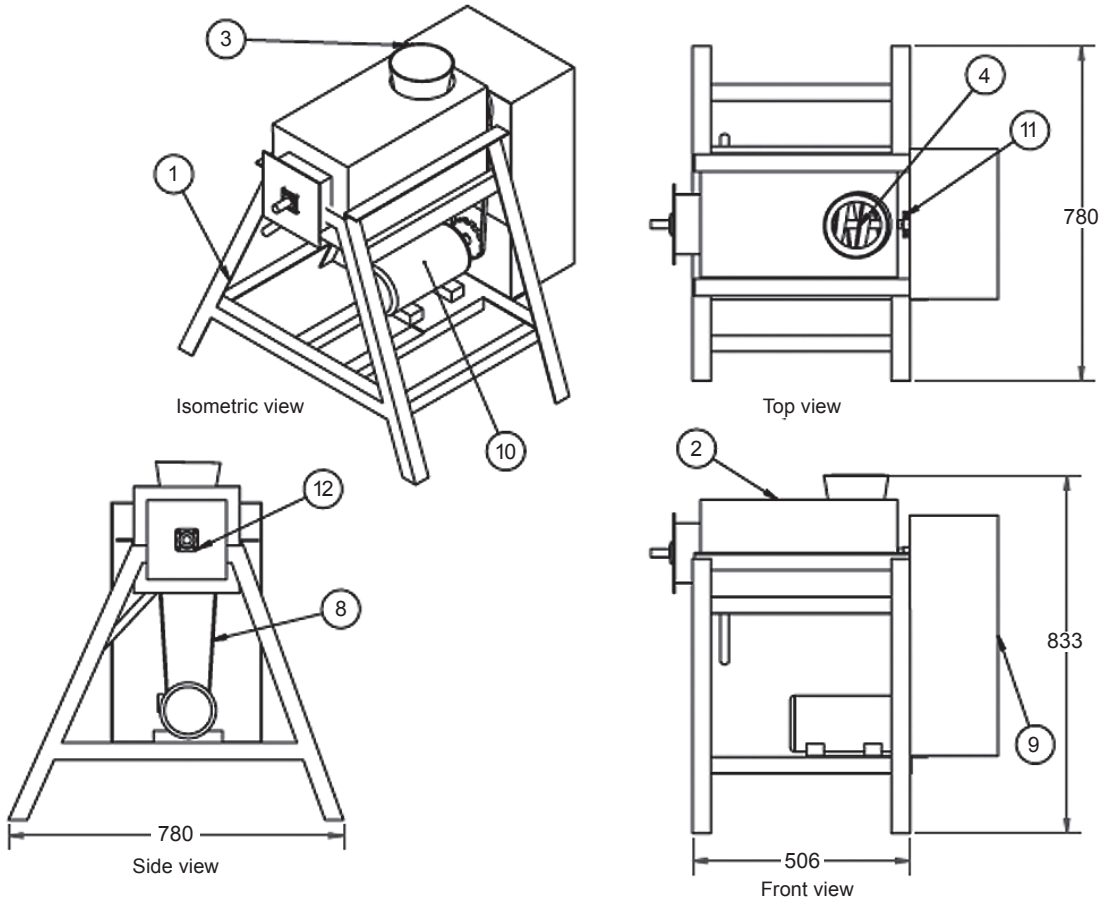
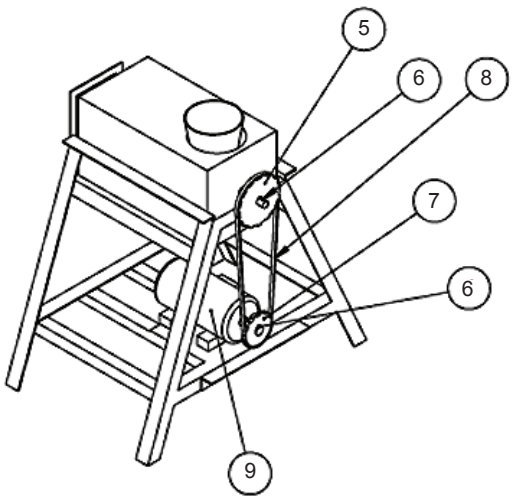


Figure 5. Production drawing of machine components (isometric and orthographic views).



Item No.	Part number	Description	Quantity
1	Frame	Stainless steel 304, 40x40x3 mm	1
2	Cover	Stainless steel 304, 3 mm thick sheet	1
3	Oil extraction cylinder	Stainless steel 304, sieve hole dia 2 mm, thickness - 3 mm	1
4	Screw shaft	20 mm dia shaft	1
5	Larger sprocket	Cast iron (90 teeth)	1
6	Key	Cast iron, 5x5 mm	2
7	Small sprocket	Cast iron (12 teeth)	1
8	Chain	Plain carbon steel, length 2280 mm	1
9	Motor	1 HP (3 phase, geared)	1
10	Bearing	Pillow block	1
11	Bearing2	4 bolt square flange mounted ball bearing	1

Figure 6. Part of machine components with part and materials list.

Machine Performance

Results of machine performance of the two extraction methods are shown in *Table 1*.

TABLE 1. PERFORMANCE EVALUATION OF THE EXTRACTION METHOD

Parameter (%)	Semi-mechanised motorised method	Traditional method
OER	16.20a ± 0.20	13.53b ± 0.46
OEE	77.13a ± 0.95	64.44b ± 2.20
MDE	81.70a ± 0.36	73.13a ± 2.02
OEL	18.30a ± 0.36	24.76b ± 1.04

Note: Each value is expressed as mean ± standard deviation (n=3). Means with different letters within rows are significantly ($p \leq 0.05$) different.
OER - oil extraction ratio, OEE - oil extraction efficiency, MDE - material discharge efficiency, OEL - oil extraction loss.

There were significant differences ($p \leq 0.05$) between OER, OEE and OEL of the two methods of extraction while MDE of the two extraction methods did not show a significant difference. The motorised extraction method using the constructed palm oil extractor had the greater mean values of OER, OEE and MDE of 16.02%, 77.13% and 81.70%, respectively (*Table 1*). This was due to the improvement in the design of the machine as compared to the traditional extraction machine that is in existence and this led to the improvement and increase in these efficiencies which was the objective of the design. The mean OEL was greater for the traditional extraction method (24.76%). This was due to the mode of extraction that involved four people operating the vertical screw press that largely depended on their working modes and individual energies that cannot be mechanically controlled as it was the case with the designed motorised extractor whose design parameters were predetermined.

Physio-chemical Quality Parameters

The physio-chemical quality parameters of the two extraction methods are presented in *Table 2*.

Results for FFA of the oil samples show a significant ($p \leq 0.05$) difference between the means, with the traditional extraction method having a higher value (6.86%) than the semi-mechanised motorised extraction method. The IV and SV values for the two methods did not show a significant ($p > 0.05$) difference between the means, while in both oil samples of the extraction methods, the PV was not detected.

The FFA value of 2.47% for the semi-mechanised method is below the maximum limit of 5% for virgin extracted palm oil (Alimentarius, 1994) which indicates good quality of this oil sample while the FFA value of 6.86% for the traditional method is above the maximum limit of 5% which indicates poor quality of this oil sample. The FFA results of this study are comparable to those of Ngando *et al.* (2011) who reported % FFA of 6.39% for traditional method and 5%-10.36% for semi-mechanised method. However, the results of this study are below those reported by Amata and Ozuor (2013) of 15.97% for the traditional method and 13.70% for the semi-mechanised method while the value of 6.86% for the traditional method is greater than that reported by Onwuka and Akaerue (2006) of 2.75% by traditional extraction method. The 2.47% FFA for this study is below that reported by Ohimain *et al.* (2013) of 8.43% for semi-mechanised processor in Bayelsa state Nigeria. FFA concentration is the most widely used criterion for determining the quality of palm oil and must not exceed 5%, presence of FFA in palm oil is an indicator of impairment of the quality of the oil (Amata and Ozuor, 2013). Since the oil extracted by both methods in this study was from the same variety (*Tenera*) and subjected to the same conditions before laboratory analysis (same shelf-life of 48 hr after extraction), it indicates that the differences in FFA values were brought about by the method of extraction.

The IV of 36.65 and 43.62 for semi-mechanised and traditional extraction methods respectively were both below the maximum limit of 55 (Alimentarius, 1994). This implies that in both oil samples, there were lower levels of deterioration, unsaturation as well as susceptibility to oxidative rancidity. The values for this study are comparable to those of Akubor and Ogu (2012) of 30.4-42.8 and those of Onwuka and Akaerue (2006) of 38.4-42.8 but below those reported by Agbaire (2012) of 55.55-53.66 and that of Ohimain *et al.* (2013) of 51.17. Much as there was no significant ($p > 0.05$) difference in the IV of the oil samples, semi-mechanised method had a lower value implying that its oil was of a higher quality as compared to that of the traditional method.

TABLE 2. PHYSIO-CHEMICAL QUALITY PARAMETERS OF OIL EXTRACTED BY THE TWO DIFFERENT METHODS

Parameter	Semi-mechanised motorised method	Traditional method
FFA (% of oleic acid)	2.47a ± 0.35	6.86b ± 0.68
IV	36.65a ± 0.30	43.62a ± 4.52
SV (mg KOH g^{-1})	193.74a ± 9.39	182.39a ± 8.32
PV (Meq O_2 kg^{-1})	ND	ND

Note: Each value is expressed as mean ± standard deviation (n=3). Means with different letters within rows are significantly ($p \leq 0.05$) different. ND - not detected.
FFA - free fatty acids, IV - iodine value, SV - saponification values, PV - peroxide value.

The SV of 193.74 and 182.39 for semi-mechanised and traditional methods respectively were both below the maximum limit of 209 given by Alimentarius (1994). The SV is an indicator of the molecular weights of the triglycerides and it is inversely proportional to the length of fatty acids (Muhammad *et al*., 2011). This can be justified by the results of this study where the traditional method had a lower SV (182.39) but a higher value of FFA (6.86%) than the semi-mechanised method. The SV of 193.74 for the semi-mechanised method is comparable to that of Ohimain *et al.* (2013) of 192.05 and that of Akubor and Ogu (2012) of 191-235 while the SV for the traditional method of 182.39 is comparable to that of Onwuka and Akaerue (2006) of 107-251. However, the results of this study are below those reported by Agbaire (2012) of 195.76-198.75.

For both the oil samples extracted by the two methods, the PV was not detected implying that no spoilage had taken place in these oil samples in terms of oxidation under storage and rancidity occurring under mild conditions (Agbaire, 2012). PV assesses the quality of cooking oils through the measurements of the amount of lipid peroxides and hydro-peroxides formed during initial stages of oxidative degradation (Ngando *et al*., 2013). The method of extraction therefore did not have an effect on the PV of the final oil product.

CONCLUSION

Results from this study indicated that the semi-mechanised method of oil extraction had greater efficiencies in terms of oil yield, oil extraction efficiency and material discharge efficiency in comparison to the traditional extraction method. In terms of the physio-chemical parameters, oil extracted by the semi-mechanised motorised method had better quality parameters with its FFA, IV and SV all being below the maximum limits given by Alimentarius (1994). The FFA value for the traditionally extracted oil was above the maximum limit which indicated a low quality in this oil samples while the IV and SV were below the maximum limits though greater than those for the semi-mechanised method which justifies that the method of oil extraction greatly affects the quality of the oil produced. The production cost of the machine (USD 1142) is affordable to smallholder palm oil farmers and hence, adopting this technology makes them competitive on the market due to the high quantity and quality of the produced oil.

REFERENCES

Agbaire, P O (2012). Quality assessment of palm oil sold in some major markets in Delta State, Southern Nigeria. *African J. Food Science and Technology, 3(9)*: 223-226.

Akubor, P and Ogu, G I (2012). Quality attributes of fresh palm oil produced from selected communities around Anyigba, Kogi State, Nigeria. *Nigerian J. Nutritional Sciences, 33(1)*: 12-17.

Alimentarius, C (1994). Joint FAO/WHO Food Standards Programme. Codex Committee on Methods of Analysis and Sampling: 19th Session, Budapest, Hungary. Criteria for Evaluating Acceptable Methods for Evaluating Acceptable Methods for Codex Purposes. http://www.fao.org/tempref/codex/Meetings/CCEXEC/CCEXEC62/ex62_05e.pdf, accessed on 22 November 2016.

Amata, I A and Ozuor, E (2013). The effect of different processing methods on the quality of crude palm oil (CPO) in Delta North Agricultural zone of Delta State, Nigeria. *J. Environmental Issues and Agriculture in Developing Countries, 5(1)*: 19-24.

Anebi, J G; Umogbai, V J and Bako, T (2014). Design and construction of a soyabean oil extracting machine. *Asian J. Science and Technology, 5(10)*: 606-611.

Aremu, A K and Ogunlade, C A (2013). Development and performance evaluation of an extractor for removing oil from soaked kenaf bast and core. *Int. J. Advanced Research Vol. 1(4)*: 196-201.

Azodo, P A; Hasan A B; Ezenwa, J and Oghan, P U (2013). Design and fabrication of motorized hydraulically operated palm oil press. *The Pacific J. Science and Technology, 14(1)*: 79-88.

Hall, A S; Holowenko, A R and Laughlin, H G (1961). *Theory and Problems of Machine Design*. New York: McGraw-Hill. 344 pp.

IFAD (2010). *Vegetable Oil Development Project. Interim Evaluation, Report No. 2195-UG*. http://www.ifad.org › documents › vodp.pdf, accessed on 19 January 2016. p. 193-209.

Khurmi, R S and Gupta, J K (2005). *A Text Book of Machine Design*. Eurasia Publishing house (PVT) Ltd., Russia. p. 576-586.

Kukeera, T; Banadda, N; Tumutegyereize, P; Kiggundu, N and Asuman, N R (2015). Extraction, quantification and characterization of oil from pumpkin seeds. *Int. J. Agricultural and Biological Engineering, 8(1)*: 98-102.

Mahmud, A; Rehrig, M and Hills, G (2010). Improving the livelihoods of palm oil smallholders:

The role of the private sector. FSG Social Impact Advisors for the World Bankgroup, Washington, DC. http://www.fsg.org/publications/improving-livelihoods-palm-oil-smallholders, accessed on 17 March 2016.

Moses, D M (2014). Performance evaluation of continuous screw press for extraction of soybean oil. *Amer. J. Science and Technology, 1(5)*: 238-242.

Mrema, G C and McNulty, P B (1985). Mathematical model of mechanical oil expression from oil seeds. *J. Agricultural Engineering Research, 31(5)*: 361-370.

Muhammad, N; Bamishaiye, E; Bamishaiye, O; Usruan, L; Salawu, M; Nafiu, N and Olayede, O (2011). Physiochemical properties and fatty acid composition of *Cyperus escalentus* (Tiger Nut). Tuber oil. *Biores Bull, 5*: 51-54.

Ngando, E G F; Mpondo, M E A and Ewane, M A (2013). Some quality parameters of crude palm oil from major markets of Douala, Cameroon. *African J. Food Science, 7(12)*: 473-478.

Ngando, E G F; Mpondo, E A; Dikotto, E E L and Koona, P (2011). Assessment of the quality of crude palm oil from smallholders in Cameroon. *J. Stored Products and Postharvest Research, 2(3)*: 52-58.

Nsiah, S A; Zu, A C S and Nimoh, F (2012). A technological and financial assessment of small scale palm oil production in Kwaebibrem District, Ghana. *J. Agricultural Science, 4(7)*: 111-120.

Ohimain, E I; Izah, S C and Fawari, A D (2013). Quality assessment of crude palm oil produced by semi-mechanized processors in Bayelsa State, Nigeria. *Discourse J. Agriculture and Food Sciences, 1(11)*: 171-181.

Okogeri, O and Otika, N F (2014). Quality parameters of crude palm oil obtained by traditional processing techniques. Department of Food Science and Technology. Ebonyi State University. http://www.cibindia.in/16052/processing-of-crude-palm-oil, accessed on 24 January 2016.

Olaniyan, A M; Yusuf, K A; Wahab, A L and Afolayan, K O (2012). Design, development and testing of a screw press expeller for palm kernel and soybean oil extraction. Post-harvest, Food and Process Engineering. *Int. Conference of Agricultural Engineering-CIGR-AgEng 2012*. Valencia, Spain. p. 1748.

Onwuala, A P; Akubuo C O and Ahaneku, I E (2006). *Fundamentals of Engineering in Agriculture*. Lagos: Immaculate Publications Ltd. p. 259-262.

Onwuka, G I and Akaerue, B I (2006). Evaluation of the quality of palm oil produced by different methods of processing. *Research J. Biological Sciences, 1(1-4)*: 16-19.

Osei-Amponsah, C; Visser, L; Adjei-Nsiah, S; Struik, P C; Sakyi-Dawson, O and Stomph, T J (2012). Processing practices of small-scale palm oil producers in the Kwaebibirem District, Ghana: A diagnostic study. *NJAS-Wageningen J. Life Sciences, 60*: 49-56.

Owalarafe, O K; Osunleke, A S and Oyebamiji, B E (2007). Effect of hydraulic press parameters on crude palm oil yield. *Int. Agrophysics, 21*: 285-291.

Pearson, D (1976). *The Chemical Analysis of Foods*. 7th edition. Longman Group Ltd. p. 626-641.

Poku, I (2002). Small scale palm oil processing in Africa. *FAO Agricultural Services Bulletin 148*. Food and Agricultural Organisation of the United Nations. Rome. 56 pp.

Razali, M H; Ssomad, A M; Halim, M A and Roslan, S (2012). A review on crop plant production and ripeness forecasting. *Int. J. Agriculture and Crop Sciences, 4(2)*: 54-63.

Shigley, J E and Mischeke, C R (2001). Ch. 18. *Mechanical Engineering Design*. 6th Edition. McGraw-Hill Higher Education. New York. p. 933-951.

Tarmizi, A H A; Razali, I and Kuntom, A (2016). Effect of frying on the palm oil quality attributes - A review. *J. Oil Palm Res. Vol. 28(2)*: 143-153.

WorldGrowth (2011). The economic benefit of palm oil to Indonesia (February). http://www.scirp.org, accessed on 13 February 2016.

Wortmann, C S and Sones, K R (2017). *Fertilizer Use Optimization in Sub-Saharan Africa*. CABI. p. 193-209.

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EFFECT OF CATALYSTS ON THE YIELD AND PROPERTIES OF LIGNIN FROM MICROWAVE-ASSISTED ACETOSOLV EXTRACTION OF OIL PALM EMPTY FRUIT BUNCH FIBRES

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ABSTRACT

Acetosolv is an enhancement organosolv technique utilising acetic acid as solvent and produces high purity of lignin. However, the limitation of this technique in the conventional heating method is the high energy consumption during a long reaction time. Therefore, the employment of microwave is used to overcome this limitation with the expectation of a lower power consumption and short reaction time. In this study, three types of catalysts, sulphuric acid (H_2SO_4), aluminium choride ($AlCl_3$), and chromium nitrate [$Cr(NO_3)_3$] were used to investigate the lignin extraction performance and its properties from microwave-assisted (MWA) acetosolv treatment of oil palm empty fruit bunches (EFB). The highest yields of lignin (76.98%) were obtained using an aqueous solution of acetic acid combined with 3.0% of H_2SO_4 under 110°C for 30 min. Meanwhile, $AlCl_3$ performed almost similar to H_2SO_4, providing lignin yield of 71.57% at the highest temperature of 110°C. The usage of microwave-assisted technique also produces a high yield and high purity of lignin. It is also proven that $AlCl_3$ can be a substitute to Bronsted acid for lignin extraction. However, $Cr(NO_3)_3$ was found not suitable for the lignin's extraction despite having a high potential for cellulose extraction.

Keywords: lignocellulose, microwave-assisted, extraction lignin.

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INTRODUCTION

In recent years, concerns over the continuous depletion of fossil resources as well as an increment in ecological awareness have drawn a remarkable interest to reduce the dependence on fossil resources as raw materials. Countless research have been conducted to overcome these issues and lignocellulosic biomass has been considered as the best candidate to replace or provide alternative to petroleum-based materials (Amran *et al.*, 2017). This reason is not only for its sustainability but the content of cellulose, lignin and hemicellulose in the biomass provide various functionalities and structures that can be tailored into desired applications. By utilising lignocellulosic biomass, the issues related to petroleum depletion can be prevented as this biomass can be obtained in large quantity at low economical and energy cost (Chen and Lee, 2018). Furthermore, this waste can also be converted into a high-value product, simultaneously solving the waste management problem (Roslan *et al.*, 2014).

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In general, the oil palm empty fruit bunches (EFB) is an organic substrate derived from harvested or processed oil palm fruit bunches (Kheong *et al.*, 2010). These organic substrate is also difficult to degrade biologically (David *et al.*, 2019). In Malaysia, EFB are the colossal solid waste from the oil palm industry and regarded as the most important lignocellulosic biomass for production of chemical and fuels (Coral *et al.*, 2018). The oil palm planted area in 2019 reached 5.90 million hectares, and the production of crude palm oil in the same year was 19.86 million tonnes (MPOB, 2020). The amount of unutilised EFB sent back to landfill is around 40% (Reeb *et al.*, 2014). There were several reports regarding to the utilisation of the EFB as raw materials for the generation of high value-added products, such as production of cellulose (Aditiawati *et al.*, 2019; Owi *et al.*, 2016), adhesives (Rohimi *et al.*, 2020), activated carbon (Choi *et al.*, 2018; Zaini and Ali, 2018) and composites (Amir *et al.*, 2018). In this study, the effective valorisation of lignin will be the focus on the pursuit of more sustainable and competitive biorefineries.

Basically, oil palm EFB fibre consists about 42%-63% cellulose, 21.9%-33% hemicellulose and 10%-36.6% lignin (Omar *et al.*, 2011). Lignin is the second most abundant terrestrial organic polymer on earth after cellulose (Fan *et al.*, 2015). Basically, it consists of three types of monolignols, which are p-coumaryl, coniferyl, and synapyl alcohol. It generally has an irregular structure with a highly condensed cross-linked polymer network (Constant *et al.*, 2015). The composition and properties of lignin are influenced by solvent, extraction technique, catalyst, and the nature of the raw material. However, it is regrettable to notice that only a small portion is currently used for value-added product (Sameni *et al.*, 2017).

As a by-product of the pulp and paper industry, lignin is typically extracted by utilising acid or alkali medium to increase the rate of delignification. The raw material is usually heated at elevated temperatures under high pressure by using high-pressure reactors or autoclaves. This method is not favourable since it consumes high energy due to the long reaction time, resulting from conductive heating for several hours (Avelino *et al.*, 2018). Therefore, in this study, microwave-assisted (MWA) extraction as the heating method will be introduced to promote the delignification process. Compared to the traditional oven or oil bath convection heating, microwave irradiation utilises the dipole rotation and ionic conduction to heat, and the heating process will occur directly to the targeted sample (Fatriasari *et al.*, 2017; Kappe, 2004; Priecel and Lopez-Sanchez, 2018). This technique can be used as a sustainable methodology for lignin extraction since it operates on low energy consumption due to reduced reaction time and simultaneously increases the efficiency and quality of the product.

In MWA technique, polar compounds must be used in order to absorb the radiation. Therefore, acetosolv technique will be employed as the method of lignin extraction. In addition, acetosolv technique was also chosen as it produces high purity of lignin compared to sulphite and Kraft processes. Sulphite and Kraft will limit the lignin suitability as a resource for future processing and disturb any application that requires 'clean' lignin (Sammons *et al.*, 2013). In this technique, acetic acid was used as a solvent to collapse the plant tissue and promote the lignin degradation from lignocellulosic biomass with the assistance of temperature and acid catalyst (Hernández-Hernández *et al.*, 2016). It can be used to delignify biomass and to remove most of the hemicellulose. Moreover, the lignin extracted from this process also shows excellent properties such as low molecular weight and solubility in organic solvents (Schwiderski *et al.*, 2014). Commonly, aqueous solutions of acetic acid and ethanol combined with low concentrations of Bronsted acids as a catalyst are used to separate lignin and carbohydrates. In general, Bronsted acid is the proton donor substance that donates proton in acid-base reactions such as H_2SO_4 and HCl. However, the concentrated H_2SO_4 has a drawback, of which expensive materials are needed to fabricate the reaction vessel for anti-corrosion purposes. Therefore, the application of Lewis acid; molecules with incomplete octet of electrons that can accept electrons, in the extraction of lignin as substitute to Bronsted acids will be the main objective in this study (Abdullah *et al.*, 2012).

Therefore, this study is aimed to evaluate the physico-chemical properties of acetosolv lignin (AL) extracted from EFB fibres via MWA acetosolv technique using different Lewis acid catalyst [$AlCl_3$ and $Cr(NO_3)_3$] and compared with commonly used Bronsted acids (H_2SO_4). The reaction time was fixed at 30 min to avoid incomplete reaction from occurring below that reaction time.

MATERIALS AND METHOD

Materials

EFB were provided by Szetech Engineering Sdn Bhd. The following chemicals were used in the study: sulphuric acid (H_2SO_4, 95%-98%, R&M Chemicals), aluminium chloride ($AlCl_3$, 98%, Sigma-Aldrich), chromium nitrate $Cr(NO_3)_3$, 98%, Sigma-Aldrich and glacial acetic acid.

MWA Acetosolv Reaction for Lignin Extraction

The MWA acetosolv was conducted in a 1L reaction flask equipped with a reflux system. Several temperatures were studied during the MWA

which are 90°C, 100°C and 110°C at a fixed 30 min reaction time in the presence of 3% catalyst; H_2SO_4, $AlCl_3$ and $Cr(NO_3)_3$. In these experiments, the EFB to solvent ratio was fixed at 1:10, and the reaction took place in a four-neck flat bottom reaction flask containing the acetosolv solution [acetic acid: water solution (9:1, v:v)] with the presence of catalysts. After the reaction completed, black liquor was obtained and filtered for separation of residue before being precipitated using deionised water. Finally, the lignin was vacuum filtered, washed with deionised water several times until reaching neutral and dried in an oven for 12 hr.

Lignin Extraction Yield and Purity

Lignin yield was calculated according to Equation (1):

$$ń = \frac{mMWAL}{mLig} \quad \text{Equation (1)}$$

where ń is the lignin yield (%); mMWAL is the mass of lignin extracted in the MWAL process (g) while mLig is the mass of lignin gained from overall biomass (g) as determined by TAPPI standards (Hames *et al.*, 2008). The purity of lignin was carried out using the National Renewable Energy Laboratory (NREL) standard biomass analytical procedure with three replication.

Characterisation of Lignin

Scanning electron microscope (SEM). The surface morphology of the raw EFB and residues, the samples were placed on specimen stub, followed by binding with double-sided carbon tape before being observed using FEI Quanta450 SEM with 1000X image magnification.

Fourier transform infrared spectroscopy (FT-IR). FT-IR was performed in a Perkin Elmer Spectrum One under KBr disc technique. Spectra were acquired between 4000 and 500 cm^{-1} with a resolution of 4 cm^{-1}. Proton nuclear magnetic resonance (^1H NMR) was used to support the structural analysis, which was recorded on 500 MHz, Bruker NMR spectrometry. The solvent used was deuterated dimethylsulfoxide, DMSO-d6 and the solvent chemical shift was set and calibrated at 2.5 ppm.

Thermogravimetric (TGA) analysis. TGA analysis was performed in a TGA Q500 from the TA instrument. The 15 mg of the sample was heated from 30°C-700°C under a nitrogen atmosphere of 40 ml min^{-1} at a scanning rate of 10°C min^{-1}.

RESULT AND DISCUSSION

Effect of Reaction Temperature and Catalyst on Yield of Crude and Purified Lignin Gained via MWA Acetosolv Treatment

Initially, two parameters were evaluated in this study; the reaction temperature and the catalyst [H_2SO_4, $AlCl_3$ and $Cr(NO_3)_3$], as shown in *Figure 1*. In acetosolv technique, acetic acid (CH_3COOH) was used to produce H_3O^+ ions, which are responsible for the protonation of ether groups present in lignin; hence, affecting the yield of lignin. However, the ability of these CH_3COOH to produce the H_3O^+ ions is low, and the presence of catalysts is vital for improving this behaviour. From *Figure 1*, the employment of $Cr(NO_3)_3$ was not able to extract any lignin. Meanwhile, $AlCl_3$ gave quite the same lignin yield as H_2SO_4 at the highest temperature (100°C) used in this study. It is proven that the yield of lignin is affected by the nature of catalysts used. From a previous study (Avelino *et al.*, 2018; Schwiderski *et al.*, 2014), $AlCl_3$ showed quite a similar yield to Bronsted acids as it has similar acidic power despite the effect of higher cation hardness compared to other Lewis acids. This acidic power leads to more efficient coordination with the oxygen atoms present in the ether groups and making them more susceptible to hydrolysis. Therefore, these results support the fact that the acidic power also has a significant influence on yield, but not so much in the lignin purity, as shown in *Figure 1*. For $Cr(NO_3)_3$, the only possible reason that can be explained to the absence of lignin is that Cr is adsorbed on the lignin fragment since lignin has a higher affinity for Cr(III) before the precipitation process being done. This high affinity eventually leads to the formation of water repellent lignin (Garcia-Reyes and Rangel-Mendez, 2009; Pandey *et al.*, 1998; Wu *et al.*, 2008).

The lignin yield based on reaction temperature was increased with the increased temperature. From *Figure 1*, it is shown that as temperature increased from 90°C-110°C, the lignin yield obtained had also increases from 43.07%-76.98%, with the highest purity of 94.15% for H_2SO_4. As for $AlCl_3$, the yield increased from 28.81%-71.57%, with an increment of purity from 83.39%-92.89%. Basically, temperature plays a vital role in lignin extraction as it enhances the degradation of cell walls while the lignin and hemicellulose were decomposed and dissolved in the organic solvent (Amran *et al.*, 2017; 2019). The higher yield obtained was also due to the extensive lignin depolymerisation which led to higher lignin solubilisation in the organic solvent, especially with the presence of microwave irradiation (Monteil-Rivera *et al.*, 2012). Theoretically, microwave irradiation provides the homogeneity of temperature in the reactor compared with conventional heating and, consequently, facilitated the cleavage of the

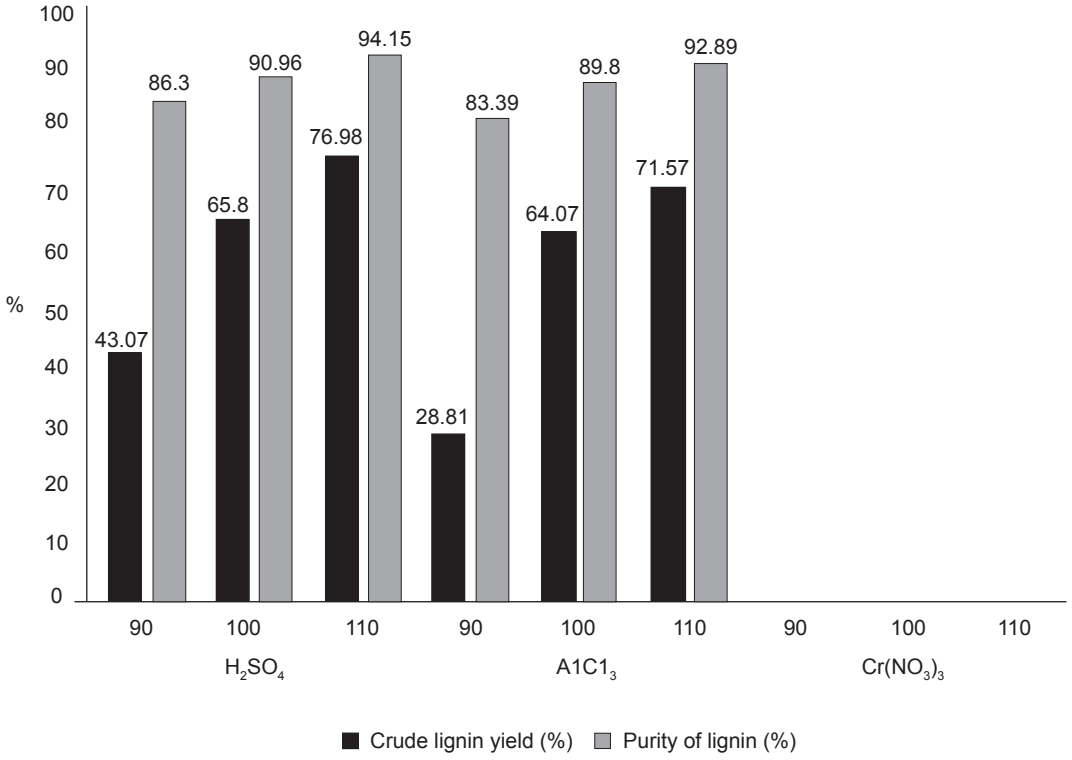

Figure 1. The percentage of crude lignin and purity of lignin by sulphuric acid (H_2SO_4), aluminium chloride ($AlCl_3$) and chromium (III) nitrate [$Cr(NO_3)_3$].

C–C bond which led to the higher yield of lignin extracted from EFB.

Morphological Observation of Oil Palm EFB Fibres Before and After MWA Acetosolv

The morphology of the EFB fibre and EFB residue are shown in *Figure 2*. In general, the SEM results showed that the EFB fibre's morphology is made up of distinct cell wall layers. For the EFB residues, the acetosolv technique has changed the morphology of EFB fibres due to the removal of lignin. At the lowest temperature (90°C), it can be seen that the morphology of the fibre undergoes a little change as the acetosolv technique had roughened the structure of cell wall surface of the fibre which disrupted the lignin's structure (Tajuddin *et al.*, 2019; Yaakob *et al.*, 2020). This generally occurred to all the catalysts used in this study. At medium temperature (100°C), the cell wall is continuously removed from the structure of the fibres for all the residues. At the highest temperature (110°C), all the residues have a different morphology based on the catalyst used. For the H_2SO_4 catalyst, it was clearly observed that the cell wall of the fibres was completely removed and exposed to the defibrated inner microfibrils, as shown in *Figure 2d*. Compared to H_2SO_4, *Figure 2g* showed the appearance of spherical droplets on the surface at the highest temperature (110°C) for $AlCl_3$ residues. The presence of this spherical droplet can be explained due to the derivation of dehydrated carbohydrates and resulting in the formation of lignin-like materials (Shen *et al.*, 2016). From *Figure 2j*, it is noticed that the separation of fibres bundles into individual fibres had taken place in $Cr(NO_3)_3$ residue. The formation of these individual fibres can be explained by the mechanism of the dissociation of Cr^{3+} ions into a coordination complex structure with water molecules (H_2O) at the initial hydrolysis stage. This finding also proves the successive hydrolysis treatment initiated by the $Cr(NO_3)_3$ in the dissolution of the less-ordered defective crystalline regions (Chen *et al.*, 2017).

Determination of Chemical Structural Using FT-IR

Basically, the structural properties of lignin are affected by the raw material origin, the environmental conditions, and the method to extract the lignin itself. *Figure 3* presented the FT-IR spectra that were used to investigate the functional group of the acetosolv lignin at various temperatures with different catalysts. In general, the spectra showed that there are no significant differences in terms of functional groups, and the spectra can be explained by dividing into two regions; the backbone region and the lignin-carbohydrates complexes regions.

In the backbone region, the functional groups related to lignin aromatic ring skeleton usually occur at the band around 1600 and 1500 cm^{-1} (Watkins *et al.*, 2015). In this study, these bands were observed at 1594 cm^{-1} and 1513 cm^{-1} by the presence of C=C stretching of the aromatic ring skeleton, 1463 cm^{-1} for the C-H deformations, and aromatic skeletal vibrations coupled with C-H in-plane deformation at 1425 cm^{-1}. As the lignin is not 100% pure, the presence of the lignin-carbohydrate complexes can be seen by the signals observed at 1714, 1269, 1165 and 1125 cm^{-1} which represent the existence of C=O stretching that is generally attributed to lignin-carbohydrate complexes (LCC) (Avelino *et al.*, 2018).

In general, there is no significant damage occurring on the lignin during the acetosolv process since the basic aromatic lignin is present as in *Figure 3*. The presence of syringyl unit can be seen at band 1329 and 1125 cm^{-1}, while at band 1513 and 1269 cm^{-1} showed the existence of guaiacyl units in the lignin and the band at 851 cm^{-1} was assigned to C-H out-of-plane in positions 2, 5 and 6 of guaiacyl units.

^{1}HNMR Analysis

Figure 4 presents the ^{1}HNMR spectra of MWA acetosolv lignin with the presence of a different acid catalyst at various temperatures. The sharp peak shown at the range of 2.33-2.51 ppm is the solvent used; DMSO-d6 to solubilise lignin for the sample preparation phase. The presence of aliphatic moiety is shown at the signals between 0.8 and 1.5 ppm (Li *et al.*, 2018). The methoxyl proton can be observed by the presence of a signal between 3.1 and 4.0 ppm. The complex signals at 3.0-4.5 also confirmed the presence of free polysaccharide moieties in the lignin (Abdelkafi *et al.*, 2011). At 3.20 ppm, it can be seen that the signal of -CH_2 linkages in the hydroxymethyl chain of the lignin was strong in all lignin except for HL100 and HL110. The possible explanation for this occurrence is due to the catalyst efficiency used at high temperature. Above 100°C, the hydrolysis reaction which occurred with the presence of H_2SO_4 are capable of breaking the lignin cellulose complex (LCC) bond effectively without leaving the CH_2

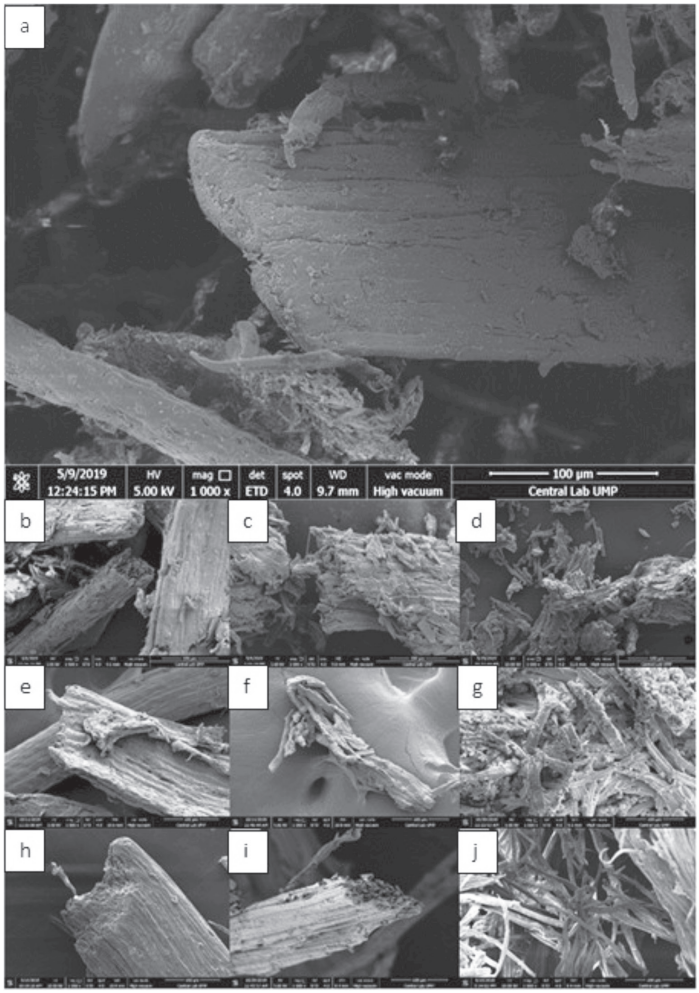

Figure 2. Scanning electron microscopy (SEM) image of a) native empty fruit bunches (EFB) and residues after microwave-assisted (MWA) acetosolv in the presence of b) at 90°C, c) H_2SO_4 at 100°C, d) H_2SO_4 at 110°C, e) $AlCl_3$ at 90°C, f) $AlCl_3$ at 100°C, g) $AlCl_3$ at 110°C, h) $Cr(NO_3)_3$ at 90°C, i) $Cr(NO_3)_3$ at 100°C and j) $Cr(NO_3)_3$ at 110°C.

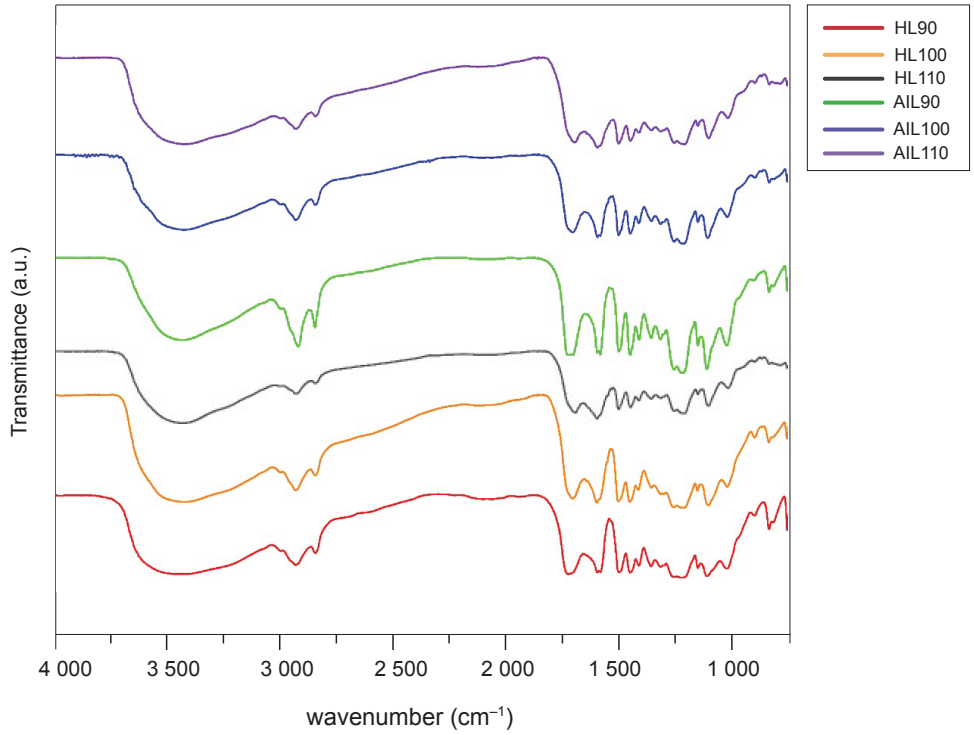

Figure 3. Fourier transform infrared (FT-IR) spectra for microwave-assisted (MWA) acetosolv lignin in the presence of H_2SO_4 at 90°C (HL90), 100°C (HL100) and 110°C (HL110) and $AlCl_3$ at 90°C (AIL90), 100°C (AIL100) and 110°C (AIL110).

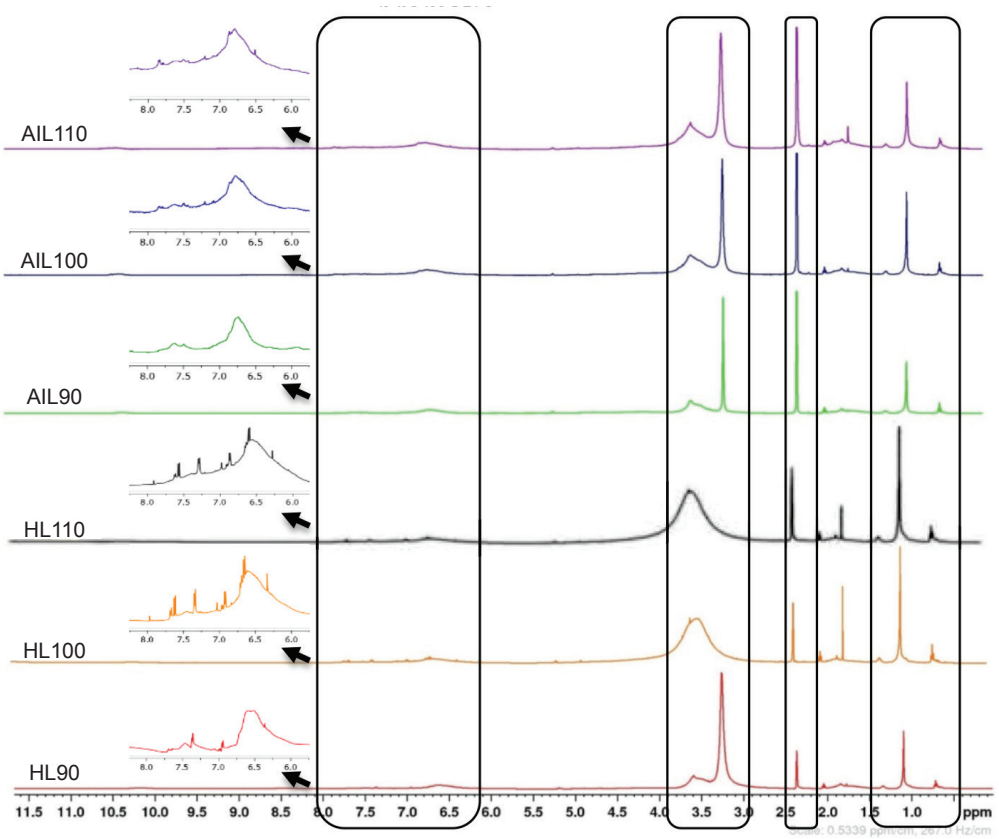

Figure 4. Proton nuclear magnetic resonance (^{1}HNMR) of microwave-assisted (MWA) acetosolv lignins.

linkage on the lignin structure. The β-O-4 structures in the lignin are shown between 5.9-6.6 ppm and the active proton at C5 position was also observed at signal around 6.96 ppm (Li *et al.*, 2018; Hussin *et al.*, 2013; Rashid *et al.*, 2018). The presence of signals between 6.0 and 8.9 ppm indicates the presence of aromatic protons in the lignin's unit (Rashid *et al.*, 2018).

Thermal Decomposition of Acetosolv Lignin

The decomposition of lignin in this work was investigated using TGA under nitrogen flow, and the percentage of lignin weight loss in relation to temperature was revealed by the TGA curves, as shown in *Figure 5*. From this result, it is shown that the decomposition stages of lignin can be divided into three stages from 30°C-800°C. The first stage started below 100°C, where the evaporation of water, carbon dioxide, and carbon monoxide occurred. The second decomposition stage referred to the remaining lignin-carbohydrate complex (LCC) and hemicellulose at the range of 200°C-350°C (Hashim *et al.*, 2016; Watkin *et al.*, 2015). The final stage occurred at a temperature above 400°C due to the cleavage of the monomeric unit bond lead to the degradation of volatile products such as phenolic and alcohol derived from lignin. Therefore, in general, the wide range of the lignin decomposition is a result of the various branching of the lignin molecular structure. In terms of catalyst effect on the thermal stability of the lignin, the TGA curve proved the ability of the catalyst in removing the bonding in LCC as thermal stability is directly proportional to the purity of the lignin. Therefore, in this study, it is observed that the thermal stability for all lignin has no significant difference as the purity of the lignin in all parameter is quite similar.

CONCLUSION

High purity lignin was successfully obtained from oil palm EFB via MWA acetosolv process with a high yield of crude lignin. It is shown that the yield of lignin obtained via utilisation of Bronsted acid (76.98%) is slightly higher compared to utilisation of Lewis acid (71.57%) and it is noted that chromium (III) nitrate is not suitable for extraction of lignin but has a high potential for production of cellulose. The extraction process with different acid catalysts produced lignin with similar structural and thermal properties. Moreover, the extraction of lignin can be done using a simple and time-saving method, as presented by the MWA acetosolv process.

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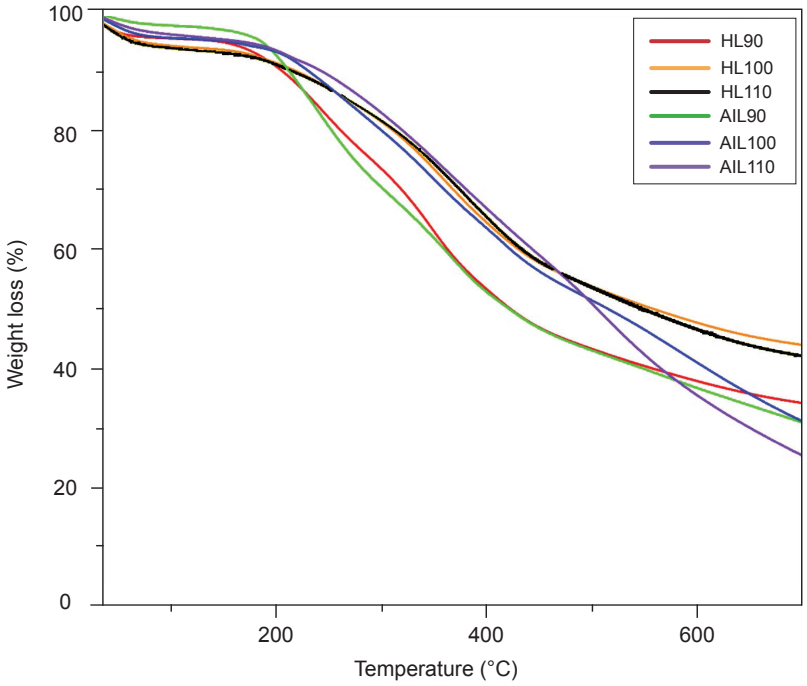

Figure 5. Thermogravimetric analysis (TGA) curves of lignins gained for different acid catalyst and at different temperatures.

REFERENCES

Abdullah, N; Mohd Ismail, H B; Abu Bakar, N H and Michael, L (2012). *College Matriculation Chemistry SK016*. SAP Education.

Abdelkafi, F; Ammar, H; Rousseau, B; Tessier, M; El Gharbi, R and Fradet, A (2011). Structural analysis of alfa grass (*Stipa tenacissima* l.) lignin obtained by acetic acid/formic acid delignification. *Biomacromolecules*, *12(11)*: 3895-3902.

Aditiawati, P; Dungani, R; Fikri; R M and Hartati, S (2019). Optimization of cellulose nanofiber production from oil palm empty fruit bunch using *Trichoderma* sp. with the solid state fermentation method. *Bioresources*, *14(2)*: 3688-3700.

Amir, S M M; Sultan, M T H; Jawaid, M; Ariffin, A H; Ishak, M R; Reussmazran Yusof, M; Mohd, S and Salleh, K A M (2018). Effect of gamma radiation on compressive properties of kevlar/oil palm empty fruit bunch hybrid composites. *Bioresources*, *13(4)*: 7628-7639.

Amran, U A; Zakaria, S; Chia, C H; Roslan, R; Jaafar, S N and Salleh, K M (2019). Polyols and rigid polyurethane foams derived from liquefied lignocellulosic and cellulosic biomass. *Cellulose*, *26(5)*: 3231-3246.

Amran, U A; Zakaria, S; Chia, C H; Fang, Z and Masli, M Z (2017). Production of liquefied oil palm empty fruit bunch based polyols via microwave heating. *Energy and Fuels*, *31(10)*: 10975-10982.

Avelino, F; Da Silva, K T; De Souza Filho, M D S M; Mazzetto, S E and Lomonaco, D (2018). Microwave-assisted organosolv extraction of coconut shell lignin by Bronsted and Lewis acids catalysts. *J. Cleaner Production, 189*: 785-796.

Chen, Y; Tan, T; Lee, H and Abd Hamid, S (2017). Easy fabrication of highly thermal-stable cellulose nanocrystals using $Cr(NO_3)_3$ catalytic hydrolysis system: A feasibility study from macro- to nano-dimensions. *Materials*, *10(1)*: 42.

Chen, Y W and Lee, H V (2018). Revalorization of selected municipal solid wastes as new precursors of 'green' nanocellulose via a novel one-pot isolation system: A source perspective. *Int. J. Biological Macromolecules*, *10*: 778-792.

Choi, M S; Park, S; Lee, H and Park, H S (2018). Hierarchically nanoporous carbons derived from empty fruit bunches for high performance supercapacitors. *Carbon Letters*, *25(1)*: 103-112.

Constant, S; Basset, C; Dumas, C; Di Renzo, F; Robitzer, M; Barakat, A and Quignard, F (2015). Reactive organosolv lignin extraction from wheat straw: Influence of Lewis acid catalysts on structural and chemical properties of lignins. *Industrial Crops and Products*, *65*: 180-189.

Coral Medina, J D; Woiciechowski, A L; Filho, A Z; Brar, S K; Magalhães Júnior, A I and Soccol, C R (2018). Energetic and economic analysis of ethanol, xylitol and lignin production using oil palm empty fruit bunches from a Brazilian factory. *J. Cleaner Production, 19*: 544–550.

David Castano, J; Camilo Crespo, C and Torres, E (2019). Evaluation of chemical and biological treatments to degrade oil palm empty fruit bunches (*Elaeis guineensis* Jacq.) and their potential use. *J. Oil Palm Res. Vol. 31(2)*: 271-280.

Fan, L; Ruan, R; Liu, Y; Wang, Y and Tu, C (2015). Effects of extraction conditions on the characteristics of ethanol organosolv lignin from bamboo (Phyllostachys pubescens Mazel). *BioResources*, *10(4)*: 7998-8013.

Fatriasari, W; Anita, S H and Risanto, L (2017). Microwave assisted acid pretreatment of oil palm empty fruit bunches (EFB) to enhance its fermentable sugar production. *Waste and Biomass Valorization*, *8(2)*: 379-391.

Garcia-Reyes, R B and Rangel-Mendez, J R (2009). Contribution of agro-waste material main components (hemicelluloses, cellulose, and lignin) to the removal of chromium (III) from aqueous solution. *J. Chemical Technology and Biotechnology*, *84(10)*: 1533-1538.

Hames, B; Ruiz, R; Scarlata, C; Sluiter, A and Templeton, D (2008). *Preparation of Sample for Compositional Analysis: Laboratory Analytical Procedures (LAP)*. 12 pp.

Hashim, S N A S; Zakaria, S; Chia, C H; Pua, F L and Syed Jaafar, S N (2016). *Chemical and Thermal Properties of Purified Kenaf Core and Oil Palm Empty Fruit Bunch Lignin*. p. 451653.

Hernández-Hernández, H M; Chanona-Pérez, J J; Vega, A; Ligero, P; Mendoza-Pérez, J A; Calderón-Domínguez, G; Terrés, E and Farrera-Rebollo, R R (2016). Acetosolv treatment of fibers from waste agave leaves: Influence of process variables and microstructural study. *Industrial Crops and Products*, *86*: 163-172.

Hussin, M H; Rahim, A A; Mohamad Ibrahim, M N and Brosse, N (2013). Physicochemical

characterization of alkaline and ethanol organosolv lignins from oil palm (*Elaeis guineensis*) fronds as phenol substitutes for green material applications. *Industrial Crops and Products, 49*: 23-32.

Kappe, C O (2004). Controlled microwave heating in modern organic synthesis. *Angewandte Chemie International Edition, 43(46)*: 6250-6284.

Kheong, L V; Rahman, Z A; Musa, M H and Hussein, A (2010). Empty fruit bunch application and oil palm root proliferation. *J. Oil Palm Res. Vol. 22(1):* 749-757.

Li, J; Zhang, J; Zhang, S; Gao, Q; Li, J and Zhang, W (2018). Alkali lignin depolymerization under eco-friendly and cost-effective NaOH/urea aqueous solution for fast curing bio-based phenolic resin. *Industrial Crops and Products*. p. 12025-33.

Monteil-Rivera, F; Huang, G H; Paquet, L; Deschamps, S; Beaulieu, C and Hawari, J (2012). Microwave-assisted extraction of lignin from triticale straw: Optimization and microwave effects. *Bioresource Technology*. p. 775-782.

MPOB (2020). Overview of the Malaysian oil palm industry 2019. Available from: http://www.mpob.gov.my.

Omar, R; Idris, A; Yunus, R; Khalid, K and Aida Isma, M I (2011). Characterization of empty fruit bunch for microwave-assisted pyrolysis. *Fuel, 90(4)*: 1536-1544.

Owi, W T; Lin, O H; Sam, S T; Chia, C H; Zakaria, S; Mohaiyiddin, M S; Villagracia, A R and Akil, H M (2016). Comparative study of microcelluloses isolated from two different biomasses with commercial cellulose. *Bioresources, 11(2)*: 3453-3465.

Pandey, K K; Chaohan, S S and Aggarwal, P K (1998). Reaction of wood with inorganic salts. *Holz als Roh- und Werkstoff, 56(6)*: 412-415.

Priecel, P and Antonio Lopez-Sanchez, J (2018). Advantages and limitations of microwave reactors: From chemical synthesis to the catalytic valorization of biobased chemicals. *ACS Sustainable Chemistry and Engineering, 7(1)*: 3-21.

Rashid, T; Gnanasundaram, N; Appusamy, A; Kait, C F and Thanabalan, M (2018). Enhanced lignin extraction from different species of oil palm biomass: Kinetics and optimization of extraction conditions. *Industrial Crops and Products, 116*: 123-136.

Reeb, C W; Hays, T; Venditti, R A; Gonzalez, R and Kelley, S (2014). Supply chain analysis, delivered cost, and life cycle assessment of oil palm empty fruit bunch biomass for green chemical production in Malaysia. *Bioresources, 9(3)*: 5385-5416.

Rohimi, N F; Roslan, R; Salim, N; Mustapha, S N H; Zakaria, S and Chia, C H (2020). Lignin-based polybenzoxazine derived from empty fruit bunch fibers with good thermal and mechanical properties. *Materials Science Forum Vol. 981*: 121-126.

Roslan, R; Zakaria, S; Chia, C H; Boehm, R and Laborie, M P (2014). Physico-mechanical properties of resol phenolic adhesives derived from liquefaction of oil palm empty fruit bunch fibres. *Industrial Crops and Products, 62*: 119-124.

Sameni, J; Krigstin, S and Sain, M (2017). Solubility of lignin and acetylated lignin in organic solvents. *BioResources, 12(1)*: 1548-1565.

Sammons, R J; Harper, D P; Labbé, N; Bozell, J J; Elder, T and Rials, T G (2013). Characterization of organosolv lignins using thermal and FT-IR spectroscopic analysis. *BioResources, 8(2)*: 2752-2767.

Schwiderski, M; Kruse, A; Grandl, R and Dockendorf, D (2014). Comparison of the influence of a Lewis acid $AlCl_3$ and a Bronsted acid HCl on the organosolv pulping of beech wood. *Green Chemistry, 16(3)*: 1569.

Shen, X -J; Wang, B; Huang, P -L; Wen, J -L and Sun, R-C (2016). Effects of aluminum chloride-catalyzed hydrothermal pretreatment on the structural characteristics of lignin and enzymatic hydrolysis. *Bioresource Technology, 22*: 657-664.

Tajuddin, A M; Harun, S; Sajab, M S; Zubairi, S I; Jahim, J; Markom, M; Nor, M T M; Abdullah, M A and Hashim, N (2019). Influence of deep eutectic solvent (DES) pretreatment on various chemical composition of empty fruit bunch (EFB). *Int. J. Engineering and Technology Vol. 8 No. 1.2*: 66–74.

Watkins, D; Nuruddin, Md; Hosur, M; Tcherbi-Narteh, A and Jeelani, S (2015). Extraction and characterization of lignin from different biomass resources. *J. Materials Research and Technology, 4(1): 26-32.*

Wu, Y; Zhang, S; Guo, X and Huang, H (2008). Adsorption of chromium(III) on lignin. *Bioresource Technology, 99(16):* 7709-7715.

Yaakob, M N A; Roslan, R; Salim, N; Mustapha, S N H; Zakaria, S; Chia, C H; Sajab, M S and Yek, P N Y (2020). Effect of temperature on the yield of lignin extracted using microwave-assisted acetosolv from empty fruit bunch fibers. *Materials Science Forum, 981*: 240-244

Zaini, M A A and Ali, A H (2018). Adsorptive characteristics and microwave dielectric properties of oil palm empty fruit bunch-based activated carbons for dye removal. *Malaysian J. Fundamental and Applied Sciences, 14(2)*: 241-245.

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USING TERRAIN ALGORITHMS ON A DIGITAL ELEVATION MODEL TO EVALUATE YIELD VARIABILITY IN OIL PALM

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ABSTRACT

Oil palm (Elaeis guineensis *Jacq.*) *plantations face strong pressure to improve fertiliser-use efficiency. Digital soil mapping methods based on topographic analysis using globally-available digital elevation models (DEM) provide an efficient means of quantifying topography-driven variability of soil properties within oil palm plantations. The shutter radar topography mission (SRTM) global digital elevation model (GDEM) was used as the basis for modeling topography across an individual oil palm plantation. Terrain algorithms were used to model terrain attributes and generate continuous soil property maps along topographic soil classes in conjunction with georeferenced soil samples as model inputs. The resulting raster layers of soil property values were evaluated for mean error and their correlation to yield variability across the plantation. Modified catchment area (MCA), an iterative measure of a landscape position represented by a grid cell's propensity to lose or gain soil water, was found to have a strong effect on yield, suggesting that soil moisture distribution was an important driver of yield variability in this system.*

Keywords: digital elevation, terrain algorithms, modified catchment area, topography, palm oil yield.

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INTRODUCTION

Advances in soil and terrain mapping provide new techniques for explaining, and perhaps predicting, in-field variation in oil palm yield. In particular, the ability to generate high-resolution digital elevation models (DEM) via remote sensing has allowed for terrain mapping previously not possible at the scale needed to distinguish in-field variability, and in turn this has made it possible to incorporate topography in mapping the spatial distribution of soil properties (Odeh *et al*., 1991). The availability of digital soil mapping methods have enabled new insights into how soil variability, including variability in soil moisture and drainage patterns, relates to in-field yield variability in different cropping systems (Iqbal *et al*., 2005).

Terrain attributes are DEM-derived environmental variables, which integrate surface-controlled processes that relate to the development of different soil properties (Odeh *et al*., 1991; Florinski, 2016). Terrain algorithms (TA) can be used to quantify terrain attributes and analyse the topographic and hydrologic properties of a target location based on a DEM (Florinski, 2016). As the use of TA to evaluate a location's topography does not rely on on-the-ground surveying, they offer a cost-effective means of evaluating a target site's topography, particularly in remote areas. Four specific terrain attributes that may be useful in topographic classification in oil palm plantations are slope, normalised height (NH), topographic wetness index (TWI), and modified catchment area

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(MCA) from the System for automated geoscientific analyses (SAGA) (Ashtekar and Owens, 2013). Slope is expressed as a percentage, ratio or angle, and describes the proportion of horizontal and vertical distances between points, while NH presents the relative terrain elevation after normalising according to the elevation range within the target site (Böhner and Selige, 2006). MCA and TWI quantitatively describe the effect of topography on hydrological processes and rely on iterative analysis of a digital elevation model to quantify the relative propensity that a grid cell will either loose or accumulate water. Both MCA and TWI are capable of estimating both water excess and water scarcity (Jenson and Domingue, 1988; Quinn *et al*., 1995).

Insufficient and excessive soil moisture both can reduce oil palm yield (Fedepalma, 2016). Oil palm grows in areas of intensive solar radiation and can exhibit high photosynthetic activity and respiration rates (Fedepalma, 2016). Oil palm production is thus only possible in areas of high precipitation (minimum 2000-2500 mm yr^{-1}) (Pirker *et al*., 2016), as transpiration rates of up to 280-350 mm $palm^{-1}$ per day are required to maintain optimal plant function (Carr, 2011). Sustaining such high transpiration levels requires constant replenishing of soil moisture, and sustained stress from insufficient soil moisture can lead to sharp drops in palm oil production (Pirker *et al*., 2016). However, oil palm cannot grow under saturated soil conditions, as its root system is ill-adapted to waterlogged conditions (Carr, 2011; Pirker *et al*., 2016). Proper drainage is therefore required to evacuate excess water.

TA that relate to water availability have been found useful for describing yield variability in several agronomic crops over the last three decades (Simmons *et al*., 1989; Kaspar *et al*., 2003; Jiang and Thelen, 2004; Maestrini and Basso, 2018), however studies in oil palm have been limited (Mfondoum *et al*., 2019). Recent efforts to simulate potential yield of oil palm have assumed optimal moisture conditions (Hoffmann *et al*., 2014), but not water excess or limitation which are dependent on landscape effects on moisture allocation as well as rainfall. The objectives of this research were to evaluate the consistency of the two predominant publicly-available DEM for describing elevation in an oil palm plantation and determine how well TA derived from a DEM predict in-field variability of oil palm yield in the Colombian Llanos region.

METHODS

Study Site and Plantation Management

The study site was a 5220 ha oil palm plantation in the Colombian Llanos, in the municipality of Villanueva, Casanare (*Figure 1*). The soils of the plantation were uniformly classified as Typic Fluvaquents, with slopes of 0%-3%, derived from recent alluvial deposits from the eastern Andes mountain range, with a depth greater than 100 cm (IGAC, 2014). In 2010-2016, yearly precipitation in the plantation regularly exceeded 2000 mm, while average temperatures were about 27°C (*Table 1*).

The studied area comprised six management zones of different size that were planted at different times (*Table 2*). Within each management zone, plantings were made in the same season and with the same genetic material at 160 palm ha^{-1}. The size and shape of management zones was determined without definite criteria as the plantation expanded and new plantings were added starting in the 1970s. Following standard practice, palms were planted in triangular patterns with 9 m between palms, and parallel rows established between palm transects alternately designated as harvest paths and undisturbed rows left unused by machinery and harvest crews (Corley and Tinker, 2008). All plantings in this area were replanted post 1990 (*Table 2*).

TABLE 1. YEARLY PRECIPITATION AND AVERAGE TEMPERATURE IN 2010-2016

Year	Precipitation (mm)	Temperature (°C)
2010	2 866	27.0
2011	2 195	26.7
2012	2 535	26.8
2013	2 265	27.0
2014	2 116	26.6
2015	1 957	27.0
2016	2 003	27.2

Note: For a 5220 ha oil palm plantation in the municipality of Villanueva, Casanare, in the Colombian llanos.

TABLE 2. MANAGEMENT ZONE SIZE, NUMBER OF HARVESTING UNITS (distinct yield points) AND YEAR OF PLANTING FOR A 5220 ha OIL PALM PLANTATION

Management zone (MZ)	ha per MZ	Harvesting units per MZ	Year of planting
1	306	12	2004
2	134	9	2004
3	205	10	2004
4	481	23	1999
5	267	10	2005
6	410	14	1990

Note: In the municipality of Villanueva, Casanare, in the Colombian Llanos. Management zones are defined by having been planted at the same time and from the same genetic material.

Management practices and input applications were identical throughout the plantation and followed best practices as defined by the Colombian National Federation of Palm Oil Growers

(Fedepalma, 2016). Palms received uniform applications of pre-mixed 13-5-27-5 (N-P-K-Mg as % of total fertiliser weight) fertiliser at the rate of 4 kg palm^{-1} from the onset of production, for a total yearly per hectare application of 83 kg ha^{-1} N, 32 kg ha^{-1} P, 173 kg ha^{-1} K, and 32 kg ha^{-1} mg. The fertiliser was a physical mixture of urea, monoammonium-phosphate, muriate of potash and magnesium oxide. Irrigation and artificial drainage were not used anywhere in the study site.

Since management zones were too large for a crew to harvest in a workday, they were divided into smaller harvesting units. There was no consistent methodology used by different plantation managers to divide management zones into harvesting units as new management zones were added over time, thus they were highly irregular in shape. Seventy-eight harvesting units existed in the study site, ranging from 10-65 ha in size.

Yield data for each harvesting unit were collected from 2013-2016. Only a single yield value was reported per harvest for each unit, so yield variability within a harvesting unit was not discernable. Yield data were digitised as vector layers of 78 yield points, one for each harvesting unit, and indexed by year. These vector layers were rasterised using the GDAL module of QGIS, which creates raster bands consistent with the target vector geometries, and co-registered to match the projection and 30 m x 30 m cell grid of the DEM and TA rasters. In this way, the vector data from these irregular polygons was reconciled with the raster soil property data for subsequent statistical analysis described later.

Satellite Data

The open-source QGIS platform was used for all geographic and terrain analyses. The corresponding sections of both the shutter radar topography mission (SRTM) and advanced spaceborne thermal emission and reflection radiometer (ASTER) global digital elevation model (GDEM) were downloaded from USGS EarthExplorer, and they were clipped to the perimeter of the plantation. Both DEM were re-projected to Magna Sirgas Colombia Bogota projection (EPSG 3116), which is based on the national geodetic reference frame used by Colombia's National Geographic Institute Agustin Codazzi. All georeferenced data were thereafter stored and processed in the Magna Sirgas Colombia Bogota projection. Since exact coordinate points for the entire perimeter were not available, the visible outline of the palms from Landsat 8 images was used to create a shapefile of the plantation outline. A shapefile of the internal boundaries of the plantation management zones was generated using waypoints from a handheld GPS device.

Terrain Algorithms

The SRTM DEM was compared against the ASTER DEM to evaluate for discrepancies using the raster calculator function of QGIS. Terrain analyses were performed using the SAGA module.

MCA was calculated via iteration, where the modified catchment area of each grid cell was calculated as a function of slope in angle β and the neighbouring maximum values MCA_{max} until results no longer changed between iterations.

$$MCA = MCA_{max}(1/15)^{bexp(15b)} \quad \text{for} \quad MCA < MCA_{max}(1/15)^{bexp(15b)}$$

TWI was calculated as

$$TWI = \ln(\alpha / \tan\beta),$$

where α is the local upslope contributing area and tan β is the local slope.

NH, TWI and slope TA were also generated from the SAGA module as inputs for the FSM model (Ashtekar and Owens, 2013) using the 30 m x 30 m SRTM DEM.

Depth to the Water Table Measurements

To have a point of comparison between computed MCA values and an in-field measurement of soil water, the depth to water table was determined at the grid cell locations predicted by the MCA terrain algorithm to be the wettest and driest areas within each management zone (*Figure 2*). Precipitation in the Piedmont region of the Llanos during the rainy season is characterised by brief but intense periods of rainfall, often lasting less than an hour, interspersed by clear skies, with average cumulative monthly precipitation reaching 500 mm (Marin and Ramirez, 2006). Over the course of the 2017 rainy season, specifically the months of May and June, water table depth from the soil surface was measured simultaneously for each selected grid cell 1 hr after five individual rain events by boring a hole to the water table with a 10 cm diameter auger and recording depth from the soil surface.

Statistical Analysis of Terrain Algorithm Raster Data

ASCII files of individual soil properties were uploaded into *R* as data rasters (Fox, 2005). For each depth sampled, matrix *X* of georeferenced soil property values was created from the individual soil property rasters. Every element X_{nk} was indexed to a 30 m x 30 m pixel *n* as defined in the SRTM DEM and an individual soil property *k*.

$$X = \begin{bmatrix} X_{11} & \cdots & X_{1k} \\ \vdots & \ddots & \vdots \\ X_{n1} & \cdots & X_{nk} \end{bmatrix}$$

where X is an (n x k) matrix of soil data, with n grid cells and k soil properties.

The X_k was calculated as the mean value for each property k, and the value at 0-20 cm compared with the values at 20-40 and 40-60 cm.

Correlation and Regression Analysis of Terrain Algorithm Raster Data

When evaluating the effect of soil properties on yield, the dimensionality of the X matrix was reduced by averaging soil property values, originally at the 30 m x 30 m resolution, to match the lower resolution of the yield data, resulting in the following reduced matrices:

$$Y = \begin{bmatrix} Y_1 \\ \vdots \\ Y_m \end{bmatrix} \qquad X = \begin{bmatrix} 1 & X_{11} & \cdots & X_{1k} \\ 1 & \vdots & \ddots & \vdots \\ 1 & X_{m1} & \cdots & X_{mk} \end{bmatrix}$$

where: Y = is an (m x 1) vector of yield values,
m = the number of harvesting units in a management zone,
X = is an [m x (k+1)] matrix of soil property data points,
and k = the number of soil variables measured.

The Pearson correlation was calculated between each soil property value and the corresponding yield value for each management zone. The correlations were performed in R using a 0.05 probability level as the threshold for statistical significance.

RESULTS AND DISCUSSION

Comparison of SRTM and ASTER DEM

When the SRTM DEM was compared to the ASTER DEM, the mean percent difference between co-registered grid cells was 2.0%, with a maximum difference for an individual pixel of 16.8% (*Figure 1*). Areas of maximum distortion between DEM were scattered around the plantation with no obvious pattern or commonality to their occurrence. The SRTM DEM was constructed using interferometric synthetic aperture radar while the ASTER DEM used stereoscopic VNIR images to calculate elevation values. Radar and VNIR light can interact differently with both the atmosphere and the ground surface, potentially arising in small differences in elevation values. The infrequent and minor discrepancies between ASTER and SRTM DEM at our study site are comparable with those observed in previous comparisons of the two DEM (Arabelos, 2000; Nikolakopoulos *et al*., 2006). Since there were generally minor differences between DEM, we chose to use the SRTM DEM as the basis for terrain analysis, generation of soil property maps and defining the grid structure of all subsequent raster data generated in this study to be consistent with previous studies in the Llanos region (Ashtekar *et al*., 2014).

Correlation of Yield with Terrain Algorithm

Management zones each contained 9-23 harvesting units, each 10-65 ha in size, which provided the individual yield points for the plantation (*Table 2*). A large amount of variability in yield existed among harvesting units within management zones (*Table 3*) despite uniform management, planting material and planting date within a management zone.

MCA had the most frequent and highest correlation with oil palm yield across the plantation of any terrain algorithm (*Table 4*), with a significant correlation in four of six management zones. However, the direction of the correlation differed among zones, with a positive correlation between MCA value and yield in MZ1 (r= 0.86) and negative correlations in MZ 2, 3 and 5 (r= 0.88, 0.79 and 0.87, respectively). The correlation of TWI and yield (*Table 4*) generally mirrored that of MCA in all management zones, which was not surprising as both predict soil moisture from topography. However, TWI correlations were not as often significant nor as high as those for MCA.

Normalised height had comparably high, but positive correlations (r= 0.77 and 0.74, respectively) with yield in MZ 2 and MZ 3 as did MCA. A high NH value indicates a higher topographic position and would thus likely also indicate a predominantly dry grid cell, suggesting that excess soil moisture reduced yield in low NH areas in MZ 2 and 3 consistent with the relationships between MCA and TWI and yield. Slope was not correlated with yield in any management zone.

TABLE 3. MEAN, MAXIMUM AND MINIMUM OIL PALM YIELD FOR SIX MANAGEMENT ZONES IN YEARS 2013-2016

Statistic	Oil palm yield (t ha^{-1})					
	Management zone					
	1	2	3	4	5	6
Mean	13.3	13.7	11.1	18.7	18.7	17.1
Maximum	20.0	18.7	13.3	24.3	21.9	18.7
Minimum	5.8	10.6	8.5	13.0	17.0	15.1

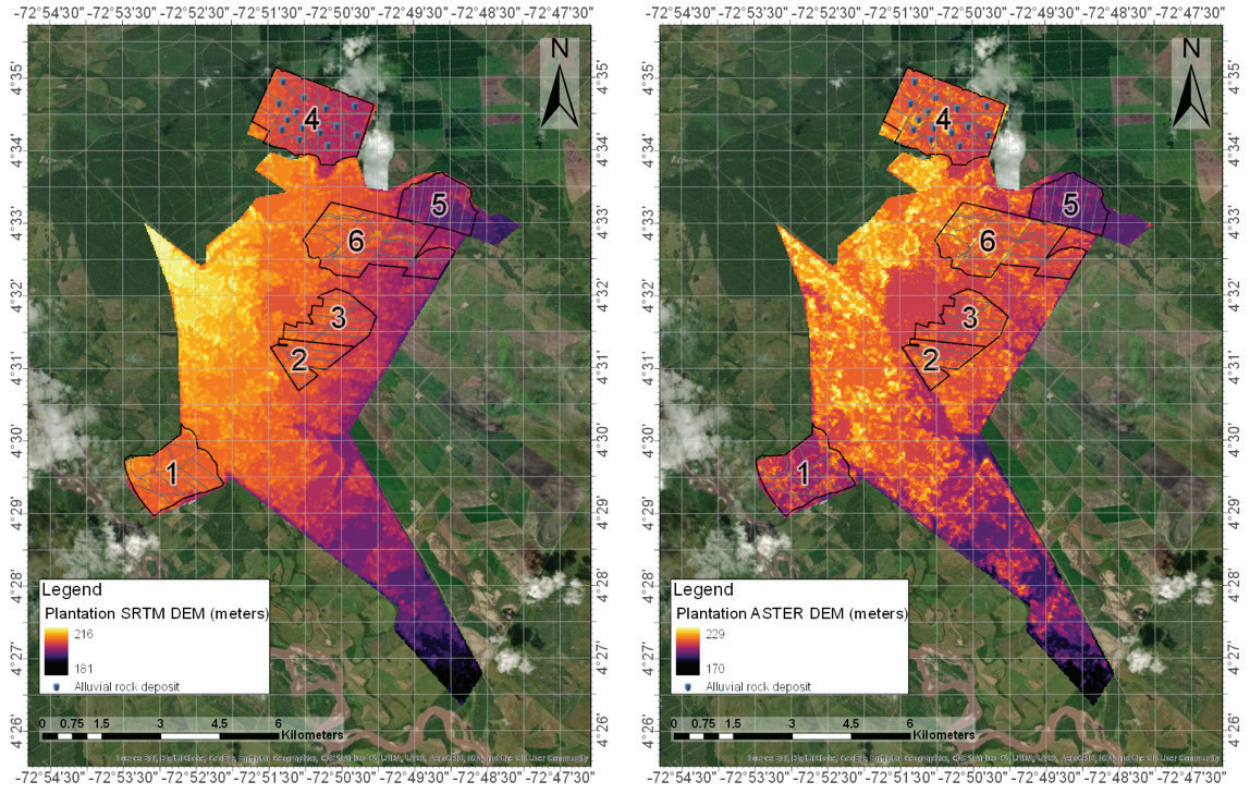

Figure 1. Shuttle radar topography mission (SRTM) and advanced spaceborne thermal emission and reflection radiometer (ASTER) 30 m digital elevation models (DEM) for the study site, a 5220 ha oil palm plantation in the municipality of Villanueva, Casanare, in the Colombian Llanos. The mean value for the SRTM and ASTER DEM respectively was 200±5.8 m and 202±7.9 m, with an overall difference between both DEM of 2%.

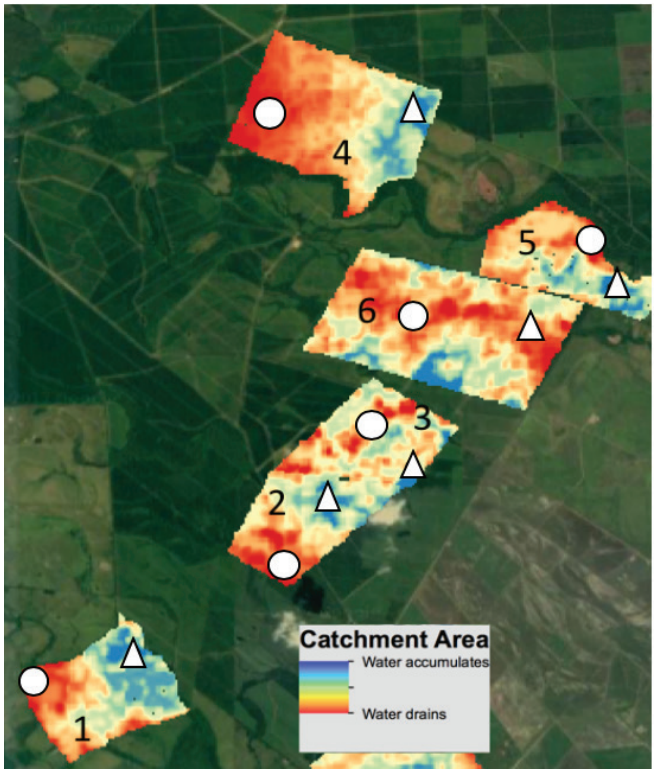

Figure 2. Modified catchment area (MCA) by management zone (MZ), sampled at the wettest and driest planted grid cells in each management zone (marked respectively by triangles and ovals). Blue indicates high MCA values/high propensity for soil moisture accumulation.

TABLE 4. CORRELATION OF TERRAIN ALGORITHMS DERIVED FROM SRTM DEM WITH OIL PALM YIELD FOR EACH OF SIX MANAGEMENT ZONES (MZ)

Terrain algorithm	MZ 1		MZ 2		MZ 3		MZ 4		MZ 5		MZ 6	
	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value	r	p-value
MCA	**0.86**	**<0.001**	**-0.88**	**<0.001**	**-0.79**	**0.004**	0.11	0.61	**-0.87**	**<0.001**	0.30	0.29
TWI	**0.78**	**0.003**	**-0.80**	**0.01**	-0.55	0.09	0.13	0.57	-0.53	0.17	0.14	0.63
NH	-0.41	0.18	**0.77**	**0.01**	**0.74**	**0.01**	**-0.47**	**0.02**	-0.50	0.21	-0.19	0.51
Slope	-0.54	0.07	0.10	0.80	0.25	0.47	-0.18	0.41	0.10	0.82	0.15	0.61

Note: Bolded values indicate significant correlations ($p < 0.05$).
MCA - modified catchment area; TWI - topographical wetness index; NH - normalised height.
DEM - digital elevation models.
SRTM - shuttle radar topography mission.

Grid cells predicted from MCA calculations to be most prone to water accumulation within each management zone had substantially shallower water tables than those predicted to be drier (*Table 5*). The negative correlation between MCA and yield in MZ 2, 3 and 5 (*Table 4*) indicated that a higher propensity for soil moisture accumulation likely reduced palm oil production in these MZ. Palm oil is susceptible to yield loss under waterlogged conditions (Lee and Ong, 2006; Henson *et al*., 2008).

The relationship between MCA and palm oil yield was very different in MZ 1 than in MZ 2, 3 and 5, showing a positive correlation between yield and MCA (r= 0.86). The positive correlation between palm oil yield and MCA in MZ 1 might indicate this part of the plantation was excessively well drained, with oil palms in grid cells with lower MCA values suffering yield losses from insufficient soil moisture (Paramananthan, 2000). Indeed, the depth to water table at the grid cell with the lowest MCA value was 127 cm, compared to 45 cm, 42 cm, and 73 cm at the grid cells with lowest MCA values in MZ 2, 3 and 5 (*Table 5*). MZ 1 protrudes out of the southwestern end of the plantation, and the harvesting units at the most southwestern edge are surrounded on three sides by a sharp drop to lower-lying terrain outside the plantation boundaries, potentially creating an excessively well-drained zone at the management zone edge. As seen in *Figure 3*, it is the harvesting units at this edge that have the lowest MCA values and the lowest yields. Additionally, yield in MZ 1 was negatively correlated to the percentage of sand (r= -0.7), which supports the argument that insufficient moisture was driving yield differences within this management zone.

TABLE 5. AVERAGE DEPTH TO WATER TABLE (DWT) AND STANDARD DEVIATION IN cm AT GRID CELLS

MZ	Driest		Wettest	
	DWT (cm)	MCA	DWT (cm)	MCA
1	127±5	22	35±2	99
2	45±4	18	7±1	52
3	42±5	21	4±0.5	35
4	39±3	17	7±1	70
5	73±2	22	35±2	41
6	47±3	38	36±3	55

Note: With driest and wettest modified catchment area (MCA) values within each management zone (MZ).

Normalised height was the only TA to correlate with yield in MZ 4. MZ 4 had a wide range of MCA values (17-70) and depth to water table (7-39 cm), yet no significant correlation between MCA, TWI or NH with yield was found for this area. An extensive deposit of large alluvial rocks near the soil surface across a large portion of this area seemed on visual inspection to have hindered palm root growth in affected areas and may have introduced an extraneous factor that obscured the effect of variable soil hydrology on palm oil yield.

None of the TA were correlated with yield in MZ 6. As can be seen in *Figure 4*, the planted areas lie along a ridge of well-drained terrain directly between two unplanted poorly-drained areas. Within this planted ridge, depth to water table varied by only 9 cm between highest and lowest MCA values. In contrast, the difference in water table height ranged from 32-92 cm between highest and lowest MCA values in the other management zones. A relative homogeneity in soil moisture in the planted area might explain why there was no significant correlation between MCA and yield for this management zone.

The Llanos region of Colombia is an area of high rainfall, particularly in the Piedmont region of the study site, where annual rainfall can reach 4000 mm (IGAC, 2014). The topographic layout of the study site, with relative topographic highs and lows spread throughout the plantation, can thus result in the rapid redistribution of large amounts of water by gravitational pull following rain events, leading to zones of disparate levels of soil moisture within close proximity (Zhang, 2004). Additionally, the marked seasonality of rainfall in the region means that palms can be exposed to very limited rainfall in the dry season followed by intense precipitation in the wet season, creating the potential for hydraulic stress

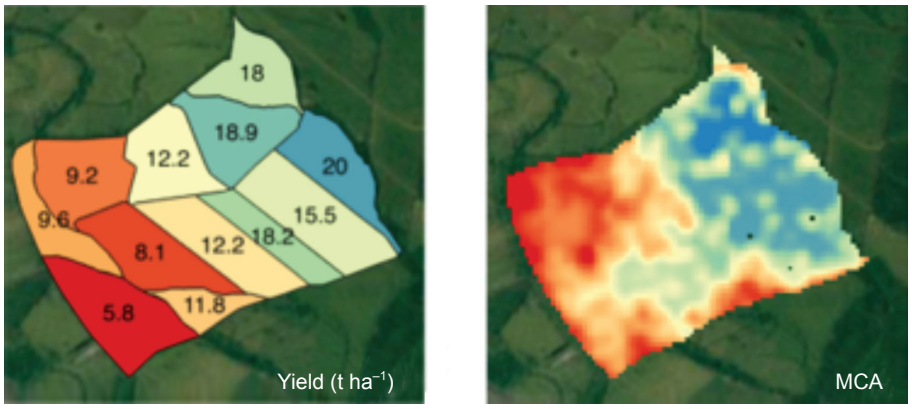

Figure 3. Yield in t ha^{-1} of fresh fruit bunches and modified catchment area (MCA) for management zone (MZ) 1. Blue indicates a higher yield in the left-hand image and a higher MCA value/greater propensity for soil moisture gain in the right-hand image. MZ 1 comprises 306 ha.

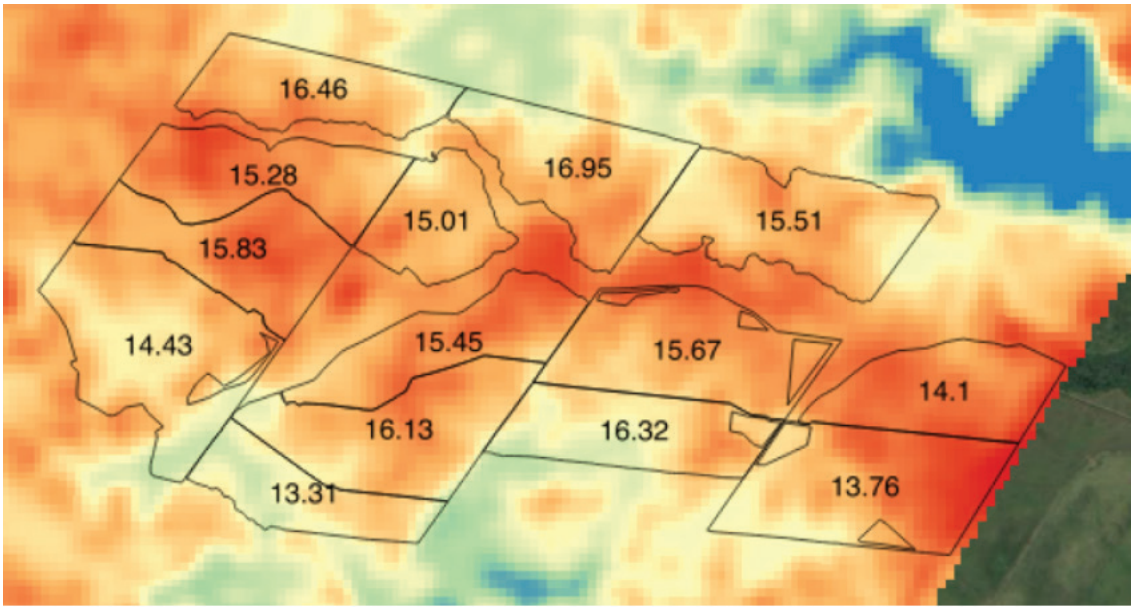

Figure 4. Average yield over seven years in t ha^{-1} of fresh fruit bunches over modified catchment area (MCA) map for management zone (MZ) 6, where blue indicates a higher MCA value/greater propensity for soil moisture gain. MZ 6 comprises 410 ha.

from both excessive and insufficient soil moisture at different times of the year, and suggesting that the ideal soil drainage class for oil palm in the plantation would balance draining excessive moisture in the wet season with the retention of sufficient moisture in the dry season (Jipp *et al*., 1998).

The study showed that the ASTER and SRTM DEM provided a similar description of the plantation topography. The study also supported the hypothesis that TA, particularly MCA, could be correlated with in-field yield variability within a Llanos oil palm plantation, suggesting that differences in water distribution could be an important driver of yield variability. MCA is meant to serve as a remote sensing proxy for water distribution across topographies, and a better understanding of the direct relationship between soil water and MCA in the study site might help explain the underlying mechanism behind the correlation between MCA and yield. This includes soil variables that also affect water distribution and availability could help produce a better model of soil water and oil palm yield. Results could be used to model future yields in established plantations in conjunction with climate change modeling. This approach could be implemented to assess land for replanting or the establishment of new plantations.

REFERENCES

Arabelos, D (2000). Intercomparisons of the global DTMs ETOPO5, TerrainBase and JGP95E. *Physics and Chemistry of the Earth, Part A: Solid Earth and Geodesy*, *25*: 89-93.

Ashtekar, J M and Owens, P R (2013). Remembering knowledge: An expert knowledge based approach to digital soil mapping. *Soil Horizons Vol*. *54*: 1-6.

Ashtekar, J M; Owens, P R; Brown, R A; Winzeler, H E; Dorantes, M; Libohova, Z; Dasilva, M and Castro, A (2014). Digital mapping of soil properties and associated uncertainties in the Llanos Orientales, South America. *GobalSoilMap: Basis of Global Spatial Soil Information System* (Arrouays, D; McKenzie, N; Hempel, J; Richer de Forges, A and McBratney, A eds.). CRC Press, Boca Raton, FL, USA. p. 367-372.

Böhner, J and Selige, T (2006). Spatial prediction of soil attributes using terrain analysis and climate regionalisation. *Göttinger Geographische Abhandlungen*, *115*: 13-28.

Carr, M K V (2011). The water relations and irrigation requirements of oil palm (*Elaeis guineensis*): A review. *Experimental Agriculture*, *47*: 629-652.

Corley, R H V and Tinker, P B (2008). *The Oil Palm*. 5th Edition. John Wiley & Sons, Hoboken, New Jersey. 674 pp.

Fedepalma, C (2016). Mejores prácticas agroindustriales para una excelente palmicultura. *Boletín El Palmicultor*, *(537 Noviem)*: 17-18.

Florinsky, I (2016). *Digital Terrain Analysis in Soil Science and Geology*. 2nd Edition. Academic Press, London, UK. 475 pp.

Fox, J (2005). Getting started with the R commander: A basic-statistics graphical user interface to R. *J. Stat. Softw.*, *14*: 1-42.

Henson, I E; Harun, M H and Chang, K C (2008). Some observations on the effects of high water tables and flooding on oil palm and a preliminary model of oil palm water balance and use in the presence of a high water table. *Oil Palm Bulletin No. 56:* 14-22.

Hoffmann, M P; Vera, A C; Van Wijk, M T; Giller, K E; Oberthür, T; Donough, C, and Whitbread, A M (2014). Simulating potential growth and yield of oil palm (*Elaeis guineensis*) with PALMSIM: Model description, evaluation and application. *Agricultural Systems*, *131*: 1-10.

Instituto Geográfico Agustín Codazzi (IGAC) (2014). *Estudio general de suelos y zonificación de tierras, Departamento de Casanare*. Bogotá, D.C., Colombia.

Iqbal, J; Thomasson, J A; Jenkins, J N; Owens, P R and Whisler, F D (2005). Spatial variability analysis of soil physical properties of alluvial soils. *Soil Sci. Soc. Am. J., 69*: 1338-1350.

Jenson, S K and Domingue, J O (1988). Extracting topographic structure from digital elevation data for geographic information system analysis. *Photogrammetric Engineering and Remote Sensing*, *54*: 1593-1600.

Jiang, P and Thelen, K D (2004). Effect of soil and topographic properties on crop yield in north-central corn-soybean cropping system. *Agron. J., 96*: 252-258.

Jipp, P H; Nepstad, D C; Cassel, D K and De Carvalho, C R (1998). Deep soil moisture storage and transpiration in forests and pastures of seasonally-dry Amazonia. *Potential Impacts of Climate Change on Tropical Forest Ecosystems*. Springer, Dordrecht. p. 255-272.

Kaspar, T C; Colvin, T S; Jaynes, D B; Karlen, D L; James, D E; Meek, D M; Pulido, D and Butler, H (2003). Relationship between six years of corn yields and terrain attributes. *Prec. Agric., 4*: 87-101.

Lee, W K and Ong, B K (2006). The unseen flood: Waterlogging in large oil palm plantations. *Jurutera (January 2006)*: 28-31.

Marin, S and Ramirez, J A (2006). The response of precipitation and surface hydrology to tropical macro-climate forcing in Colombia. *Hydrological Processes*, *20*: 3759-3789.

Maestrini, B and Basso, B (2018). Drivers of within-field spatial and temporal variability of crop yield across the US Midwest. *Sci. Reports, 8*: 1-9.

Mfondoum, A H N; Mfondoum, R B N; Wokwenmendam, P N; Gbetkom, P G and Ntengo, M (2019). Modeling best oil palm site planting in Njimom, West-Cameroon: A GIS-analysis combining weighted linear combination, fuzzy analytical hierarchy process and utility function. *J. Geographic Info. Syst.*, *11*: 138-165.

Nikolakopoulos, K G; Kamaratakis, E K and Chrysoulakis, N (2006). SRTM *vs*. ASTER elevation products. Comparison for two regions in Crete, Greece. *Int. J. Remote Sensing, 27*: 4819-4838.

Odeh, I O A; Chittleborough, D J and McBratney, A B (1991). Elucidation of soil-landform interrelationships by canonical ordination analysis. *Geoderma*., *49*: 1-32.

Paramananthan, S (2000). Soil requirements of oil palm for high yields. *Managing Oil Palm for High Yields: Agronomic Principles*. Malaysian Society of Soil Science and Param Agricultural Surveys, Kuala Lumpur. p. 18-38.

Pirker, J; Mosnier, A; Kraxner, F; Havlík, P and Obersteiner, M (2016). What are the limits to oil palm expansion? *Global Environmental Change*, *40*: 73-81.

Quinn, P; Beven, K and Lamb, R (1995). The In (a/tan/β) index: How to calculate it and how to use it within the Topmodel framework. *Hydrological Processes*, *9*: 161-182.

Simmons, F W; Cassel, D K and Daniels, R B (1989). Landscape and soil property effects on corn grain yield response to tillage. *Soil Sci. Soc. Am. J.*, *53*: 534-539.

Zhang, X N (2004). Comparative analysis of drainage networks derived from grid-based DEM. *Advances in Science and Technology of Water Resources*, *3*: 110-115.

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EXTRACTION AND PURIFICATION OF PHYTOSTEROLS MIXTURE FROM PALM FATTY ACID DISTILLATE (PFAD) USING MULTISTAGE EXTRACTION PROCESSES

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ABSTRACT

Phytosterols are among the bioactive compounds naturally present in vegetable oils and their by-products or derivatives. A phytosterol resource (PSR), solid by-product from the extraction of vitamin E in palm fatty acid distillate (PFAD), contains 2%-4% (w/w) total sterols. Therefore, the extraction of phytosterols from the PSR in a mini-pilot scale involving multistage extraction processes was developed to recover the valuable minor component. The multistage extraction and purification processes comprised of solid-liquid extraction (SLE) with hexane at 35°C in 1 hr, saponification reaction at the reflux temperature of 80°C for 1 hr, liquid-liquid extraction (LLE) with hexane and water, and crystallisation and vacuum filtration at -5°C for 20 hr. On average, gas chromatographic (GC) analysis showed the phytosterols recovered from the extraction and purification process had more than 80% purity. The recovery of total sterols from the PSR was 84% composed of β-sitosterol (21%-22%), campesterol (13%-20%) and stigmasterol (59%-64%). This extraction process is technically feasible to extract and produce crude phytosterols from a PFAD by-product.

Keywords: palm fatty acid distillate (PFAD), phytosterols, multistage extraction, palm oil by-product.

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INTRODUCTION

Phytosterols (plant sterols) are one of the bioactive compounds that occur naturally in vegetable oils and their by-products or derivatives. Phytosterols can be found in olive oil, sunflower oil and pistachios. Several types of phytosterols have been identified and reported but only β-sitosterol, campesterol and stigmasterol exist in significant amount in these resources (Moreau *et al*., 2018; Massimo *et al*., 2019). Other types of phytosterols such as brassicasterol, Δ^5-avenasterol, Δ^7-avenasterol, sitostanol, campstanol and Δ^7-stigmasterol can also be found in small quantities (Fernandes and Cabral, 2007). Furthermore, Moreau *et al*. (2018) have determined other types of phytosterols, which are stanols and their conjugates in foods.

Consumption of phytosterols has been reported to be able to reduce cholesterol absorption and lowering total serum and low density lipoprotein (LDL) cholesterol levels in animals and humans (Jones *et al*., 1999; Tasan *et al*., 2006). Phytosterols also showed anti-cancer property toward breast, colon and prostate cancer cell lines (Awad and Fink,

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2000). These important beneficial characteristics rendered phytosterols to have wide applications in nutraceuticals and functional food industries. Therefore, phytosterols can be incorporated into various types of food for enrichment to provide enough phytosterols for daily intake of 2 g per day (Devaraj and Jialal, 2006). Examples of functional food products containing phytosterols are margarine, butter, cereals, milk and spreads products, which are enriched with plant-derived sterols and their esters (Kowalski, 2017).

Commercial phytosterols are extracted from soyabean oil, corn oil, rapeseed oil, sunflower oil as well as tall oil. In palm oil, sterols can be found as minor component; together with tocotrienols, tocopherols, carotene, coenzyme Q10 and squalene. The sterols content in crude palm oil (CPO) ranges from 250 to 730 ppm (Chandrasekaram, 2009). Phytosterols are also present in the by-products of palm oil mill and refinery such as palm pressed fibre oil (PPFO) and palm fatty acid distillate (PFAD) in various concentrations depending on the processes (Ab Gapor, 2010; Lau *et al*., 2008). In Malaysia, 53 palm oil refineries are in operation with a total annual refining capacity of 27.33 million tonnes and thus about 765 000 t of PFAD are also being generated from the refining process (Kushairi *et al*., 2018). On average, PFAD contains about 0.4% phytosterols (Ab Gapor, 2010) and thus, it is estimated that about 3000 t of phytosterols are available to be extracted from the PFAD.

Extraction of phytosterols can be conducted using several methods and extraction technologies depending on the source of raw materials (Fernandes and Cabral, 2007). The common recovery processes of phytosterols from oil are multistage processes of esterification, saponification, molecular distillation, crystallisation and filtration (Choo *et al*., 2005). Sterols in raw materials are partially found in ester form. As such, pre-treatments involving chemical modification such as saponification and hydrolysis are required to convert a component in the substance into different properties to allow for easier separation. These processes will convert sterol esters into unsaponifiable free sterols and also convert fatty acids and their esters into soap matrix (Fernandes and Cabral, 2007). Selective adsorption and desorption method using styrene-diviynlbenzene type adsorbent with various type of solvents type such as methanol, isopropanol and hexane is also applied to enrich the phytonutrient content in CPO and PPFO (Phoon *et al*., 2018). A greener technology such as supercritical fluid extraction (SFE) was introduced to extract oil enriched with phytonutrient from PPFO, olive oil deodouriser and PFAD (Akgun, 2011; Lau *et al*., 2008; Norhidayah *et al*., 2012; Sugihara *et al*., 2010). The SFE is able to simultaneously extract carotene, vitamin E, squalene and phytosterols from the oil, which are then collected as fractional products from SFE based on the temperature and pressure of the supercritical carbon dioxide set during the operation. Ng and Choo (2013) developed a method using flash chromatography to recover phytonutrients such as carotenes from palm oil. Aqueous enzymatic method has also been found to be able to increase phytonutrients content in PPFO (Noorshamsiana *et al*., 2017). All of these green systems are able to recover phytonutrients from the feedstock. However, phytosterols concentration recovered were low, below 2%. Therefore, additional processes are required in order to obtain high purity of individual phytonutrients. Moreover, as compared to conventional processes, these new technologies incur high capital cost and need skilled manpower.

Previous studies mainly focused on phytosterols extraction directly from CPO and deodourised distillates. Hence, few oil palm by-products from palm oil mills and refinery have been collected and analysed for their phytosterols content. This includes a solid by-product of the commercial vitamin E extraction from PFAD that has huge amount of unrecovered phytosterols. Suitable extraction and purification processes have yet to be properly developed to recover the phytosterols from this particular resource. Therefore, the aim of this study is to extract and purify the phytosterols from the solid by-product in a mini-pilot scale multistage extraction process. The recovery of this valuable component from the by-product of oil palm processing will help the oil palm industry to generate additional income and subsequently sustain the palm oil industry in Malaysia.

MATERIALS AND METHOD

Materials Preparation

CPO, PPFO, sludge palm oil (SPO) and oil palm empty fruit bunch (OPEFB) residual oil are collected from various sampling points at several palm oil mills in Peninsular Malaysia. All samples were stored at 4°C prior to analysis. PFAD was purchased from an oil refinery company (MOI Foods Malaysia Sdn Bhd, Selangor, Malaysia) and stored in a stainless steel drum before the extraction processes.

PFAD was then subjected to several processes, consisting of esterification, transesterification, distillation, crystallisation and ion exchange adsorption as depicted in *Figure 1*, adapted from vitamin E extraction method by Ab Gapor *et al*. (1993). The solid by-product after the crystallisation process known as phytosterol resources (PSR) was collected and analysed for its phytonutrients content.

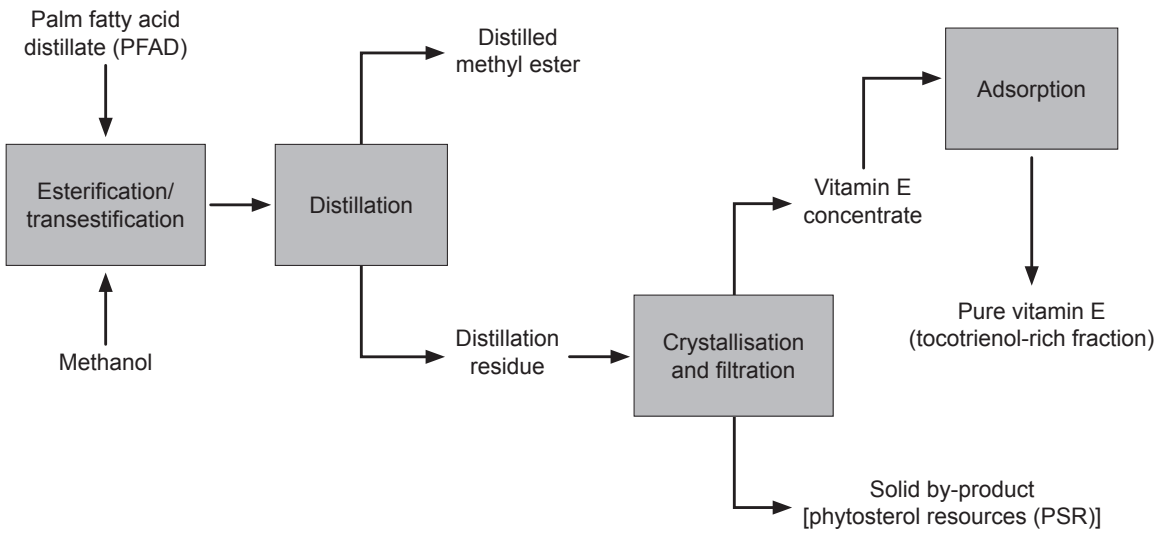

Figure 1. Extraction of vitamin E from palm fatty acid distillate (PFAD).

Extraction of Phytosterols in Laboratory Scale

A sample of 5 g PSR was mixed with 50 ml of ethanol and 2.5 g of potassium hydroxide (KOH), and then subjected to saponification reaction in 250 ml round bottom flask equipped with reflux condenser. The reaction was conducted at the ethanol reflux temperature for 1 hr. The reacted mixture was then extracted five times with hexane until a colourless organic layer was obtained. The extracted organic layer was then washed with distilled water until the neutral pH was obtained. Excess solvent was evaporated and the sample was then mixed with various solvents namely hexane, ethanol, acetone and methanol before being subjected to purification stage. A 1 g of extracted sample phytosterols-rich fraction (PSRF) added with 10 ml of solvent are heated to 60°C and then crystallised in deep freezer at temperature of -5°C for 20 hr. The formed crystal was filtered and dried in oven. The final product was expected in a form of pure phytosterols mixture.

Extraction of Phytosterols in Mini-pilot Scale Multistage Extraction Processes

Extraction of phytosterols mixture. All the PSR samples were subjected to solid-liquid extraction (SLE) process using an overflow method with two different temperatures, 35°C and 50°C. The SLE unit (SOLTEQ®) was equipped with a 20-litre solvent pot with reboiler, distillation column, distillate condenser, extraction vessel and solvent collection vessel.

About 200 g of extracted product was subjected to saponification reaction for the recovery of unsaponifiable material (USM). The extract was then mixed with 100 g KOH and ethanol (10 x sample weight), refluxed at 80°C to 90°C for 1 hr to 4 hr. The reaction was conducted in a 10-litre multipurpose glass reactor equipped with a condenser, which was purchased from Buchiglasuster, Switzerland.

The reaction mixture was then subjected to liquid-liquid extraction (LLE) by mixing the reaction mixture with hexane and water at the ratio of 10:10:1 (hexane:water:sample) in order to extract the USM. The USM extracts or known as PSRF was collected in the light phase together with hexane, leaving the water-soluble compound in the heavy phase. The process was repeated for five cycles. The LLE process was conducted in a DN50 1500 mm borosilicate column (25 litres capacity), manufactured by SOLTEQ®, Malaysia.

Purification of phytosterols mixture. The PSRF extract was subsequently subjected to crystallisation process in a 5 litre crystallisation vessel equipped with vacuum filtration (Buchiglasuster, Switzerland) in order to further purify the phytosterols present in PSR samples. Different types of solvents (hexane, ethanol, methanol and acetone) were mixed with the PSRF with solvent to PSRF ratio (volume to weight) of 10:1. Then, the mixture was heated at 60°C for 1 hr and cooled down to -5°C for 19 hr. Finally, the mixture was filtered under vacuum condition, while the temperature was maintained at -5°C during filtration. The solid and filtrate were analysed for total sterols content and quantified for product yield and recovery. The multistage extraction processes flow is illustrated in *Figure 2*.

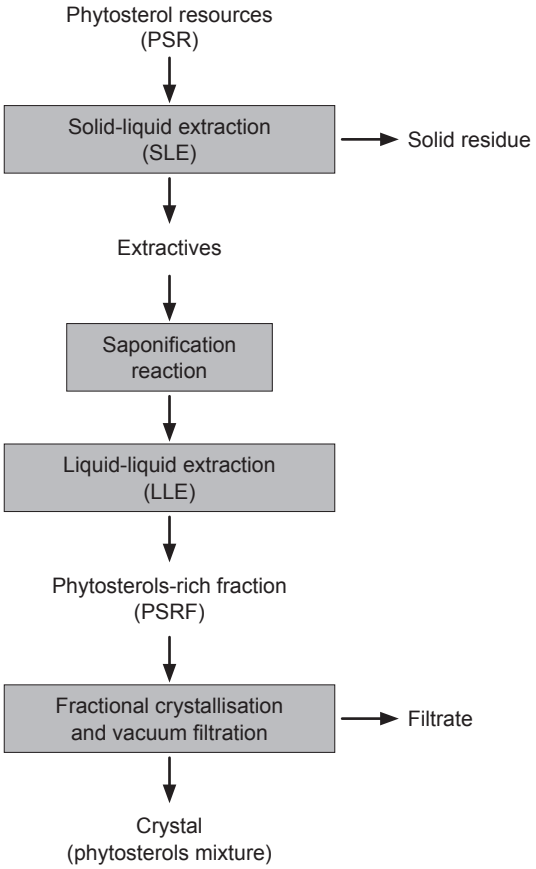

Figure 2. Multistage extraction processes of phytosterols mixture from the phytosterols resources (PSR).

Analysis of Phytonutrients

Samples preparation. All samples (except for PSRF and phytosterols mixture) were pre-treated via saponification prior to the analysis of sterols based on MPOB Test Method (MPOB, 2004). Samples weighing 5.0 g were mixed with 2.5 g 10% (w/v) KOH in an ethanolic solution and then refluxed for 1 hr at temperature between 70°C to 80°C. Then, hexane was used to extract the USM from the saponified mixture and the extracts were washed using distilled water until the neutral pH was obtained. The remaining moisture was removed using sodium sulphate ($NaSO_4$) and hexane was then removed using rotary evaporator. The USM was weighed to quantify the recovery yield as in Equation (1).

$$\text{Yield of USM recovery} = \frac{\text{Weight of PSR - Weight of dried USM}}{\text{Weight of PSR}} \times 100 \qquad \text{Equation (1)}$$

Phytosterols, squalene and vitamin E determination using gas chromatography (GC) analyser. The samples were dissolved and diluted in 100% n-hexane to an appropriate concentration. High purity samples such as PSRF and phytosterols mixture are directly dissolved in warm n-hexane without undergoing any pre-treatment. All the diluted sample were analysed using GC Autosystem XL, Perkin Elmer as a platform for the sterols separation equipped with flame ionisation detector (FID) for peak detection. The samples were separated in the capillary column purchased from Supelco SAC™-5 (Sigma) with the length of 30 m and internal diameter of 0.25 mm, bonded with a 0.25 µm film of 5% phenyl/95% dimethylpolysiloxane in the presence of helium gas as mobile phase. The initial oven temperature, maximum oven temperature and detector temperature were set at 270°C, 320°C and 270°C, respectively. Identification of compounds was achieved by comparing their retention times in GC spectra with those of standards (*Table 1*). For quantitative analysis, calibration curves were prepared by analysing different concentrations of squalene, vitamin E and sterol standards and by representing peak area versus concentration.

TABLE 1. RETENTION TIME FOR SQUALENE, VITAMIN E AND STEROLS DURING GAS CHROMATOGRAPHY-FLAME IONISATION DETECTOR (GC-FID) ANALYSIS

Components	Retention time (min)
Squalene	5.20
Vitamin E	
α-tocopherol	7.60
α-tocotrienol	8.60
γ-tocotrienol	9.30
δ-tocotrienol	10.92
Sterols	
Cholesterol	8.80
Campestrols	10.80
Stigmasterols	11.40
β-sitosterols	12.70

Fourier transform infrared (FTIR) analysis. The infrared spectrum of sterols samples was recorded using Perkin-Elmer Spectrum One FTIR Spectrometer with wavelength in the range of 4000-650 cm^{-1}. The spectrums were compared with the individual sterols standard.

Nuclear magnetic resonance (NMR) analysis. Samples were dissolved in 200 µl deuterated chloroform, shaken and placed in a 5 mm NMR capillary tube. The ^{1}H and ^{13}C experiments were performed using 600 MHz JEOL Spectrometer for compound identification [adapted from Teh *et al.* (2017)].

RESULTS AND DISCUSSIONS

Phytosterols Composition in Palm Oil and its By-products

Various oil palm by-products were analysed for their phytosterols content, The composition of extracted USM and total phytosterols content

in palm oil and its by-products are as tabulated in *Table 2*. Results showed that OPEFB residual oil and CPO have low USM content of 0.82%-0.83%. These two samples composed of only 5.48%-6.19% of phytosterols in USM, with only 450-500 ppm of total phytosterols composition in these PSR. Another component of USM might be carotene, which makes the oil reddish in colour (Rusnani *et al*., 2012; Md Yunos *et al*., 2015). Other than these two samples, the phytosterols content in SPO was recorded at 800-1100 ppm, followed by PPFO with 1700-4100 ppm phytosterols. USM recovery percentage of both samples were less than 2%. It was observed that the phytosterols composition in this SPO (800-1000 ppm) was higher than that in the slurry obtained from the heavy phase of CPO clarification tank (508.7 ppm). This might be due to the dilution of the sludge with water from CPO clarification process (Teh *et al*., 2017). Meanwhile, the SPO composed of only oil recovered and concentrated from that particular sludge. Besides, it was reported that most of USM in POME composed from carotene, squalene and vitamin E (Sangkharak *et al*., 2016; Teh *et al*., 2017). As expected, the highest phytosterols content of 20 300-141 000 ppm was found in solid by-product from vitamin E extraction process. This PSR sample contained 3.82%-15.15% USM. The phytosterols content in this PSR sample is relatively higher than palm phytonutrient concentrate produced from palm methyl ester as reported by Chandrasekaram (2009). The higher value of phytosterols in this sample was due to the fact that most of the esters and glycerides were primarily removed during the distillation process for vitamin E extraction. The remaining esters, squalene and vitamin E were also extracted in liquid fraction during the crystallisation and filtration process. This sample also had the highest total phytosterols content in USM of more than 50%, which allowed simpler purification step to obtain phytosterols as compared to other PSR samples. Besides high total phytosterols content, relatively low fatty acids content reduces the requirement of the equipment size towards the downstream stages of the extraction processes (Fernandes and Cabral, 2007).

Extraction of Phytosterols Mixture from PSR

Process for extraction of vitamin E from PFAD produced 1.50% (w/w) solid by-product. This solid by-product PSR composed of up to 15.15% USM that mostly consisted of phytosterols. This contributed to high content of phytosterols in the PSR samples amounting to 14.10%. The extraction of phytosterols from this PSR was conducted through SLE using 100% n-hexane at 35°C and 50°C. Temperature of 35°C was selected to represent the extraction at constant room temperature adapted from the maceration method (Azwanida, 2015). Warm solvent at 50°C enhanced the extraction of phytosterols. However, the temperature for extraction must be below hexane boiling point in order to avoid solvent evaporation. Solvent extraction at 35°C yielded 47% extractives (*Table 3*). The yield was increased to more than 90% when extraction temperature was set at 50°C. The extractive yield was lower but the extract contained higher USM, which consequently increased the total sterols content in the extract. Solubility of fatty acids and methyl ester in hexane is lesser at lower temperature (Calvo *et al*., 2009). Therefore, solvent temperature at 35°C is preferred for extraction of phytosterols in order to minimise the carry over of fatty acids and methyl ester in the extract. Different solvent temperatures will give different products and recovery yields due to the differences in component solubility in hexane.

After the SLE process, saponification was carried out to convert all sterol esters into free sterol and all glycerides into water-soluble fatty acid soap. The reaction was conducted in laboratory scale by varying the duration of reaction time between 1 hr to 4 hr in order to determine the highest USM recovery. It was found that as the reaction time increased, the recovery of USM reduced from 43.70% at 1 hr to 23.60% at 4 hr (*Figure 3*). The longer reaction time has continuously exposed samples to heat and it may have destroyed some phytonutrients in USM such as sterols and squalene (Lau *et al*., 2005).

TABLE 2. PHYTOSTEROLS CONTENT IN VARIOUS PALM OIL AND ITS BY-PRODUCTS

Phytosterols resources	Unsaponifiable matter recovery (%)	Phytosterols composition in unsaponifiable matter form (%)	Phytosterols composition (ppm)
Crude palm oil*	0.83	6.19	500
Palm fatty acid distillate	1.86 - 2.87	2.39 - 15.33	600 - 4 200
Palm pressed fibre oil	0.96 - 1.58	17.99 - 25.93	1 700 - 4 100
Oil palm empty fruit bunch residual oil*	0.82	5.48	450
Sludge palm oil	1.15 - 1.17	7.16 - 9.04	800 - 1 100
Solid by-product of vitamin E extraction process	3.82 - 15.15	50.35 - 93.12	20 300 -141 000

Note: *Analysis of one sample.

TABLE 3. TOTAL STEROLS AND ITS COMPOSITION DURING SOLID-LIQUID EXTRACTION

Solvent temperatures	35°C			50°C		
Extracted components	**PSR**	**Extractives**	**Solid residue**	**PSR**	**Extractives**	**Solid residue**
USM (%)	19.45	41.27	0.07	16.10	16.97	6.82
Total sterols in samples (%)	13.75	25.35	0.01	8.55	8.61	5.82
Sterols composition in USM (%)*						
Cholesterol	1.39	4.12	4.12	1.42	1.58	1.43
Campesterol	22.66	23.53	20.29	22.72	23.26	23.73
Stigmasterol	14.96	16.60	21.32	15.55	15.67	19.33
β-sitosterol	60.99	55.75	54.26	60.32	59.49	55.51
Yield (%)	-	47	53	-	91.5	8.5
Sterols recovery (%)	-	99.95	-	-	94.09	-

Note: PSR - phytosterol resources; USM - unsaponifiable matter.
*Normalised to 100%.

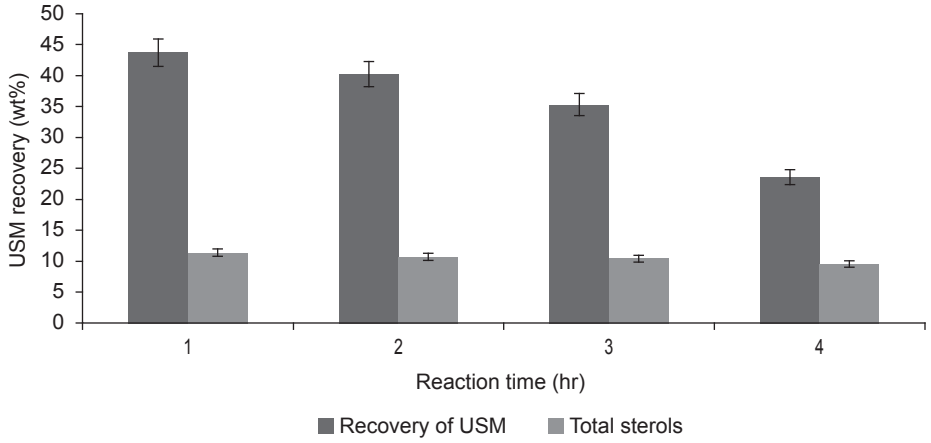

Figure 3. Effect of reaction time on the recovery of unsaponifiable matter (USM) from solid residue of vitamin E extraction.

As such, the mini-pilot test was conducted using optimised saponification reaction time of 1 hr. The amount of solvent used in mini-pilot liquid-liquid extraction was fixed at a ratio of 10:1 in order to maximise the extraction of phytosterols. The USM recovery in mini-pilot test was in the range of 14.32%-19.95% after five runs. The average sterols content was 47.64%±4.78%. Apart from phytosterols, the USM also contained squalene and traces of unreacted glycerides. Sterols and squalene have great differences in melting point and hence, solvent-assisted crystallisation process was used to isolate the phytosterols from the squalene and other USM mixtures. The separation of free phytosterols and tocopherols is usually performed through fractional crystallisation as free phytosterols tend to precipitate at a low temperature (Moreira and Baltanás, 2004).

Purification of Phytosterols Mixture

Phytosterols mixture extract was further purified using crystallisation. Crystallisation is one of the best and cheapest methods available for the purification of solids from impure solutions. There are several types of solvents and solvent mixtures suitable for the crystallisation procedure. However, a simple purification process should use only one solvent instead of mixed solvents for recovery and recycling purposes. In this study, four types of solvents have been tested in laboratory scale crystallisation; they were ethanol, methanol, acetone and hexane (*Table 4*). From this experiment, crystallisation of USM with hexane gave the highest crystal yield of 62% with pure phytosterols (100% purity), followed by ethanol and then acetone. The lowest crystal yield obtained was when methanol was used as solvent, producing 25% crystal yield with phytosterols content of 48.25%. Other solvents such as benzene, toluene and cyclohexane may give higher sterols purity, but with lower yield and highly toxic (Yan *et al*., 2011). Thus, these solvents are not desirable for crystallisation of phytosterols.

The crystallisation process was further tested at mini-plant scale, using hexane and ethanol as solvents. The yield of crystal and filtrate are tabulated in *Table 5*. The crystal yield and sterol content for mini-pilot scale were lower than that

of laboratory scale crystallisation. This was due to higher phytosterols loss in the filtrate. The purity of phytosterols obtained was also lower than that of laboratory scale extraction. However, the crude phytosterols purity was maintained at more than 80%. This was due to the presence of other impurities such as unreacted glycerides that have close melting point as sterols.

Characterisation of Phytosterols Mixture

Phytosterols mixture after the purification process was a waxy solid and white in colour. *Table 6* shows the individual sterols composition in PFAD, phytosterols mixture (phytosterols-mix) extracted in laboratory and mini-pilot scale in comparison with commercial vegetable oil sterols. It was found that β-sitosterol content in palm-based sterols is higher than commercial sterols, while stigmasterols content is lower. The β-sitosterol has advantages to treat men's health problems such as increased urinary flow rates, decreasing the amount of urine left in the bladder after urinating and improving the overall quality of life (Wilt *et al*., 1999).

Apart from GC analysis, the extracted phytosterols were further analysed using FTIR and NMR to confirm their characteristics. FTIR spectrum (*Figure 4*) showed the absorption bands at 3345.68 cm^{-1} (OH), 2920.02 cm^{-1} (CH_2) and 2850.54 cm^{-1} (CH). The absorption at 1641.6 cm^{-1} for the olefinic bond in stigmasterol was also sighted. However, the band was weakly absorbed due to the C=C stretching. A bending frequency of cyclic (CH_2) and $-CH_2(CH_3)\gamma$ were observed at 1461.81 cm^{-1} and 1377.95 cm^{-1} respectively. The absorption frequency at 1051.89 cm^{-1} and 958.79 cm^{-1} were that of trisubstituted olefin, which were usually referred to as β-sitosterol and stigmasterol (Jamaluddin *et al*., 1994).

The ^{13}C NMR spectrum (*Figure 5*) shows that the presence of the compound in phytosterols mixture is in a form of steroid skeleton due to similar chemical shift obtained as compared in the literature (Jain and Bari, 2010; Jamaluddin *et al*., 1994). The main difference between the three main sterols is the presence of double bond at C22=C23 in stigmasterols with a chemical shift at 138.404 and 129.247 ppm, while β-sitosterols and campesterol both have a chemical shift at 34.020 and 26.150 ppm corresponding to C22-C23 single bond. The ^{13}C NMR spectrum for campesterol and β-sitosterols were identical, thus, the presence of those two compounds were confirmed.

Based on mini-pilot plant trial, it was estimated that one tonne of PFAD will produce 15 kg by-product of PSR during the vitamin E extraction process. After multistage extraction process, 1.08 kg phytosterols mixture with average purity of 87.23% was obtained with overall sterols recovery from the PSR of about 84%. Overall mass balance on the multistage production of phytosterols mixture from PFAD is given in *Figure 6*.

TABLE 4. PURIFICATION OF PHYTOSTEROLS-RICH FRACTION (PSRF) THROUGH CRYSTALLISATION WITH DIFFERENT TYPES OF SOLVENTS IN LABORATORY SCALE

Solvent type	Samples	Sterols composition (%)				Total sterols (%)	Yield (%)
		Cholesterol	Campesterol	Stigmasterol	β-Sitoterol		
Ethanol	Crystal	7.02	18.58	16.37	58.03	93.92	43
	Filtrate	0.00	24.32	0.00	75.68	22.24	
Methanol	Crystal	10.20	3.42	28.81	57.57	48.25	25
	Filtrate	12.57	23.29	0.00	64.14	11.41	
Acetone	Crystal	5.39	17.32	18.31	58.98	100.00	32
	Filtrate	11.12	29.06	19.62	40.20	22.19	
Hexane	Crystal	4.87	19.18	17.16	58.78	100.00	62
	Filtrate	8.47	20.43	0.00	71.11	32.97	

TABLE 5. PURIFICATION OF PHYTOSTEROLS-RICH FRACTION (PSRF) THROUGH CRYSTALLISATION IN MINI-PILOT SCALE

Solvent type	Samples	Phytonutrient composition (%)				Yield of phytosterols mixture (%)
		Sterol	Squalene	Vitamin E	Others	
Ethanol	Crystal	84.47	0.21	N.D	18.32	37
	Filtrate	68.40	20.97	N.D	10.63	
Hexane	Crystal	87.23	0.66	N.D	12.11	47
	Filtrate	59.62	13.40	N.D	26.98	

Note: N.D - not detected.

TABLE 6. INDIVIDUAL STEROLS COMPOSITION IN PALM FATTY ACID DISTILLATE (PFAD), PHYTOSTEROLS MIXTURE FROM PFAD AND COMMERCIAL STEROLS

Sample/ Material	Individual sterols compositions (%)				Total sterols (%)
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol	
PFAD	3-9	23-25	13-14	53-60	0.2 – 0.4
Phytosterol-mix (laboratory)	1-5	19-22	17-20	57-59	84 - 100
Phytosterol-mix (mini-pilot plant)	<2	20-22	13-20	59-63	84 - 94
Commercial sterol*	<3**	20-28	16-23	40-58	>90

Note: *Corowise™ sterols. **Other minor sterols.

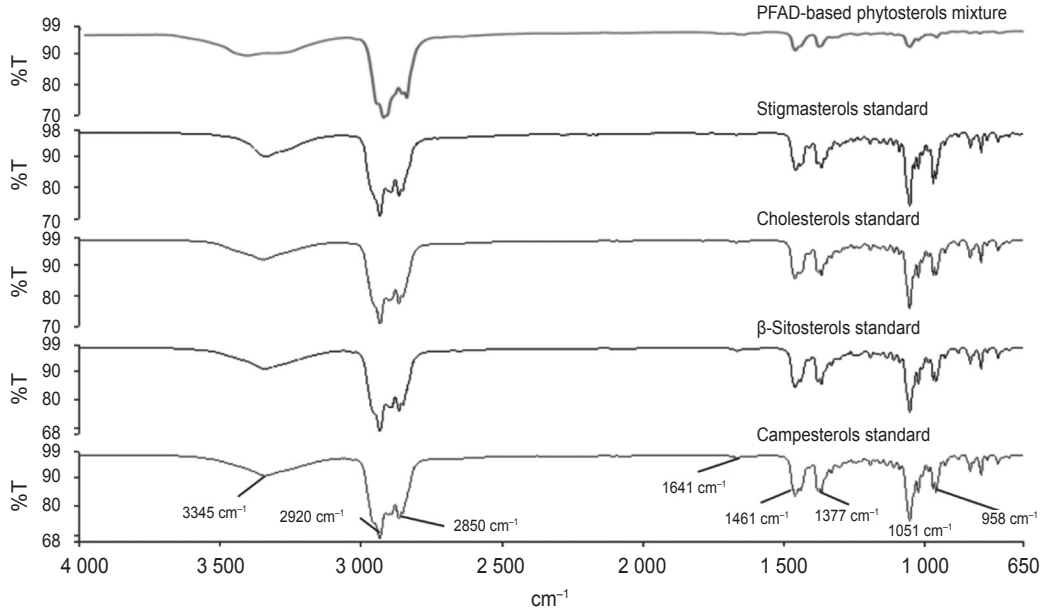

Figure 4. IR spectrum of stigmasterols, β-sitosterols, campesterols and cholesterol standard and phytosterols mixture extracted from palm fatty acid distillate (PFAD).

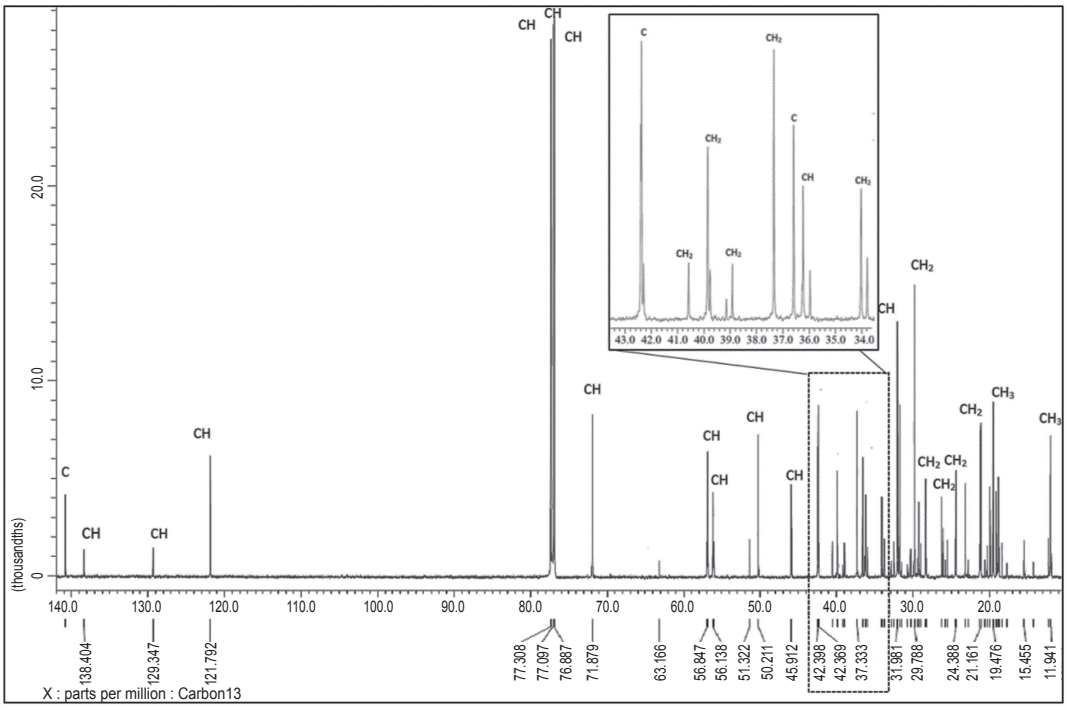

Figure 5. The ^{13}C NMR spectrum of phytosterols mixture extracted from palm fatty acid distillate (PFAD).

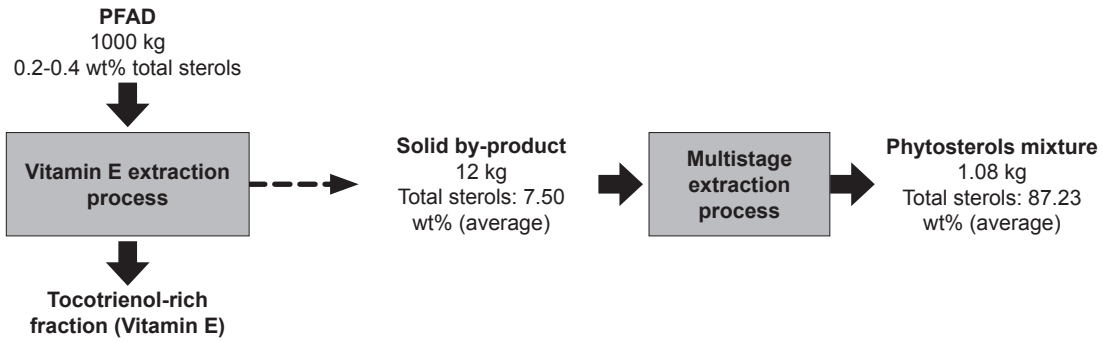

Figure 6. The overall mass balance on the multistage production of phytosterols mixture from palm fatty acid distillate (PFAD).

CONCLUSION

Extraction and purification of phytosterols from solid residue obtained after vitamin E extraction from PFAD have been successfully conducted using multistage extraction processes in laboratory scale and mini-pilot scale. Technically, the multistage extraction and purification methods in mini-pilot scale which comprised of SLE, saponification reaction, LLE, crystallisation and filtration, were capable to produce phytosterols mixture with purity of up to 94% (w/w) with individual sterols compositions of β-sitosterol (21%-22%), campesterol (13%-20%) and stigmasterol (59%-64%). The overall recovery for sterol from the PSR was 84%. FTIR, NMR and GC analysis confirmed the presence of phytosterols in the extract. This extraction process is technically feasible to extract and produce crude phytosterols from a PFAD by-product and the extraction of this minor component will adds value to the oil palm industry.

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REFERENCES

Ab Gapor, M T (2010). Production and utilization of palm fatty acid distillate (PFAD). *Lipid Technol.,* 22: 11-13.

Ab Gapor, M T; Leong, W L; Augustine, O S H; Kawada, T; Watanabe, H and Tsuchiya, N (1993). Production of high concentration tocopherols and tocotrienols from palm oil by-products. US patent No. 5 190 618.

Akgun, N A (2011). Separation of squalene from olive oil deodorizer distillate using supercritical fluids. *Eur. J. Lipid Sci. Technol*., *113*: 1558-1565.

Awad, A B and Fink, C S (2000). Phytosterols as anticancer dietary components: Evidence and mechanism of action. *J. Nutr., 130*: 2127-2130.

Azwanida, N N (2015). A review on the extraction methods use in medicinal plants, principle, strength and limitation. *Med. Aromat. Plants, 4*: 196.

Calvo, B; Collado, I and Cepeda, E A (2009). Solubilities of palmitic acid in pure solvents and its mixtures. *J. Chem. Eng. Data, 54*: 64-68.

Chandrasekaram, K (2009). *Analysis of Phytonutrients from Palm Concentrates by High Performance Liquid Analysis of Phytonutrients from Palm Concentrates by High Performance Liquid*. M.Sc thesis. Universiti Malaya.

Choo, Y M; Lau, H L N; Ma, A N and Yusof, B (2005a). Extraction of palm vitamin E, phytosterols and squalene from palm oil. US patent No. 20050250953A1.

Devaraj, S and Jialal, I (2006). The role of dietary supplementation with plant sterols and stanols in the prevention of cardiovascular disease. *Nutr. Rev., 64*: 348-354.

Fernandes, P and Cabral, J M S (2007). Phytosterols: Applications and recovery methods. *Bioresour. Technol., 98*: 2335-2350.

Jain, P S and Bari, S B (2010). Isolation of lupeol, stigmasterol and campesterol from petroleum ether extract of woody stem of *Wrightia tinctoria. Asian J. Plant., 9*: 163-167.

Jamaluddin, F; Mohamed, S and Lajis, N (1994). Hypoglycaemic effect of *Paria speciosa* seeds due to the synergistic action of β-sitosterol and stigmasterol. *Food Chem., 49*: 339-345.

Jones, P J H; Ntanios, F Y; Raeini-sarjaz, M and Vanstone, C A (1999). Cholesterol-lowering efficacy of a sitostanol-containing phytosterol mixture with a prudent diet in hyperlipidemic men. *Am. J. Clin. Nutr., 69*: 1144-1150.

Kowalski, R E (2017). Plant sterols: A natural way to lower cholesterol. http://www.corowise.com/articles/07 plantsterols.html., accessed on 14 December 2017.

Kushairi, A; Loh, S K; Elina, H; Meilina, O; Mohd Noor Izuddin, Z B; Razmah, G; Sundram, S and Parveez, G K A (2018). Oil palm economic performance in Malaysia and R&D progress in 2017. *J. Oil Palm Res. Vol. 30(2)*: 163-195.

Lau, H L N; Puah, C W; Choo, Y M; Ma, A N and Chuah, C H (2005). Simultaneous quantification of free fatty acids, free sterols, squalene, and acylglycerol molecular species in palm oil by high-temperature gas chromatography – Flame ionization detection. *Lipids, 40*: 3-8.

Lau, H L N; Choo, Y M; Ma, A N and Chuah, C H (2008). Selective extraction of palm carotene and vitamin E from fresh palm-pressed mesocarp fiber (*Elaeis guineensis*) using supercritical CO_2. *J. Food Eng., 84*: 289-296.

Massimo, L; Laura, D and Ginevra, L B (2019). Phytosterols and phytosterol oxides in Bronte's pistachio (*Pistacia vera L.*) and in processed pistachio products. *Eur. Food Res. Technol*., *246*: 307-314.

Md Yunos, N; Samsu Baharudin, A; Md Yunos, K; Hafid, H; Busu, Z; Mokhtar, M and Md Som, A (2015). The physicochemical characteristics of residual oil and fibers from oil palm empty fruit bunches. *BioResources, 10*: 14-29.

Moreau, R A; Nyström, L; Whitaker, B D; Winkler-Moser, J K; Baer, J; Gebauer, S K and Hicks, K B (2018). Phytosterols and their derivatives: Structural diversity, distribution, metabolism, analysis, and health-promoting uses. *Prog. Lipid Res., 70*: 35-61.

Moreira, E A and Baltana´s, M A (2004). Recovery of phytosterols from sunflower oil deodorizer distillates. *J. Amer. Oil Chem. Soc., 81*: 161-167.

MPOB (2004). *MPOB Test Methods*. MPOB, Bangi. 395 pp.

Ng, M H and Choo, Y M (2013). Isolation and recovery of phytonutrients in palm by isocrated and isobaric flash chromatography. *J. Oil Palm Res. Vol. 25*: 165-169.

Noorshamsiana, A W; Astimar, A A; Iberahim, N I; Nor Faizah, J; Anis, M; Hamid, F A and Kamarudin, H (2017). The quality of oil extracted from palm pressed fibre using aqueous enzymatic treatment. *J. Oil Palm Res. Vol. 29(4)*: 588-593.

Norhidayah, S; Baharin, B S; Hamed, M and Zaidul, I S M (2012). Squalene recovery from palm fatty acid distillate using supercritical fluid extraction. *Int. Food Res. J., 19*: 1661-1667.

Phoon, K Y; Ng, H S; Zakaria, R; Yim, H S and Mokhtar, M N (2018). Enrichment of minor components from crude palm oil and palm-pressed mesocarp fibre oil via sequential adsorption-desorption strategy. *Ind. Crops Prod., 113*: 187-195.

Rusnani, A M; Mohammad, A W and Choo, Y M (2012). Properties of residual palm pressed fibre oil. *J. Oil Palm Res. Vol. 24*: 1310-1317.

Sangkharak, K; Pichid, N and Yunu, T (2016). Separation of carotenes and vitamin e from palm oil mill effluent using silica from agricultural waste as an adsorbent. *Walailak J. Sci. and Tech., 13*: 939-947.

Sugihara, N; Kanda, A; Nakano, T; Nakamura, T; Igusa, H and Hara, S (2010). Novel fractionation method for squalene and phytosterols contained in the deodorization distillate of rice bran oil. *J. Oleo. Sci., 59*: 65-70.

Tasan, M; Bilgin, B; Gecgel, Ü and Demirci, A Ş (2006). Phytosterols as functional food ingredients chemical structures of phytosterols. *J. Tekirdag Agric. Fac., 3*: 153-159.

Teh, S S; Hock Ong, A S and Mah, S H (2017). Recovery and utilization of palm oil mill effluent source as value-added food products. *J. Oleo Sci., 66*: 1183-1191.

Wilt, T J; Ishani, A; MacDonald, R; Stark, G; Mulrow, C D and Lau, J (1999). Beta-sitosterols for benign prostatic hyperplasia. *Cochrane Database of Systematic Reviews 1999 Issue 3*. Art. No. CD001043. DOI: 10.1002/14651858.CD001043.

Yan, F; Yang, H, Wu, D; Huo, M and Li, X (2011). Recovery of phytosterols from waste residue of soybean oil deodorizer distillate. *Soybean - Applications and Technology* (Ng, T ed.). p. 329-340..

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POST-TREATMENT OF PALM OIL MILL EFFLUENT USING ZEOLITE AND WASTEWATER

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ABSTRACT

The palm oil mill effluent (POME) is one of the most important ecosystems hazard and can become a crucial environmental burden if discharged without any treatment to nature. The present study aimed to develop a fast method for post-treatment of POME. To enhance treatment process, the domestic wastewater (DWW) and zeolite were added to the sequencing batch reactor (SBR) as the available microbial source and new adsorbent, respectively. The results indicate that the chemical oxygen demand (COD), biochemical oxygen demand (BOD), total suspended solids (TSS), ammonia nitrogen (AN) and colour removal rates were in the range of 95.34%-98.31%, 88.79%-91.44%, 95.47%-98.95%, 96.19%-98.30% and 56.94%-81.64%, respectively. Moreover, SBR with both DWW and zeolite addition was able to remove high percentage of all pollution compared to only DWW addition (with a removal percentage of 77% TSS, 74% COD, 76% colour and 90% AN) or zeolite (23% TSS, 10% COD, 9.6% colour and 80% AN). The response surface methodology (RSM) was used to elucidate response surface and optimise the independent variables. The highest desirability of POME treatment (0.988) was achieved in optimum operation conditions. Under these conditions, COD, BOD, colour, AN and TSS removal rates were 96.80%, 90.1%, 69.90%, 98.20% and 97.20%, respectively.

Keywords: zeolite, wastewater, palm oil mill effluent, adsorption, sequencing batch reactor.

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INTRODUCTION

The industry of palm oil has rapidly developed over the last decades. In Malaysia, the palm oil is considered as a major part of agro-economy with a production of 39% in the world (Moradi *et al*., 2015; Wong *et al*., 2015). However, a great amount of palm oil mill effluent (POME) is generated due to high production of palm oil. Ng *et al*. (2012) indicated that with production of 94 million tonnes of fresh fruit bunch (FFB), more than 60 million tonnes POME is produced. The POME with high total solids (43 635 mg litre^{-1}), average chemical oxygen demand (COD) and biochemical oxygen demand (BOD) of 70 000 and 30 000 mg litre^{-1}, respectively can be considered as the main reason for ecosystem and environmental hazard if it is discharged without any treatment (Chan *et al*., 2012; Ma *et al*., 1993). Liew *et al*. (2015) showed that COD, BOD, total suspended solids (TSS), and colour are the most serious contaminants in POME. A range of BOD/COD ratio between 0.29 and 0.62 indicates that the POME is a highly polluted agro-industry

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wastewater that should be treated using biological methods (Mohan and Karthikeyan, 1997; Chan *et al*., 2010; Vijayaraghavan *et al*., 2007; Metcalf and Eddy, 2014; Khemkhao *et al*., 2015).

Generally, several treatment systems have been applied to improve POME quality such as sequencing batch reactor (SBR), up-flow anaerobic sludge bioreactor, added chemical and biochemical productions, enhanced waste stabilisation ponds (aerated lagoon), membrane bioreactor, waste stabilisation pond, extended aeration activated sludge and membrane filters (Aziz *et al*., 2011a, b). However, the open tank digester and ponding methods are the most popular and commonly used systems to treat POME. Although the ponding systems were suggested as the cost-effective techniques for treatment of POME, several disadvantages were observed in these systems such as the big size of the digester, off odour, long treatment duration, insufficient effluent quality, colour, large variation in effluent quality, and large footprint. In order to overcome the problems and improve POME treatment, a high-rate anaerobic bioreactor system has been recommended with smaller foot print and high efficiency such as up-flow anaerobic sludge bioreactor (Najafpour *et al*., 2006), improved anaerobic baffled bioreactor (Setiadi and Husaini, 1996; Faisal and Unno, 2001) and anaerobic fluidised bed bioreactor (Borja and Banks, 1995). Yacob *et al*. (2006) reported that compared to conventional methods, the anaerobic bioreactors remove more pollution at shorter hydraulic retention time and provide better treatment efficiencies.

Although, the anaerobic pond is one of the most commonly used methods for POME treatment, the effluent of this method can hardly fulfil the standard level of discharge defined by the Malaysian Department of Environment (DOE) (Chan *et al*., 2010). In order to meet the DOE levels, an appropriate post-treatment is essential for POME discharge. Ho and Tan (1983) reported that the aerobic technologies should be applied as a post-treatment system to reduce pollution of POME within the standards rate. Nasrullah *et al*. (2017) proposed a post-treatment method for colour removal of POME using electrocoagulation. They reported that this method was effective and slightly expensive for POME treatment. Moreover, phytoremediation was recommended as another laboratory-scale and plant-based post-treatment that is often used for anaerobically treated and diluted POME (Darajeh *et al*., 2014). For treatment of the industrial and municipal wastewater, the anaerobic-aerobic processes using reactors have been recommended as an effective method with less sludge production, low energy and higher treatment efficiency (Jenícek *et al*., 1999; Garbossa *et al*., 2005; Chan *et al*., 2010; 2011). Although, the conventional anaerobic-aerobic methods have been able to successfully treat the POME, their low organic loading rate (OLR), long hydraulic retention time (HRT), and large space requirement are the disadvantage of these methods. Chou *et al.* (2016) reported that only a few studies are available in literature related to aerobic post-treatment of anaerobically digested POME and more effort is required to understand the performance of these treatments.

Since the conventional method have no high potential to treat POME, various methods have been proposed to solve this problem such as adsorption, advanced oxidation processes, membrane filtration and coagulation/flocculation. Recently, SBR with a simple configuration of the tank was recommended as a new system with the low-space requirement and effective technique for treatment of industrial and municipal wastewater (Aziz *et al*., 2012). Ahmad *et al*. (2003) indicated that flocculation is one of the effective method to remove turbidity in POME. Moussavi *et al.* (2011) showed that zeolite has high ability in coagulation and flocculation of suspended particle. Recently, SBR with a simple configuration of the tank was recommended as a new system with the low-space requirement and effective technique for treatment of industrial and municipal wastewater (Aziz *et al*., 2012). Compared to other biological treatment methods, the SBR has greater process flexibility to treat wastewater and leachate (Lim *et al*., 2014; Mojiri *et al*., 2014). This treatment is highly recommended as a cost-effective eco-friendly green technology. As shown in *Table 1*, the SBR with high efficiency in COD, TSS, and BOD_5 removal has been employed for POME treatment in previous studies. Moreover, the SBR with activated sludge has been used frequently in a laboratory scale for POME aerobic treatment (Chin *et al*., 1987; Ma and Ong, 1988; Chin *et al*., 2013; Chou *et al*., 2016). Although the efficiency of the SBR method is close to DOE standards, operational cost, activated sludge requirement, and extended period of treatment are drawbacks of this system for POME treatment (Chan *et al*., 2011). Therefore, a cost-effective and fast method is necessary to improve removal efficiency of aerobic SBR system.

The raw POME, acidic media with a temperature of 80°C-90°C (Chin *et al*., 2013), will cause low content of microbial population. However, the low biodegradability of raw POME makes the digestion process longer and decreases pollutant removal efficiency (Oswal *et al*., 2002). Zahrim *et al*. (2009) indicated that the environmental adaptation of microorganisms supplied from activated sludge is a time-consuming process with an average of 60 days. Moreover, low efficiency and high energy consumption were reported when extending the period of treatment (Fun *et al*., 2007; Vijayaraghavan *et al*., 2007; Chou *et al*.,

TABLE 1. EFFECTIVENESS OF THE SBR TREATMENT FOR POME

Parameters	Fu (2007)	Chin and Ma (1987)	Zahrim *et al.* (2009)	Chan (2010)	Chan *et al.* (2011)	Chou *et al.* (2016)
Post-treatment	SBR activated sludge	SBR activated sludge	SBR activated sludge	SBR activated sludge	SBR activated sludge	SBR activated sludge*
Pre-treatment	Anaerobic digested	Adsorbent pre-treated	Anaerobic digested	Anaerobic digested	Anaerobic digested	Anaerobic digested
Influent COD (mg litre^{-1})	-	1 550	1 141	13 650	13 532	10 030
Influent BOD_5 (mg litre^{-1})	-	700	-	1 355	1 355	-
Biodegradability	-	0.45	-	0.1	0.1	-
COD removal (%)	82	31-50	70	95-96	63-86	75-93
BOD_5 removal (%)	-	50-70	-	97-98	65-87	-
TSS removal (%)	62	-	-	98-99	79.2-89.1	81-95
Colour removal (%)	-	50	41	-	-	-
Temperature (°C)	-	42	-	28	50	30
pH	-	-	7.7 - 8.3	7.4	7.4	7.7
Treatment time	14 days	25 days	60 days	30 days	> 11 days	7.2-18 days

Note: *Equal inoculum used as activated sludge.
SBR - sequencing batch reactor; COD - chemical oxygen demand; BOD - biochemical oxygen demand; TSS - total suspended solids; POME - palm oil mill effluent.

2016). Many studies reported that a cost-effective natural adsorbent can be used for enhancing the efficiency of POME decontamination and biological treatment of domestic wastewater (DWW) (Aziz *et al*., 2011b; 2012; Ismail *et al*., 2013). The combination of municipal wastewater for enhancing microbial degradation of pollutants, such as BOD_5, COD, TSS and AN, is reported frequently for landfill leachate (Mojiri *et al*., 2014; Aziz *et al*., 2011a, b). The POME is a non-toxic agricultural effluent with an extremely high concentration of BOD_5, COD, TSS and colour and low concentration of microbial community. Therefore, municipal or DWW as a low cost material with high microbial source can be used for the treatment of POME. However, a gap of knowledge can still be founded in the literature, particularly to decrease time and cost of POME treatment in SBR system as well as activated sludge requirement (Mansor *et al*., 2017).

The aim of this study is to develop a fast method to treat POME and improve removal efficiency of aerobic SBR technique. The DWW and zeolite were employed as an available microbial source and new adsorbent, respectively to enhance the SBR treatment process. To achieve a high efficiency of aeration, a new aeration system was developed for the SBR through a couple bulb bottom with opposite direction of aeration. In this reactor, the couple bottom aeration was employed for both mixer and oxygen supplier. The removal of COD, BOD, TSS, AN and colour were measured to evaluate removal efficiency of the suggested technique. The central composite design (CCD) and response surface methodology (RSM) were used to elucidate response surface and optimise the independent variables including contact time, aeration rate and DWW/POME ratio, as well as their dependent variables.

MATERIALS AND METHODS

POME Sampling

In the present study, eight raw POME samples were collected during 15 October 2014 to 18 May 2015 from an anaerobically ponding treatment system of United Oil Palm (UOP) located at 100° 30′ 27.90″ E and 5° 9′ 13.63″ N. As shown in *Figure 1*, the pond number 8 which is located at the end of the POME treatment system (before algae pond), was selected for the data sampling. The collected samples were transported to a cooling room with temperatures of 4°C to minimise the chemical and biological reactions. The characteristics of the collected POME are listed in *Table 2*.

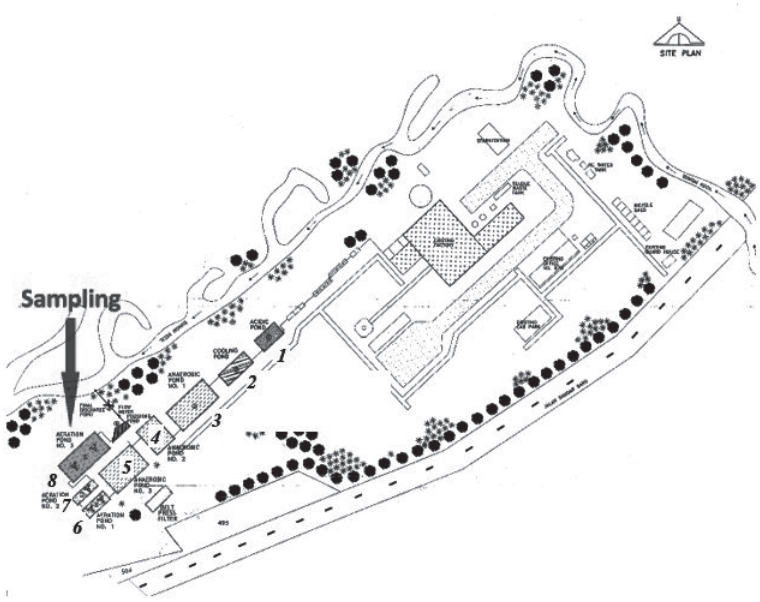


Figure 1. Anaerobically treated palm oil mill effluent (POME) sampling (pond number).

TABLE 2. CHARACTERISTICS OF POME, DWW AND STANDARD DISCHARGE LIMIT FOR POME

Parameters	POME			DWW			Standard discharge limit for POME
	Min	Max	Ave	Min	Max	Ave	
Temperature	28.5	32	30.2	29	31	29.3	45.0
pH	6.7	7.6	7.32	6.6	7	6.8	5.0-9.0
Total suspended solids (mg litre^{-1})	2 890	4 570	4 310	4.7	5.2	5.0	200.0
Colour (ADMI)	1 750	3 350	2 550	68	75	77	100.0
BOD_5 (mg litre^{-1})	995	1 685	1 230	91	102	100	20.0
COD (mg litre^{-1})	8 225	13 555	11 090	225	260	240	50*
BOD_5/COD	0.1	0.116	0.11	0.38	0.46	0.41	-
Total phosphorus (mg litre^{-1})	97	153	124.5	7.2	9.8	9	-
Ammonia nitrogen (AN) (mg litre^{-1})	82	107	94.5	11	21	14	10.0
Total nitrogen (mg litre^{-1})	275	440	360	16.8	30.5	22.9	10.0
Total iron (mg litre^{-1})	0.34	0.55	0.4	1.39	1.55	1.4	-
Total manganese (mg litre^{-1})	135	153	146.5	5.5	5.8	5.7	-
Total calcium (mg litre^{-1})	38	43	40.9	18.5	23.2	22.1	-
Turbidity (NTU)	4362	790	6012	8	13.3	11.9	-

Note: *Requirement set by the Malaysia Sewage and Industrial Effluent Discharge Standard, Deparment of Environment (DOE).
POME - palm oil mill effluent; DWW - domestic wastewater; BOD - biochemical oxygen demand; Ave - average; ADMI - American Dye Manufacturers Institut; COD - chemical oxygen demand; NTU - nephelometric turbidity units.

Domestic Wastewater (DWW) Sampling

DWW was collected from the Indah Water Konsortium Regional Treatment Plant located at 100° 27′ 10.8″ E and 5° 20′ 25.5″ N. The samples were directly send to a cool room (4°C) and kept in a white high density polyethylene (HDPE) container. Before using the samples, they were shaken until their temperature reached the laboratory temperature (31.2°C). The characteristics of DWW and POME standards discharge suggested by DOE is shown in *Table 2*.

Reactor Characteristics

In this research, a new SBR (new-SBR) was developed to improved removal efficiency of traditional reactors. The new-SBR was composed of a transparent column and a plexiglas plate to observe the settling process. To achieve a high efficiency of aeration, a novel system was developed for aeration of reactors through a couple bulb bottom with opposite direction of aeration. In the new-SBR, the couple bottom aeration was used for both mixer and oxygen supplier (*Figure 2*). No

need for any mixing system can be considered as a novelty of the new-SBR. Twenty of the new-SBR reactor were created for experiments and they run at the same time under the same environmental conditions. Aeration was prepared using two air pumps (air volume, 60 litre min^{-1}; LP-60A Model, Yasunaga, Air Pump Inc., China) where each pump was connected to 10 new-SBR using a valve. To adjust the flow rate, a manual air flow meter was used. Easy application and can be simultaneously run is the attraction of this new system.

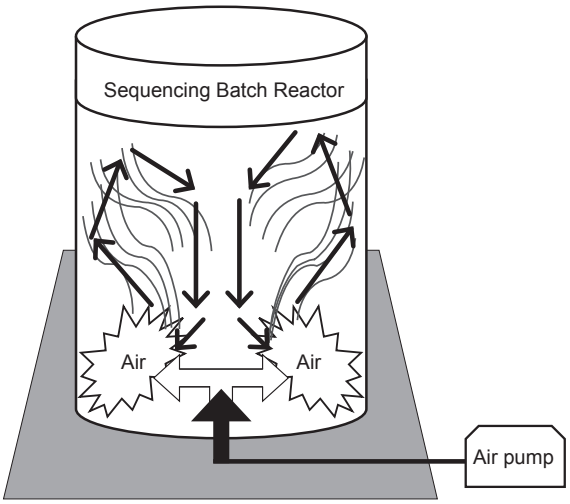


Figure 2. New-sequencing batch reactor without a mixer system.

Zeolite Preparation

In the present study, powdered natural zeolite (PNZ) was used as an adsorbent in the new-SBR. The sieves No. 100 and 200 were chosen for all experiment to provide the suitable powder PNZ in range of 75-150 µm (Mojiri *et al*., 2014). To prepare zeolite for each treatment, at first, 15 g $litre^{-1}$ of zeolite was dried in the oven for 24 hr at 105±2°C. *Table 3* shows the features of the PNZ with the autosorb test. *Figure 3* and *Table 4* indicate the results of the X-ray fluorescence (XRF) and scanning electron microscope (SEM) analyses for PNZ, respectively. The SEM affords a magnified 3D view of natural zeolite surface with a great depth of focus. The SEM images from the surface of zeolite with magnified of 10 000 and 20 000 is shown in *Figure 3*. The pores on the surface of zeolite is a good reason for low density of zeolite and indicates that it is a suitable adsorbent with high-quality.

The energy dispersive X-ray spectroscopy (EDS) was carried out to illustrate the structural compound and elements existing in the zeolite. *Table 5* shows the weight and atomic percentage of natural zeolite elements before treatment. The result indicates that zeolite is an aluminosilicate mineral with a low carbon content in its structure. Furthermore, the Si/Al with a value of 3.41 (19.52/5.71=3.41, *Table 5*) is within the range of 2.7 to 5.3, therefore the natural zeolite applied in this study can be considered as Clinoptilolite (Kesraoui-Okui *et al*., 1994). The percentages of the different structural elements of the zeolite were also determined from the spectrum diagram in *Figure 4*. The spectrum of the natural zeolite shows sharp peaks without shoulders and separated elements in a thin basement line. The results indicate that the carbon content in the zeolite is very low with a little impurity of unknown elements.

TABLE 3. POWDERED NATURAL ZEOLITE CHARACTERISTICS

Parameters	Unit	Value
Single point	$m^2 g^{-1}$	23.76
Multi point BET	$m^2 g^{-1}$	23.88
Langmuir surface area	$m^2 g^{-1}$	36.10
T method micro pore surface area	$m^2 g^{-1}$	10.03
T method external surface area	$m^2 g^{-1}$	13.85
Total pore volume for pore	ml g^{-1}	0.0052

Note: BET – Brunauer-Emmet-Teller.

TABLE 4. RESULTS OF X-RAY FLUORESCENCE (XRF) FOR ZEOLITE

Compounds	Percentage
Silicon dioxide (SiO_2)	73.30
Aluminium oxide (Al_2O_3)	16.69
Calcium oxide (CaO)	2.59
Potassium oxide (K_2O)	2.54
Iron (III) oxide (Fe_2O_3)	2.17
Sodium oxide (Na_2O)	0.52
Magnesium oxide (MgO)	1.53
Titanium dioxide (TiO_2)	0.24
Manganese oxide (MnO)	0.10
Others	0.32

TABLE 5. WEIGHT AND THE ATOMIC PERCENTAGE OF NATURAL ZEOLITE ELEMENTS (SEM-EDS)

Element	Weight % before treatment	Atomic % before treatment
Oxygen (O)	56.72 ± 0.72	66.94
Silicon (Si)	29.04 ± 0.67	19.52
Carbon (C)	3.92 ± 0.44	6.16
Aluminium (Al)	8.16 ± 0.42	5.71
Magnesium (Mg)	2.15 ± 0.26	1.67
Total	100.00	100.00

Note: SEM – scanning electron microscope; EDS – energy dispersive X-ray spectroscopy.

Analytical Method

In this study, the standard methods of the American Public Health Association (APHA) 2017 were used for all tests of wastewater and water. A Multiprobe system of YSI 556 was used to record the total dissolved solids (TDS, mg $litre^{-1}$), pH, and dissolved oxygen (DO, mg $litre^{-1}$).

A spectrophotometer (DR 2800, 2100 N and DR 2500 HACH) was used to determine the suspended solids (mg litre^{-1}), contents of colour (Pt. Co.), total phosphorus (mg litre^{-1}), total nitrogen (mg litre^{-1}), AN (mg litre^{-1}), nitrate (mg litre^{-1}), nitrite (mg litre^{-1}), and COD (mg litre^{-1}). The inductively coupled plasma (ICP Varian, OES 715) was utilised to determine the concentration of metallic elements, such as iron (Fe, mg litre^{-1}), magnesium (Mg, mg litre^{-1}), and calcium ($CaCO_3$, mg litre^{-1}).

The New-SBR Operation

The new-SBR system involves the following phases: fill, react, settling, and draw and idle. The durations of fill and mix (20 min), settling (156 min), and draw and idle (8 min) were considered to be fixed in all of the experiments. Various aeration rates of 0.5, 4 and 7.5 litres min^{-1}; different duration for contact times (2 hr, 12 hr and 22 hr); and different rates of DWW to POME (DWW/POME) such as 20%, 50%, and 80% were applied to evaluate treatment of the new-SBR. Twenty Plexiglas beakers with a working volume of 1 litre and a final volume of 1700 ml were used to avoid errors caused by operational or environmental factors. As mentioned, the developed new-SBR was used in all of the 20 reactors. Based on the preliminary experiments and before aeration, 15 g litre^{-1} PNZ was used in each new-SBR for the adsorption of pollutants. The pollution parameters such as BOD, TSS, COD, AN and colour were measured after and before the treatment process. The Equation (1) was used to determine the removal efficiency of the new-SBR systems:

$$Removal\ (\%) = \frac{C_i - C_f}{C_i} \times 100 \qquad \text{Equation (1)}$$

where C_i is initial and C_f is final concentration of pollution parameters.

Data Analysis and Experimental Design

To determine the optimum conditions for the independent parameters and demonstrate the nature of the response surface in the experimental design, both CCD and RSM were utilised in this study. The Design Expert software was used to estimated CCD

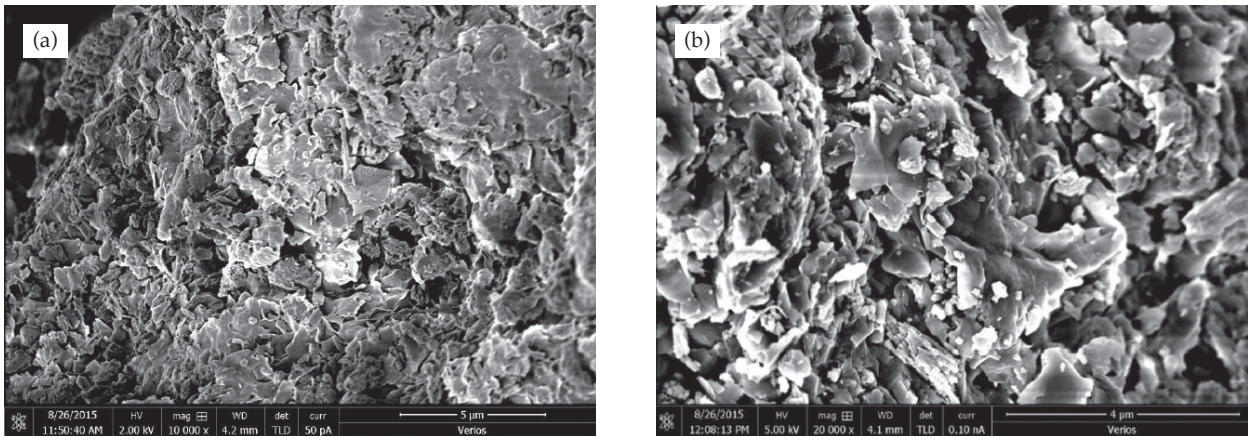


Figure 3. Scanning electron microscope (SEM) images from the surface of zeolite with magnified of (a) 10 000 and (b) 20 000.

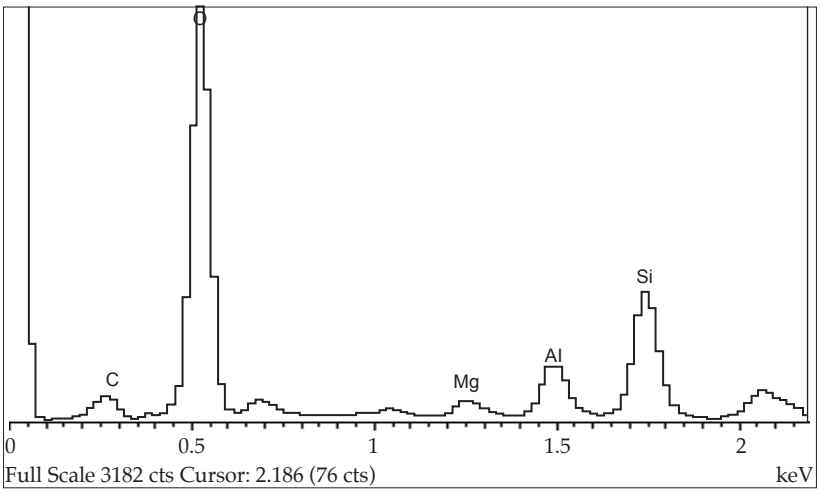


Figure 4. Natural zeolite spectrum (energy dispersive X-ray spectroscopy of the plotted area) before treatment.

and RSM. The polynomial equation with second-order, as expressed in Equation (2), was selected to evaluate the system performance:

$$Y = \beta_0 + \Sigma_{i=1}^{k} \beta_i X_i + \Sigma_{i=1}^{k} \beta_{ii} X^2 + \Sigma_{i<j}^{k} \Sigma_{j}^{k} \beta_{ij} X_i X_j + \cdots + e \quad \text{Equation (2)}$$

where Y represents the response; the variables are represented by X_i and X_j; β_0 represents a constant coefficient; numbers of studied factors are represented by k; the linear quadratic and second-order coefficients are represented by β_j, β_{ij}, and β_{ii}; and error is represented by e. The provided results were evaluated through analysis of variance (ANOVA) technique. As mentioned, k is the number of variables; thus, k^2 is equal to the factorial point supported by a centre point and $2k$ axial points. To fit the second-order polynomial models and calculate the experimental error, six replicates were used at the central points. Three levels were chosen to evaluate the three operating variables namely, high (+1), medium (0), and low (−1). The RSM and CCD were applied to determine the best value of responses and for optimising the appropriate circumstances of the operation. The contact time (2 hr, 12 hr and 22 hr), aeration rate (0.5, 4 and 7.5 litres min^{-1}), and DWW/POME ratio (20%, 50%, and 80%; V/V) were chosen as independent variables and their response (COD, BOD, TSS, AN and colour) were selected as dependent variables in this study (*Table 6*).

RESULTS AND DISCUSSION

COD, AN, Colour and BOD Removal

Table 2 indicates the maximum, minimum and average values of measured parameters of POME, DWW and standard discharge limit suggested by DOE. Understanding the characteristics of pollutants is crucial in the selection of treatment method. According to Ho and Tan (1983), centrifuge technique (depending on gravity force and operation time) can reduce the total amount of POME pollutants, such as TSS, BOD_5, COD, total nitrogen and AN. Consequently, POME contains high concentrations of real colour particles. Therefore, the parameters of BOD, COD, AN and colour are chosen for discussion in this section. The POME contains high concentrations of TSS (4310 mg $litre^{-1}$) COD (11 090 mg $litre^{-1}$) and high intensive colour (2550 Pt. Co.). The value of BOD_5 (1230 mg $litre^{-1}$) and BOD_5/COD (0.11) show the low biodegradability in the POME (*Table 2*). Metcalf and Eddy (2014) reported that for a wastewater with biodegradability index (BOD_5/COD ratio) bigger than 0.5, the wastewater can be considered for biological treatment. These results indicated that the anaerobic treatment of POME still contains high pollution and this method can hardly reach to the standard level of discharge defined by DOE. Therefore, an appropriate post-treatment is essential in order to meet the DOE levels. Previous

TABLE 6. EXPERIMENTAL VARIABLES AND RESULTS FOR THE NEW- SEQUENCING BATCH REACTOR (SBR)

Run No.	Aeration* (litre min^{-1})	Aeration duration (hr)	DWW/ POME (%)	TSS removal (%)	COD removal (%)	BOD removal (%)	Colour removal (%)	Ammonia removal (%)	BOD/COD
1	0.5	2	20	98.21	95.91	90.16	64.66	98.2	0.94
2	4.0	22	50	98.37	97.07	91.44	73.05	98.09	0.94
3	4.0	12	50	96.61	96.67	90.56	71.88	98.09	0.94
4	7.5	12	50	96.14	96.35	89.47	66.70	98.20	0.93
5	0.5	22	80	98.95	98.31	90.08	81.64	96.93	0.92
6	7.5	22	20	98.86	95.92	89.98	69.52	97.98	0.94
7	4.0	12	50	96.79	96.48	90.03	65.84	97.88	0.93
8	4.0	12	80	98.90	98.23	89.67	80.43	98.09	0.91
9	4.0	12	50	98.23	96.40	89.10	67.17	98.29	0.92
10	0.5	2	80	95.87	96.32	89.51	59.60	97.98	0.93
11	7.5	2	20	96.31	95.34	90.01	56.94	97.67	0.94
12	7.5	22	80	98.32	98.10	90.69	75.25	98.30	0.92
13	0.5	22	20	97.91	95.70	89.30	57.52	97.21	0.93
14	4.0	12	50	96.12	96.37	88.79	64.70	98.20	0.92
15	7.5	2	80	98.32	98.07	90.29	80.70	98.20	0.92
16	0.5	12	50	95.47	96.33	89.46	62.66	97.67	0.93
17	4.0	12	20	97.74	97.85	89.52	79.05	97.98	0.91
18	4.0	2	50	97.88	96.50	89.20	67.25	96.19	0.92
19	4.0	12	50	97.23	96.74	88.95	70.39	97.67	0.92
20	4.0	12	50	97.09	96.91	89.57	74.15	98.09	0.92

Note: *Before aeration, 15 g $litre^{-1}$ zeolite (based on the volume of POME) was used in each new-SBR.
DWW - domestic wastewater; POME – palm oil mill effluent; TSS - total suspended solids; BOD - biochemical oxygen demand; COD – chemical oxygen demand.

studies indicated that high concentration of COD, NH_3-N and low biodegradability index decreased the efficiency of SBR (Aziz *et al*., 2011; Kamarudzaman *et al*., 2011). However, adding absorbent into the SBR can efficiently remove pollutants at an improved rate (Neczaj *et al*., 2007).

In this study, the PNZ and wastewater were used as a cost-effective biological co-treatment materials to improve the efficiency of the new-SBR and to decrease the environmental impacts of discharging caused by POME. *Table 6* shows the removal efficiency of the new-SBR for the different variable of POME. As mentioned, before aeration and for the adsorption of pollutants, 15 g litre^{-1} PNZ was used in the new-SBR. The results are reported based on different aeration rate, aeration duration and percentage of DWW to POME (DWW/POME). As shown in *Table 6*, the variation of COD removal is between 95.34% and 98.31%. The highest removal of COD (98.31%) was achieved under the contact time of 22 hr, aeration rate of 0.5 litre min^{-1} and DWW/POME ratio of 80%. The minimum COD removal (95.34%) was at 7.5 litres min^{-1} aeration rate, 2 hr contact time, and 20% of DWW/POME. The 2D contour plot and 3D response surface plot for COD removal are illustrated in *Figure 5*. An optimal COD removal (98.362%) was provided at the aeration rate of 2.930 litres min^{-1}, contact time of 13.617 hr, and DWW/POME ratio of 78.476%. The results are consistent with those in previous studies (Aziz *et al*., 2011; Mojiri *et al*., 2014).

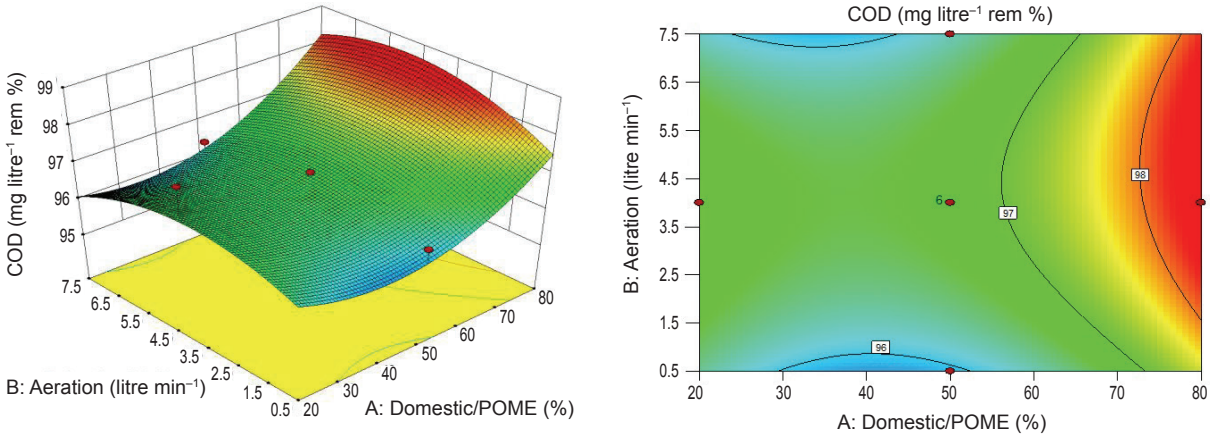
In the conventional biological treatment of wastewater, the AN is a significant inhibitor compound. Ammonia decreases biodegradability ratio and prohibits microbial growth. Furthermore, eutrophication accelerates and DO decreases with increasing concentration of AN (Li *et al*., 1999). Aziz *et al*. (2013) showed that most AN can be removed biologically. A combination of biological degradation and zeolite adsorption was used in the new-SBR for AN removal through microbial biofilm formation on the surface of the zeolite. As shown in *Table 6*, the ammonia removal increased from 96.19%-98.30%. The lowest ammonia removal occurred under contact time of 2.0 hr, the aeration rate of 4.0 litres min^{-1}, and DWW/POME ratio of 50%. The highest ammonia removal was observed under the following conditions, 7.5 litres min^{-1} aeration rate, 22 hr contact time, and 80% DWW/POME.

Figure 6 shows the 3D response surface plot for AN removal. The results showed that the new-SBR system can reach to the optimum removal efficiency of ammonia (98.33%) at a contact time of 18.32 hr, an aeration rate of 4.81 litres min^{-1}, and DWW/POME ratio of 76.97%. This finding means that the optimal desirable operation can be achieved through POME treatment in the new-SBR system under the aforementioned operation conditions.

In medium strength wastewater, approximately 75% of suspended solids and 40% of filterable solids are the organic materials (Metcalf and Eddy, 2014). The major organic compounds present in wastewater, detergents, carbohydrates, greases and oil, and proteins. Drinan and Spellman (2012) reported that around 30% of the organic compounds are not biodegradable. The POME is a high-strength colloidal agroindustry effluent with a high value of TSS (4310 mg litre^{-1}, *Table 2*), then a chemical adsorption is necessary to remove TSS. Chemical adsorption plays a supplementary role in microbial growth and adsorption, and zeolite is a promising adsorbent for suspended solids. According to Montalvo *et al*. (2012), a 3D structure of zeolite presents a high specific surface area (SSA). Erdem *et al*. (2004) reported that the zeolite with 41.5% porosity, 2.27 g cm^{-3} appearance density and 1.32 g cm^{-3} weight per unit volume is recognised as a suitable adsorbent with high hollow micropores and macropores. These properties provide a high surface for the monolayer adsorption of organic pollutants and a cation exchangeable capacity that can act as a capable chemical adsorption of macro and micro elements. *Table 6* indicates that the efficiency of the new-SBR with zeolite and DWW in TSS removal increased from 95.47% (contact time of 12 hr, aeration flow of 0.5 litre min^{-1}, and DWW/POME ratio of 50%) to 98.95% (contact time of 22 hr, aeration rate of 0.5 litre min^{-1} and DWW/POME ratio of 80%).

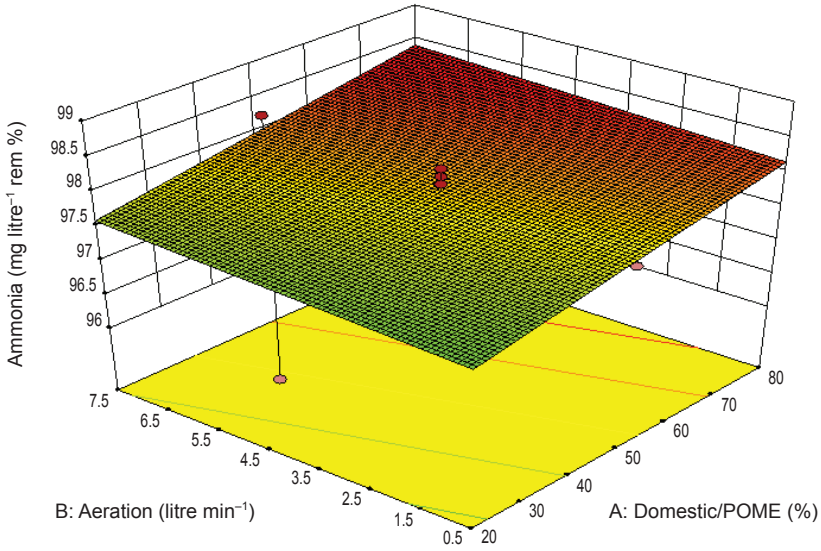
TSS removal and biodegradability index (BOD_5/COD ratio) are bigger than 95% and 0.5, respectively for all cases in *Table 6*. High BOD_5/COD increases the percentage of degradation and functions as an acceptable indicator of enhanced TSS removal as an organic pollutant. Therefore, the efficiency of the new-SBR in POME treatment increases by enhancing POME biodegradability. Results of TSS removal indicated that the combination of raw DWW as a microbial biodegradation supplier in the presence of zeolites for coagulation is an effective treatment method for the decontamination of biodegradable and non-biodegradable suspended solids. *Figure 7* shows the 2D contour plot and 3D response surface plot for TSS removal. The optimum TSS removal (99.16%) was observed at DWW/POME of 22.06%, aeration rate of 5.16 litres min^{-1} and a contact time of 21.6 hr. This result means that the new-SBR system could reach the highest achievable desirability under the aforementioned conditions.

Table 2 shows that collected POME from UOP contained a high intensity of colour (2550 Pt. Co.). According to Liew *et al.* (2015), colour is a critical pollutant in POME. Several methods have been applied to remove colour such as, the SBR systems (Zahrim *et al*., 2009), anaerobic reactions (Zhang *et al*., 2008), banana peel (Mohammed and Chong, 2014), boiler fly ash (Igwe *et al*., 2010) and activated carbon (Zahrim *et al*., 2009). The low declorisation efficiency



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

Figure 5. The 3D surface plots and 2D contour plot for COD removal.



Note: POME – palm oil mill effluent.

Figure 6. The 3D surface plots of ammonia nitrogen (AN) removal.

and high-cost treatments are the two main problems that limit the applications of commonly used methods. The biological and adsorption phenomena are the main factors influencing colour removal. The zeolite contains a high SSA which provides a specific suitable surface for microbial film forming (adsorbing and biodegradation). Micropores and macropores could be considered as suitable spaces for colour particles absorbing. For colour removal of landfill leachate, the aerobic SBR with adsorbent and wastewater as the augmentations was recommended as a cost-effective method (Aziz *et al*., 2011a). *Table 6* illustrates that in the new-SBR system, the minimum and maximum colour removal are 56.94% (contact time of 2 hr, DWW/POME of 20%, and aeration rate of 7.5 litres min^{-1}) and 81.64% (contact time of 22 hr, DWW/POME of 80%, and aeration rate of 0.5 litre min^{-1}) respectively.

Based on the Brunauer-Emmet-Teller (BET) test, the SSA of zeolite were decreased significantly after treatment process which may illustrate the action of zeolite as a suitable adsorbent. Moreover, the SEM-EDX indicates that natural zeolite fully covered by pollutants after the treatment process. Therefore, the suggested new-SBR can be employed as a cost effective declorisation system because of the positive effect of zeolite and DWW that enhances biological treatment. The POME treatment in the new-SBR could reach to optimum colour removal at DWW/POME ratio of 77.296%, aeration rate of 4.298 litres min^{-1} and contact time of 15.506 hr. Under these conditions, optimum decolourisation was observed to be 82.028%.

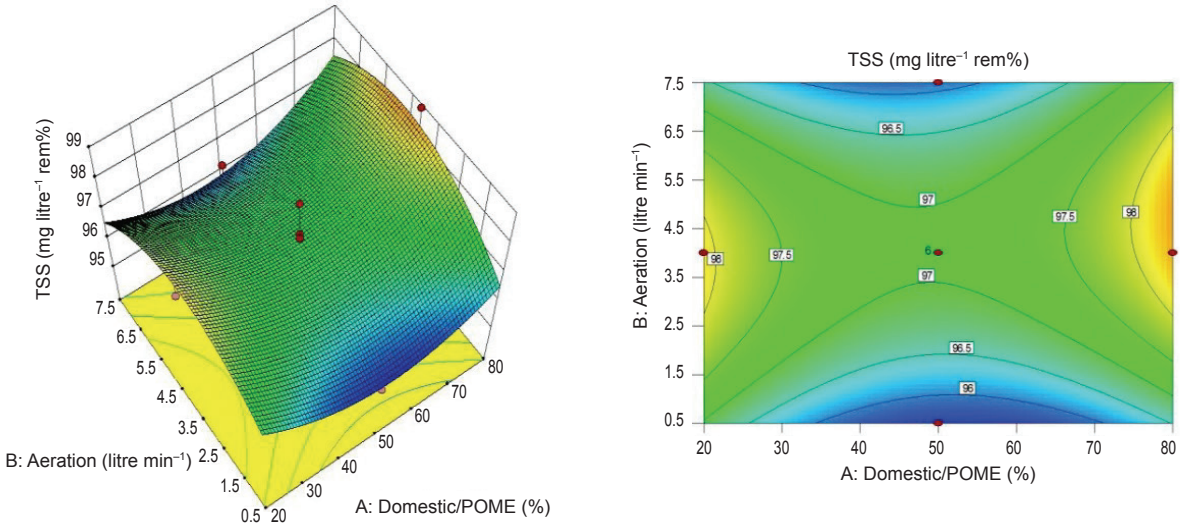
Estimation of BOD in the wastewater is one of the best methods to determine organic content. Gerardi (2011) showed that reducing oil, fats and

grease decreases BOD significantly. The BOD adsorption is recognised as particle diffusion-controlled mechanism and can be used to treat POME. In the new-SBR, the BOD removal varied from 88.79%-91.44% (*Table 6*). The lowest BOD removal was observed at a DWW/POME of 50%, contact time of 12 hr, and aeration rate of 4 litres min^{-1} while the highest one occurred at a DWW/POME of 50%, contact time of 22 hr and aeration rate of 4 litres min^{-1}. The results indicate that there is no significant different between the highest and lowest BOD removal efficiencies (88.79%-91.44%). It seems that the presence of DWW even in very low concentration provides high performance of BOD removal. For Run 1 (*Table 6*), although the DWW is low (DWW/POME=20%), the BOD removal is 90.16%. More researches required to specifying the minimum DWW as augmentation for POME treatment in the aerobic new-SBR system. The duration of aeration is another parameter which directly effects on BOD removal. A comparison between Run 12 and 15 with a same DWW/POME of 80% and aeration of 7.5 litres min^{-1} indicates that BOD removal increases from 90.29%-90.96% with increase in the aeration contact time from 2.0-22 hr, respectively. Furthermore, a comparison between Run 5 and 12 shows that BOD removal enhances by raising the aeration rate. Therefore, high aeration rate and long contact time did improve BOD removal efficiency through the new-SBR systems. Similar results were reported by Sahu *et al*. (2009). The RSM and CCD analysis indicate that the optimum removal efficiency of BOD (90.67%) was attained at 6.74 litres min^{-1} aeration rate, 1.99 hr contact time, and 66.39% DWW/POME ratio.

Microbiological treatment can be determined from biodegradability index (BOD/COD ratio). However, decontamination of wastewater increases with enhancing biodegradability. As shown in *Table 2*, by considering the value of BOD_5/COD as the biodegradability index in POME (0.11) and DWW (0.41), DWW is more biodegradable in comparison with anaerobically treated POME. After treatment of POME in the new-SBR, the results indicate that the ratio of BOD_5/COD is bigger than 0.9 for all cases (*Table 6*). It can be concluded the new-SBR with DWW and zeolite as the augmentations was achieved to successfully improve the biodegradability of POME. A 3D surface plots and 2D contour of POME biodegradability is shown in *Figure 8*. The optimum POME biodegradability was obtained for 12 hr contact time, 4 litres min^{-1} aeration and the DWW/POME of 80%.

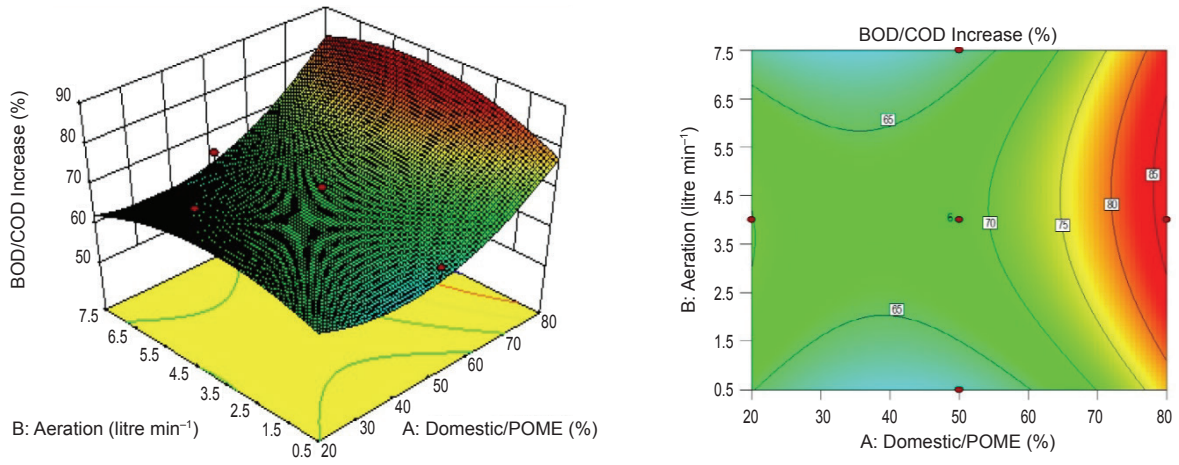
Evaluation of the New-SBR with Zeolite and DWW

The aim of this part of the research is to identify the most influenced augmentation for POME treatment in the new-SBR system. As shown in *Table 7*, several experiments were conducted to evaluate removal efficiency of the new-SBR for TSS, COD, colour and AN. Previous studies reported that augmentation of natural adsorbents can enhance pollutant removal efficiency (Halim *et al*., 2010; Kalló, 2001; Shavandi *et al*., 2012). Moreover, Vijayaraghavan *et al*. (2007) found that microbial communities exert a positive effect on POME treatment. Therefore, different conditions were considered to compare the ability of the new-SBR to improve POME such as 1) the blank new-SBR without any extra material as a case for control, 2) the new-SBR with adding zeolite, 3) the new-SBR with adding DWW and 4) the new-SBR with adding both zeolite and DWW. The experiments were performed under the similar optimum conditions and three replication runs were conducted to provide actual assessment.



Note: POME – palm oil mill effluent; TSS – total suspended solids.

Figure 7. The 3D surface plots and 2D contour plot for TSS removal.



Note: POME – palm oil mill effluent; BOD - biochemical oxygen demand.

Figure 8. The 3D surface plots and 2D contour plot for POME biodegradability.

TABLE 7. REMOVAL POME COMPONENTS IN THE NEW-SBR UNDER DIFFERENT CONDITION

Parameters	POME (control) removal (%)	Zeolite + POME removal (%)	DWW+ POME removal (%)	Zeolite + DWW+ POME removal (%)
TSS (mg litre^{-1})	-22	23	77	98.95
COD (mg litre^{-1})	9	10	74	98.31
Colour (Pt. Co.)	-30	9.6	76	81.64
AN (mg litre^{-1})	69	80	90	98.30

Note: POME - palm oil mill effluent; SBR - sequencing batch reactor; DWW - domestic wastewater; TSS – total suspended solids; COD – chemical oxygen demand; AN – ammonia nitrogen.

Table 2 illustrates that the anaerobic treatment of POME did not successfully removed colour (2550 Pt. Co.), AN (94.5 mg litre^{-1}), COD (11 090 mg litre^{-1}) and TSS (4310 mg litre^{-1}). As shown in *Table 7*, the negative values of TSS (-22) and colour (-30) show that the TSS and colour were increased in the POME due to strong mixing power of the aeration system on the suspended solids. However, in other cases, the presence of adsorbent (natural zeolite) and microbial degradation source (DWW) is a reason to increase removal efficiency of TSS (from -22% in POME to 23% in PMOE + zeolite, 77% in PMOE + DWW and 98.9% in PMOE + zeolite+ DWW) and colour (from -30% in POME to 9.6% in PMOE + zeolite, 76% in PMOE + DWW and 81.6% in PMOE + zeolite + DWW). The results indicate that removal of TSS, AN, COD and colour in the new-SBR with DWW (POME + DWW) is higher than those in both blank new-SBR (POME) and the new-SBR with zeolite (POME + zeolite). Therefore, DWW has a significantly higher influence on POME decontamination compared to the zeolite. The results indicate that the new-SBR with augmentation of DWW and zeolite (POME + DWW + zeolite) was able to remove a high percentage of all pollutants compared to the new-SBR with the only DWW. In sum, the removal efficiency of the new-SBR systems can be sorted as the following order: DWW + POME + zeolite > POME + DWW > POME + zeolite > POME. In can be concluded that using both zeolite and DWW improves biodegradability and shortens treatment duration of POME treatment.

Optimisation of Experimental Conditions and Statistical Analysis

In this section, the RSM and CCD were employed to determine the optimum conditions of the independent factors. In this analysis, DWW/POME ratio, aeration rate (litre min^{-1}), and the contact time (hr) were chosen as independent variables. To conduct a sufficient analysis for the aerobic method of the new-SBR, five dependent variables of TSS, BOD, COD, AN and colour were considered as responses. The effects of operational factors and responses of RSM modeling are presented in *Table 8*. The RSM results illustrated the specific effect of the independent parameters as well as the influence of the interactive effects on the selected responses. These results indicated that DWW/POME ratio has a significant positive effect on COD, AN and colour removal with P-value < 0.05 and a low effect on BOD_5 (P-value= 0.5681) and TSS (P-value= 0.6456). Moreover, a P-value of 0.0007 for BOD_5/COD indicates that POME biodegradability is significantly affected by DWW/POME.

TABLE 8. RESPONSES AND THE EFFECTS OF OPERATIONAL FACTORS FOR RSM MODELING

Responses	Intercept	A*	B	C	AB	AC	BC	A^2	B^2	C^2
TSS	97.0674	0.1330	0.1540	0.5820	0.3463	0.1038	-0.0288	1.1691	-1.3459	0.9741
P-value**		0.6456	0.5951	0.0648	0.2954	0.7476	0.9288	0.0537	0.0306	0.0986
BOD_5	89.5549	0.1270	0.1930	0.2320	0.1075	0.2325	0.0825	-0.0423	-0.1723	0.6827
P-value		0.5681	0.3908	0.3063	0.6645	0.3566	0.7387	0.9200	0.6835	0.1271
COD	96.7792	0.8310	0.1210	0.2960	0.2363	0.2063	-0.1463	0.9845	-0.7155	-0.2705
P-value		0.0007	0.4959	0.1145	0.2453	0.3066	0.4625	0.0130	0.0532	0.4268
Colour	69.4014	4.9930	2.3030	2.7830	1.3038	1.3938	-0.9713	8.0191	-7.0409	-1.5709
P-value		0.0337	0.2830	0.2004	0.5783	0.5528	0.6778	0.0651	0.0989	0.6934
AN	97.8850	0.4000	0.1260	0.1050	-	-	-	-	-	-
P-value		0.0096	0.3680	0.4514	-	-	-	-	-	-
BOD_5/COD	68.6273	8.2000	0.6000	2.2000	2.0000	1.2500	-1.7500	9.6818	-6.3182	-4.3182
P-value		0.0007	0.7318	0.2253	0.3181	0.5262	0.3795	0.0138	0.0802	0.2130

Note: RSM - response surface methodology.
*A - MWW/POME ratio, B - aeration rate and C - contact time.
**P-value < 0.05 - significant and P-value > 0.05 - no significant.
TSS - total suspended solids; COD - chemical oxygen demand; AN - ammonia nitrogen; BOD - biochemical oxygen demand.

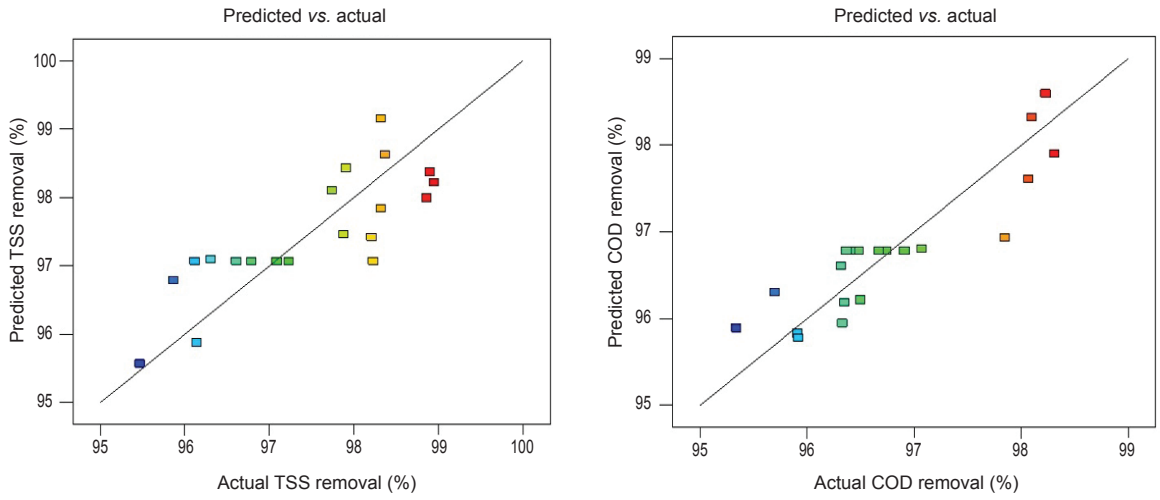
TABLE 9. DEVELOPED EQUATIONS AND ANOVA RESULTS FOR RESPONSE PARAMETERS

Responses*	Modified equations with significant terms**	Prob.***	R^2	Adjusted R^2	Adequate precision	SD	CV	Press****
TSS	$99.47+0.80A-0.18C-7.14AC-0.11A^2+9.5C^2$	0.24	0.71	0.66	3.53	1.16	1.19	121.3
BOD_5	$90.15+0.09A+-0.17C+2.86AC+-0.02A^2-+6C^2$	0.67	0.54	0.45	2.72	0.74	0.82	23.5
COD	$96.07+0.63A+0.09C-6.42AC-0.08A^2+-2.62C^2$	0.35	0.54	0.45	5.81	0.89	0.92	15.12
Colour	$65.61+6.04A+0.88C-0.02AC-0.711A^2-0.03C^2$	0.44	0.68	0.62	3.26	9.08	13.02	7 472.5
AN	$95.61-0.06A-0.06C+3.57AC+0.02A^2+4.18C^2$	0.10	0.82	0.77	5.18	0.5	0.51	12.11
BOD_5/COD	$63.05+5.04A+1.65C-0.05AC-0.65A^2-0.06C^2$	0.18	0.86	0.83	5.53	6.97	10.15	3 026

Note: *All removal units are mg $litre^{-1}$ except BOD_5/COD (unit less).
**In final equations, where A - DWW/POME (%); B - the aeration rate (litre min^{-1}); C - contact time (hr).
***Prob. - the probability of error; R^2 - coefficient of determination; SD - standard deviation; CV - coefficient of variance;
****Press - predicted residual error sum of square. ANOVA - analysis of variance.
TSS - total suspended solids; COD - chemical oxygen demand; AN - ammonia nitrogen; BOD - biochemical oxygen demand.

The second-order functions of pollutant removal including the results of ANOVA analysis are shown in *Table 9*. These equations express the relationship between independent parameters and the reduction of TSS, BOD, COD, AN, colour and BOD_5/COD. It was noted that the regression model for the reduction of pollutant was significant at a confidence level of 95% ($p < 0.05$) with R^2 equal to 0.86, 0.82, 0.71, 0.68 0.54, 0.54 and for BOD_5/COD, AN, TSS, colour, BOD and COD respectively. The coefficient of determination (R^2) indicates that although the regression model was able to successfully predict BOD_5/COD (R^2=0.86) and AN (R^2=0.82), other techniques such as soft computing methods can be recommended to prodive higher accuracy for other parameters (Zakaria *et al*., 2010; Mohammadpour *et al*., 2014; 2018; 2019; Ghani and Mohammadpour, 2015; Mohammadpour, 2017). A comparison between actual and predicted TSS and COD removal is illustrated in *Figure 9*.

Finding the optimum point of each element (aeration rate, contact time and DWW/POME ratio) could produce a clear picture for achieving the highest performance of decontamination. Based on the predicted model, the highest desirability of treatment (0.988) could be achieved in optimum operation conditions (contact time of 17.9 hr, DWW/POME ratio of 58.7%, and aeration rate of 6.85 litre min^{-1}). Under these conditions, COD, BOD, colour, AN and TSS removal rates were 96.80%, 90.1%, 69.90%, 98.20% and 97.20%, respectively. This research highlights that the recommended system can be successfully used as a cost-effective methods for post-treatment of POME. This system is able to highly remove the pollution within a short time (less than a day). Therefore, it can be commonly used as a rapid, economic and highly reliable technique to remove pollution and treat wastewater at any aquatic system worldwide.



Note: TSS - total suspended solids; COD - chemical oxygen demand.

Figure 9. Experimental vs. *predicted (a) TSS removal; (b) COD removal.*

CONCLUSION

The POME with high pollution components can be considered as an ecosystem and environmental hazard if it is discharged without any treatment to the environment. SBR is recommended to biologically treat wastewater and POME. In this study, a novel and cost-effective aeration system namely the new-SBR with opposite direction of aeration was developed to achieve a high efficiency of aeration in SBR. The DWW and zeolite were employed as an available microbial source and new adsorbent, respectively to improve the new-SBR treatment duration. A high pollution removal was obtained using the new-SBR. The COD, BOD, TSS, AN and colour removal rates were found in the range of 95.34%-98.31%, 88.79%-91.44%, 95.47%-98.95%, 96.19%-98.30% and 56.94%-81.64%, respectively. Several extra experiments have been conducted with and without zeolite and DWW to determine the ability of these materials in POME treatment. The results indicate that the new-SBR with adding both DWW and zeolite (POME+DWW+zeolite) is able to remove a high percentage of all pollution in compare to the new-SBR with only DWW or zeolite. In sum, the removal efficiency of the new-SBR systems can be sorted as the following order: POME + DWW + zeolite > POME +DWW > POME + zeolite > POME. It can be concluded that using both zeolite and DWW improves biodegradability and shortens treatment duration of POME treatment. The RSM and CCD methodology have been used to determine the optimum values of the independent parameters, including contact time (hr), aeration rate (litre min^{-1}), and ratio of domestic wastewater to POME (DWW/POME; v/v), as well as their dependent parameters (COD, BOD, colour, AN and TSS). The highest desirability of POME treatment (0.988) was achieved in optimum operation conditions (DWW/POME ratio of 58.7%, aeration rate of 6.85 litre min^{-1}, and contact time of 17.9 hr). Under these conditions, COD, BOD, colour, AN and TSS removal rates were 96.80%, 90.1%, 69.90%, 98.20% and 97.20%, respectively. Finally, an ANOVA analysis indicated that DWW/POME ratio has a significant positive effect on COD, AN and colour removal and low effect on BOD_5 and TSS removal.

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REFERENCES

Ahmad, A L; Suzylawati Ismail and Subhash Bhatia (2003). Water recycling from palm oil mill effluent (POME) using membrane technology. *Desalination, 157(1)*: 87-95.

APHA (2017). *Standard Methods for the Examination of Water and Wastewater.* 23st Edition. American Water Works Association/American Public Works Association/Water Environment Federation.

Aziz, S Q; Aziz, H A; Yusoff, M S and Bashir, M J (2011a). Landfill leachate treatment using powdered activated carbon augmented sequencing batch

reactor (SBR) process: Optimization by response surface methodology. *J. Hazardous Materials, 189*: 404-413.

Aziz, S Q; Aziz, H A and Yusoff, M S (2011b). Optimum process parameters for the treatment of landfill leachate using powdered activated carbon augmented sequencing batch reactor (SBR) technology. S*eparation Science and Technology, 46*: 2348-2359.

Aziz, S Q; Aziz, D; Abdul, H; Yusoff, M S; Mojiri, A and Amr, S A (2012). Adsorption isotherms in landfill leachate treatment using powdered activated carbon augmented sequencing batch reactor technique: Statistical analysis by response surface methodology. *Int. J. Chemical Reactor Engineering, 10(89)*: 21.

Aziz, S Q; Aziz, H A; Mojiri, A; Bashir, M J and Abu Amr, S (2013). Landfill leachate treatment using sequencing batch reactor (SBR) process: Limitation of operational parameters and performance. *Int. J. Scientific Research in Knowledge*, *1*: 34-43.

Borja, R and Banks, C J (1995). Comparison of an anaerobic filter and an anaerobic fluidized bed reactor treating palm oil mill effluent. *Process Biochem., 30*: 511-521.

Chan, Y J; Chong, M F and Law, C L (2012). An integrated anaerobic-aerobic bioreactor (IAAB) for the treatment of palm oil mill effluent (POME): Start-up and steady state performance. *Process Biochemistry, 47(3)*: 485-495.

Chan, Y J; Chong, M F and Law, C L (2011). Optimization on thermophilic aerobic treatment of anaerobically digested palm oil mill effluent (POME). *Biochemical Engineering J., 55(3)*: 193-198.

Chan, Y J; Chong, M F and Law, C L (2010). Biological treatment of anaerobically digested palm oil mill effluent (POME) using a lab-scale sequencing batch reactor (SBR). *J. Environmental Management*, *91*: 1738-1746.

Chin, K K; Ng, W J and Ma, A N (1987). Palm oil refinery effluent treatment by sequencing batch reactors. *Biological Wastes, 20(2)*: 101-109.

Chin, M J; Poh, P E; Tey B T; Chan, E S and Chin, K L (2013). Biogas from palm oil mill effluent (POME): Opportunities and challenges from Malaysia's perspective. *Renewable and Sustainable Energy Reviews*, *26*: 717-726.

Chou, K W; Tan, S W; Morad, N; Tow, T T; Kadir, M O and Ismail, N (2016). Aerobic post-treatment of different anaerobically digested palm oil mill effluent (POME). *Int. J. Environmental Science and Development, 7(7)*: 511.

Darajeh, N; Idris, A; Truong, P; Abdulaziz, A; Abubakar, R and Cheman, H (2014). Phytoremediation potential of vetiver system technology for improving the quality of palm oil mill effluent. *Advances in Materials Science and Engineering, Vol. 2014 (4)*: 1-10.

Drinan, J E and Spellman, F (2012). *Water and Wastewater Treatment: A Guide for the Nonengineering Professional*. CRC Press. 300 pp.

Erdem, E; Karapinar, N and Donat, R (2004). The removal of heavy metal cations by natural zeolites. *J. Colloid and Interface Science, 280(2)*: 309-314.

Faisal, M and Unno, H (2001). Kinetic analysis of palm oil mill wastewater treatment by a modified anaerobic baffled reactor. *Biochem. Eng. J., 9*: 25-31.

Fun, C W; Haq, M R U and Kutty, S R M (2007). Treatment of palm oil mill effluent using biological sequencing batch reactor system. *WIT Transactions on Ecology and the Environment Vol. 104*. Southampton, United Kingdom. WIT Press. p. 511-518.

Ghani, A A and Mohammadpour, R (2015). Temporal variation of clear-water scour at compound abutments. *Ain Shams Engineering J., 7*: 1045-1052.

Garbossa, L H P; Lapa, K R; Zaiat, M and Foresti, E (2005). Development and evaluation of a radial anaerobic/aerobic reactor treating organic matter and nitrogen in sewage. *Brazilian J. Chemical Engineering, 22(4)*: 511e519.

Gerardi, M H (2011). *Troubleshooting the Sequencing Batch Reactor*. John Wiley & Sons. 216 pp.

Halim, A A; Aziz, H A; Johari, M A M and Ariffin, K S (2010). Comparison study of ammonia and COD adsorption on zeolite, activated carbon and composite materials in landfill leachate treatment. *Desalination, 262(1)*: 31-35.

Ho, C C and Tan, Y K (1983). Centrifugal fractionation studies on the particulates of palm oil mill effluent. *Water Research*, *17(6)*: 613-618.

Igwe, J C; Onyegbado, C O and Abia, A A (2010). Studies on the kinetics and intraparticle diffusivities of BOD_5, colour and TSS reduction from palm oil mill effluent (POME) using boiler fly ash. *African J. Environmental Science and Technology, 4(6)*: 392-400.

Ismail, M H S; Dalang, S; Syam, S and Izhar, S (2013). A study on zeolite performance in waste treating ponds for treatment of palm oil mill effluent. *J. Water Resource and Protection, 5(07)*: 18.

Jenícek, P; Dohányos, M and Zábranská, J (1999). Combined anaerobic treatment of wastewaters and sludges. *Water Science and Technology, 40(1)*: 85-91.

Kalló, D (2001). Applications of natural zeolites in water and wastewater treatment. *Reviews in Mineralogy and Geochemistry, 45(1)*: 519-550.

Kamarudzaman, A N; Aziz, R A and Jalil, M (2011). Removal of heavy metals from landfill leachate using horizontal and vertical subsurface flow constructed wetland planted with Limnocharis flava. *Int. J. Civil and Environmental Engineering, 11*: 85-91.

Kesraoui-Ouki, S; Cheeseman, C R and Perry, R (1994). Natural zeolite utilisation in pollution control: A review of applications to metals' effluents. *J. Chemical Technology and Biotechnology: Int. Research in Process, Environmental and Clean Technology, 59(2)*: 121-126.

Khemkhao, M; Techkarnjanaruk, S and Phalakornkule, C (2015). Simultaneous treatment of raw palm oil mill effluent and biodegradation of palm fiber in a high-rate CSTR. *Bioresource Technology, 177*: 17-27.

Li, X; Zhao, Q and Hao, X (1999). Ammonium removal from landfill leachate by chemical precipitation. *Waste Management, 19*: 409-415.

Liew, W L; Kassim, M A; Muda, K; Loh, S K and Affam, A C (2015). Conventional methods and emerging wastewater polishing technologies for palm oil mill effluent treatment: A review. *J. Environmental Management, 149*: 222-235.

Lim, Y L; Ho, Y C and Alkarkhi, A F M (2014). Application of optimization in wastewater treatment. Wastewater engineering: Types, characteristics and treatment technologies. IJSR Publications. https://books.google.com.my/books?id=sv6MAwAAQBAJ, p. 225-237.

Ma, A N and Ong, A S H (1988). Treatment of palm oil steriliser condensate by an anaerobic process. *Biological Wastes, 23(2)*: 85-97.

Ma, A; Cheah, S; Chow, M and Yeoh, B (1993). Current status of palm oil processing waste management. *Waste Management in Malaysia: Current Status and Prospects for Bioremediation* (Yeoh, B S *et. al*). p. 111-136.

Mansor, U Q; Yahya, A; Shafie, N F; Roslan, Q I; Manshor, N M; Som, A M; Nour, Z H and Yunus, R M (2017). An overview of anaerobic treatment processes performance treating palm oil mill effluent (POME) past, present and future. *Advanced Science Letters, 23(5)*: 4179-4183.

Metcalf and Eddy (2014). *Wastewater Engineering-Treatment and Reuse.* 4th Edition. McGraw Hill, Inc.

Mohammed, R R and Chong, M F (2014). Treatment and decolorization of biologically treated palm oil mill effluent (POME) using banana peel as novel biosorbent. *J. Environ. Manage., 132*: 237-249.

Mohammadpour, R; Shaharuddin, S; Chang, C; Zakaria, N; Ghani, A and Chan, N (2014). Prediction of water quality index in constructed wetlands using support vector machine. *Environmental Science and Pollution Research, 22(1)*: 6206-6219.

Mohammadpour, R (2017). Prediction of local scour around complex piers using GEP and M5-Tree. *Arabian J. Geosciences, 10*: Article No. 416.

Mohammadpour, R; Asaie, Z; Shojaeian, M R and Sadeghzadeh, M (2018) A hybrid of ANN and CLA to predict rainfall. *Arabian J. Geosciences, 11*: 533.

Mohammadpour, R; Ghani, A A; Sabzevari, T and Fared Murshed, M (2019). Local scour around complex abutments. *ISH J. Hydraulic Engineering*. DOI: 10.1080/09715010.219.1607783.

Mohan, S V and Karthikeyan, J (1997). Removal of lignin and tannin colour from aqueous solution by adsorption onto activated charcoal. *Environ. Pollu., 97(1)*: 183-187.

Mojiri, A; Aziz, H A; Zaman, N Q; Aziz, S Q and Zahed, M A (2014). Powdered ZELIAC augmented sequencing batch reactors (SBR) process for co-treatment of landfill leachate and domestic wastewater. *J. Environ. Manage., 139*: 1-14.

Montalvo, S; Guerrero, L; Borja, R; Sánchez, E; Milán, Z; Cortés, I and De la la Rubia, M A (2012) Application of natural zeolites in anaerobic digestion processes: A review. *Applied Clay Science, 58*: 125-133.

Moradi, A; Teh, B S C; Goh, K J; Husni, M H A and Fauziah, I C (2015). Effect of four soil and water conservation practices on soil physical processes in a non-terraced oil palm plantation. *Soil Tillage. Res., 145*: 62-71.

Moussavi, G; Talebi, S; Farrokhi, M and Sabouti, R M (2011). The investigation of mechanism,

kinetic and isotherm of ammonia and humic acid co-adsorption onto natural zeolite. *Chemical Engineering J., 171(3)*: 1159-1169.

Nasrullah, M; Singh, L; Mohamad, Z; Norsita, S; Krishnan, S; Wahida, N and Zularisam, A W (2017). Treatment of palm oil mill effluent by electrocoagulation with presence of hydrogen peroxide as oxidizing agent and polialuminum chloride as coagulant-aid. *Water Resources and Industry, 17*: 7-10.

Najafpour, G D; Zinatizadeh, A A L; Mohamed, A R; Hasnain Isa, M and Nasrollahzadeh, H (2006). High-rate anaerobic digestion of palm oil mill effluent in an upflow anaerobic sludge-fixed film bioreactor. *Process Biochemistry, 41(2)*: 370-379.

Neczaj, E; Kacprzak, M; Lach, J and Okoniewska, E (2007). Effect of sonication on combined treatment of landfill leachate and domestic sewage in SBR reactor. *Desalination, 204(1)*: 227-233.

Ng, F Y; Yew, F K; Basiron, Y and Sundram, K (2012). A renewable future driven with Malaysian palm oil-based green technology. *J. Oil Palm, Environment and Health (JOPEH), 2*: 1-7.

Oswal, N; Sarma, P M; Zinjarde, S S and Pant, A (2002). Palm oil mill effluent treatment by a tropical marine yeast. *Bioresource Technology, 85(1)*: 35-37.

Sahu, J; Agarwal, S; Meikap, B and Biswas, M (2009). Performance of a modified multi-stage bubble column reactor for lead (II) and biological oxygen demand removal from wastewater using activated rice husk. *J. Hazardous Materials, 161*: 317-324.

Shavandi, M A; Haddadian, Z; Ismail, M H S and Abdullah, N (2012). Continuous metal and residual oil removal from palm oil mill effluent using natural zeolite-packed column. *J. Taiwan Institute of Chemical Engineers, 43*: 934-941.

Setiadi, T and Husaini, A (1996). Palm oil mill effluent treatment by anaerobic baffled reactors: Recycle effects and biokinetic parameters. *Water Sci. Technol., 34*: 59-66.

Vijayaraghavan, K; Ahmad, D and Aziz, M A (2007). Aerobic treatment of palm oil mill effluent. *J. Environmental Management, 82*: 24-31.

Wong, W H; Lee, W X; Ramanan, R N; Tee, L H; Kong, K W; Galanakis, C M; Sun, J and Prasad, K N (2015). Two level half factorial design for the extraction of phenolics, flavonoids and antioxidants recovery from palm kernel by-product. *Industrial Crops and Products, 63*: 238-248.

Yacob Hassan, M A; Shirai, Y; Wakisaka, M and Subash, S (2006). Baseline study of methane emission from anaerobic ponds of palm oil mill effluent treatment. *Sci. Total Environ., 366*: 187-196.

Zahrim, A; Rachel, F; Menaka, S; Su, S; Melvin, F and Chan, E (2009). Decolourisation of anaerobic palm oil mill effluent via activated sludge-granular activated carbon. *World Appl. Sci. J., 5*: 126-129.

Zakaria, N A; Azamathulla, H M; Chang, C K and Ghani, A A (2010). Gene expression programming for total bed material load estimation - A case study. *Science of the Total Environment, 408*: 5078-5085.

Zhang, Y; Li, Y A N; Xiangli, Q I A O; Lina, C H I; Xiangjun, N I U; Zhijian, M E I and Zhang, Z (2008). Integration of biological method and membrane technology in treating palm oil mill effluent. *J. Environmental Sciences, 20(5)*: 558-564.

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PALM TOCOTRIENOLS CAUSE CLEAVAGE OF POLY-(ADP)-RIBOSE POLYMERASE ENZYME AND DOWN-REGULATION OF CYCLOOXYGENASE-2 PROTEIN LEVEL IN HUMAN BREAST CANCER CELLS

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ABSTRACT

Breast cancer is a leading cause of cancer-related deaths in women globally. The anti-cancer effects of various forms of vitamin E from palm oil [tocotrienol-rich fraction (TRF): natural form of vitamin E complex in palm oil, tocotrienol-enriched fraction (TEF), and major vitamin E homologues in palm oil: α-tocopherol (αToc) and tocotrienols (T3) (α, δ or γ)] were tested on two human breast cancer cell lines [MDA-MB-231 (triple negative) and MCF-7 (oestrogen-dependent)]. Chronic inflammation plays a key role in tumourigenesis. Both cell lines used express high levels of poly-(ADP)-ribose polymerase-1 (PARP-1) and cyclooxygenase-2 (COX-2), which are key mediators of inflammation. Tocotrienols exerted marked anti-proliferative by promoting apoptosis in both MDA-MB-231 and MCF-7 cells. In addition, T3 also induced time-dependent inactivation of PARP-1 as well as inhibited expression of COX-2 in both MDA-MB-231 and MCF-7 cells. The rate of T3 uptake was found to be comparable to the anti-proliferative and apoptotic activities observed. In conclusion, T3 induced marked anti-proliferative ($p<0.05$) and pro-apoptotic ($p<0.05$) effects, which were most likely associated with PARP-1 inactivation and COX-2 down-regulation in these human breast cancer cells.

Keywords: vitamin E, tocotrienol (T3), tocopherols (Toc), breast cancer, anti-inflammation, PARP-1, COX-2.

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INTRODUCTION

Breast and lung cancers are reported to be the leading cause of cancer-related deaths in women globally (World Health Organisation, 2018). Breast cancer (BC) is the major cancer that affect women from diverse ethnic backgrounds all over the world (World Health Organisation, 2018). This is also the case in Malaysian women (Hisham and Yip, 2004); where 1:19 women is at risk of being diagnosed with BC (Lee *et al*., 2019). One of the widely acclaimed risk factors for BC are inherited genetic factors. It is not possible to change the genetic make-up of one at risk of developing cancer. However, studies have shown that only 10%-15% of BC incidence was related to heredity factors such as mutations in the *BRCA1* and *BRCA2* genes (Kotsopoulos *et al*., 2014). This means that the majority of BC is attributed to non-genetic factors, which are mostly modifiable risk factors.

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Some of these non-genetic risk factors include reproductive factors such as exposure to endogenous and exogenous hormones or life-style factors such as alcohol consumption, overweight and obesity, and physical inactivity (World Health Organisation, 2018). So, it may be possible to prevent cancer by changing some of these modifiable risk factors. In this regard, it would benefit all of mankind if cancer can be prevented. It is always better to prevent onset of a disease rather than trying to find a cure once it sets in. Nutritional intervention could be one of the ways that can be used to achieve this goal.

Several natural bioactive compounds have been shown to possess anti-cancer activities, including tocotrienols (Aggarwal *et al*., 2019; Fu *et al*., 2019; Tung and Ng, 2019), genistein (Zhang *et al*., 2019; Chae *et al*., 2019) and others (Cavalcanti *et al*., 2019). Consuming some of these bioactive compounds may be beneficial to reduce risk of cancer and its mortality rate. Vitamin E exists naturally as two families namely, tocopherols (Toc) and tocotrienols (T3). Each vitamin E family exists naturally in four isoforms namely alpha (α), beta (β), delta (δ) and gamma (γ). Palm oil is a rich source of vitamin E. Vitamin E in palm oil is known as tocotrienol-rich fraction (TRF). The main vitamin E isoforms present in palm oil are γT3 (46%), αToc (20%), δT3 (22%) and βT3 (12%) (Loganathan *et al*., 2013). Tocotrienols are structurally similar to Toc, except that the side-chain of T3 have three unsaturated bonds and one chiral centre, which are reported to be key enablers that allow T3 to enter tissues lipids freely and perform more efficient metabolic functions compared to Toc (Ahsan *et al*., 2014). Palm TRF has met the standard of reasonable certainty of no harm and thus is considered safe within the terms of the American Federal Food, Drug and Cosmetic Act and has been given the Generally Recognised as Safe (GRAS) status (US Food and Drug Administration, 2009). There is now compelling evidence, which shows that vitamin E, especially T3 have several health-enhancing effects such as antioxidant (Aggarwal *et al*., 2019; Fu *et al*., 2019; Tung and Ng, 2019), anti-inflammatory (Yang and Jiang, 2019), anti-obesity (Fukui *et al*., 2019) and anti-cancer (Montagnani Marelli *et al*., 2019).

Poly-(ADP)-ribose polymerase-1 (PARP-1) is an enzyme reported to play an important role in several cellular processes involving deoxyribonucleic acid (DNA) repair and programmed cell death (Pinto *et al*., 2009). Breast cancer cells are reported to express high levels of PARP-1 (Ma *et al*., 2019). Cleavage of PARP-1 is shown to reduce inflammatory responses as well as induce apoptosis, which is mediated through activation of caspases (Ma *et al*., 2019). When apoptosis is initiated, PARP-1 is activated when it binds to DNA ends or nicks; and subsequently gets inactivated following its cleavage. Cleavage of nuclear PARP occurs through activation of caspases, which cleave between Asp 216 and Gly 217, separating the 116 kDa PARP protein into one large (90 kDa) and one small (26 kDa) fragments (Satoh *et al*., 2003). The smaller fragment contains a zinc finger motif essential for DNA binding; whilst the larger fragment has the auto-modification and catalytic domains (Satoh *et al*., 2003). Cleavage of PARP prevents depletion of energy by nicotinamide adenine dinucleotide (NAD) and adenosine triphosphate (ATP), which is reported to be required for later stages of apoptosis (Satoh *et al*., 2003).

Many human cancers exhibit elevated prostaglandin (PG) levels owing to up-regulation of cyclooxgense-2 (COX-2). The COX-2 levels are reported to be elevated in about 40% of aggressive BC cases; with a higher incidence in pre-invasive ductal carcinoma *in situ* (Howe, 2007). Elevated levels of COX-2 in BC inhibit apoptosis, enhance angiogenesis; increase invasiveness; promote cell growth and produce mutagens (Divvela *et al*., 2010). The COX-2-prostaglandin E2 (PGE2) pathway is reported to play an important role in helping cancer cells adapt to the tumour microenvironment (Divvela *et al*., 2010) such as becoming resistant to programmed cell death. Non-steroidal anti-inflammatory drugs (NSAID), which are COX-2 inhibitors, are often prescribed to relief pain, fever and inflammation (Jahnavi *et al*., 2019).

The present study was conducted to compare the effects of various forms of vitamin E from palm oil on the effects on PARP-1 cleavage and COX-2 inhibition using two human breast cancer cell lines namely MDA-MB-231 (triple negative) and MCF-7 (oestrogen-dependent).

MATERIALS AND METHODS

Preparation of Vitamin E Treatments

TRF [γT3 (29%); αToc (32%); αT3 (25%); δT3 (14%)] (Golden Hope Plantations Bhd, Selangor, Malaysia); tocotrienol-enriched fraction (TEF), which is enriched with tocotrienols [αT3 (45.3%); δT3 (25.3%); γT3 (29.4%)] and free from αToc (Davos Life Sciences Pte Ltd, Singapore); pure T3 isoforms (αT3, δT3 and γT3) (Eisai Food and Chemicals Co., Ltd, Tokyo, Japan) and αToc (Aldrich Chemical Company, Inc., Milwaukee, USA). The T3 preparations (10 μg ml^{-1}) used for the various assays were quantified using high-performance liquid chromatography (HPLC).

Cell Lines

It has been shown previously that T3 selectively inhibit proliferation of cancer cells (Loganathan *et al*., 2013; 2015) but not normal cells. To further demonstrate that T3 are safe for normal cells,

the effect of culturing murine splenocytes in the presence of serial concentrations of different isoforms of vitamin E was also examined. Briefly, spleen was aseptically removed from six-week old female albino (BALB/c) mice and placed in a sterile Petri dish (10 cm) containing 10 ml of ice-cold complete Roswell Park Memorial Institute medium (RPMI 1640): fetal bovine serum (FBS): L-glutamine: penicillin-streptomycin at 97:5:1:1 ratio. Splenocytes were released by gently teasing out the spleen and recovered via centrifugation at 4000 rpm for 10 min. The cells were resuspended in complete medium at 1×10^6 cells ml^{-1} and 100 µl per well was added to a 96-well plate. Cells were left at 37°C in a humidified 5% CO_2 incubator for 1 hr prior to treatment. Upon cell culture stabilisation, vitamin E treatments (0-10 µg ml^{-1}) were added and the cells were incubated in a humidified atmosphere of 5% CO_2 in air at 37°C for 24 hr. Untreated splenocytes served as negative control whilst lipopolysaccharides (LPS) (Sigma Aldrich, USA) stimulated murine splenocytes were used as positive controls.

The highly aggressive triple negative human breast cancer cells, MDA-MB-231 and oestrogen-dependent human breast cancer cells, MCF-7 were obtained from the American Type Culture Collection (ATCC) (ATCC, Manassas, Virginia, USA). The MDA-MB-231 cells were cultured as monolayer in culture flasks (Orange Scientific, USA) in Dulbecco's Modified Eagle Medium (DMEM) supplemented FBS, L-glutamine: penicillin-streptomycin at 88:10:1:1 ratio. The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 incubator. The MCF-7 cells were grown using the same condition and medium except that 10^{-8} M of β-oestradiol was also added to the medium. The culture medium was changed routinely every alternate day.

Cell Proliferation Assay

The MTT assay (Chemicon International Inc., USA) was conducted to measure cell viability. Briefly, 10 µl of the MTT solution was added to all wells and incubated for 4 hr at 37°C. Then, 100 µl of 4 mM HCl-acid isopropanol stop mixture was added into each well, shaken on orbital shaker for 15 min and mixed thoroughly by pipetting the liquid to dissolve the black formazan, which results in a homogeneous blue solution. Absorbance at 570 nM was measured on microplate reader (Sunnyvale, USA).

Cell Death Detection-DNA Fragmentation Assay

The cells were seeded at a density of 1×10^4 cells/well and incubated for 1 hr at 37°C in a humidified atmosphere of 5% CO_2. Following this, 100 µl of the test compounds at desired concentrations, positive control, and control with and without vehicle were added to the respective wells. The plate was incubated in the incubator for the specified period of time (6 hr, 15 hr, 24 hr or 48 hr). At the end of each incubation period, cytoplasmic and nuclear histone/DNA fragments from cells were extracted and quantified using a commercial cell death detection kit (Roche Diagnostic Gmbh, Mannheim, Germany). The principle of this assay is based on sandwich-enzyme-immunosorbent whereby mouse monoclonal antibodies are directed against DNA and histones, respectively. Briefly, the nuclear and cytoplasmic fractions were incubated in a streptavidin-coated plate that was provided with the kit. Subsequently, an immune-reagent containing biotin-labelled anti-histone-biotin peroxidase conjugated anti-DNA-POD was added for the detection of histone-associated DNA fragments. Colour change, indicating binding was detected following the addition of ABTS, which is the substrate for the peroxidase. Spectrometric absorbance at 405 nM was measured. The results are presented as enrichment factor of mono and oligonucleosomes as described previously (Loganathan *et al*., 2013; 2015).

COX-2 Protein Concentration

The amount of COX-2 expressed in the human breast cancer cells (MCF-7 or MDA-MB-231 cells) treated with various forms of vitamin E was analysed using a commercial human COX-2 enzyme-linked immunosorbent assay (ELISA) kit. Briefly, the human breast cancer cells were seeded at a density of 1×10^7 in 10 cm Petri dishes. The cells were pre-treated with various forms of vitamin E (10 µg ml^{-1}) for 24 hr or 48 hr at 37°C in a humidified 5% CO_2 incubator. Following this, the cells were exposed to 1 nM of tumour necrosis factor-alpha (TNF-alpha) for 30 min. Untreated cells served as control. At the end of the incubation period, the cells were harvested, and a cell lysate was prepared as previously described (Loganathan *et al*., 2015). Protein content in the cell lysate was estimated by DC protein assay (Bio-Rad Laboratories). The amount of COX-2 in the cell lysate samples was quantified using a commercial human COX-2 ELISA kit as recommended by the manufacturer (Alpha Diagnostic International, USA). Briefly, the cell lysates were incubated for 1 hr with mouse monoclonal antibody coated plate. Following subsequent wash, horseradish-peroxidase (HRP)-conjugated antibody against COX-2 was added to the wells. The plate was left at room temperature for half an hour before addition of the 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate. The solution turned yellow once the stop solution was added to the wells. Absorbance at 450 nM of the samples were measured. The concentration of COX-2 in each cell lysate sample was generated based on the standard curve and calculated based on protein concentration.

Determination of PARP Cleavage

The amount of PARP cleavage induced in the human breast cancer cells (MCF-7 or MDA-MB-231 cells) treated with various forms of vitamin E was analysed using a commercial human PARP cleavage ELISA kit. Briefly, the human breast cancer cells were seeded at a density of 1 x 10^7 in 10 cm Petri dishes. The cells were pre-treated with various forms of vitamin E (10 μg ml^{-1}) for 6 hr, 15 hr, 24 hr and 48 hr at 37°C in a humidified 5% CO_2 incubator. Following this, the cells were exposed to 1 nM of TNF-alpha for 30 min. Untreated cells served as control. At the end of the incubation period, the cells were harvested, and a cell lysate was prepared. The amount of cleaved PARP (Asp214) in the cell lysate samples was quantified using a commercial human cleaved PARP (Asp214) ELISA kit using the manufacturer (PathScan, Cell Signalling Technology Inc.) recommended protocol as described previously (Loganathan *et al*., 2013; 2015).

Vitamin E Cellular Accumulation Analysis

Cellular accumulation of vitamin E was determined according to published protocols (Sen *et al*., 2000) with some modifications. Briefly, the human breast cancer cells (MCF-7 or MDA-MB-231 cells) were treated with vitamin E (10 μg ml^{-1}) for 72 hr. Following this, the cells were washed twice with cold phosphate-buffered saline (PBS) and subjected to a trysinisation step. The cells were recovered by centrifugation. The cell pellet was dried under nitrogen gas to estimate the dry weight. Then, 0.925 ml of PBS containing 1 mM EDTA disodium salt, 0.025 ml of 10 mg ml^{-1} butylated hydroxytoluene, and 0.5 ml of 0.1 M sodium dodecyl sulphate (SDS) was added to the dry pellet. This was followed by addition of 1 ml ethanol and 5 ml hexane. The mixture was vigorously vortexed for 1 hr and centrifuged at 3000 g for 15 min. The hexane layer was used for analysis with the HPLC. Quantification and characterisation of the various forms of vitamin E were done using a normal phase HPLC equipped with fluorescence spectrophotometer (HP Agilent 1100 HPLC G1321A FLD Detector, USA) and ChemStation Rev. A.06.0x (Agilent, USA). The mobile phase consisted of 970 μl hexane; 25 μl dioxane and 5 μl isopropyl alcohol (v/v). The mobile phase was delivered at 1 μl min^{-1} flow rate through a silica column [Phenomenex® Luna 5 m silica column (dimension: 250 x 4.6 mm I.D, 5 μM)]. A standard solution of 10 μg ml^{-1} vitamin E was injected prior to sample injection for calibration purposes. Fluorescence detector was set at emission (λem = 325 nm) and excitation (λex = 295 nm) spectra. The samples were then injected onto HPLC and analysed with Agilent Chemstation in duplicates.

Equation:

$$\text{Percentage of uptake (\%)} = \text{Concentration of vitamin E in cells/media} \times 100$$

Statistical Analysis

Data were expressed as the average of mean ± standard deviation of triplicates. Experimental data were processed by one-way analysis of variance (ANOVA) test Tukey HSD post-hoc test were used to compare treatment means. The P-value of < 0.05 was considered statistically significant.

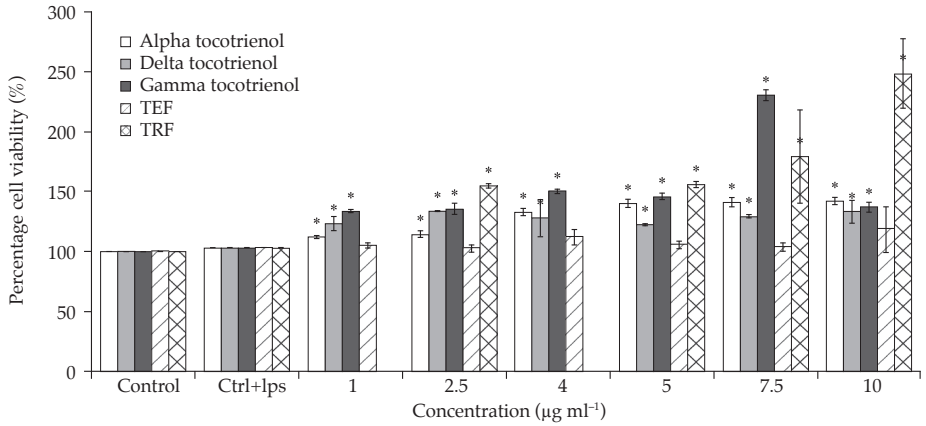
RESULTS

Effects of Palm Vitamin E on Normal Cells

None of the vitamin E treatments at 0-10 μg ml^{-1} concentrations inhibited proliferation of the lipopolysaccharide (LPS)-stimulated murine splenocytes (*Figure 1*). In fact, treatments with αT3, δT3 or γT3 at 1 μg ml^{-1} significantly ($P<0.05$) increased viability of the murine splenocytes. Similar results were observed when higher concentrations of these vitamin E isoforms were used. The findings imply that T3 may have less impact on the proliferation of normal cells. In order to confirm that T3 isoforms are not cytotoxic to normal cells, the LPS-stimulated murine splenocytes were treated with a fixed concentration of 10 μg ml^{-1} of various forms of vitamin E. The cytoplasmic and nuclear histone/DNA fragments from these cells were harvested and analysed using a cell death ELISA kit. The concentration chosen was previously shown to induce apoptosis in human breast cancer cells (Loganathan *et al*., 2013). None of the test compounds showed any signs of inducing apoptosis in the murine splenocytes (*Figure 2*). These findings showed that T3 isoforms did not have cytotoxic effects on the murine splenocytes, *i.e.* normal primary cells.

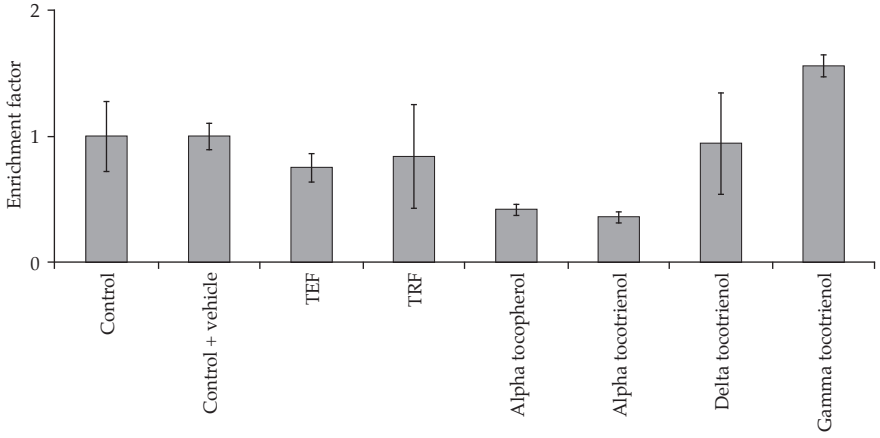
Induction of Apoptosis Due to Down-regulation of COX-2

Protein concentration of COX-2 was down-regulated in MDA-MB-231 (*Figure 3a*) and MCF-7 (*Figure 3b*) human breast cancer cells following treatment with tocotrienols. In control cells, the concentration of COX-2 was elevated in both human breast cancer cells, even before the addition of TNF-alpha (*Figure 3*). The results showed that tocotrienols caused significant ($P<0.05$) reduction in protein concentration of COX-2 in the human breast cancer cells. The ability of the vitamin E isomers to reduce COX-2 expression in the MDA-MB-231 after 24 hr was found to be higher when compared to 48 hr. A similar trend was observed with the MCF-7 cells.



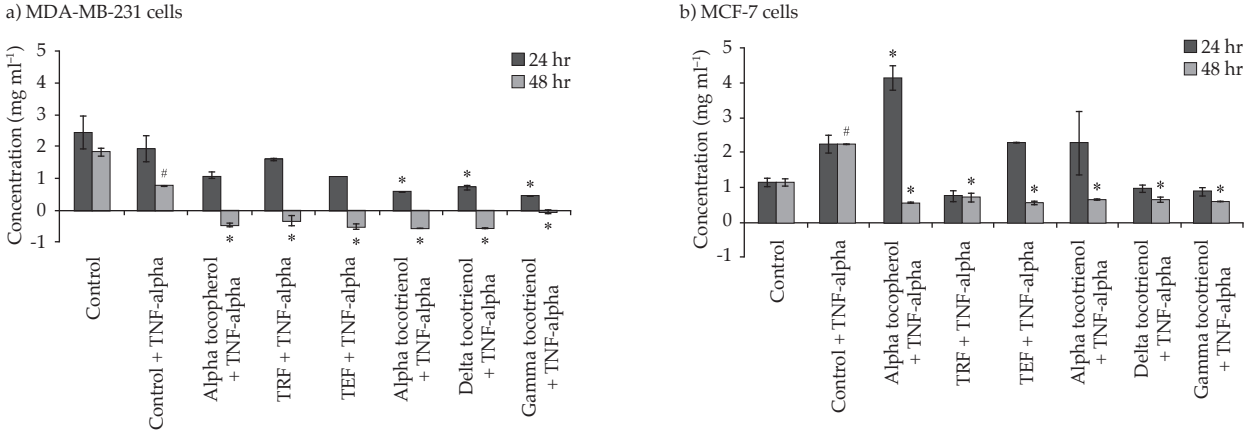
Note: There are no data available for tocotrienol-rich fraction (TRF) at 1 μg and 4 μg ml^{-1} as these concentrations were not tested.
TEF - tocotrienol-enriched fraction.
SD - standard deviation.

*Figure 1. The effect of tocotrienol isomers on the proliferation on 1 μg ml^{-1} lipopolysaccharide (LPS) stimulated murine splenocytes. Points represent the mean of three readings/well ± SD for triplicates in each treatment group are shown. * P<0.05 compared with control group.*



Note: TRF - tocotrienol-rich fraction.
TEF - tocotrienol-enriched fraction.
SD - standard deviation.

Figure 2. Effect of palm vitamin E isoforms (10 μg ml^{-1}) in inducing apoptosis in Con-A stimulated murine splenocytes. The rate of apoptotic event is presented as the enrichment factor. Points represent the mean of three readings/well ± SD for triplicates in each treatment group are shown. No significant difference compared to control.



Note: TNF - tumour necrosis factor.

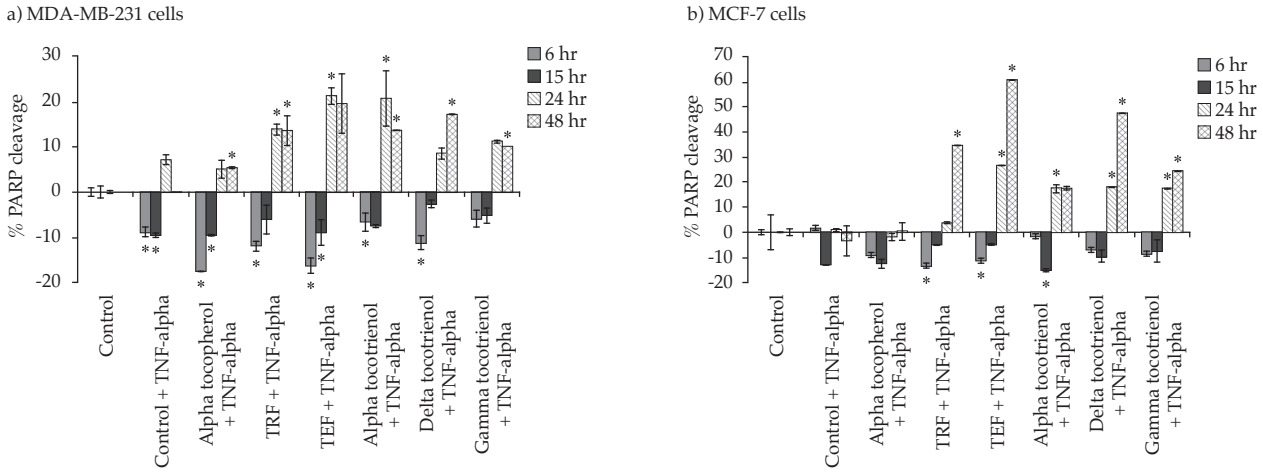
*Figure 3. The effect of tocotrienol isomers on cyclooxygenase-2 (COX-2) expression of 1 nM TNF-alpha stimulated (a) MDA-MB-231 and (b) MCF-7 human breast cancer cells for 24 hr or 48 hr. Points represent the mean ± SD for triplicates in each treatment group are shown. # Values are significantly different (p<0.05) from control group. *Values are significantly different (p<0.05) from control + TNF-alpha group.*

Induction of Apoptosis Due to PARP Cleavage

The MDA-MB-231 (*Figure 4a*) and MCF-7 (*Figure 4b*) were treated with the various forms of 10 μg ml^{-1} palm vitamin E and PARP cleavage activity was determined over a period of time at fixed intervals. The percentage of PARP cleavage was generally not detectable after 6 hr and 15 hr of exposure to the palm vitamin E in (a) MDA-MB-231 (*Figure 4a*) or (b) MCF-7 (*Figure 4b*) human breast cancer cells. This period corresponds to the PARP activation stage. However, the percentage of PARP cleavage increased ($P<0.05$) when the exposure period to vitamin E was increased to 24 hr and 48 hr, indicating PARP inactivation in both (a) MDA-MB-231 (*Figure 4a*) or (b) MCF-7 (*Figure 4b*) human breast cancer cells. The findings show a time-dependent cleavage of PARP with treatments with all forms of palm vitamin E on both the cell lines except for α-tocopherol on the MCF-7 cells (*Figure 4b*).

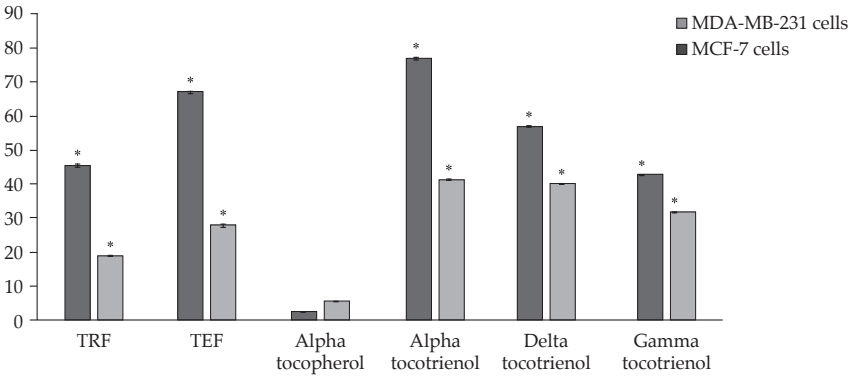
Cellular Uptake of Vitamin E

Cellular accumulation of vitamin E should be demonstrated in order to compare cellular affinity, bioavailability and physiological concentrations. The results show that cellular uptake of T3 isoforms were more efficient than αToc in both the human breast cancer cell lines (MCF-7 and MDA-MB-231). The uptake of vitamin E isoforms decreased in the following order: αT3 > TEF > δT3 > TRF > γT3 > αToc in MDA-MB-231 cells whilst in MCF-7 cells, the order was αT3 > δT3 > γT3 > TEF > TRF > αToc (*Figure 5*).



Note: TNF - tumour necrosis factor.
SD - standard deviation.

*Figure 4. Apoptosis induction by poly-(ADP)-ribose polymerase (PARP) cleavage in time-dependent manner following vitamin E treatment on a) MDA-MB-231 and b) MCF-7 cells. Points represent the mean ± SD for triplicates in each treatment group are shown. *Values are significantly different (p<0.05) from control+TNF-alpha group.*



Note: TRF - tocotrienol-rich fraction.
TEF - tocotrienol-enriched fraction.
SD - standard deviation.
ANOVA - analysis of variance.

*Figure 5. Cellular uptake of palm vitamin E. Results are shown as the mean ± SD from duplicate cultures. *Values are significantly different (p<0.05) from alpha tocopherol group (one-way ANOVA).*

DISCUSSION

Current study shows T3 induced selective anti-proliferative and pro-apoptotic effects, which were most likely associated with PARP-1 inactivation and COX-2 down-regulation in MDA-MB-231 and MCF-7 human breast cancer cells.

TRF was used in most of the initial studies involving palm T3. This bioactive compound has a standardised composition of 32% αToc, 25% αT3, 29% γT3, and 14% δT3. This has raised certain queries regarding the effect of αToc in the observed response. So, the efficacy of TRF, T3 isoforms (α, δ and γ), αToc as well as TEF was compared in this study. The TEF is a tocopherol-free preparation of TRF, which contains a α-, δ- and γ-tocotrienols.

Based on our previous growth inhibition studies and IC_{50} values, the anti-proliferative activity of vitamin E on the MDA-MB-231 cells was found to reduce in the following order: TEF (3.7 μg ml^{-1}) > γT3 (4.7 ± 0.8 μg ml^{-1}) > δT3 (6.9 ± 0.3 μg ml^{-1}) > TRF (8.5 ± 0.2 μg ml^{-1}) > αT3 (9.6 ± 1.1 μg ml^{-1}) whilst for MCF-7 cells, it reduced as: TEF (3.4±0.2 μg ml^{-1}) > TRF (4.55±0.7 μg ml^{-1}) > γT3 (6.35±0.15 μg ml^{-1}) > δT3 (6.8±0.3 mg ml^{-1}) > αT3 (11.05±0.45 μg ml^{-1}). In contrast, no inhibition of cell proliferation was reported on αToc concentrations tested (0-20 μg ml^{-1}) (Loganathan *et al*., 2013). In the current study, we found no adverse effects on normal LPS-stimulated murine splenocytes with similar test compounds. Hence, the data confirms that T3 exert selective anti-proliferative effects on breast cancer cells without any adverse effect on normal cells at the concentrations tested (0-10 μg ml^{-1}).

Induction of apoptosis is one of the most potent defence against progression of cancer. Hence, many of the currently used chemotherapeutic drugs are developed to target apoptotic cell death (Ricci and Zong, 2006). However, a key stumbling block of many of these anti-cancer drugs is that many of these drugs can also damage normal cells. Thus, novel molecules that can selectively induce apoptosis in cancer cells whilst sparing normal cells would be a preferred approach for cancer treatment. Several studies have confirmed that T3 selectively induce apoptosis in cancer cells but do not affect normal cells (McIntyre *et al*., 2000a). The results from the present study reaffirms that T3 selectively target cancer cells and exerted no or little toxicity.

Increased levels of COX-2 have been reported in aggressive breast cancers (Howe, 2007). Elevated levels of COX-2 could result in certain kinds of cancer cells becoming resistant to programmed cell death mostly due to the impaired ability of the cell to undergo intrinsic cell death. In the present study, the protein concentration of COX-2 was down-regulated following treatment with T3. This is an important observation as COX-2 is a NF-κB regulated gene product associated with cell proliferation. This effect was also observed in both the human breast cancer cell that were pre-treated with various forms of vitamin E (10 μg ml^{-1}) for 24 hr before these cells were exposed to 1 nM of TNF-alpha for 30 min. As shown in *Figure 3*, the protein concentration of COX-2 was elevated in both the human breast cancer cells that were not subjected to pre-treatment with the vitamin E isomers prior to exposure to the TNF-alpha. The results showed that tocotrienols significantly ($p<0.05$) reduced the protein concentration of COX-2 in these cells. The ability of the vitamin E isomers to reduce protein concentration of COX-2 in the MDA-MB-231 was in the following order γT3 > αT3 > δT3 > TEF > αToc > TRF and in the following order: TRF > γT3 > δT3 for MCF-7 cells. This finding is based on the concentration of COX-2 following 24 hr of pre-treatment. TEF and αT3 required more than 24 hr to reduce protein concentration of COX-2 in the MCF-7 cell lines. When the cells were pre-treated with the vitamin E isomers for 48 hr, the ability of the vitamin E isomers to reduce protein concentration of COX-2 in the MDA-MB-231 reduces in the following order αT3 > δT3 > TEF > αToc > TRF > γT3 and in the following order TEF > αToc > γT3 > αT3 > δT3 >TRF for MCF-7 cells.

Chronic inflammation plays a role in several steps associated with tumourigenesis; such as cellular transformation, promotion, survival, proliferation, invasion, angiogenesis and metastasis. Besides cancer, chronic inflammation also contributes to development of many degenerative disorders like cardiovascular diseases, neurogenerative disorders, arthritis and diabetes. Knocking down inflammation or inhibiting COX-2 are good targets for anti-cancer treatments (Jahnavi *et al*., 2019). NSAID are commonly prescribed drugs that act as COX inhibitors, which are commonly used to relief pain, fever and inflammation (Jahnavi *et al*., 2019). Vitamin E, especially T3 have potent free radical scavenging activities and thus could serve as anti-inflammatory therapeutic agent. Synergism between low concentrations of γT3 and celecoxib was reported to induce growth inhibition, which was associated with a decrease in PGE_2 synthesis, COX-2, phospho-Akt (active), and phospho-NF-κB (active) levels in breast cancer patients while avoiding the toxicity associates with high-dose COX-2 inhibitor monotherapy (Shirode and Sylvester, 2010). The mechanisms underlying the potent anti-inflammatory activity of TRF observed on LPS-induced human monocytes have been attributed to inhibition of iNOS, COX-2 and NF-κB but not inhibition of COX-1 (Wu *et al*., 2008). Dietary γT3 was reported to inhibit COX-2 activity in LPS-activated macrophages and IL-1β-stimulated human epithelial cells (Wu *et al*., 2008).

Tocotrienols appear to be better anti-inflammatory agents when compared to αToc; and the most effective form was reported to be δT3 in LPS-stimulated RAW264.7 macrophages (Yam *et al*., 2009). Current results indicate that T3 can down-regulate protein concentration of COX-2 on both human breast cancer cell lines (MCF-7 and MDA-MB-231) in a time-dependent manner (*Figure 3*).

PARP-1 inhibitors are proteins that plays a major role in a number of cellular processes involving mainly DNA repair and programmed cell death (Pinto *et al*., 2009). Cleavage of PARP proteins indicate presence of an apoptotic event. This was the principle applied in the commercial ELISA kit used in this study; where the kit detected the cleaved fragment (89 kDa) that consisted mainly of the catalytic domain of PARP. The PARP molecule is a protein involved in a number of cellular processes involving mainly DNA repair and NF-κB is one of its acceptor proteins. Majority of breast carcinomas were found to express high level of PARP-1 (Domagala *et al*., 2011). Both cell lines used in the present study (MCF-7 and MDA-MB-231) are aggressive human breast cancer cell lines that express high levels of PARP-1, which enable these cells to recruit and induce activation of NF-κB; a protein that is closely related to inflammation and upregulation of COX-2. Our results show that the expression of PARP-1 (*Figure 4*) and protein concentration of COX-2 (*Figure 3*) were down-regulated in both human breast cancer cells (MCF-7 and MDA-MB-231) treated with T3. Hence, a dual role of apoptosis and anti-inflammation could be observed with T3 treatment. Previously, we have reported that the pro-apoptotic effects of T3 was associated with DNA fragmentation and PARP-1 cleavage (Loganathan *et al*., 2013).

In this article, we report the mechanism of PARP-1 cleavage. In the event of apoptosis cleavage of the nuclear PARP-1 occurs through a cascade of caspases between Asp 216 and Gly 217 separating the 116 kDa PARP-1 protein into two fragments of 26 kDa and 90 kDa. Activation of PARP-1 was found at 6-15 hr corresponding to its binding to DNA ends or nicks; and subsequently the inactivation of PARP-1 occurs by its cleavage at 24-48 hr (*Figure 5*). In human, tocotrienols possess a half-life of 3.5 hr and its clearance from hepatic blood flow is within 24 hr (Aggarwal *et al*., 2010), this article may provide a benchmark of 24 hr to study apoptotic activity of tocotrienols. Besides, it is also evident cleavage of PARP-1 reduces inflammatory responses and cell death mediated by apoptosis. The cleavage of PARP-1 prevents the induction of necrosis during apoptosis and ensures appropriate execution of caspase-mediated programmed cell death. It is evident as previously we have shown that the apoptotic death by DNA fragmentation were notably higher compared to necrotic death with tocotrienols treatment (Loganathan *et al*., 2013).

The level of saturated phytyl chain and methylated chromanol ring in T3 may influence its mobility and distribution into the cells, thereby reflecting its biopotency (Palozza *et al*., 2006). There are various evidence to support the potent free radical scavenging activity of T3; including biochemical reactions towards radicals as well as cellular uptake or distribution, concentration and mobility at the microenvironment (Yoshida *et al*., 2003). It was reported that αT3 was better incorporated in human erythrocytes when compared to αToc and it resulted in providing better protection against oxidation and deformability (Begum and Terao, 2002). Tocotrienols uptake was reported to be more efficient than αToc in HT4 neuron cells in culture (Sen *et al*., 2000).

According to McIntyre *et al.* (2000a, b), T3 displayed significantly higher bio-potency than Toc as these are more easily or preferentially taken up by normal, pre-neoplastic (CL-SI), neoplastic (-SA) and highly malignant (+SA) mammary epithelial cells (McIntyre *et al*., 2000a, b). Twenty-four times greater concentration of α, γ and δToc was required to attain similar concentration of α, γ and δT3 in the pre-neoplastic and neoplastic mammary epithelial cell lines. Interestingly, in primary cultures it was found that the rate of uptake of vitamin E isoforms was significantly higher in tumour cells as compared to normal mammary epithelial cells. McIntyre *et al.* (2000a) reported the order of cellular accumulation of T3 in mammary epithelial to be as δT3 > γT3 > αT3; which appeared to have a direct correlation between relative bio-potency and observed effects. Decrease in the level of chromanol ring methylation (α > γ > δ) also corresponds to a decrease in partition coefficient of the compound. This causes a reduction in lipophilicity; thus, enhances cellular accumulation (McIntyre *et al*., 2000a). The finding from the present study also implies that αT3 is best absorbed by the cells, which corresponds to our previous report on the apoptotic activity of vitamin E from palm oil (Loganathan *et al*., 2013). The preferential uptake of T3 can somewhat explain the reasons behind the higher anti-cancer bio-potency of T3 when compared to αToc.

Status of estrogen receptor (ER) is an important factor to be considered for the prognosis of cancer (Nesaretnam *et al*., 2012). ER-beta (ER-β) is more widely expressed in breast cancer cells compared to of estrogen receptor-alpha (ER-α) (Nesaretnam *et al*., 2012). The MDA-MB-231 breast cancer cells express ER-β (Comitato *et al*., 2009) whereas MCF-7 breast cancer cells express both ER-α and ER-β (Comitato *et al*., 2010). *In silico* simulations and *in vitro* competitive binding assays have shown that T3 have higher binding activity for ER-β compared to ER-α (Comitato *et al*., 2010; 2009). In the present

study, there was higher uptake of T3 by the MDA-MB-231 cells compared to MCF-7 cells (*Figure 3*), which may be related to T3's selective affinity towards ER-β.

CONCLUSION

The findings from the current study show that T3 induced apoptosis in the two human breast cancer cells (MDA-MB-231 and MCF-7) through PARP-1 inactivation and down-regulation of COX-2. The extent of the apoptotic activity correlated with the rate of vitamin E uptake by these cancer cells.

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REFERENCES

Aggarwal, B; Sundaram, C; Prasad, S and Kannappan, R (2010). Tocotrienols, the vitamin E of the 21st century: Its potential against cancer and other chronic diseases. *Biochem. Pharmacol., 80*: 1613-1631.

Aggarwal, V; Kashyap, D; Sak, K; Tuli, H S; Jain, A; Chaudhary, A; Garg, V K; Sethi, G and Yerer, M B (2019). Molecular mechanisms of action of tocotrienols in cancer: Recent trends and advancements. *Int. J. Mol. Sci., 20*: 656-674.

Ahsan, H; Ahad, A; Iqbal, J and Siddiqui, W A (2014). Pharmacological potential of tocotrienols: A review. *Nutr. Metab., 11*: 1-22.

Begum, N and Terao, J (2002). Protective effect of α-tocotrienol against free radical-induced impairment of erythrocyte deformability. *Biosci. Biotech. Biochem., 66*: 398-403.

Cavalcanti, É B; Félix, M B; Scotti, L and Scotti, M T (2019). Virtual screening of natural products to select compounds with potential anticancer activity. *Anticancer Agents Med. Chem., 19*: 154-171.

Chae, H-S; Xu, R; Won, J-Y; Chin, Y-W and Yim, H (2019). Molecular targets of genistein and its related flavonoids to exert anticancer effects. *Int. J. Mol. Sci., 20*: 2420-2438.

Comitato, R; Leoni, G; Canali, R; Ambra, R; Nesaretnam, K and Virgili, F (2010). Tocotrienols activity in MCF-7 breast cancer cells: Involvement of ERβ signal transduction. *Mol. Nutr. Food. Res., 54*: 669-678.

Comitato, R; Nesaretnam, K; Leoni, G; Ambra, R; Canali, R; Bolli, A; Marino, M and Virgili, F (2009). A novel mechanism of natural vitamin E tocotrienol activity: Involvement of ERβ signal transduction. *Am. J. Physiol. Endocrinol. Metab., 297*: E427-E437.

Divvela, A K C; Challa, S R and Tagaram, I K (2010). Pathogenic role of Cyclooxygenase-2 in cancer. *J. Health Sci., 56*: 502-516.

Domagala, P; Huzarski, T; Lubinski, J; Gugala, K and Domagala, W (2011). PARP-1 expression in breast cancer including BRCA1-associated, triple negative and basal-like tumors: possible implications for PARP-1 inhibitor therapy. *Breast Cancer Res. Treat., 127*: 861-869.

Fu, J-Y; Maniam, G; Wong, F-S; Tan, D M-Y; Meganathan, P and Chuah, L-H (2019). Tocotrienols: From bench to bedside. *InVitamin E*: 12-31.

Fukui, K; Shirai, M; Ninuma, T and Kato, Y (2019). Anti-obesity effects of tocotrienols and bran in high-fat diet-treated mice. *Nutrients, 11*: 830-842.

Hisham, A N and Yip, C H (2004). Overview of breast cancer in Malaysian women: A problem with late diagnosis. *Asian J. Surg., 27*: 130-133.

Howe, L R (2007). Cyclooxygenase/prostaglandin signaling and breast cancer. *Breast Cancer Res. Treat., 9*: 210-219.

Jahnavi, K; Reddy, P P; Vasudha, B and Narender, B (2019). Non-steroidal anti-inflammatory drugs: An overview. *J. Drug Deliv. Ther., 9*: 442-448.

Kotsopoulos, J; Metcalfe, K; Alston, J; Nikitina, D; Ginsburg, O; Eisen, A; Demsky, R; Akbari, M; Zbuk, K and Narod, S A (2014). Prospective study of high-risk, BRCA1/2-mutation negative women: The 'negative study'. *BMC Cancer, 14*: 221-231.

Lee, M-S; Ma'ruf, C A A; Izhar, D P N; Ishak, S N; Jamaluddin, W S W; Ya'acob, S N M and Kamaluddin, M N (2019). Awareness on breast cancer screening in Malaysia: A cross sectional study. *BioMedicine, 9(3)*: 19-25.

Loganathan, R; Radhakrishnan, A K; Selvaduray, K R and Nesaretnam, K (2015). Selective anti-cancer effects of palm phytonutrients on human breast cancer cells. *RSC Adv., 5*: 1745-1753.

Loganathan, R; Selvaduray, K R; Nesaretnam, K and Radhakrishnan, A K (2013). Tocotrienols promote apoptosis in human breast cancer cells by inducing poly(ADP-ribose) polymerase cleavage and inhibiting nuclear factor kappa-B activity. *Cell Prolif., 46*: 203-213.

Ma, X; Dang, C; Min, W; Diao, Y; Hui, W; Wang, X; Dai, Z; Wang, X and Kang, H (2019). Downregulation of APE1 potentiates breast cancer cells to olaparib by inhibiting PARP-1 expression. *Breast Cancer Res. Treat., 176*: 109-117.

Mcintyre, B S; Briski, K P; Gapor, A and Sylvester, P W (2000a). Antiproliferative and apoptotic effects of tocopherols and tocotrienols on preneoplastic and neoplastic mouse mammary epithelial cells. *Proc. Soc. Exp. Biol. Med., 224*: 292-301.

Mcintyre, B S; Briski, K P; Tirmenstein, M A; Fariss, M W; Gapor, A and Sylvester, P W (2000b). Antiproliferative and apoptotic effects of tocopherols and tocotrienols on normal mouse mammary epithelial cells. *Lipids, 35*: 171-180.

Montagnani Marelli, M; Marzagalli, M; Fontana, F; Raimondi, M; Moretti, R M and Limonta, P (2019). Anticancer properties of tocotrienols: A review of cellular mechanisms and molecular targets. *J. Cell. Physio., 234*: 1147-1164.

Nesaretnam, K; Meganathan, P; Veerasenan, S D and Selvaduray, K R (2012). Tocotrienols and breast cancer: The evidence to date. *Genes Nutr., 7*: 3-9.

Palozza, P; Verdecchia, S; Avanzi, L; Vertuani, S; Serini, S; Iannone, A and Manfredini, S (2006). Comparative antioxidant activity of tocotrienols and the novel chromanyl-polyisoprenyl molecule FeAox-6 in isolated membranes and intact cells. *Mol. Cell Biochem., 287*: 21-32.

Pinto, L; Sloan, A; Persaud, I and Narain, N R (2009). Normalization of BCL-2 family members in breast cancer by coenzyme Q10. *Ethnicity and Disease, 19*: 17-18.

Ricci, M S and Zong, W-X (2006). Chemotherapeutic approaches for targeting cell death pathways. *Oncologist, 11*: 342-357.

Satoh, T H; Surmacz, T A; Nyormoi, O and Whitacre, C M (2003). Inhibition of focal adhesion kinase by antisense oligonucleotides enhances the sensitivity of breast cancer cells to camptothecins. *Biocell., 27*: 47-55.

Sen, C K; Khanna, S; Roy, S and Packer, L (2000). Molecular basis of vitamin E action tocotrienol potently inhibits glutamate-induced pp60c-Src kinase activation and death of ht4 neuronal cells. *J. Biol. Chem., 275*: 13049-13055.

Shirode, A B and Sylvester, P W (2010). Synergistic anticancer effects of combined gamma-tocotrienol and celecoxib treatment are associated with suppression in Akt and NFkappaB signaling. *Biomed. Pharmacother., 64*: 327-32.

Tung, Y-H and Ng, L-T (2019). Effects of nitrogen fertilization rate on tocopherols, tocotrienols and γ-oryzanol contents and enzymatic antioxidant activities in rice grains. *Physio. Mol. Bio. Plants, 25*: 189-195.

U.S. Food and Drug Administration (2009). Generally Recognized as Safe (GRAS) determination for the use of palm tocotrienol rich fractions (TRF) as ingredients in food. www.accessdata.fda.gov/scripts/fcn/gras_notices/grn_307.pdf, accessed on 20 October 2012.

World Health Organisation (2018). Cancer. https://www.who.int/news-room/fact-sheets/detail/cancer, accessed on 8 May 2019.

Wu, S-J; Liu, P-L and Ng, L-T (2008). Research article tocotrienol-rich fraction of palm oil exhibits anti-inflammatory property by suppressing the expression of inflammatory mediators in human monocytic cells. *Mol. Nutr. Food Res., 52*: 921-929.

Yam, M-L; Hafid, S R A; Cheng, H-M and Nesaretnam, K (2009). Tocotrienols suppress proinflammatory markers and cyclooxygenase-2 expression in RAW264. 7 macrophages. *Lipids, 44*: 787-797.

Yang, C and Jiang, Q (2019). Vitamin E δ-tocotrienol inhibits TNF-α-stimulated NF-κB activation by up-regulation of anti-inflammatory A20 via modulation of sphingolipid including elevation of intracellular dihydroceramides. *J. Nut. Biochem., 64*: 101-109.

Yoshida, Y; Niki, E and Noguchi, N (2003). Comparative study on the action of tocopherols and tocotrienols as antioxidant: Chemical and physical effects. *Chem. Phys. Lipids, 123*: 63-75.

Zhang, Q; Bao, J and Yang, J (2019). Genistein-triggered anticancer activity against liver cancer cell line HepG2 involves ROS generation, mitochondrial apoptosis, G2/M cell cycle arrest and inhibition of cell migration. *Arch. Med. Sci., 15*: 1001-1011.

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FRYING OIL QUALITY IN FAST FOOD RESTAURANTS IN EAST COAST OF MALAYSIA: A PRELIMINARY SURVEY

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ABSTRACT

Fresh, in-use regardless the days of frying and discarded oils were collected from fast food restaurants in Kuala Terengganu, Terengganu, Malaysia to investigate the quality of the frying oil used in the commercial frying industries. The fresh oils used in these restaurants were palm olein and/or palm oil. Oxidative stability index (OSI), smoke point, colour, free fatty acid (FFA), polar compound, peroxide value (PV) and ρ-anisidine value (ρ-AV) were evaluated. The fresh oils showed quality that is within specification of standards and references. The in-use oils showed a high degree of deterioration by having a lower OSI, smoke point, and darker colour. High levels of FFA showed that 42.9% of in-use oils and 100% of discarded oils had exceeded the maximum limit of 1%. Polar compound of 42.9% of the in-use oils and 50% of the discarded oils had exceeded the maximum limit (>27%). As high as 81% of the in-use oils and 75% of the discarded oil collected having ρ-AV of more than 10 which suggests that inspection by the local authority should be carried out to monitor the quality of the cooking oil used in the fast food restaurants.

Keywords: fast food restaurants, frying oil, oil quality.

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INTRODUCTION

Fried food products are popular nowadays with good acceptance and demand globally. Deep frying is a cooking method whereby the food is immersed in hot oil or fat. Deep frying method used for the production of fried food products has been used for decades and the preferable method of cooking nowadays as it is simple, fast and convenient providing distinctively good sensory properties (*i.e.* texture, aroma and flavour). The crispy outer crust with moist inner part enhances palatability of the food. Besides domestic cooking, deep frying method is being used commercially in restaurants (including fast food restaurants), mass catering and various food industries (including snack industry). Fast food restaurants have been experiencing exponential growth worldwide, especially in big cities due to changes in lifestyles when people have limited time to prepare daily meals (Shaharudin *et al.*, 2011). The popularity of fast food restaurants among the younger generation is undeniable as they offer menus with affordable prices, easy to access and convenient (Habib *et al.*, 2011), complemented with good marketing and advertising strategies.

Numerous types of lipids, in the form of liquid, solid and semi-solid at room temperature are available in the market for deep frying. These lipids act as a medium for heat transfer to the food. Despite being simple, fast and convenient, deep frying is a complex process and may result in physical and chemical changes when the lipids are being used repeatedly at a high temperature of between 150°C-200°C (thermal degradation) (Ahmad Tarmizi

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et al., 2016). This degradation depends on the type of lipids, products, temperature, time as well as frying process (batch or continuous). Thermal degradation may include oxidative, hydrolytic and polymerisation reactions as listed in *Table 1*, that produce displeasing breakdown products which may affect organoleptic properties and nutritive value of the food (Andrikopoulos *et al*., 2003; Gertz, 2000). Toxic compounds which are being produced during frying at prolonged time and repeated use of oils are likely to increase at high concentrations (Ghobadi *et al*., 2018). About 4%-14% of frying oils and fats are absorbed into the food structure during frying (Andrikopoulos *et al*., 2002; 2003; Goburdhum and Jhuree, 1995). Thus, the quality of fried food products is largely affected by the quality of lipids used in the frying. The undesirable breakdown products from thermal degradation may affect human health (Innawong *et al*., 2004).

TABLE 1. PHYSICAL AND CHEMICAL PROPERTIES CHANGES OF FRYING OILS AND FATS DURING FRYING

Category	Reaction	Changes
Physical	Aeration, vapourisation, foaming, smoke, solubilisation, colouration	Viscosity, density, tension, dielectric constant and conductivity
Chemical	Hydrolysis, auto-oxidation, dehydration, polymerisation, cyclisation and Maillard reactions	Polar compound, polymer compound, free fatty acid, fatty acid composition, rancidity and peroxides

Source: Ahmad Tarmizi *et al.* (2016).

Various methods are used to evaluate the quality of the frying oils used in the fast food restaurants. A simple and easy method is visual inspection of oil for physical changes in the colour and odour and check for excessive smoking and foaming. However, colour changes in the oil is not an accurate method and specification of the exact time for oil replacement based on colour change is difficult as it depends on human judgement (Ghobadi *et al*., 2018; Ibrahim *et al*., 2019). Moreover, the changes are only visible when the oils are already unsafe to be consumed. Chemical parameters include the total polar compounds, polymers, free fatty acids or acid value and viscosity are more reliable as the extent of the oils degradation are evaluated (Ibrahim *et al*., 2019).

To date, there is no reference on the quality of the frying oils used in fast food restaurants in Malaysia. The objective of this survey was to study the quality of oils used for frying in four selected fast food restaurants in east coast of Malaysia. Fresh, in-use and discarded frying oils from the fast food restaurants were analysed for their oxidative stability index (OSI), smoke point, colour (redness), free fatty acid (FFA), polar compound, peroxide value (PV), ρ-anisidine value (ρ-AV) and total oxidation (TOTOX) value.

MATERIALS AND METHODS

On-site Sampling

Fresh, in-use and discarded oils were collected from four fast food restaurants. *Table 2* describes the type of oils, usage of the fats and oils and days of frying. Frying procedures at all restaurants were different from each other in terms of the type of oil, food, fryers and frying conditions. Restaurants A and D used refined, bleached and deodourised (RBD) palm olein, and Restaurant C used RBD palm oil for frying of all products in their outlets. Restaurant B used palm olein to fry fries, whereas palm oil was used for frying other products. Fresh oil was taken directly from the storage container. The in-use oil was taken straight from the fryers, regardless the type of food being fried and the days of frying. Discarded oil was taken from the designated discard oil drums (oils from all fryers). One kg of each type of cooking oils (samples) was filled into Amber glass bottles. The samples were filled about 2 cm from the opening of the bottles as this method could avoid the photo-oxidation and headspace oxidation during transportation and storage of the oils before the analysis.

Samples Preparation

All oil samples were melted in an oven at 60°C and filtered using filter cloth to remove sediments. The oil was transferred to a clean Amber bottle, sparged with nitrogen and stored at -18°C for further analyses.

FFA

FFA was analysed according to the Association of Official Analytical Chemists' Official Method Ca 5a-40 (AOCS, 2009). The 20 g of oil samples were dissolved in 50 ml isopropanol and 2 ml phenolphthalein was added as the indicator solution. The mixture was titrated with 0.1 M sodium hydroxide until the first permanent pink colour appeared and remained for at least 30 s. The FFA was expressed as the percentage of palmitic acid.

Smoke Point

The smoke point of oils was analysed according to AOCS Official Method CC 9a-48 (2012). The oil

TABLE 2. TYPES, USAGE AND DAYS OF FRYING OF FATS AND OILS FROM FOUR FAST FOOD RESTAURANTS IN EAST COAST OF MALAYSIA

Restaurant	Types of oils/fats	Usage	Days of frying
A	Palm olein	Fresh	-
		Fries	6
		Fries	6
		Chicken (original)	6
		Chicken (spicy)	6
		Chicken (spicy)	6
		Chicken (spicy)	6
	Discarded		-
B	Palm oil	Fresh	-
		Fries	2
		Chicken	3
		Chicken mixed (patty/fillet/nugget/bites)	3
	Discarded		-
C	Palm olein	Fresh	-
		Fries	6
	Palm oil	Fresh	-
		Sweet dessert	6
		Chicken nugget	6
		Chicken patty	6
		Chicken patty	6
		Chicken patty	6
		Chicken (spicy)	6
		Chicken (original)	6
	Discarded		-
D	Palm olein	Fresh	-
		Chicken (original)	3
		Chicken (spicy)	3
		Chicken mixed (patty/fillet/nugget)	3
		Fries	3
	Discarded		-

was heated rapidly to within 42°C of the smoke point followed by regulated heating 5°C-6°C per minute until the presence of a thin and continuous bluish smoke.

OSI

OSI was evaluated according to the Association of Official Analytical Chemists' Official Method Cd 12b-92 (AOCS, 2009) using Rancimat 743 (Metrohm, Switzerland). The oil samples were melted at 60°C in an oven before being weighed directly in the reaction tubes. The reaction tubes were placed in the heating block and the analysis started immediately. The evaluation parameters were: sample weight, 3.0 ± 0.1 g; heating temperature, 110°C; gas flow, 20 litre hr^{-1}; and absorption solution, 60 ml. OSI result was translated as induction time.

Colour

Colour was determined according to ISO 15305 (1998) using a Tintometer Model E (Lovibond, Amesbury, England). The oil samples were heated to 60°C in an oven and placed in glass cells, 5.25 inches and 1 inch (13.3 and 2.5 cm) path lengths. The cell containing the oil sample was placed within the lighting cabinet. The lid of the lighting cabinet was closed and colour of the cooking oil sample was determined immediately using colour racks.

Polar Compound

Polar compound was analysed gravimetrically according to IUPAC 2.507 (IUPAC, 1992). One gram of oil sample was dissolved in 8 ml of 90% petroleum ether (PE) and 10% diethyl ether (DE) mixture. The mixture was then transmitted to a glass chromatography column, which was packed with 7 g of Silica Gel 60 suspended in the PE-DE mixture, and covered with a layer of sea sand (1 g). The column was rinsed with 100 ml of PE-DE mixture for 60-70 min to separate the non-polar fraction. The polar fraction was eluted using approximately 100 ml of DE within 60-70 min. Both polar and non-polar fractions were collected in separate flasks. Solvent was evaporated from the polar fraction using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland). Nitrogen was purged into the system from a rubber bulb before the termination of distillation. The residues were dried to constant weight at 105°C using an oven. The mass of polar compounds was determined from the residue obtained from the polar fraction.

PV

The PV was measured according to procedures described in ISO 3960 (2001). The oil samples were dissolved in the mixture of isooctane and glacial acetic acid (40:60) and allowed to react with freshly prepared saturated solution of potassium iodide (KI) for 60 s. Free iodine (I_2) was specified by titration of the desired mixture with standard sodium thiosulfate (Na_2S2O_3; 0.01 M) in presence of starch solution (1 g/100 ml) as the indicator.

ρ-AV

The ρ-AV was measured according to the procedures described in AOCS Official Method Cd 18-90 (AOCS, 2011). About 0.5 g oil sample was dissolved in iso-octane until a total volume of 25 ml was obtained. Five ml of the solution was drawn out and further reacted with 1 ml of ρ-anisidine solution containing ρ-anisidine and glacial acetic acid. The ρ-AV was analysed based on colour intensity using a CECIL Spectrophotometer 1000 Series (CECIL Instruments Ltd, Cambridge, United Kingdom) at a

wavelength of 350 nm. Colour intensity is reflected as yellowish colour formed from the reaction solution which correlates with the presence of aldehydic components in the sample.

TOTOX

TOTOX was calculated as TOTOX = 2PV + ρ-AV, where PV and ρ-AV represent peroxide value and ρ-anisidine value, respectively (Sebastian *et al.*, 2014).

Data Analysis

Results were tabulated as mean values ± standard deviation of three replicates.

RESULTS AND DISCUSSION

FFA

FFA is one of the most broadly used parameter to determine the quality of the frying oil (Chen *et al.*, 2013). It is measured based on the amount of potassium hydroxide required to compensate the FFA present in 1 g of oil. FFA is formed from the decomposition of hydroperoxides due to the presence of moisture and air at high temperature during frying process (Nayak *et al.*, 2016). Malaysia has set the maximum limit of FFA at 0.1% for both RBD palm oil and palm olein (Malaysian Standard, 2007). Ismail (2005) reported that the maximum threshold allowed for the FFA is differed by the type of fried food. For example, the FFA threshold of oil for potato chips frying on the industrial production scale is 0.5%, whereas the threshold of 1% of FFA was set for the processors of pre-fried French fries. However, a higher threshold (2.0%-2.5%) is tolerated for the oils used to fry battered and breaded products (Ahmad Tarmizi *et al.*, 2013). Some countries have set the regulation on the maximum limit of FFA threshold before discarding the frying oil. These include Germany (1%), Austria (1.25%), Japan (1.25%), Netherlands (2.25%) (Rossell, 1997) and United States (2%) (Sebastian *et al.*, 2014).

The FFA of fresh oils from all restaurants was in the range of 0.03%-0.1%, which complies with the maximum FFA limit set by the Malaysian Standard (*Table 3*). The FFA of the in-use oils was higher than those of fresh oil, varied between 0.41%-8.41% (*Table 4*). The FFA values increased as the time of frying increased, suggested that the triacylglycerol molecules in the frying oil have undergone hydrolytic deterioration. A similar result was reported by Ibrahim *et al.* (2019). They expected that the difference in the FFA value was due to the polymerisation and hydrolysis reaction in the oil that take place during frying. The in-use oils to fry fries in Restaurants A, B and D, and to fry breaded products by Restaurants C and B were beyond the maximum threshold of 0.5% and 2.5%, respectively. The highest FFA was noted in the in-use oil from Restaurant C which was used to fry chicken. Ali *et al.* (2014) and Enríquez-Fernández *et al.* (2011) reported that the FFA of oil used to fry chicken nuggets was higher compared to the oil used to fry french fries. However, a contrary observation was detected in the in-use oils used to fry fries in Restaurants A, C and D where the FFA was higher compared to the in-use oils used to fry chicken products. The finding was similar with Pokorny (1998) where the frying oil used to fry starch oxidised faster than the frying oil used to fry protein substrates. This was due to the reaction of the lipid peroxides with the remainder of the amino acid in the protein. The FFA of the discarded oil was in the range of 1.47%-7.72% (*Table 5*). The highest FFA level in the in-use oil and discarded oil was 8.41 and 7.72, respectively, which have exceeded the regulatory guidelines from any countries mentioned above. Ahmad Tarmizi and Siew (2008) reported that the development of FFA is highly correlated with the smoke point in which higher FFA content lowers the smoke point. A similar pattern of results was observed in the present studies (*Tables 4* and *5*). The smoke point of the in-use and discarded oils was decreased along with the increase of FFA.

Smoke Point

Smoking is the signal that fat has broken down chemically to produce glycerol and fatty acids. The glycerol is then further broken down to acrolein, a main component of the smoke (McGill, 1980). A survey on the worldwide regulation of frying fat and oil revealed that some countries had set their legal minimum limit for the smoke point of frying oil (Firestone, 1993). Austria, Belgium, Finland, Germany, Japan and Switzerland had set a limit of 170°C, whilst Hungary had set a limit of 180°C. Frying fats and oils with smoke point of more than 200°C are recommended for frying as the fats and oils will not produce smoke albeit first used (Mba *et al.*, 2015). Eyres (2015) stated that a higher smoke point is a beneficial property of cooking oil. Smaller, more volatile components, especially fatty acids reduce the smoke point and the reduction is maintained during the time of frying until the oil is no longer usable. At this point, the smoke point is less than 170°C. Typically, the smoke point of fresh palm oil is 230°C (Mba *et al.*, 2015) and palm olein is 220°C (Fan *et al.*, 2013). In the present study, the temperature of 170°C was used as the minimum limit of smoke point of the oils. The smoke point of fresh oils used in all restaurants was in the range of 214.7°C-237.3°C (*Table 3*) which indicates that the fresh palm oil and palm olein have excellent smoke

point. The in-use oils was noticed to have lower range of smoke point between 135.6°C-205°C (*Table 4*). The results describe that 12 samples (57.1%) of the in-use oils have smoke point below of 170°C. The results indicate that the frying operation lowers the smoke point of the frying oils. During frying, FFA forms from the hydrolysis of triglycerides and breakdown of hydroperoxide occurs at high temperature in the presence of moisture and air (Nayak *et al.*, 2016). The formation of aldehydes, which is the major products of FFA, induces the formation of acrolein (Katragadda *et al.*, 2010), which is the major component of the smoke. The smoke point of the discarded oils from all restaurants was in the range of 140.3°C-161.3°C, below the minimum limit of 170°C (*Table 5*).

Oxidative Stability

Rancimat is the most common accelerated method used to determine the oxidative stability of fats and oils and fat-containing food (Hadorn and Zurcher, 1974). Rancimat method evaluates the stability of the oil towards oxidation based on the induction time. The longer the induction time relates to the higher stability of the oil (Kowalski *et al.*, 2004; Farhoosh *et al.*, 2008). OSI of fresh oils in terms of induction time is tabulated in *Table 3*. The OSI of the fresh oils used by the fast food restaurants ranged from 20.8-54.8 hr. Fresh oils from Restaurants A and B had the highest OSI which was 54.4 and 54.8 hr, respectively. Fresh oils from Restaurants C and D had OSI that resembled the OSI of RBD palm oil and/or palm olein, which was 20.6-33.9 hr. High OSI of the fresh oils from Restaurants A and B was most probably due to the addition of permitted synthetic antioxidants such as tert-butylhydroquinone (TBHQ), butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). These synthetic antioxidants are commonly being used in the edible oil by the industries such as Malaysia, China, Peru, Australia and America, due to being widely available, cost effective and able to delay the oxidative rancidity in the oil (Liu *et al.*, 2016). The OSI of the in-use oils was in the range of 5.0-36.8 hr (*Table 4*). The stability of the oils collected from the fast food restaurants towards oxidation decreased gradually as the frying commenced, parallel with the findings reported by Ahmad Tarmizi *et al.* (2016). According to Ahmad Tarmizi *et al.* (2016), the induction time of palm olein and blended oils was decreasing for the first five days of frying due to the deterioration of vitamin E in the oil. From these results it is clear that higher induction hour in frying breaded products which contained chicken might be due to the saturated fatty acids content in the chickens leaching into the frying oil compared to fries which was mainly the carbohydrate.

Colour

Colour is a common parameter to determine oil quality. According to Maskan (2003), changes in oil colour during frying is the result of oxidation, polymerisation and other chemical changes. The rapid changes of oil colour from a light yellow to a dark orange-brown indicates that the oil has undergone deterioration. The oil colour darkening can also be affected by the foods being fried as the colour of the frying food tends to diffuse in the oil (Melton *et al.*, 1994). The maximum colour of RBD palm oil (MS 814:2007) and palm olein (MS 816:2007) set by the Malaysian Standard (2007) is 3.0R based on the 5.25″ Lovibond cell. The colour of fresh oils from all restaurants was in the range of 1.7 to 2.1R, showing that the fresh oils complied with the standard (*Table 3*). Drastic increase in the redness and yellowness values (darkening) was observed in oils used for frying food in all restaurants (*Table 4*). The colour of the in-use oils from all restaurants was in the range of 7.4 to 29.9R. The colour of the discarded oils was in the range of 14.3 to 28.8R (*Table 5*).

The changes in colour may be associated with the thermal and auto-oxidation by the existence of phospholipids which darkens upon heating (Latha and Nasirullah, 2014). The darkening of the oil might be due to the presence of pieces of food which accelerate the oil darkening (Lin *et al.*, 2001) and are affected by the type of food being fried and the time of frying (Ahmad Tarmizi *et al.*, 2013; Enríquez-Fernández *et al.*, 2011) and products of Maillard reaction which took place in the presence of sugar and amino acids also lead to the darkening of oil (Nayak *et al.*, 2016). Enríquez-Fernández *et al.* (2011) observed that the darkening of oil was higher in the oil used for frying chicken nuggets compared to the fries, due to the fat leaching out from the nugget into the oil. Ahmad Tarmizi *et al.* (2013) reported that the darkening (more reddish and yellowish) of oil was increased as the frying progressed. The darkening of the colour of frying oil is also highly affected by the type of oil used for frying. A study by Abdulkarim *et al.* (2007) on colour changes in different oils during frying affirmed that the colour of palm olein was the highest on Day 1 of the frying but the rate of the darkening slowed down and became constant throughout the frying study. After five days of frying, the colour of palm olein was lighter than the colour of canola and soyabean oils. A comparative study by Razali *et al.* (1999) found that the darkening of palm olein was faster than the high oleic sunflower oil. However, the colour of the fried products was not affected by the darker colour of palm olein. Irwandi *et al.* (2000) suggested that the increase in the darkness of oils was contributed by the oxidation and polymerisation of unsaturated fatty acids in the frying oil. The darkening of the

oil after frying was also influenced by the build-up of non-volatile decomposition products such as free fatty acids and oxidised triacylglycerols (Abdulkarim *et al.*, 2007). Nayak *et al.* (2016) suggested that the oil colour darkening can be a beneficial indicator to prevent further frying activities of the oil which has endured extreme deterioration. However, it must not be the main parameter of the exact time to change the oil as this is not an accurate method (Ghobadi *et al.*, 2018).

Polar Compound

A study by Bansal (2010) suggested that polar compound can be considered as the primary parameter that indicates the quality of the frying oils. The development of polar compounds indicates the deterioration of the oil which is strongly associated with both primary and secondary oxidation during frying activity (Karakaya and Simsek, 2011). The non-volatility of polar compound reflects major reactions from the degraded oils and fats (Warner and Gupta, 2003). Many countries have set the maximum level of polar compound obliged in the frying oil. For example, France, Italy, Belgium, Chile, Spain and South Africa have set the maximum level of 25% for the polar compound while Austria and Germany have set a maximum limit to 27% (Berger, 2005). In the present study, the maximum limit of 27% was used as the limit of polar compound.

The polar compound of fresh oils collected from the fast food restaurants ranged from 4.44%-7.76% (*Table 3*). These values indicate that the oils used are fresh. The polar compound of the in-use frying oils ranged from 6.57%-47.77% (*Table 4*). It is alarming when polar compound in most of the in-use oils from Restaurants C and D had exceeded the recommended maximum limit of 27%. Surprisingly, the polar compound of the in-use oils from Restaurant D (*Table 4*) had exceeded the maximum level at Day 3 of frying. This restaurant discards the oils use for frying every six days. Low polar compound in the in-use oils from Restaurants A and B can be associated with the addition of synthetic antioxidants in the fresh oil. In recent study, Díaz-Sánchez *et al.* (2019) reported that the palm olein with the addition of antioxidants had lower polar compound than palm olein without the addition of antioxidants in industrial continuous frying. Banu *et al.* (2016) also claimed that the polar compound was lower in the frying oils with the addition of 200 ppm of TBHQ compared to those without TBHQ. Polar compound of the discarded oils from these fast food restaurants ranged from 17.32%-40.01% (*Table 5*). Polar compound of the discarded oils from Restaurants C and D exceeded the maximum allowance of 27%. However, polar compound analysis does not provide an accurate estimation of the oils' degradation; therefore, other decisive factors such as ρ-AV should be considered too (Esfarjani *et al.*, 2019).

PV

The PV is an indicator of the primary oxidative state of oils. PV indicates products from the initial reaction of lipid oxidation such as alkanes, alkenes, hydroperoxides, ketones, epoxides, aldehydes and carboxylic acids (Qin, 2011; Tian, 2013). The hydroperoxides are further broken down at high temperature to form secondary oxidation products. Generally, freshly refined oils have PV of less than 1 meq kg^{-1} oil. The oil is regarded as rancid at PV of more than 10 meq kg^{-1} oil (Gunstone, 2008). It is clear that there were variation in the PV of fresh oil, from 0.34 to 1.28 meq kg^{-1}, with the PV of fresh oils from Restaurants B and D above the limit of 1 meq kg^{-1} (*Table 3*). The PV of the in-use oils ranged from 0.54-7.27 (*Table 4*). These results clearly indicated that none of the samples had PV above the limit of 10 meq kg^{-1}. A similar study done by Sebastian *et al.* (2014) on the quality of frying oils used in restaurants in Toronto, Canada, found that the PV of fresh frying oils from all restaurants in their study was above the limit of 1 meq kg^{-1} and 35% of the in-use oils have PV above the limit of 10 meq kg^{-1}. Ghobadi *et al.* (2018) reported that high PV was detected in the frying oils collected from the fast food restaurants in Shiraz, Iran. Enríquez-Fernández *et al.* (2011) claimed that the final PV of palm olein used to fry carbohydrate-based product was lower than protein-based product. The finding was in contrast with the PV in this study. We encountered that the PV of oils used to fry fries was higher than chicken products. The PV of the discarded oils ranged from 0.47-4.79 (*Table 5*) meq kg^{-1}, which is within the maximum limit of 10 meq kg^{-1}. Similar research by Esfarjani *et al.* (2019) reported that the mean of PV of the discarded oils collected from the fast food restaurants in Tehran, Iran, was 3.06 meq kg^{-1}. Nonetheless, PV can only detects the primary oxidation. PV may surge even after the oil is taken from the fryer. Thus, it is not a decisive method to conclude the oxidation state occurred inside the oil (Man and Hussain, 1998). For this rationale, the ρ-AV was determined to measure the secondary oxidation products as to access the quality of these oils.

ρ-AV

The ρ-AV measures the amount of aldehyde in oil, particularly 2,4-dienals and 2-alkenals. These products are produced during the secondary oxidation of lipids (Tompkins and Perkins, 1999). The products produced during the secondary oxidation are more stable compared to the peroxide

TABLE 3. PHYSICAL AND CHEMICAL ANALYSIS OF FRESH FRYING OILS

Restaurant	Induction time (hr)	Smoke point (°C)	Lovibond colour	Free fatty acid (%)	Polar compound (%)	Peroxide value (mg kg^{-1})	ρ-anisidine value	TOTOX
A	54.4 ± 0.6	237.3 ± 0.6	1.7R + 16.9Y	0.10 ± 0.00	4.44 ± 0.12	0.34 ± 0.06	1.24 ± 0.02	1.92
B	54.8 ± 0.3	230.5 ± 0.7	2.0R + 23Y	0.07 ± 0.00	5.88 ± 0.19	1.01 ± 0.10	0.51 ± 0.06	2.53
C	25.9 ± 0.7	214.7 ± 1.1	2.0R + 29.0Y	0.06 ± 0.01	6.22 ± 0.63	0.86 ± 0.05	1.38 ± 0.01	3.10
	33.9 ± 1.0	234.6 ± 0.5	2.1R + 23.1Y	0.03 ± 0.00	4.45 ± 0.29	0.54 ± 0.06	1.06 ± 0.06	2.14
D	20.6 ± 0.2	220.7 ± 0.5	2.1R + 21.2Y	0.04 ± 0.00	7.76 ± 0.20	1.28 ± 0.06	1.73 ± 0.24	4.29

Note: TOTOX – total oxidation.

TABLE 4. PHYSICAL AND CHEMICAL ANALYSIS OF IN-USE OILS

Restaurant	Induction time (hr)	Smoke point (°C)	Lovibond colour	Free fatty acid (%)	Polar compound (%)	Peroxide value (mg kg^{-1})	ρ-anisidine value	TOTOX
A	16.5 ± 1.3	166.7 ± 1.2	19.1R + 60.0Y +3.6B	1.78 ± 0.00	15.46 ± 0.30	0.97 ± 0.00	25.14 ± 0.60	27.08
	16.4 ± 1.2	162.7 ± 0.6	18.8R + 68.8Y + 2.5B	1.68 ± 0.00	13.45 ± 0.26	0.97 ± 0.21	23.83 ± 0.47	25.77
	36.8 ± 0.2	167.0 ± 1.0	19.9R + 49.0Y + 3.4B	0.59 ± 0.00	7.36 ± 0.25	0.88 ± 0.05	7.91 ± 0.57	9.67
	34.8 ± 1.0	171.7 ± 0.6	16.7R + 48.8Y +2.6B	0.71 ± 0.00	6.57 ± 0.26	0.74 ± 0.12	7.91 ± 0.34	9.39
	35.2 ± 0.9	165.3 ± 1.2	20.7R + 48.6Y + 5.6B	0.89 ± 0.01	7.76 ± 0.19	0.89 ± 0.09	7.16 ± 0.18	8.94
	32.7 ± 0.1	164.7 ± 1.1	19.8R + 48.5Y + 5.7B	0.99 ± 0.01	8.39 ± 0.24	0.54 ± 0.05	8.27 ± 0.16	9.35
B	25.8 ± 1.1	174.3 ± 0.6	21.2R + 21.2Y + 3.3B	0.95 ± 0.01	12.59 ± 0.34	1.31 ± 0.00	31.21 ± 0.23	33.83
	34.5 ± 0.7	159.0 ± 1.0	22.7R + 47.2Y + 8.4B	2.75 ± 0.01	17.19 ± 0.28	1.52 ± 0.01	15.58 ± 0.20	18.62
	25.0 ± 0.7	190.3 ± 0.6	12.1R + 21.6Y + 0.9B	0.41 ± 0.00	8.76 ±0.18	1.58 ± 0.07	27.08 ± 0.64	30.24
C	14.8 ± 0.7	205.0 ± 1.0	7.4R + 77.4Y	0.16 ± 0.00	10.14 ± 0.16	7.27 ± 0.29	49.15 ± 0.61	63.69
	5.1 ± 0.1	180.0 ± 1.0	23.8R + 32Y + 2.1B	0.71 ± 0.02	27.70 ± 0.36	4.42 ± 0.08	55.31 ± 0.60	64.15
	7.7 ± 0.5	159.7 ± 0.6	22.9R + 78.8Y + 7.8B	1.72 ± 0.02	37.63 ± 0.51	4.97 ± 0.17	54.86 ± 0.51	64.80
	13.0 ± 0.9	166.6 ± 1.2	29.9R + 50.0Y + 11.0B	1.82 ± 0.01	47.77 ± 0.06	5.21 ± 0.13	57.71 ± 0.44	68.13
	34.1 ± 0.8	151.0 ± 1.0	17.5R + 47.9Y + 18.9B	2.74 ± 0.02	43.40 ± 0.20	4.35 ± 0.19	48.08 ± 0.16	56.78
	13.0 ± 0.7	149.7 ± 0.6	22.4R + 47.9Y + 10.4B	1.82 ± 0.01	36.77 ± 0.25	4.30 ± 0.23	52.84 ± 0.38	61.44
	10.9 ± 0.3	166.7 ± 1.2	13.8R + 49.9Y + 0.4B	0.98 ± 0.01	21.24 ± 0.55	3.76 ± 0.17	42.04 ± 0.61	49.56
	7.1 ± 1.0	135.6 ± 0.5	22.3R + 39.9Y + 13.3B	8.41 ± 0.02	32.60 ± 0.36	0.90 ± 0.10	11.07 ± 0.27	12.87
D	7.3 ± 0.2	183.0 ± 1.0	17.2R + 35.0Y +1.4B	0.65 ± 0.00	23.56 ± 0.28	4.46 ± 0.09	59.64 ± 0.43	68.56
	5.2 ± 0.1	184.7 ± 0.6	13.5R + 24.7Y + 0.7B	0.53 ± 0.00	27.59 ± 0.38	4.58 ± 0.13	63.62 ± 1.05	72.78
	5.3 ± 0.6	174.0 ± 0.0	21.7R + 44.8Y + 1.5B	0.98 ± 0.00	38.19 ± 0.19	4.38 ± 0.04	77.93 ± 0.13	86.69
	5.0 ± 0.1	165.3 ± 1.2	21.9R + 44.9Y + 1.8B	1.66 ± 0.00	35.85 ± 0.18	4.62 ± 0.14	71.98 ± 0.10	81.22

Note: TOTOX – total oxidation.

TABLE 5. PHYSICAL AND CHEMICAL ANALYSIS OF DISCARDED OILS

Restaurant	Induction time (hr)	Smoke point (°C)	Lovibond colour	Free fatty acid (%)	Polar compound (%)	Peroxide value (mg kg^{-1})	ρ-anisidine value	TOTOX
A	31.3 ± 0.8	140.3 ± 2.1	19.9R + 31.8Y + 14.7 B	4.02 ± 0.03	17.32 ± 0.28	0.47 ± 0.06	5.99 ± 0.34	6.93
B	8.1 ± 1.1	161.3 ± 1.2	28.8R +26.9Y + 2.5B	1.47 ± 0.01	22.60 ± 0.36	4.79 ± 0.11	59.92 ± 0.30	69.50
C	14.9 ± 0.8	145.7 ± 0.6	14.3R + 23.9Y + 25.3B	7.72 ± 0.07	33.94 ± 0.41	1.81 ± 0.09	27.34 ± 0.44	30.96
D	31.9 ± 2.4	147.3 ± 1.2	22.4R + 40.4Y + 15.5B	3.98 ± 0.05	48.01 ± 0.22	3.88 ± 0.14	61.64 ± 0.49	69.40

Note: TOTOX – total oxidation.

compounds produced in the primary oxidation of lipids (Sebastian *et al.*, 2014). Gupta (2005) described that the ρ-AV of a good quality fresh oil should be less than 4.0 with not more than 6.0. Oils with ρ-AV of more than 6.0 is classified as extremely oxidised. However, Rossell (1989) suggested that the ρ-AV should be less than 10 for good quality oil. The ρ-AV of the fresh oils from the fast food restaurants participated in this study was in the range of 0.51-1.73. Based on the results obtained, none of the fresh oil had ρ-AV of more than 6, although the PV of the fresh oil from Restaurants B and D was more than 1 meq kg^{-1} (*Table 3*) indicating that the fresh oils used in all restaurants were good quality oil. There was a tremendous increase in ρ-AV in the in-use oils compared to the fresh oils. As high as 81% of the collected in-use oils having ρ-AV of more than 10 (*Table 4*). The lowest ρ-AV was noted from Restaurant A with ρ-AV of 7.16 while the highest was from Restaurant D with ρ-AV of 77.93. Karimi *et al.* (2017) reported that ρ-AV of the in-use oils collected from 11 commercial vendors around Nairobi city, Kenya, ranged from 7.7-42.1. Aladedunye and Przybylski (2009) explained that the ρ-AV increases along with the frying time. A similar finding was reached by Ahmad Tarmizi *et al.* (2013). They found that the ρ-AV increased gradually after five days of frying fries. Mahboubifar *et al.* (2016) also reported that the ρ-AV of the studied vegetable oils was increased moderately within 24 hr of frying and after that, the ρ-AV increased significantly. As mentioned earlier, the PV of oils used to fry fries was higher than the chicken products, and we encountered the same trend in ρ-AV. The ρ-AV of the chicken products in Restaurant A was the lowest compared to the other restaurants. It may be suggested that Restaurant A might practice a good oil management system in their frying procedures. It is very important to maintain a good oil management in the restaurant such as to have a schedule in the replenishment of the frying oil with the fresh oil. Aladedunye and Przybylski (2014) observed an elevated ρ-AV of the frying oils for the first two days of frying. However, the ρ-AV of the oils reached a plateau after that point. They suggested that the replenishment of frying oil with the fresh oil every day of frying contributed to the leveling of the ρ-AV. The ρ-AV of the discarded oils was in the range of 5.99-61.64 (*Table 5*). Similar research by Esfarjani *et al.*(2019) stated that the ρ-AV of the discarded samples collected in Tehran was 58.

TOTOX

TOTOX is typically used to explicit the overall oxidation state of the frying oils which combines the PV and ρ-AV. Generally, the recommended level of TOTOX value is less than or equal to 19.5 meq kg^{-1}, which increases linearly with both PV and ρ-AV (De Abreu *et al.*, 2010). Wai *et al.* (2009) noted that the lower the TOTOX value, the better quality of the oil as it is less susceptible to oxidative deterioration. TOTOX value of fresh oils from all restaurants was in the range of 1.92-4.29 (*Table 3*). It was observed that the values increased in the in-use oils. The TOTOX value of the in-use oils was in the range of 8.94-86.69 (*Table 4*). The highest TOTOX value was noted in the in-use oil from Restaurant D which was used to fry mixed products only after three days of frying. The lowest TOTOX value was observed in the in-use oil from Restaurant A which was used to fry original fried chicken indicating that the oil used in the restaurant was stable towards oxidative rancidity. The TOTOX value of the discarded oil was in the range of 6.93-69.50 with the highest from Restaurant B while the lowest from Restaurant A (*Table 5*).

CONCLUSION

The fast food restaurants in this study used RBD palm olein and/or palm oil as the frying medium for their food products. The fresh oils from all restaurants were of good quality for frying which were all below the maximum limit of smoke point, colour, FFA, polar compound, ρ-AV and have high oxidative stability index, making these oils good choice as the frying oils. Undoubtedly, frying activities deteriorated the quality of these oils by decreasing the smoke point and OSI, darkened

the colour and increased the levels of FFA, polar compound, PV and ρ-AV. The fast food restaurants in this study continued with their frying activities regardless of the state of the oils. Regardless the addition of synthetic antioxidants in the fresh oil, the oxidation state of the oils were alarming especially in Restaurant D where the oxidation state was the highest although the oils were used on Day 3. To ensure the safety of the consumption of these frying products from the fast food restaurants, it is suggested that inspection of the quality of the oils used in the fast food industry be implemented regularly as to maintain the quality of the products as well as the health of the consumers. On top of that, adequate training on the proper oil management can be given to the operators who are involved directly and indirectly with the frying activities in the premises to ensure that the fast food operators do not abuse the frying oils for their commercial frying activities. Based on the results of this study, it is crucial for an extensive study to be done to investigate the quality and the safety of the oils used for frying from various premises which operate the frying activities as this kind of studies could give a broad perspective of the oxidation process in frying oil related to the frying conditions.

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REFERENCES

Abdulkarim, S M; Long, K; Lai, O M; Muhammad, S K S and Ghazali, H M (2007). Frying quality and stability of high-oleic *Moringa oleifera* seed oil in comparison with other vegetable oils. *Food Chem., 105*: 1382-1389.

Ahmad Tarmizi, A H and Siew, W L (2008). Quality assessment of palm products upon prolonged heat treatment. *J. Oleo Sci., 57(12)*: 639-648.

Ahmad Tarmizi, A H; Niranjan, K and Gordon, M (2013). Physicochemical changes occurring in oil when atmospheric frying is combined with post-frying vacuum application. *Food Chem., 136*: 902-908.

Ahmad Tarmizi, A H; Karimah, A; Miskandar, M S and Choo, Y M (2016). Rancimat test for measuring oxidative stability of cooking oils upon prolonged frying: Short communication. *J. Oil Palm Res. Vol. 28(4)*: 531-535.

Aladedunye, F and Przybylski, R (2009). Degradation and nutritional quality changes of oil during frying. *J. Amer. Oil Chem. Soc., 86*: 149-156.

Aladedunye, F and Przybylski, R (2014). Performance of palm olein and modified rapeseed, sunflower, and soybean oils in intermittent deep-frying. *Euro J. Lipid Sci. Tech., 116*: 144-152.

Ali, M A; Daud, A S M; Latip, R A; Othman, N H and Islam, M A (2014). Impact of chicken nugget presence on the degradation of canola oil during frying. *Intl. Food Res. J., 21(2)*: 1119-1124.

Andrikopoulos, N K; Dedoussis, G V Z; Falirea, A; Kalogeropoulos, N and Hatzinikola, H S (2002). Deterioration of natural antioxidant species of vegetable oils during the domestic deep-frying and pan-frying of potatoes. *Intl. J. Food Sci. Nutr., 53*: 351-363.

Andrikopoulos, N K; Boskou, G; Dedoussis, G V Z; Chiou, A; Tzamtzis, V A and Papathanasiou, A (2003). Quality assessment of frying oils and fats from 63 restaurants in Athens, Greece. *Food Service Technology, 3*: 49-59.

AOCS (2012). *Official Methods and Recommended Practices of the American Oil Chemist Society*. AOCS Press, Champaign, IL, USA.

AOCS (2011). *Official Methods and Recommended Practices of the American Oil Chemist Society*. AOCS Press, Champaign, IL, USA.

AOCS (2009). *Official Methods and Recommended Practices of the American Oil Chemists' Society*. AOCS Press, Champaign, IL, USA.

AOCS (1992). *Official Methods and Recommended Practices of the American Oil Chemists' Society*. AOCS Press, Champaign, IL, USA.

Bansal, G; Zhou, W; Barlow, P J; Joshi, P S; Hui, L L and Chung, Y K (2010). Review of rapid tests available for measuring the quality changes in frying oils and comparison with standard methods. *Crit. Rev. Food Sci., 50(6)*: 503-514.

Banu, M; Prasad, N and Siddaramaiah (2016). Effect of antioxidant on thermal stability of vegetable oils by using ultrasonic studies. *Intl. Food Res. J., 23(2)*: 528-536.

Berger, K G (2005). *The Use of Palm Oil in Frying*. Malaysian Palm Oil Promotion Council. 113 pp.

Chen, W A; Chiu, C; Cheng, W C; Hsu, C K and Kuo, M I (2013). Total polar compounds and acid

values of repeatedly used frying oils measured by standard and rapid methods. *J. Food Drug. Anal., 21(1)*: 58-65.

Díaz-Sánchez, F; Santos-López, E M; Sosa-Morales, M E; Ramírez-Corona, N; Palou, E and López-Malo, A (2019). Stability of palm olein with or without antioxidants during industrial continuous deep-fat frying of wheat snacks. *J. Oil Palm Res. Vol. 31(3)*: 286-293.

De Abreu, D P; Losada, P P; Maroto, J and Cruz, J (2010). Evaluation of the effectiveness of a new active packaging film containing natural antioxidants (from barley husks) that retard lipid damage in frozen Atlantic salmon (Salmo *salar* L.). *Food Res. Int., 43(5)*: 1277-1282.

Enríquez-Fernández, B E; Yañez, L Á D L C Y and Sosa-Morales, M E (2011). Comparison of the stability of palm olein and a palm olein/canola oil blend during deep-fat frying of chicken nuggets and French fries. *Intl. J. Food Sci. Tech., 46*: 1231-1237.

Esfarjani, F; Khoshtinat, K; Zargaraan, A; Mohammadi-Nasrabadi, F; Salmani, Y; Saghafi, Z; Hosseini, H and Bahmaei, M (2019). Evaluating the rancidity and quality of discarded oils in fast food restaurants. *Food Sci. Nutr., 7*: 2302-2311.

Eyres, L (2015). Frying oils: Selection, smoke points and potential deleterious effects for health. *Food New Zealand, 15(1)*: 30.

Fan, H Y; Sharifudin, M S; Hasmadi, M and Chew, H M (2013). Frying stability of rice bran oil and palm olein. *Intl. Food Res. J., 20(1)*: 403-407.

Farhoosh, R; Niazmand, R; Rezaei, M and Sarabi, M (2008). Kinetic parameter determination of vegetable oil oxidation under Rancimat test conditions. *Euro J. Lipid Sci. Tech., 110*: 587-592.

Firestone, D (1993). Worldwide regulation of frying fats and oils. *INFORM, 4(12)*: 1366-1371.

Gertz, C (2000). Chemical and physical parameters as quality indicators of used frying fats. *Euro J. Lipid Sci. Tech., 102*: 566-572.

Ghobadi, S; Akhlaghi, M; Shams, S and Mazloomi, S M (2018). Acid and peroxide values and total polar compounds of frying oils in fast food restaurants of Shiraz, Southern Iran. *Intl. J. Nutr. Sci., 3(1)*: 25-30.

Goburdhum, D and Jhuree, B (1995). Effect of deep fat fry on fat oxidation in soybean oil. *Intl. J. Food Sci. Nutr., 46*: 363-371.

Gunstone, F D (2008). *Oils and Fats in the Food Industry* (First ed.). Wiley-Blackwell. United Kingdom. 160 pp.

Gupta, M K (2005). Frying oils. *Bailey's Industrial Oil and Fat Products* (Shahidi, F ed.). Volume 4. John Wiley and Sons. New Jersey. p. 1-31.

Habib, F Q; Abu Dardak, R and Zakaria, S (2011). Consumers' preference and consumption towards fast food: Evidence from Malaysia. *Business and Management Quaterly Review, 2(1)*: 14-27.

Hadorn, H and Zurcher, K (1974). Zur Bestimmung der Oxydations- stabilitat von O¨ len und Fetten. *Deutsche Lebensmittel-Rundschau, 70*: 57-65.

Ibrahim, N U A; Abd Aziz, S; Hashim, N; Jamaludin, D and Khaled, A Y (2019). Dielectric spectroscopy of palm olein during batch deep frying and their relation with degradation parameters. *J. Food Sci., 84(4)*: 792-797.

Innawong, B; Mallikarjunan, P and Irudayaraj, J (2004). The determination of frying oil quality using a chemosensory system. *J. Food Sci. Technol., 37*: 35-41.

Irwandi, J; Cheman, Y B and Kitts, D D (2000). Synergistic effect of rosemary and sage extracts and citric acid on fatty acid retention of RBD palm olein during deep-fat frying. *J. Amer. Oil Chem. Soc., 77*: 527-533.

Ismail, R (2005). Palm oil and palm olein frying applications. *Asia Pac. J. Clin. Nutr., 14(14)*: 414-419.

ISO 15305 (1998). *Animal and Vegetable Fats and Oils - Determination of Lovibond Colour*. 7 pp.

ISO 3960:2001 (2001). *Animal and Vegetable Fats and Oils - Determination of Peroxide Value*. 6 pp.

IUPAC (1992). *Standard Methods for the Analysis of Oils, Fats and Derivatives*. Oxford: International Union of Pure and Applied Chemistry. 151 pp.

Karakaya, S and Şimşek, S (2011). Changes in total polar compounds, peroxide value, total phenols and antioxidant activity of various oils used in deep fat frying. *J. Amer. Oil Chem. Soc., 88*: 1361-1366.

Karimi, S; Wawire, M and Mathooko, F M (2017). Impact of frying practices and frying conditions on the quality and safety of frying oils used by street vendors and restaurants in Nairobi, Kenya. *J. Food Compos. Anal., 62*: 239-244.

Katragadda, H R; Fullana, A; Sidhu, S and Barrachina, A A C (2010). Emissions of volatile

aldehydes from heated cooking oils. *Food Chem., 120*: 59-65.

Kowalski, B; Ratusz, K; Kowalska, D and Bekas, W (2004). Determination of the oxidative stability of vegetable oils by differential scanning calorimetry and Rancimat measurements. *Euro J. Lipid Sci. Tech., 106*: 165-169.

Latha, R B and Nasirullah, D R (2014). Physico-chemical changes in rice bran oil during heating at frying temperature. *J. Food Sci. Technol., 51(2)*: 335-340.

Lin, S; Akoh, C C and Reynolds, A E (2001). Recovery of used frying oils with adsorbent combinations: Refrying and frequent oil replenishment. *Food Res. Int., 34(2-3)*: 159-166.

Liu, C; Li, J; Bi, Y; Wang, X; Sun, S and Yang, G (2016). Thermal losses of tertiary butylhydroquinone (TBHQ) and its effects on the qualities of palm oil. *J. Oleo Sci., 65(9)*: 739-748.

Mahboubifar, M; Yousefinejad, S; Alizadeh, M and Hemmateenejad, B (2016). Prediction of the acid value, peroxide value and the percentage of some fatty acids in edible oils during long heating time by chemometrics analysis of FTIR-ATR spectra. *J. Iran Chem Soc., 13(12)*: 2291-2299.

Malaysian Standard (2007). *Palm Olein Specification (second revision)*. Department of Standards Malaysia. Cyberjaya, Selangor. 13 pp.

Man, C and Hussain, W (1998). Comparison of frying and performance of refined bleached and deodorised palm olein and coconut oil. *J. Food Lipids, 5*: 197-210.

Maskan, M (2003). Change in colour and rheological behaviour of sunflower seed oil during frying and after adsorbent treatment of used oil. *Euro Food Res. Tech., 218*: 20-25.

Mba, O I; Dumont, M J and Ngadi, M (2015). Palm oil: Processing, characterization and utilization in the food industry - A review. *Food Biosci., 10*: 26-41.

McGill, E A (1980). The chemistry of frying. *Bakers Digest, 62(6)*: 38-42.

Melton, S L; Jafar, S; Sykes, D and Trigiano, M K (1994). Review of stability measurements for frying oils and fried food flavour. *J. Amer. Oil Chem Soc., 71(12)*: 1301-1308.

Nayak, P K; Dash, U; Rayaguru, K and Krishnan, K D (2016). Physico-chemical changes during repeated frying of cooked oil - A review. *J. Food Biochem, 40*: 371-390.

Pokorny, J (1998). Substrate influence on the frying process. *Grasas y Aceites, 49 (3-4)*: 265-270.

Qin, X (2011). *Thermal Desorption Studies of Corn Oil at Frying Temperatures: Thermal Scission Versus Autoxidation*. Ph.D thesis, The State University of New Jersey, New Brunswick. 172 pp.

Razali, I; Fauziah, A and Nor Aini, S (1999). Quality of potato chips fried in palm olein and high oleic sunflower oil during batch frying. *Proc. of the PORIM International Palm Oil Congress*. PORIM, Bangi. p. 99-103.

Rossell, J B (1989). Measurement of rancidity. *Rancidity in Foods* (Allen, J C and Hamilton, R J eds.). Elsevier Applied Science Publication, London, United Kingdom. p. 27-30.

Rossell, J B (1997). *Factors Affecting the Quality of Frying Oils in New Developments in Industrial Frying*. Bridgwater: P J Barnes and Associates.

Sebastian, A; Ghazani, S M and Marangoni, A G (2014). Quality and safety of frying oils used in restaurants. *Food Res Int., 64*: 420-423.

Shaharudin, M R; Wan Mansor, S and Elias, S J (2011). Food quality attributes among Malaysia's fast food customer. *Intl. Business and Management, 2(1)*: 198-208.

Tian, X (2013). *Chemical Factors Affecting Degradation Processes of Vegetable Oils During Frying*. Ph.D thesis. The State University of New Jersey, New Brunswick. 165 pp.

Tompkins, C and Perkins, E G (1999). The evaluation of frying oils with the ρ-anisidine value. *J. Amer. Oil Chem. Soc., 76(8)*: 945-947.

Wai, T W; Saad, B and Lim, P B (2009). Determination of TOTOX value in palm olein using a F1-potentiometric analyser. *Food Chem., 11*: 285-290.

Warner, K and Gupta, M (2003). Frying quality and stability of low and ultra-low-linolenic acid soybean oils. *J. Amer. Oil Chem. Soc., 80(3)*: 275-280.

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LIFE CYCLE ASSESSMENT FOR THE PRODUCTION OF PALM BIODIESEL

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ABSTRACT

A gate-to-gate life cycle assessment (LCA) for the production of palm biodiesel was performed. The LCA study was conducted using SimaPro software version 8.5, and the impact assessment was performed according to ReCiPe 2016 methodology. A three-year (2015-2017) inventory data was obtained from five commercial palm biodiesel producers in Malaysia. Methanol, acids and sodium methoxide (catalyst) were identified as three major contributors to the environmental impacts. Impact assessment showed that replacement of fossil-based methanol with biomethanol produced from biogas is the most preferred option, saving up to 63% fossil resources and 22% reduction in global warming impact. Allocation based on economic value was found more suitable compared to mass or energy content. This is because both palm biodiesel and crude glycerol differ in terms of economic value and being used in different applications.

Keywords: LCA, palm biodiesel, transesterification.

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INTRODUCTION

Biodiesel is an important industry for Malaysia. The commercial production of palm biodiesel in Malaysia began in 2006 with the setting up of three commercial biodiesel plants, registering a total production of 54 981 t (Harrison, 2018). With 15 plants in commercial production in 2018, the annual production volume of palm biodiesel had then exceeded one million tonnes. Out of the total biodiesel produced, 515 000 t were sold, generating an export earnings of RM 1.43 billion, while 429 000 t were utilised for the biodiesel programme implemented locally (MPOB, 2019; Unnithan, 2019).

Refined, bleached and deodourised (RBD) palm oil is the typical feedstock for biodiesel production in Malaysia. During biodiesel manufacturing, RBD palm oil is reacted with methanol in the presence of an alkaline catalyst to produce palm methyl ester (PME) or palm biodiesel (*Figure 1*). The transesterification reaction is carried out at 60°C under an atmospheric pressure (Van Gerpen and Knothe, 2010). Commercially, the reaction is typically performed in two or three reactors with a continuous flow system. Glycerol, the by-product of transesterification, is separated from the methyl ester phase in settling tanks by gravity or using a centrifuge to expedite the phase separation. Upon removal of the glycerol phase, acid is used for neutralisation of the residual catalyst in the methyl ester phase and at the same time to split any soap that is formed between the alkaline catalyst and free fatty acids. Soap reacts with acid to form water-soluble salts which will be removed in the water washing process. Excess methanol is removed, recovered and reused in the transesterification reaction. Any remaining catalyst, soaps, salts, methanol and free glycerol are further removed from methyl ester during water washing. Lastly, water is removed by a vacuum dryer. PME with water content below 500 mg kg^{-1} is stored at a bulk storage facility and ready to be used as biodiesel. The glycerol produced is also subjected to a series of purification, *i.e.* acidulation, neutralisation and methanol recovery to produce

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crude glycerol with a purity of 80%-85%. Crude glycerol is typically sold to glycerol refiners for further purification before it is used for downstream applications.

The environmental evaluation of biodiesel, particularly palm biodiesel using life cycle assessment (LCA) approach has been conducted by various parties globally for the past decades. These LCA studies were mostly cradle-to-gate or cradle-to-grave type which emphasised mainly on greenhouse gas (GHG) emissions (Yee *et al.*, 2009; De Souza *et al.*, 2010; Choo *et al.*, 2011; Mohd Nor Azman *et al.*, 2011; Silalertruksa and Gheewala, 2012; Norfaradila *et al.*, 2014; Kittithammavong *et al.*, 2014; Siregar *et al.*, 2015; Maharjan *et al.*, 2017). It was reported that the main contributors to the environmental impacts were the production and utilisation of chemical fertilisers in oil palm plantations and biogas (mainly methane) emission from palm oil mill effluent (POME). Hence, these studies suggested various rectification steps within these sectors to minimise the environmental impacts. Thus far, there were very little information reported on the evaluation of activities in biodiesel production. Furthermore, some of the studies were conducted solely based on secondary data, with several assumptions that did not reflect the actual activities of the industry. For example, most of the biodiesel produced in Malaysia was from RBD palm oil (Unnithan, 2019) and not crude palm oil (CPO), as assumed in several studies (Yee *et al.*, 2009; Silalertruksa and Gheewala, 2012; Siregar *et al.*, 2015). Some of the researchers also assumed that both palm oil and palm kernel oil were mixed and used for the production of biodiesel (Arvidsson *et al.*, 2012). Hence, there was an obviously wrong assumption and some data gaps due to the missing inventory data on palm oil refining, which is an important stage of the palm oil and palm biodiesel industry in the country.

The current study was conducted with a specific aim to evaluate the environmental performance of the production of palm biodiesel on various impact categories which focus specifically on the activities in the biodiesel plant. It was also aimed to provide an up-to-date information on the palm biodiesel production in Malaysia, after the last published study conducted a decade ago (Puah *et al.*, 2010). Some of the parameters might have changed, as the numbers of biodiesel producers, and the total production volume have increased tremendously, based on the improvements of the various technology providers. In general, the LCA study was conducted in accordance to ISO standards, namely ISO 14040 Environmental management – Life cycle assessment – Principles and framework and ISO 14044 Environmental management – Life cycle assessment – Requirements and guidelines (ISO, 2006a, b).

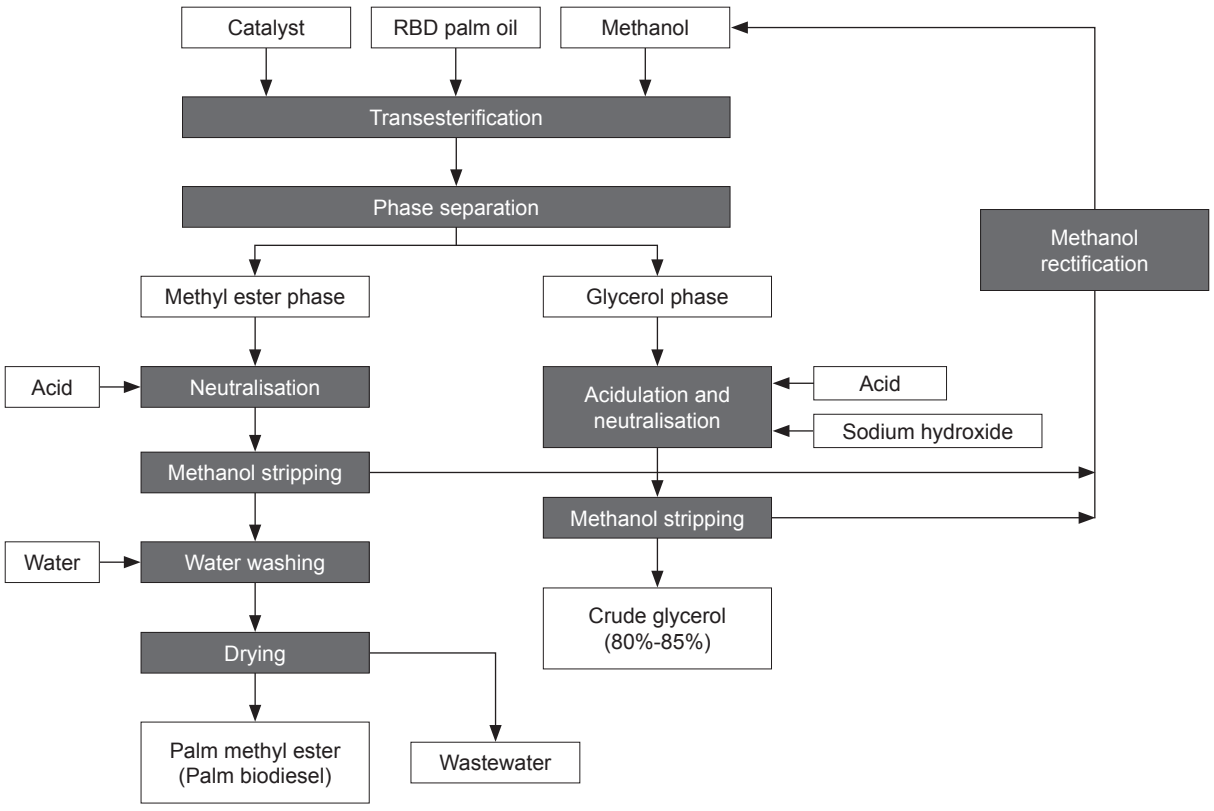


Figure 1. Process flow chart for the production of palm biodiesel.

MATERIALS AND METHODS

Goal and Scope Definition

The scope of the study focused on gate-to-gate production of palm biodiesel in commercial plants located in Malaysia. Transesterification using alkaline catalyst was the chemical process involved, and the main feedstock was RBD palm oil.

The goals of the study were to provide an up-to-date life cycle inventory data for the production of palm biodiesel in Malaysia, to perform a life cycle impact assessment (LCIA), and finally to evaluate the proposed options for environmental improvement within the scope of the study and the system boundary.

Functional Unit

The main product in a biodiesel plant is biodiesel that meets the Malaysian Standard Specification for PME, MS2008:2014 (Department of Standards Malaysia, 2014). Crude glycerol with a purity of 80%-85% is a by-product of biodiesel production. Both biodiesel and crude glycerol are commercially traded in tonne (mass) basis. Thus, the appropriate functional unit is the production of 1 t of products, *i.e.* palm biodiesel and crude glycerol.

System Boundary

The inventory data obtained were based on a gate-to-gate basis, starting from transportation of feedstock, *i.e.* RBD palm oil from palm oil refineries to biodiesel plants, transesterification of RBD palm oil to produce PME, purification of methyl ester and glycerol to produce palm biodiesel according to the standard specification and crude glycerol.

Inventory Data Collection

The inventory data for the production of palm biodiesel were gathered from five active biodiesel producers in Malaysia, three in Peninsular Malaysia, one each in Sabah and Sarawak. A three-year (2015-2017) factory operation data were collected for this purpose through dissemination of survey questionnaires. Data clarification and verification were carried out through e-mail and telephone communications. Besides site-specific foreground data, background data were obtained from the databases available in the SimaPro software, *i.e.* Ecoinvent 3.4, Agri-footprint and USLCI databases (PRé Consultants, 2018). The background process for the production of chemicals used in the transesterification process, *i.e.* methanol, sodium methoxide 30% (catalyst), sodium hydroxide (neutralising agent), acids, electricity supply from the Malaysian power grid, fossil fuels supply for steam production and water supply were referred to Ecoinvent 3.4 database. The emissions for transportation of RBD palm oil from refineries to biodiesel plants and the combustion of fossil fuels for steam production were referred to Agri-footprint and USLCI databases, respectively.

Co-products Allocation

Both palm biodiesel and crude glycerol are commercially traded according to their economic value in open market. Hence, allocation based on economic value was used in the study. The annual trading prices for palm biodiesel and crude glycerol were obtained from Thomson Reuters commodity platform to derive the 10-year average prices of palm biodiesel and crude glycerol. These prices were used to generate the allocation ratio of 96.8:3.2 for palm biodiesel and crude glycerol. Allocation methods based on mass and energy value were also evaluated and discussed in the sensitivity analysis of the study.

LCIA

Impact assessment is a process to link the inventory data to respective environmental impacts. In this study, the impact assessment was conducted using SimaPro software version 8.5 (PRé Consultants, 2018). ReCiPe 2016 (Hierarchist) methodology was used for the impact assessment at midpoint level (Huijbregts *et al*., 2017). Environmental impacts on global warming, ionising radiation, ozone formation, terrestrial acidification and fossil resource scarcity were analysed and discussed.

Exclusion

The data for capital goods such as building structures of biodiesel plants, equipment and machinery used were excluded in the study because of difficulty in getting reliable data. Besides, these data have no significant environmental impacts as reported in the previous studies (Puah *et al*., 2010; Malca and Freire, 2011; Schneider and Finkbeiner, 2013). Treatment of wastewater was also excluded in the current study for similar reasons.

RESULTS AND DISCUSSION

Inventory Analysis

The total volume of palm biodiesel produced by the five producers was 802 112 t or 42.3% of the total biodiesel production in Malaysia from 2015-2017. The weighted average data for the production of 1 t palm biodiesel are presented in *Table 1*. The inventory data are presented as tonne

of palm biodiesel produced, differing from the data reported by Puah *et al.* (2010) which focussed on the production and use of 1 MJ palm biodiesel in diesel vehicles or engines. In the previous study, the biodiesel production data were only obtained from two biodiesel producers. However, the major feed materials for biodiesel production in Malaysia were similar, *i.e.* RBD palm oil and methanol. In general, the predominant feedstock for biodiesel production in Malaysia is still RBD palm oil (94.5%) after more than a decade of commercial production. This is mainly due to its availability, as most of the CPO produced here is refined into RBD palm oil before being used for downstream edible and non-edible applications (Yung *et al.*, 2020). Furthermore, RBD palm oil is easily handled in biodiesel plants compared to CPO, as no pre-treatment or additional acid esterification is required for the former to get rid of its free fatty acids due to its low acidity. Other cheaper feedstocks in use are RBD palm stearin (3.8%) and palm fatty acid distillate (PFAD) (1.7%), producing small volume of biodiesel which were catered for the export market. Methanol is commonly used simply because it is the least expensive alcohol available globally.

On average, approximately 1 t of palm oil is required to react with 109 kg of methanol to produce 1 t of palm biodiesel and 127 kg of crude glycerol (*Table 1*). The catalyst used by all five producers for transesterification process in this study was sodium methoxide with 30% concentration, which differed from the sodium hydroxide as reported by Puah *et al.* (2010). Sodium methoxide is widely available in the commercial market at the present day and its global market value was estimated at around USD 0.3 billion (MarketWatch, 2019). It is more convenient for biodiesel producers in handling and storing sodium methoxide compared to sodium hydroxide. Furthermore, the on-site preparation of sodium hydroxide in liquid solution will potentially produce water which might adversely affect the transesterification reaction if the water is not removed from the solution (Van Gerpen and Knothe, 2010). Nevertheless, sodium hydroxide solution is used instead as a neutralising agent at the glycerol polishing stage. Acids in the form of hydrochloric acid, citric acid and acetic acid are commonly used by the producers to facilitate separation of crude glycerol from methyl ester phase. Similar to palm oil refineries, fossil fuels are used for steam production in the boiler house. Natural gas is used by producers in Peninsular Malaysia and Sarawak while petroleum diesel is the only option for the producer in Sabah. The average water consumption reported in this study was 603 litres t^{-1} of palm biodiesel produced, 66% higher than the value reported by Puah *et al.* (2010). In general, biodiesel plants are located at industrial areas near palm oil refineries. In fact, some of the biodiesel plants are part of the downstream activities next to the refinery complexes. Hence, the distance between the supply of RBD palm oil to biodiesel plant is very minimal. From the inventory data collected, less than 10 tkm was reported for transportation of feedstock to biodiesel plants.

TABLE 1. INVENTORY OF BIODIESEL PRODUCTION (per tonne of palm biodiesel produced)

Item	Unit	Amount
Input		
Refined, bleached and deodourised (RBD) palm oil	t	0.9406
RBD palm stearin	t	0.0380
Palm fatty acid distillate (PFAD)	t	0.0166
Total feed material	t	0.9952
Methanol	kg	108.8932
Sodium methoxide 30% (catalyst)	kg	9.4371
Hydrochloric acid	kg	9.5788
Citric acid	kg	0.8725
Acetic acid	kg	0.1396
Sodium hydroxide (neutralising agent)	kg	0.7853
Electricity	kWhr	37.1409
Boiler fuel		
Natural gas	m^3	6.0749
Diesel	kg	0.0081
Fuel oil	kg	0.1348
Water	litre	603.1306
Average distance from palm oil refineries to biodiesel plant	km	9.2864
Transport of feed oil to biodiesel plant	tkm	9.2418
Output		
Palm biodiesel	t	1.0000
Crude glycerol	kg	127.4327

Note: Weighted average data calculated from five palm biodiesel producers for year 2015-2017.

LCIA

The characterised LCIA for the production of palm biodiesel at midpoint level is shown in *Figure 2*. For all impact categories, the top three significant contributors were methanol, acids and the catalyst. This was followed by the production of fossil fuels and its combustion in the boiler house for steam production. The use of sodium hydroxide as a neutralising agent, water, transportation and electricity from power grid barely played any significant role, <5% to all the impact categories except for water scarcity. Approximately 30% of the impact on water scarcity was caused by the amount of water consumed in the biodiesel plant.

Methanol has significant impact to all the 18 midpoint impact categories, in particular, to the fossil and mineral resources scarcity, ozone formation (both effects on human health and terrestrial ecosystems), global warming, human non-carcinogenic toxicity, marine and freshwater ecotoxicity and stratospheric ozone depletion. More than 50% of the impact in these categories was due to methanol, followed by acids with substantial impact on marine eutrophication and land use. The use of sodium methoxide as the catalyst was significant in contributing to ionising radiation, freshwater eutrophication, terrestrial ecotoxicity and human carcinogen toxicity. Combustion of fossil fuels for steam production was an important contributor to terrestrial acidification, fine particulate matter formation and global warming. Water required for steam generation, cooling and biodiesel purification (water washing) only impacted the water consumption impact category.

Methanol is one of the most common chemicals supplied and shipped in the world with a total production which exceeded 95 billion litres every year (Hobson and Marquez, 2018). It is commercially produced mainly via syngas conversion using natural gas as feedstock.

Methanol was the single major contributor to the total GHG emitted, 77.8 kg CO_2 eq t^{-1} of palm biodiesel produced or 60% of the overall emissions (*Figure 3*). Replacement of fossil-based methanol with bioethanol or biomethanol is suggested to reduce the impact on global warming (Sampattagul *et al.*, 2011; Noorazah *et al.*, 2017). However, no detailed analysis is attainable thus far. Commercial production of biomethanol has gained recent attention as seen with the construction of several commercial biomethanol plants in Europe and North America (Hobson and Marquez, 2018).

Replacement of fossil-based methanol with biomethanol was evaluated in this study. Three scenarios were simulated, namely (1) biomethanol produced from biomass in Switzerland (Ecoinvent 3.4 database) and shipped to Malaysia, (2) biomethanol produced in Malaysia, modified from (1) with Malaysian utilities and (3) biomethanol produced from biogas by replacing natural gas with bio-compressed natural gas (bio-CNG) produced from POME. For scenario 1, although production of biomethanol from biomass could significantly lower the global warming effect (*Figure 3*), the long distance required to transport biomethanol to Malaysia has offset most of the GHG savings earned. Overall, the total GHG emitted was recorded at 112.5 kg CO_2 eq t^{-1} of palm biodiesel produced or equivalent to a saving of 13.2% compared to fossil-based methanol (*Figure 3*). No significant change was observed for scenario 2, mainly due to higher carbon emissions utilities in Malaysia compared to those in Switzerland. For scenario 3, the substitution of fossil-based methanol with biomethanol deriving from POME contributed to the highest GHG emissions reduction with a saving of 28.9 kg CO_2 eq for every tonne of palm biodiesel produced (*Figure 3*). This, in fact is indicative of bio-CNG as an environmental-friendly chemical which does not contribute to any environmental burden since it is derived from a waste material in palm oil mills.

On the other hand, it was observed that the impact on ionising radiation was 5.7 times higher if fossil-based methanol was replaced by biomethanol produced in Switzerland (*Figure 4*). This was mainly attributed to the use of nuclear-based electricity for the production of syngas from biomass and subsequently biomethanol production. A simulated biomethanol production using local utilities as for scenario 2 significantly lowered the ionising radiation impact. No significant difference was observed for scenario 3 compared to fossil-based methanol.

The impact of ozone formation on human health and terrestrial ecosystems was again dominated by methanol (61.8%), catalyst (14.2%) and acids (14.2%), as shown in *Figure 5*. This impact correlated strongly with transportation mode of methanol. The long distance transportation of biomethanol produced in Switzerland to Malaysia raised the total impact on ozone formation to 2.4 times higher than that using fossil-based methanol. However, there was no significant change for scenarios 2 and 3 as the distance involved was assumed to be relatively shorter. The same was observed for terrestrial acidification impact category with 1.9 times higher score mainly due to long distance transportation (*Figure 6*).

For fossil resource scarcity impact category, significant savings of fossil resources, *i.e.* natural gas, was observed for all scenarios if fossil-based methanol was replaced by biomethanol (*Figure 7*). Savings of 59.9 kg oil eq to 65.3 kg oil eq (more than 50% savings) per tonne of palm biodiesel produced was recorded for all three biomethanol scenarios.

Based on the LCIA analysis, changing of feed materials from fossil-based to natural resources may not always resolve the associated environmental impacts. It happened that only a few impact categories were affected.

Sensitivity Analyses - Allocation of Co-products

Evaluation of different allocation methods, *i.e.* allocation based on economic value, energy content and mass value to that of no allocation with all the environmental burden assigned to palm biodiesel only was conducted (*Figure 8*). Both palm biodiesel and crude glycerol are the main product and co-product from biodiesel plants. They are traded at different market prices. Based on the inventory data collected, 88.7% of the total products, measured by mass, was palm biodiesel, and the remaining 11.3% was crude glycerol. Allocation ratio of 96.8:3.2 was derived from the average 10-year prices of palm biodiesel and crude glycerol, whereas allocation ratio of 94.4:5.6 was based on energy content assuming that the calorific values of palm biodiesel and crude glycerol are 40.0 MJ kg^{-1} and 18.5 MJ kg^{-1}, respectively.

Figure 8 shows that the environmental impacts attributed to palm biodiesel was reduced by 11% based on smaller mass value assigned to the crude glycerol portion. This allocation is very straight forward and can be easily performed based on systematic record of the production figures of biodiesel and crude glycerol by all producers. However, the appropriateness of such allocation ratio is always questioned as similar environmental burden will be obtained for the production of 1 t of palm biodiesel and 1 t of crude glycerol although it is known that these two products are different in terms of economic value and energy content. It might be more appropriate to assign the environmental burden based on their economic value and energy content. As such, 3.2% and 5.6% of the overall environmental burden were allocated to 127 kg of crude glycerol produced. Based on the economic value, the environmental burden for the production of 1 t of palm biodiesel has become 3.9 times higher than that for 1 t of crude glycerol. If crude glycerol were to be used as an energy source, *e.g.* as a fuel for boiler or industrial burner, its environmental burden will be slightly less than half that of 1 t of palm biodiesel produced.

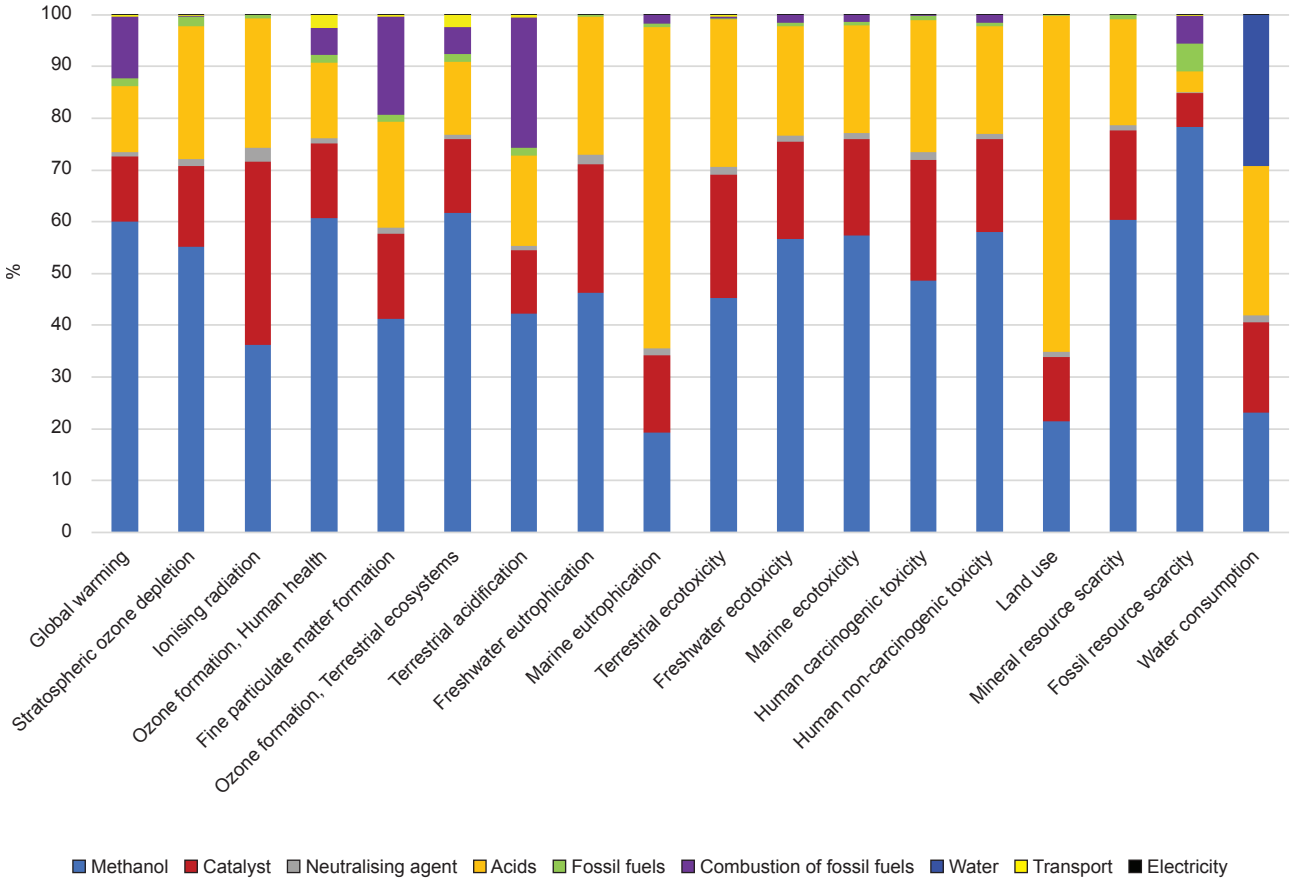
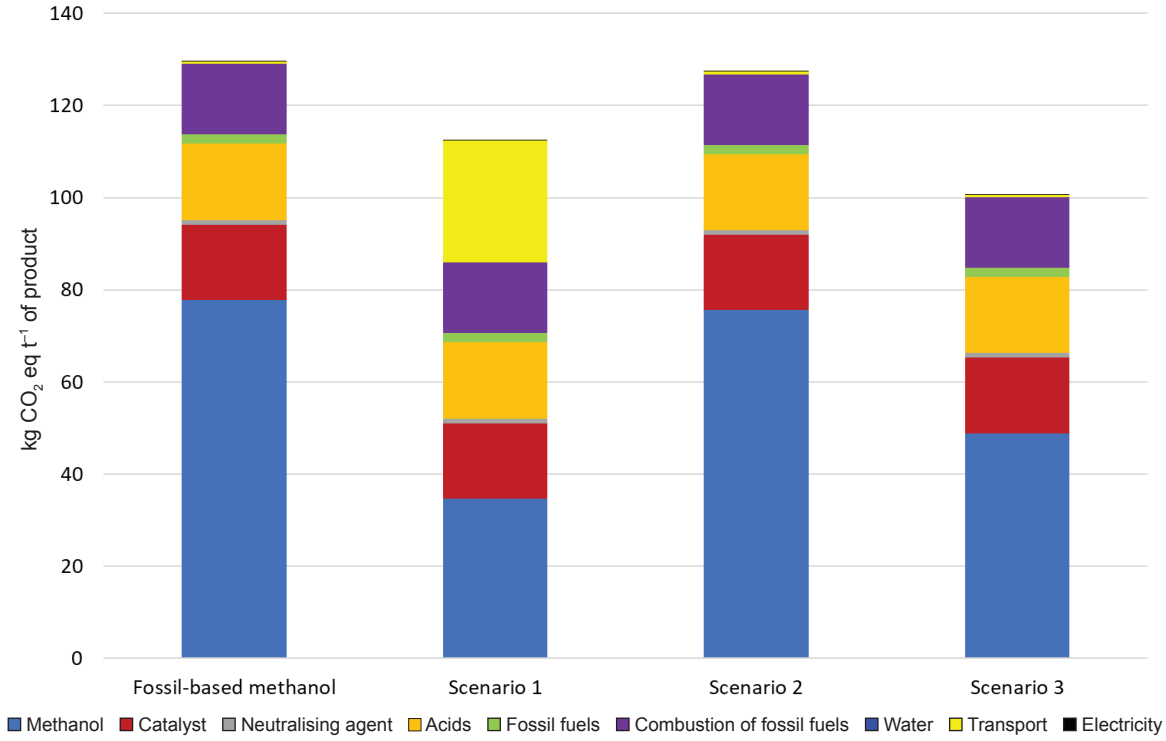
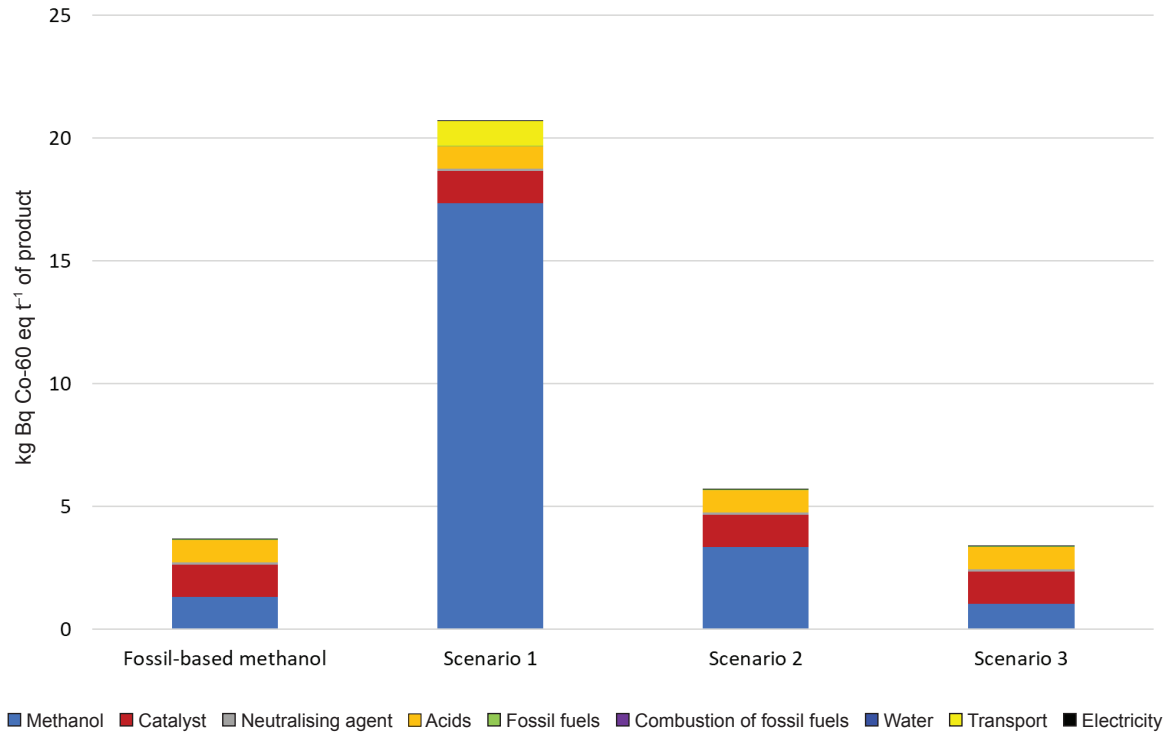


Figure 2. Characterised life cycle impact assessment (LCIA) for the production of palm biodiesel.



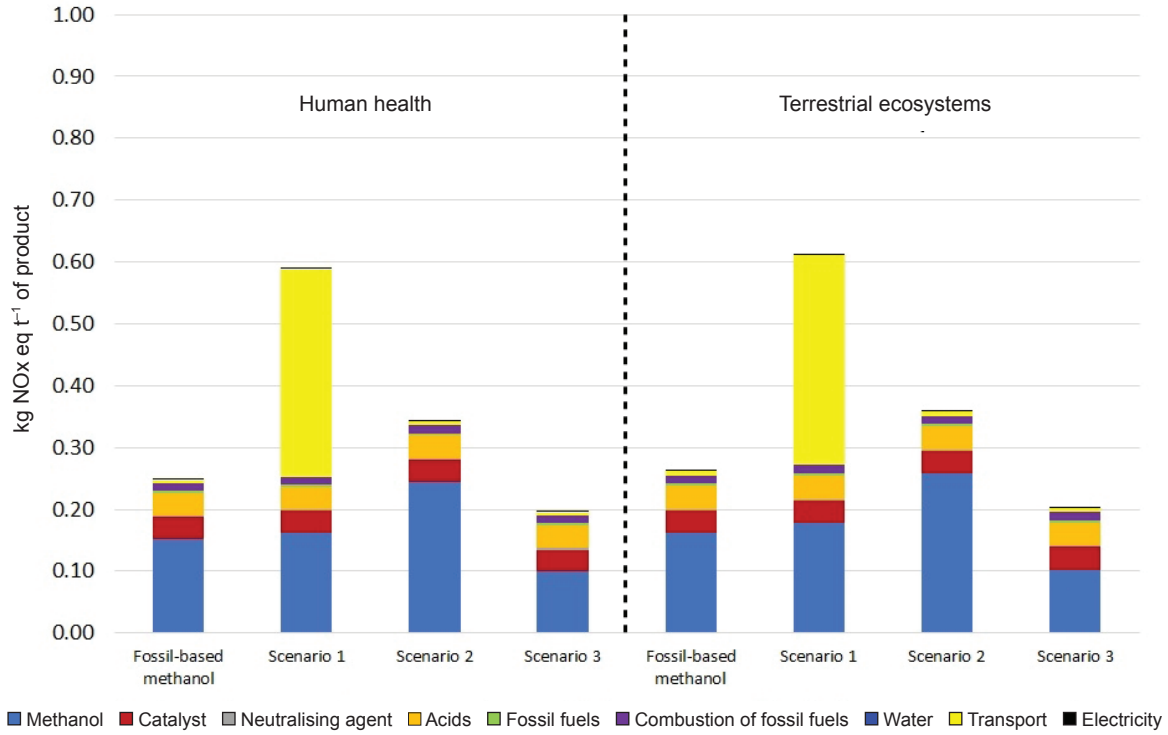
Note: Scenario 1: Replacement of fossil-based methanol with biomethanol produced from biomass in Switzerland; Scenario 2: Replacement of fossil-based methanol with biomethanol produced from biomass in Malaysia; Scenario 3: Replacement of fossil-based methanol with biomethanol produced from biogas in Malaysia.

Figure 3. Global warming for the production of 1 t of palm biodiesel.



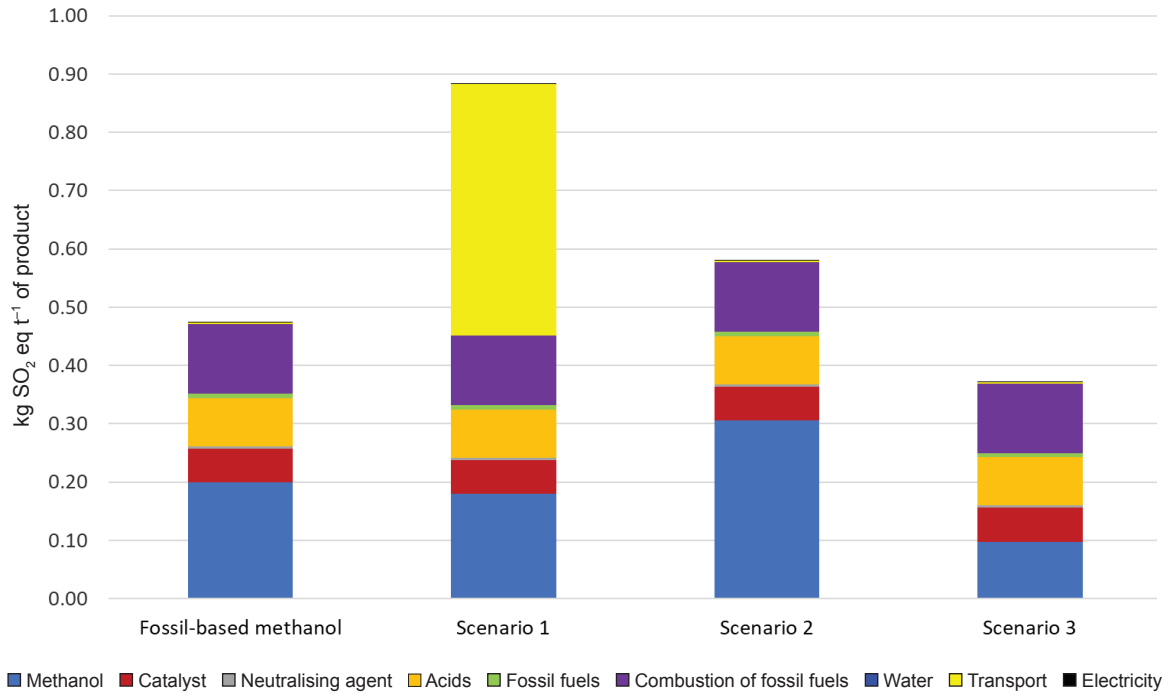
Note: Scenario 1: Replacement of fossil-based methanol with biomethanol produced from biomass in Switzerland; Scenario 2: Replacement of fossil-based methanol with biomethanol produced from biomass in Malaysia; Scenario 3: Replacement of fossil-based methanol with biomethanol produced from biogas in Malaysia.

Figure 4. Ionising radiation for the production of 1 t of palm biodiesel.



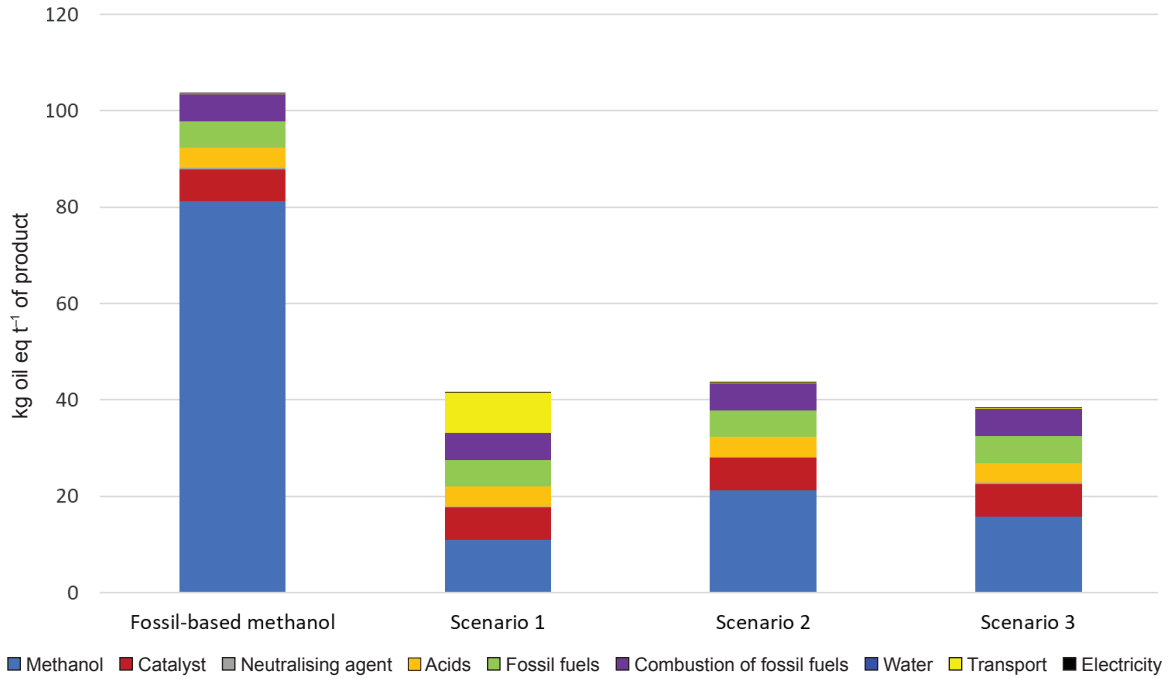
Note: Scenario 1: Replacement of fossil-based methanol with biomethanol produced from biomass in Switzerland; Scenario 2: Replacement of fossil-based methanol with biomethanol produced from biomass in Malaysia; Scenario 3: Replacement of fossil-based methanol with biomethanol produced from biogas in Malaysia.

Figure 5. Ozone formation (human health and terrestrial ecosystems) for the production of 1 t of palm biodiesel.



Note: Scenario 1: Replacement of fossil-based methanol with biomethanol produced from biomass in Switzerland; Scenario 2: Replacement of fossil-based methanol with biomethanol produced from biomass in Malaysia; Scenario 3: Replacement of fossil-based methanol with biomethanol produced from biogas in Malaysia.

Figure 6. Terrestrial acidification for the production of 1 t of palm biodiesel.



Note: Scenario 1: Replacement of fossil-based methanol with biomethanol produced from biomass in Switzerland; Scenario 2: Replacement of fossil-based methanol with biomethanol produced from biomass in Malaysia; Scenario 3: Replacement of fossil-based methanol with biomethanol produced from biogas in Malaysia.

Figure 7. Fossil resource scarcity for the production of 1 t of palm biodiesel.

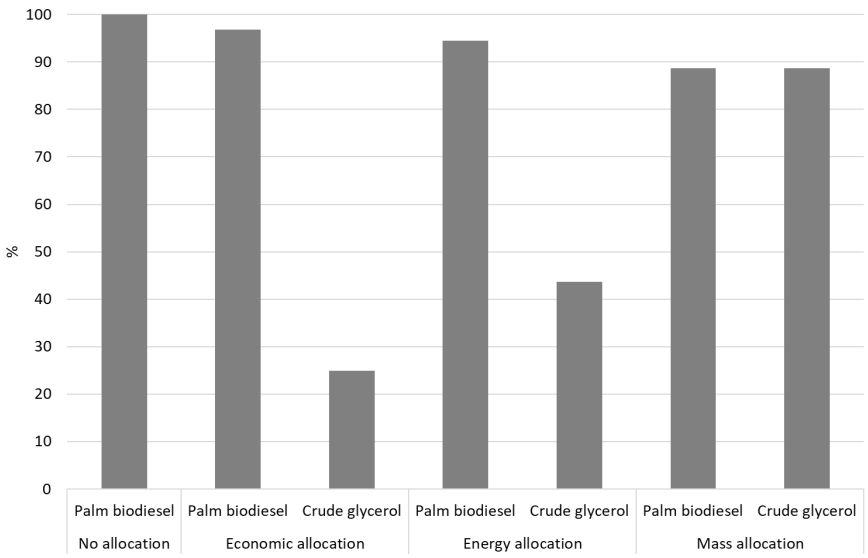


Figure 8. Environmental impacts per tonne of palm biodiesel and per tonne of crude glycerol produced, comparison of different allocation methodologies.

CONCLUSION

Based on the LCA conducted for commercial palm biodiesel production, methanol, catalyst and acids were the main contributors to the environmental impacts. Replacement of fossil-based methanol with biomethanol was able to lower the overall environmental impact. However, not all the biomethanol sources would have positive contribution to the environmental impact. Biomethanol derived using bio-CNG from POME is the most preferred as it has a positive contribution to the environment, in particular, global warming and fossil scarcity impact categories. As allocation based

on mass value does not reflect the actual differences of both products (palm biodiesel and crude glycerol) and the amount of crude glycerol used as fuel substitute is insignificant, allocations based on economic value can be more appropriate and relevant as both products are traded commercially in open market at different prices.

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REFERENCES

Arvidsson, R; Fransson, K; Froling, M; Svanstrom, M and Molander, S (2012). Energy use indicators in energy and life cycle assessments of biofuels: Review and recommendations. *J. Cleaner Prod.*, *31*: 54-61.

Choo, Y M; Halimah, M; Zulkifli, H; Vijaya, S; Puah, C W and Tan, Y A (2011). Determination of GHG contributions by subsystems in the oil palm supply chain using the LCA approach. *Int. J. Life Cycle Assess.*, *16*: 669-681.

Department of Standards Malaysia (2014). MS 2008 Malaysian Standard specification for automotive fuels - palm methyl ester (PME) for diesel engines – Requirement and test methods. Department of Standards Malaysia, Ministry of Science, Technology and Innovation, Cyberjaya.

De Souza, S P; Pacca, S; De Avila, M T and Borges, J L B (2010). Greenhouse gas emissions and energy balance of palm oil biofuel. *Renewable Energy, 35*: 2552-2561.

Harrison, L L N (2018). Status of biodiesel implementation in Malaysia and biodiesel specifications. Paper presented at the 2nd APEC Workshop on Guidelines toward High Biodiesel Blend Diesel (*e.g.* B20) Specification in the APEC Region. Hilton Waikiki Beach Hotel, Hawaii, USA. 19 March 2018. https://www.egnret.ewg.apec.org/sites/default/files/geektic/web/workshop/2nd-Biodiesel%20workshop/4_Biodiesel%20Specification-%20Malaysia.pdf, accessed on 22 August 2019.

Hobson, C and Marquez, C (2018). *Renewable Methanol Report*. Methanol Institute. https://www.methanol.org/wp-content/uploads/2019/01/MethanolReport.pdf, accessed on 30 October 2019.

Huijbregts, M A J; Steinmann, Z J N; Elshout, P M F; Stam, G; Verones, F; Veira, M D M; Hollander, A; Zijp, M and Van Zelm, R (2017). ReCiPe 2016 v1.1 A harmonized life cycle assessment method at midpoint and endpoint level. National Institute of Public Health and the Environment, BA Bilthoven, The Netherlands. https://www.rivm.nl/documenten/recipe2016v11, accessed on 23 January 2019.

ISO (2006a). ISO 14040. Environmental management - Life cycle assessment - Principles and framework. The International Organisation for Standardisation, Switzerland.

ISO (2006b). ISO 14044. Environmental management - Life cycle assessment - Requirements and guidelines. The International Organisation for Standardization, Switzerland.

Kittithammavong, V; Arpornpong, N; Charoensaeng, A and Khaodhiar, S (2014). Environmental life cycle assessment of palm oil-based biofuel production from transesterification: greenhouse gas, energy and water balances. *Proc. of the 2014 International Conference on Advances in Engineering and Technology*. p. 615-621.

Maharjan, S; Wang, W and Teah, H Y (2017). Life cycle assessment of palm-derived biodiesel in Taiwan. *Clean Techn. Environ. Policy, 19*: 959-969.

Malca, J and Freire, F (2011). Life-cycle studies of biodiesel in Europe: A review addressing the variability of results and modelling issues. *Renewable and Sustainable Energy Reviews, 15*: 338-351.

MarketWatch (2019). Sodium methoxide solution as a biodiesel catalyst market static represent 3.1% CAGR prospects 2017 to 2018. https://marketresearch.biz/report/sodium-methoxide-solution-as-a-biodiesel-catalyst-market/#details, accessed on 30 October 2019.

Mohd Nor Azman, A H; Jaramillo, P and Griffin, W M (2011). Life cycle GHG emissions from Malaysian oil palm bioenergy development: The impact on transportation sector's energy security. *Energy Policy, 39*: 2615-2625.

MPOB (2019). *Malaysian Oil Palm Statistics 2018*. 38th Edition. MPOB, Bangi.

Norfaradila, J; Norela, S; Salmijah, S and Ismail, B S (2014). Life cycle assessment (LCA) for the production of palm biodiesel: A case study in Malaysia and Thailand. *Malays. Appl. Biol., 43(1)*: 53-63.

Noorazah, Z; Zulina, A M; Razmah, G and Zainab, I (2017). Environmental assessment on methyl ester production from palm feedstock: A case study. *J. Oil Palm Res. Vol. 29(3)*: 414-423.

PRé Consultants (2018). What's new in SimaPro 8.5. https://www.pre-sustainability.com/download/manuals/SimaPro85WhatIsNew.pdf, accessed on 20 February 2018.

Puah, C W; Choo, Y M and Ma, A N (2010). Life cycle assessment for the production and use of palm biodiesel (part 5). *J. Oil Palm Res. Vol. 22*: 927-933.

Sampattagul, S; Nutongkaew, P and Kiatsiriroat T (2011). Life cycle assessment of palm oil biodiesel production in Thailand. *Int. J. Renewable Energy, 6*: 1-14.

Schneider, L and Finkbeiner, M (2013). Life cycle assessment of EU oilseed crushing and vegetable oil refining. https://www.fediol.eu/data/1521124882Full%20FEDIOL%20LCA%20report_05062013_CR%20statement.pdf, accessed on 24 August 2018.

Silalertruksa, T and Gheewala, S H (2012). Environmental sustainability assessment of palm biodiesel production in Thailand. *Energy, 43*: 306-314.

Siregar, K; Tambunan, A H; Irwanto, A K; Wirawan, S S and Araki, T (2015). A comparison of life cycle assessment on oil palm (*Elaeis guineensis* Jacq.) and physic nut (*Jatropha curcas* Linn.) as feedstock for biodiesel production in Indonesia. *Energy Procedia, 65*: 170-179.

Unnithan, U R (2019). Global development of biodiesel and challenges. *Proc. of the PIPOC 2019 International Palm Oil Congress – Chemistry, Processing Technology and Bio-energy Conference*. MPOB, Bangi. p. 129-141.

Van Gerpen, J V and Knothe, G (2010). Basic of transesterification reaction. *The Biodiesel Handbook*. 2nd Edition. AOCS Press, Illinois. p. 31-46.

Yee, K F; Tan, K T; Ahmad, Z A and Lee, K T (2009). Life cycle assessment of palm biodiesel: Revealing facts and benefits for sustainability. *Applied Energy, 86*: S189-S196.

Yung, C L; Vijaya, S and Sumiani, Y (2020). Life cycle assessment for palm oil refining and fractionation. *J. Oil Palm Res. Vol. 32(2)*: 341-354.

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LIFE CYCLE ASSESSMENT OF PALM OIL CLINKER AS A BINDER AND AGGREGATE REPLACEMENT IN CONCRETE

SAZNIZAM SAZMEE SINOH*; ZAINAH IBRAHIM* and FARIDAH OTHMAN*

ABSTRACT

Palm oil clinker (POC) is a by-product derived from crude palm oil (CPO) production. Many studies have examined POC in concrete and it has often been stated as being environmentally sustainable. However, evidence to support these claims are not abundant in the literature. Therefore, this study aimed to assess the environmental impact of POC using a comparative, midpoints life cycle assessment approach based on 13 impact categories from ReCiPe2016. The use of POC as a binder replacement, fine aggregate and coarse aggregate was considered. Only production of cement, sand, gravel, POC and transportation were included in the system boundary. The construction, service and end-of-life phases were excluded. A volume of 1 m^3 mortar or concrete with similar compressive strength was used as the functional unit. Life cycle inventory data was obtained from the literature, Malaysia Life Cycle Inventory Database (MY-LCID) and Ecoinvent database. Economic and mass allocation factors were calculated for POC. Calculations indicated that the use of POC in mortar and concrete showed reductions in all impact categories when economically allocated. When mass allocated, POC contributed minimally to all impact categories except 'Freshwater Eutrophication' and 'Human Toxicity'. Despite these drawbacks, results show that use of POC resulted in an overall improvement for the environmental sustainability.

Keywords: life cycle assessment, palm oil clinker, sustainable concrete, binder replacement, aggregate replacement, environment impact.

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INTRODUCTION

Oil palm (*Elaeis guineensis*) is a species of palm originating from West Africa which was brought into Malaya and planted commercially at Tennamaram Estate, Selangor, Malaya for the first time in 1917 and later became one of the country's key economic sectors (Nambiappan *et al.*, 2018). A complex process transforms oil palm fruit into crude palm oil (CPO) which undergoes further refinement as cooking oil or as biodiesel (Hansen *et al.*, 2014). Malaysia is the world's second largest producer of CPO, the largest exporter of CPO and the leading contributor for technical-based palm oil studies (Hansen *et al.*, 2015). The production of CPO of this magnitude creates a proportionally large quantity of waste. This creates an opportunity for Malaysia to be the leading proponent for the use of this waste in the local construction industry.

Figure 1 shows a schematic diagram of the CPO production process and its by-products based on literature and on-site investigation. Palm oil mills utilise energy recovery by burning solid wastes from the production of CPO. This involves incinerating empty fruit bunches (EFB), oil palm shells (OPS) and mesocarp fibre (MF) in a boiler at more than 500°C to produce steam. This steam is used for electricity generation and sterilisation of fresh fruit bunches (FFB) in the CPO production process (Subramaniam *et al.*, 2008). After incineration is complete, a grey, clumpy, hard and solid compound remains which is known as palm oil clinker (POC) (Ahmad and Nurazuwa, 2007).

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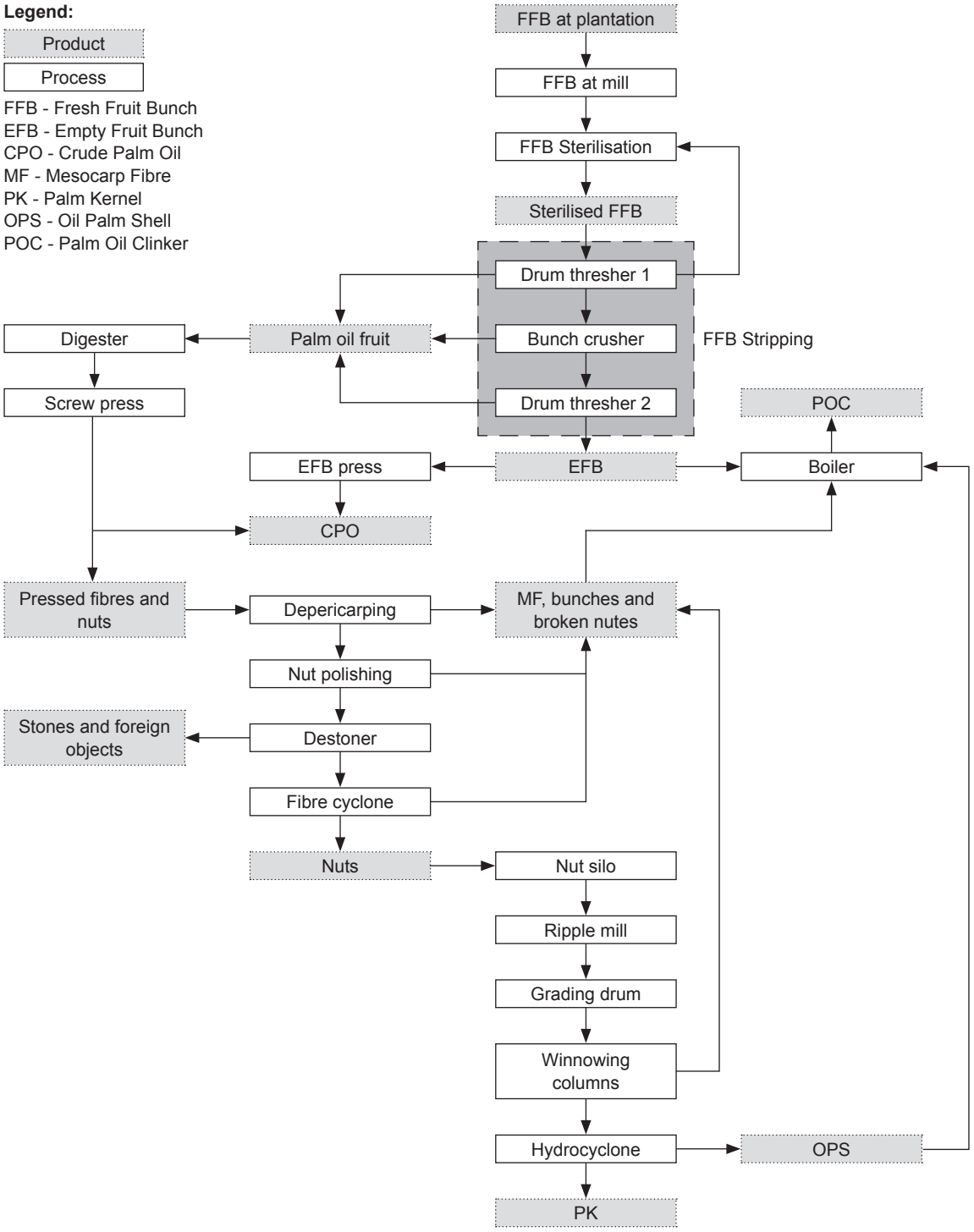


Figure 1. Production of crude palm oil (CPO) and its by-products.

POC is produced differently to another palm oil by-product (POBP) commonly utilised in concrete which is palm oil fuel ash (POFA). Fuel ash or fly ash is driven out of the boiler together with other flue gases and collected by particle filtration or electrostatic precipitators (Black, 2016). On the other hand, ash which settles towards the bottom of the boiler and fuses together is known as clinker (Sims and Brown, 1998). Thus, POC usually requires crushing and sizing before it can be used as an aggregate in concrete while POFA only requires homogenisation using a rotary pan mixer (Karim *et al.*, 2018).

Conventionally, POC is used as a filler for mill roads or is disposed as waste (Subramaniam *et al.*, 2008). However, fundamental principles of waste management dictate that disposal should be a last resort (Abdullah and Sulaiman, 2013).

Thus, researchers have studied the use of POC as a construction material. Early work on POC have shown that it can be used in mortar and concrete as either a binder or aggregate replacement. However, most of these studies on POC only address its engineering performance, but not its environmental impact.

Palm oil has received considerable attention in the past 25 years among the academic community. An exponential increase in palm oil related publications was observed from 355 publications in 2004 to 1796 in 2013 (Hansen *et al.*, 2015). Many of the studies that contributed towards this increase were from the use of POBP as a green construction material. In fact, POBP have a diverse range of novel applications (Hamada *et al.*, 2018). For example, Alengaram *et al.* (2016) studied the use of OPS to improve the blast resistance of concrete. Apart from that, Mazlan *et al.* (2020) used cellulose nanocrystals extracted from EFB as a mortar admixture. These studies are just a handful of research showing the potential of POBP which were considered as waste products several decades earlier.

Research into the use of POBP in concrete started as early as the 1990s with the introduction of POFA as a pozzolanic material in concrete (Tay, 1990). Ahmad *et al.* (2007) found that the use of POC as coarse aggregate in concrete was able to produce compressive strengths which were satisfactory for use as structural members. Beginning in the 2010s, research into the use of POBP diversified with its use in concrete mixes to produce lightweight concrete (Alengaram *et al.*, 2011) and to produce structural members (Mo *et al.*, 2016).

Most of the studies for POBP in concrete were related to its physical and durability properties. Studies on the sustainability aspect of concrete containing POBP, specifically POC, were not abundant in the literature. For example, Kanadasan and Abdul Razak (2015a) reported the reduction in carbon emissions relative to POC content in concrete but no other impact categories were included in their environmental impact assessment. Kanadasan *et al.* (2015) reported the environmental impacts of POC concrete but was limited to carbon dioxide (CO_2) emissions and engineering environmental index (EEI) only. Aslam *et al.* (2016) mentioned the potential of concretes containing POC as a step closer to achieving sustainable development but did not perform any environmental assessment. Likewise, Ahmmad *et al.* (2017) discussed the feasibility of using POC in lightweight concrete but this was limited to engineering aspects and did not include environmental impacts. Yet, some studies did report the cost efficiency of using palm oil by-products in concrete which showed that use of POC reduces the overall cost of the concrete and may be advantageous in terms of financial savings (Shafigh *et al.*, 2014; Kanadasan and Abdul Razak, 2015a).

Technical, social, economic and environmental aspects associated with the use of POC as a construction material should be addressed in a balanced manner for academic research to contribute to the holistic sustainable development of both the palm oil and construction industries. Subsequently, systematic studies on the environmental performance of POC as a construction material were not abundant in the literature. Thus, the goal of this study was to evaluate the environmental impacts of incorporating POC as a binder or aggregate replacement. Life cycle assessment (LCA) was used as the framework for environmental impact assessment of POC. Using LCA provides a detailed and systematic analysis of the environmental impacts of concrete in a variety of impact categories which are lacking in other impact evaluation methods.

LCA of Concrete

The LCA is a systematic method to quantify the inputs and outputs associated with the manufacture of a product. Studies relating to LCA in the construction industry are fragmented due to the variety of case studies, functional units, system boundaries, material selection, geographic locations, construction processes, building design and building use (Abd Rashid and Yusoff, 2015). There are two types of LCA commonly used in the construction industry: (1) comprehensive building LCA (also known as whole building LCA) which assesses the environmental impact of a building over its life span; and (2) comparative LCA which compares the environmental impacts of construction materials. This study used the comparative LCA method in which the environmental impacts of conventional mortar and concrete were compared to their counterparts containing POC.

Investigation into the environmental impacts of concrete gained popularity in the 1990s as awareness of the environment started to take hold in the public mindset and the construction industry began shifting towards more sustainable practices (Nielsen and Glavind, 2007). Much attention has shifted to concrete since it is the most abundantly used construction material and its production alone represents approximately 6%-7% of all anthropogenic carbon dioxide (Akan *et al.*, 2017). The term 'green' implies an environmental-friendly alternative to conventional concrete which often have reduced cement content, incorporate renewable or sustainable materials and possess reduced environmental impacts. Numerous studies have used LCA to systematically assess the environmental impacts of various materials in concrete such as recycled aggregate (Dobbelaere *et al.*, 2016). Concrete incorporating POC also represents a green concrete which has been comprehensively studied in terms of its technical performance, but less so in terms of its environmental impact.

MATERIALS AND METHODOLOGY

Mix Design

The mortar and concrete mix designs used in the study were obtained from the literature. Mortar mixes were obtained from Sumesh *et al.* (2018) and Kanadasan and Abdul Razak (2015b). Concrete mixes were obtained from Abutaha *et al.* (2016) and Mohd Hilton *et al.* (2008). These mixes are summarised in *Table 1*. The number in the mix designation indicates the percent replacement of either binder or aggregate. M-0 denotes the reference mix for mortar and M-40 denotes a replacement of 40% binder with palm oil clinker powder (POCP). For C-0, C-100C and C-100F, cement content remained constant but with different ratios of sand, gravel, coarse POC and fine POC. Here, C-0 was the reference mix for concrete. From *Figure 2*, compressive strengths at 28 days of the mortar mixes (M-0 and M-40) and concrete mixes (C-0, C-100C and C-100F) are shown. Although these mixes were designed for various purposes, they were selected to show that POC mixes can achieve comparable compressive strength with conventional mortar or concrete. This is also consistent with the functional unit used in the study.

TABLE 1. MIXED PROPORTIONS OF MORTAR, CONCRETE AND PALM OIL CLINKER

Mix	Cement	Water-to-binder ratio	Sand	Gravel	Palm oil clinker
M-0[a]	550	0.32	1 650	0	0
M-40[b]	520	0.29	1 140	0	350[e]
C-0[c]	420	0.53	760	1 007	0
C-100C[d]	420	0.55	621	0	729[f]
C-100F[c]	420	0.53	0	1 007	614[g]

Note: All units in kg m^3
[a]Sumesh *et al*. (2018).
[b]Kanadasan and Abdul Razak (2015b).
[c]Abutaha *et al*. (2016).
[d]Mohd Hilton *et al*. (2008).
[e]Palm oil clinker powder.
[f]Coarse palm oil clinker.
[g]Fine palm oil clinker.

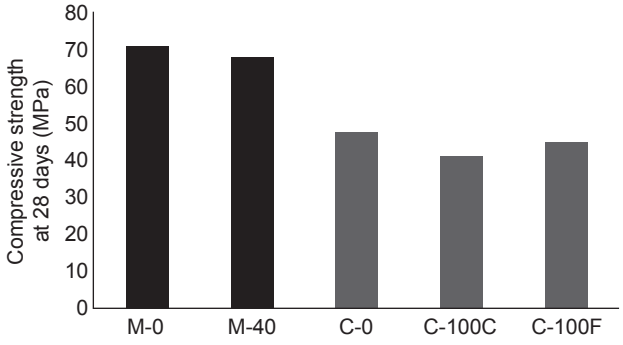


Figure 2. Compressive strength of mortar and concrete.

LCA Framework

The LCA in this study followed ISO 14040 (2006a) and ISO 14044 (2006b) which respectively describe the principles and framework of an LCA. These standard documents have been used as the standard framework for LCA for over 20 years. However, they do not specify the mechanics of the LCA such as methods for data collection which are left to the practitioner to decide. This allows LCA to be used for environmental impact assessment in different applications. As shown in *Figure 3*, an LCA is comprised of four stages: goal and scope definition, inventory analysis, impact assessment, and interpretation. In this study, the Results and Discussion section was used as the Impact Assessment and Interpretation phases of the LCA.

Functional Unit

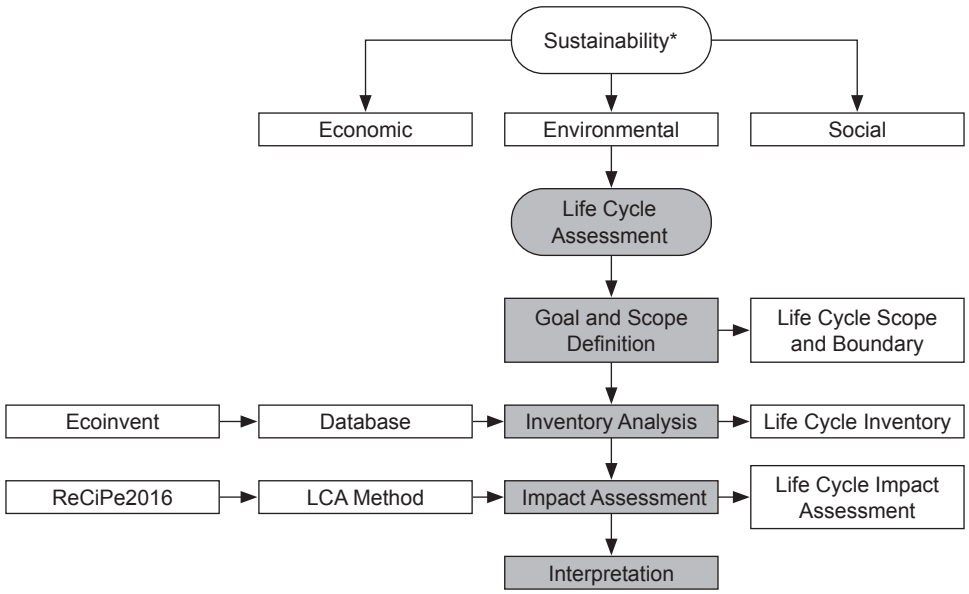
The LCA requires a functional unit (FU) to be defined as a unit of reference between different LCA studies. It is necessary to define that all concrete fulfil the same functional requirements (Marinkovic *et al.*, 2016). In other words, their mechanical properties and durability must be similar. It was desirable to use a FU which included strength and durability characteristics of the concrete which was employed in some studies (Van den Heede and De Belie, 2014). However, studies on the long-term durability properties of POC concrete are not abundant in the literature. Furthermore, it was assumed that exposure conditions were non-aggressive. Therefore, this study used a FU based on volume and compressive strength only. From *Figure 2*, it was observed that the compressive strengths for mortar mixes were within the range of 68 MPa to 71 MPa. In addition, the concrete mixes were within the range of 41 MPa to 47 MPa. Thus, the FU used in this study were as follows:

- Mortar mixes: 1 m^3 with 70 MPa compressive strength at 28 days.
- Concrete mixes: 1 m^3 with 45 MPa of compressive strength at 28 days.

Goal and Scope Definition

The system boundary of the LCA was defined based on the goal of the study which was to evaluate the environmental impacts of incorporating POC into cement mortar and concrete. The system boundary in this study included the production and transport of the following materials: cement, aggregate (coarse and fine) and POC (coarse, fine and powder). The concrete mixing process, transport to construction site, maintenance and demolition phases were excluded from the LCA. The effects of these phases may be excluded from an LCA if

two compared products are in the same geographic location and fulfil the same role. Marinkovic *et al.* (2010) noted that to compare two different concrete types, the exposure conditions must be the same. Under this assumption, they concluded that the impact of construction, use and end-of-life phases were expected to be approximately equal. This study used similar assumptions to Marinkovic *et al.* (2010). Therefore, the resulting system boundary for the LCA is shown in *Figure 4*.



Note: *Definition of sustainability according to UNESCAP (2015).

Figure 3. Framework of the study.

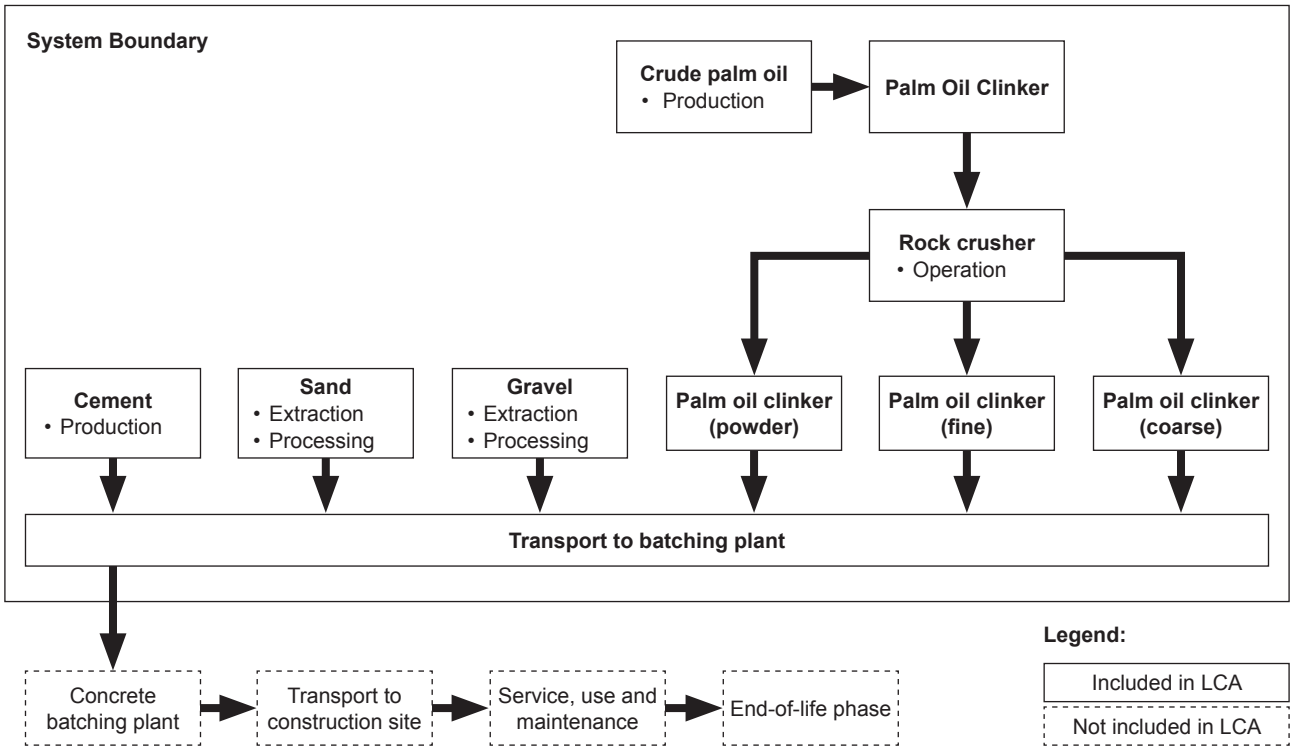


Figure 4. System boundary for the life cycle assessment (LCA) in this study.

Impact Assessment Methodology

The impact assessment was carried out using the ReCiPe 2016 (hierarchist) framework which is a midpoints (*i.e*. problem) oriented approach (Huijbregts *et al*., 2016). The hierarchist perspective was selected due to its balance between short- and long-term damaging effects. This framework includes 13 midpoint impact categories which are described in *Table 2*. Midpoints are the links in the cause-effect chain (environmental mechanism) of an impact category to the endpoints, which is where characterisation factors are derived to reflect the relative significance of emissions. In other words, midpoints describe the point between the source of pollution and the resulting damage to either humans or the ecosystem. Midpoints are useful to identify emission targets and areas of specific environmental concern. On the other hand, endpoints have less certainty, but they are more relevant to decision support. However, determining cause-effect environmental impact relationships was a desirable outcome of this study as opposed to decision-support. Therefore, this study carried out the life cycle impact assessment using a midpoints-oriented approach.

Inventory Analysis

Various sources were used to construct the life cycle inventory (LCI) used in this study. LCI data for production of cement, sand and gravel were obtained from the Malaysia Life Cycle Inventory Database (MY-LCID) to enhance the geographical representativeness of the LCA results and make more accurate conclusions within the Malaysian context (MY-LCID, 2020). The LCI data for sand and gravel were equal since these were considered to be produced from the same process (Marinkovic *et al*., 2010). On the other hand, LCI data for production of CPO and emissions from transportation were obtained from the Ecoinvent database (Martínez-Rocamora *et al*., 2016). The CPO dataset was geographically representative for Malaysia, but the transportation dataset was global. The Ecoinvent database was selected due to its integrity, usability and dedicated resources (Martínez-Rocamora *et al*., 2016). The crushing process for the POC clinker was based on the operation of a rock crusher from Landfield and Karra (2000). The crushing process was assumed to produce powder, coarse and fine POC and the energy required for all processes were considered to be the same (Marinkovic *et al*., 2010). Other studies have also used multiple LCI databases to supplement any data limitations (Onn *et al*., 2019).

A truncated inventory from the various materials in the study are shown in *Table 3*. Calculations were carried out using generic LCA tools, in this case Microsoft Excel. This method was selected to provide full control over the input data and calculations (Nemecek *et al*., 2010). It was also selected to provide simple integration with building information modelling (BIM) tools. BIM is a 3-dimensional (3D), parametric modelling software for construction (Anton and Diaz, 2014). BIM software can output information such as quantity of building materials directly to Microsoft Excel spreadsheets which may be readily used for LCA calculations (Shin and Cho, 2015).

Transport Distances

Transport distances were estimated using geographical information system (GIS) software using average road travel distance. A theoretical concrete construction site located in the centre of Kuala Lumpur was established. The average transport distances of concrete constituent materials to the construction site are shown in *Table 4*. Transport distances were multiplied by a factor of two to represent the return journey of the lorry after transportation of materials. A locality map showing the location of the construction site in relation to material sources are shown in *Figure 5*. In addition to the consumption of fuel and emission of greenhouse gases, this study considered the impacts from production, maintenance and use of the lorries. The type of lorry considered for transportation of all materials was considered to be a 16-32 t lorry with EURO3 emissions standard.

Allocation Factors

Some processes produce more than one product. Thus, environmental impacts must be distributed or 'allocated' among the multiple products. In this case, the production of CPO produces various co-products in addition to POC. It is possible to consider POC as a waste since it is discarded by most palm oil mills. However, according to the European Union (2008) Directive 2008/98/EC it may also be classified as a useful co-product since it was used as a replacement for cement, sand and gravel in this study. This study used two common methods for allocation: (1) mass allocation and (2) economic allocation. Marinković *et al.* (2017) found that mass allocation resulted in unreasonably high environmental impacts for fly ash and opted to use economic allocation instead. Van den Heede and De Belie (2012) also recommended the use of economic allocation over mass allocation for cement-replacement materials. If mass allocation were used, the high allocated impacts would be a negative factor against their use in the construction industry. However, the use of mass allocation served as a form of sensitivity analysis and was included in the study. Therefore, the main method of allocation was by economic value and supplemented by mass allocation as sensitivity analysis.

TABLE 2. OVERVIEW OF IMPACT CATEGORIES IN THE STUDY

Impact category	Midpoint characterisation factor (CFm)	CFm abbrev.	Unit
Climate change	Global warming potential	GWP	kg CO_2 eq.
Ozone depletion	Ozone depletion potential	ODP	kg CFC-11 eq.
Ionising radiation	Ionising radiation potential	IRP	kBq Co-60 eq.
Fine particulate matter formation	Particulate matter formation potential	PMFP	kg $PM_{2.5}$ eq.
Photochemical oxidant formation	Photochemical oxidant formation potential	POFP	kg NO_x eq.
Terrestrial acidification	Terrestrial acidification potential	TAP	kg SO_2 eq.
Freshwater eutrophication	Freshwater eutrophication potential	FEP	kg P eq.
Ecotoxicity	Ecotoxicity potential	ETP	1,4-DCB eq.
Human toxicity	Human toxicity potential	HTP	1,4-DCB eq.
Water use	Water consumption potential	WCP	m^3 water consumed
Land use	Agricultural land occupation potential	LOP	m^2 x annual crop eq.
Mineral resource scarcity	Surplus ore potential	SOP	kg Cu eq.
Fossil resource scarcity	Fossil fuel potential	FFP	kg oil eq.

Note: Impact categories are based on the ReCiPe2016 methodology (Huijbregts *et al.*, 2016).
Chemical abbreviations: CO_2 - carbon dioxide; CFC - chlorofluorocarbon; Co-60 - cobalt-60; $PM_{2.5}$ - particulate matter (less than 2.5 µm diameter); NO_x - nitrogen oxides; SO_2 - sulphur dioxide; P - phosphorous; 1,4-DCB - 1,4-Dichlorobenzene; Cu - copper.

TABLE 3. SELECTED EMISSIONS INVENTORY FOR MATERIALS IN THE STUDY

Emissions to air	Cement[c]	Crude palm oil[c]	Sand/gravel[c]	Crushing[d]	Transport[e]
Ammonia (NH_3)	4.57E-06	9.36E-06	3.887E-08	9.48E-09	2.09E-06
Carbon dioxide (CO_2)	9.82E-01	5.03E-02	2.87E-03	1.36E-05	1.39E-01
Methane (CH_4)	1.21E-03	1.97E-08	6.73E-06	4.46E-11	3.72E-09
Nitrogen oxides (NO_x)	2.24E-03	3.42E-04	1.94E-05	1.25E-07	1.04E-03
Nitrous oxide (N_2O)	4.03E-06	1.80E-06	3.16E-8	5.98E-09	1.60E-06
Non-methane volatile organic compounds (NMVOC)	2.91E-05	5.02E-05	1.57E-06	2.75E-08	1.05-E4
Particulate matter (PM2.5)	2.30E-05	9.62E-09	3.98E-07	9.62E-09	5.83E-05
Phosphorus (P)	5.97E-07	3.70E-05	4.59E-08	4.91E-10	2.65E-07
Water Consumption[a]					
Agriculture	-	9.91E-02	-	-	-
Industry	1.88E+00	-	1.44E-02	2.36E-06	1.70E-04
Land Use Change[b]					
From primary forest	5.68E-03	4.84E-03	1.64E-04	5.63E-09	2.99E-07
Fossil Fuel Use					
Crude oil	7.03E-03	3.05E-02	3.85E-04	1.16E-05	5.12E-02

Note: [a]Total surface water and groundwater.
[b]Land transformation.
[c]Units for emissions of cement, crude palm oil, sand and gravel are in kg of emissions per kg of material.
[d]Units for emissions of crushing are in kg of emissions per kg of material crushed.
[e]Units for emissions of transport are in kg of emissions per tonne x km of material transported.

TABLE 4. TRANSPORT DISTANCES OF MATERIALS

Material	Origin	Destination	Distance (km)	
			Scenario 1[a]	Scenario 2[b]
Cement	Cement plant	Concrete batching plant	70 x 2 = 140	100 x 2 = 200
Sand and gravel	Quarry	Concrete batching plant	50 x 2 = 100	100 x 2 = 200
Palm oil clinker	Palm oil mill	Concrete batching plant	110 x 2 = 220	100 x 2 = 200

Note: [a]Scenario based on estimated average transport distances.
[b]Scenario assuming equal transport distances.

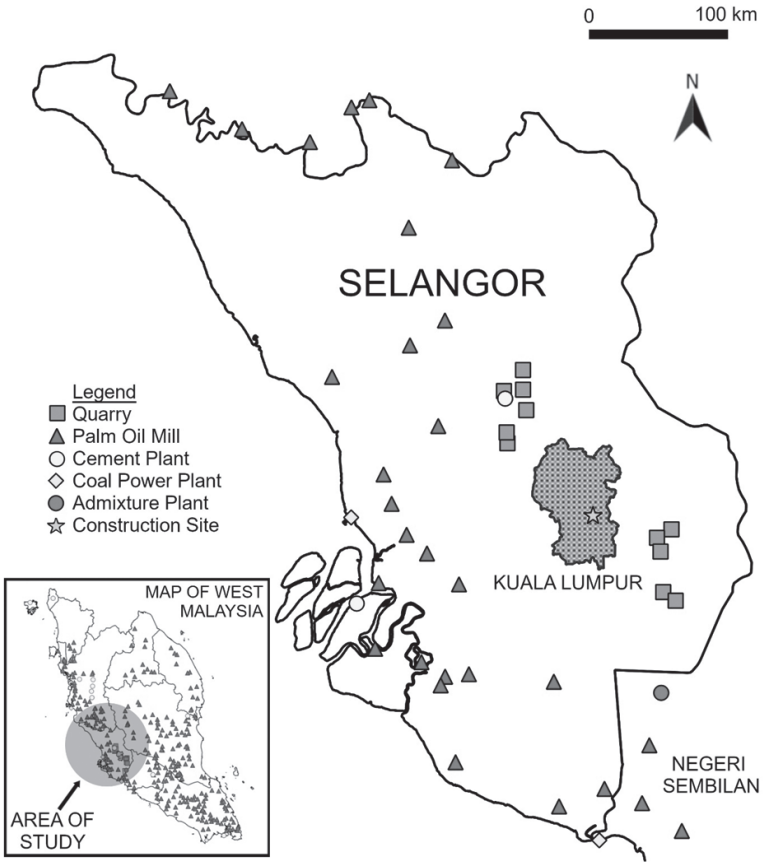


Figure 5. Locations of palm oil mills, cement plants, quarries and admixture plants located near the theoretical construction site in Kuala Lumpur.

Figure 6 shows the product system for production of CPO and its co-products. FFB from the oil palm plantation is processed at the oil palm mill to produce CPO, PK, OPS, MF and EFB. In addition, OPS and MF are fed into the on-site boiler to produce steam for the CPO production process. POC is the residue from the boiler furnace. This study allocated environmental impacts between CPO, PK, EFB and POC. This is because OPS and MF are the main source of fuel for the on-site boiler (Subramaniam *et al.*, 2008). As such, these materials are rarely available outside the oil palm mill (Aghamohammadi *et al.*, 2016). Moreover, they are intermediate materials to POC. Thus, OPS and MF may not be considered useful co-products to CPO. Though some mills may use EFB as fuel in their boilers, most are returned to the plantations for mulching thereby replenishing some nutrients of the soil (Chiew and Shimada, 2013). Also, PK contains residual oil and is put through additional processes to extract palm kernel oil (Subramaniam *et al.*, 2008). Therefore, EFB and PK may be considered as useful co-products to CPO.

The economic allocation factor, C_e was calculated according to Equation (1). On the other hand, the mass allocation factor, C_m was given by Equation (2). Both equations were defined by Chen *et al.* (2010) in their study on the LCA of green concretes. Ringgit Malaysia (RM) is the cost per unit material and m is the mass of material produced during its production process. Kanadasan and Abdul Razak (2015a) estimated the cost factor for POC as RM 20 t^{-1}. Salleh (2018) reported the cost of EFB as RM 295.64 t^{-1}. In 2015, the average price of CPO and PK were reported as RM 2153.50 t^{-1} and RM 1527.50 t^{-1} respectively (MPOB, 2015). In 2015, RM fluctuated between RM 3.80 and RM 4.35 per US Dollar (USD) (Quadry *et al.*, 2017). To avoid variations due to the high instability of the RM against the USD in 2015, prices were stated in terms of RM. Subramaniam *et al.* (2008) reported that for every 1 t of CPO produced, there are 0.41 t of PK, 1.17 t of EFB and 0.02 t of POC produced. Thus, the allocation factors included in the study were calculated as shown in *Table 5*.

$$C_e = \frac{(\text{RM} \times m)_{\text{by-product}}}{(\text{RM} \times m)_{\text{main-product}} + (\text{RM} \times m)_{\text{total-by-products}}} \quad \text{Equation (1)}$$

$$C_m = \frac{m_{\text{by-product}}}{m_{\text{main-product}} + m_{\text{total-by-products}}} \quad \text{Equation (2)}$$

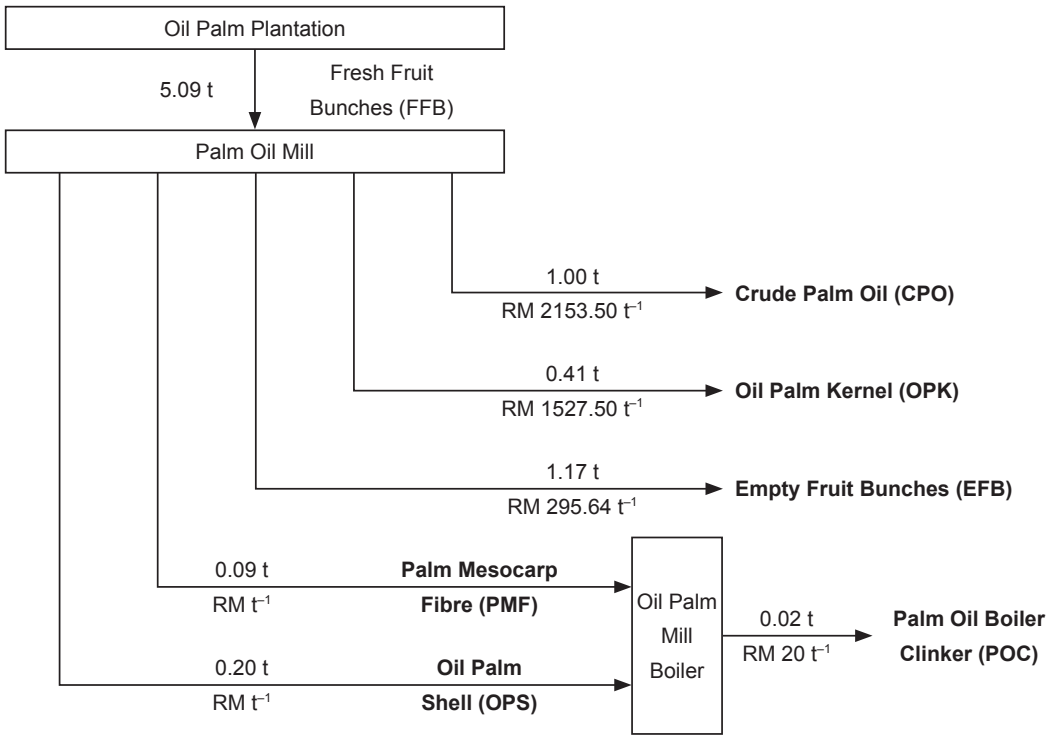


Figure 6. Product system for crude palm oil (CPO) and its co-products.

TABLE 5. ALLOCATION FACTORS BASED ON MASS AND ECONOMIC VALUES

Product	Mass produced (t)	Market price (RM t^{-1})	Allocation by mass value (%)	Allocation by economic value (%)
Crude palm oil (CPO)	1.00	2 153.50	38.46	68.89
Oil palm kernel (PK)	0.41	1 527.50	15.77	20.03
Empty fruit bunches (EFB)	1.17	295.64	45.00	11.06
Palm oil boiler clinker (POC)	0.02	20.00	0.77	0.0128
Total	2.60	3 996.64	100.00	100.00

Land Use Scenarios

The total planted area for oil palm in Malaysia reached 5.9 million hectares in 2019 which was approximately 18% of the country's total land area (MPOB, 2020). Oil palm plantations in Malaysia are primarily established on state land which consists mostly of degraded forest and are characterised by low carbon stocks (Hashim *et al.*, 2018). Despite the clearing of forest for oil palm cultivation, Malaysia still manages to maintain more than 50% of forest cover (Hamid and Rahman, 2016). Before oil palms are planted, the degraded forest is cleared, and the remaining biomass is left to be broken down by litter-feeding invertebrates. Thus, nutrients from forest biomass are returned to the soil and enrich it for future cultivation. The time period required for complete decomposition of this biomass is assumed to be 25 years (Germer and Sauerborn, 2008).

This study used a similar methodology to Hansen *et al.* (2014) in which environmental impacts of land conversion from forest to plantation were allocated to the first generation of plantation use. Since oil palm plantations are replanted every 25 years, then only the plantations established in the past 25 years were considered to contribute to land use change impacts (Kongsager and Reenberg, 2012). Hansen *et al.* (2014) reported that approximately 30% of all oil palm plantations were first generation plantations established within 25 years. Therefore, this study multiplied the land use change (LUC) factor for POC by a factor of 0.3. It must be noted that the ReCiPe2016 methodology used in this study focuses on the relative biological species loss due to LUC (Huijbregts *et al.*, 2016). The methodology does not include emission of greenhouse gases due to decomposition of cleared forest biomass.

RESULTS AND DISCUSSION

Material Impacts

This section is part of the LCA Impact Assessment and Interpretation phases. *Table 6* shows results for the 13 impact categories considered in the study when POC was economically allocated with values in brackets indicating the impacts when POC was mass allocated. As per the FU used in the study, these impact values are for the production of 1 m^3 of mortar or concrete respectively. The results in *Table 6* are not inclusive of impacts from transportation of materials. From these impact values, *Table 7* was created to show the percent change of POC mixes relative to their conventional mortar or concrete mixes. The mixes containing POC were compared to their respective conventional mixes within each impact category. For example, in the Global Warming Potential (GWP) impact category, the total emissions of M-40 is 6% lesser relative to M-0 and the emissions of C-100C is 1% lesser compared to C-0.

From *Table 7*, the use of economically allocated POC as a powder, coarse aggregate and fine aggregate showed reductions in all impact categories. When mass allocated, POC shows reductions in the majority of impact categories. However, some categories showed significant increase compared to the reference mixes. Particularly, the high impacts in the Freshwater Eutrophication (FEP) impact category of all POC mixes may be attributed to fertiliser application during oil palm cultivation. FEP in ReCiPe2016 is mainly influenced by phosphorous (P).

Malaysia possesses 2.5 million hectares of peat which is approximately 7% of the total land area of the country (Rahman *et al*., 2014). An estimated 13% of oil palm plantations are located on peat soils in Malaysia (Hashim *et al*., 2017). Peat soil has been reported to be low in various nutrients such as P and application of P fertiliser is desirable to sustain optimal agricultural yields (Mutert *et al.* 1999). Thus, P from the fertiliser may leach into nearby water bodies thereby influencing FEP. Hashim *et al.* (2017) also reported P runoff during oil palm cultivation as a significant contributor to eutrophication.

Another impact category with high contribution from POC is Human Toxicity (HTP). ReCiPe2016 uses 1,4-dichlorobenzene (1,4-DCB) and nickel (Ni) as the reference substances used in calculation of HTP. Since both MY-LCID and Ecoinvent lack data on 1,4-DCB emissions, then Ni was used as the main reference substance for calculation of HTP in this study. Ni occurs naturally in the soil which is accumulated in the biomass of oil palm fruit (Ooi *et al*., 2014). Zarcinas *et al.* (2004) found that Ni concentrations among fresh weight Malaysian food crops was highest among oil palm seed pulp. In addition, Said *et al*. (2016) demonstrated good potential for recovery of Ni from POFA which suggests that a good amount of Ni is contained within oil palm biomass. The burning of this biomass releases Ni into the atmosphere which poses carcinogenic risk to human health (Zambelli and Ciurli, 2013). Furthermore, operation and maintenance of oil palm plantations are supported by the use of heavy vehicles to transport biomass around the mill which may contribute to Ni emissions (Subramaniam *et al*., 2010).

Contribution Analysis

For each of the mixes in the study, the contribution of each material towards the total environmental impact are represented graphically using horizontal bar charts in *Figures 7*, 8 and 9. Contribution analysis reveals which material contributes the most towards a particular impact category.

Figures 7 to *9* show that in almost all impact categories, the material with the most dominant environmental impacts is cement. This is consistent with findings from previous literature where Braga *et al.* (2017) found that cement was the main governing factor for the environmental impact of concrete. In all mixes, economically allocated POC in all forms (*i.e.* powder, coarse and fine) contributed a relatively small percentage (less than 1%) to the total environmental impact in all impact categories. When mass allocated, POC in all forms contributed a minority of the percentage to the total environmental impact in all but a few impact categories. *Figures 7c, 7d* and *7e* show that mass allocated POC contributes highly to FEP and HTP.

POC is a product from the agricultural industry but contributed minimally to the 'Water Use' impact category. Agricultural products require water to produce biomass (Safitri *et al*., 2018). However, multiple studies have found that rainwater was sufficient to satisfy oil palm growth with minimal need for irrigation (Muhammad-Muaz and Marlia, 2014; Suttayakul *et al*., 2016; Safitri *et al*., 2018; Subramaniam and Hashim, 2018). On the other hand, the processing of sand requires a significant amount of water for washing and sizing (Grbes, 2015). Cement was the highest contributor to the 'Water Use' impact category. This is due to the energy intensive processes involved in cement production for which the water demand for energy generation is high (Hosseinian and Nezamoleslami, 2018).

POC also contributes minimally to the 'Land Use' impact category. Oil palm was found to be more sustainable compared to other crops such as soyabean, rapeseed or corn (De Vries *et al*., 2010). In fact, oil palm cultivation may encourage forest reversion and reduce carbon emissions due to oil

palm plantation sequestration being higher than other crops (Sayer *et al.*, 2012; Villoria *et al.*, 2013). The high output of oil palm plantations reduces the need for excessive transformation of land (Barcelos *et al.*, 2015). This explained the low contribution of POC towards the Land Use impact category despite being an agricultural product. Cement is also the highest contributor to this category. The high impact of cement may be attributed to construction and operation of cement factories which negatively affects the surrounding vegetation and water bodies (Dalil *et al.*, 2017). Besides that, extraction of mineral aggregates such as sand and gravel have relatively high impacts compared to POC due to the negative impacts of quarries on the surrounding landscape (Allacker *et al.*, 2014).

TABLE 6. MIDPOINT ENVIRONMENTAL IMPACTS OF MATERIALS

Impact category	M-0	M-40	C-0	C-100C	C-100F	Units
Climate change	569	536 (537)	436	432 (433)	434 (434)	kg CO_2 eq.
Ozone depletion	2.50E-05	2.35E-05 (2.35E-05)	1.92E-05	1.89E-05 (1.90E-05)	1.90E-05 (1.91E-05)	kg CFC-11 eq.
Ionising radiation	4.30E-03	3.72E-03 (3.72E-03)	3.72E-03	2.75E-03 (2.76E-03)	3.08E-03 (3.09E-03)	kBq Co-60 eq.
Fine particulate matter formation	0.529	0.499 (0.499)	0.406	0.401 (0.401)	0.403 (0.403)	kg $PM_{2.5}$ eq.
Photochemical oxidant formation	1.270	1.192 (1.193)	0.979	0.957 (0.959)	0.965 (0.966)	kg NO_x eq.
Terrestrial acidification	1.756	1.655 (1.656)	1.348	1.333 (1.335)	1.338 (1.339)	kg SO_2 eq.
Freshwater eutrophication	1.33E-04	1.20E-04 (1.53E-04)	1.09E-04	9.33E-05 (1.61E-04)	9.90E-05 (1.56E-04)	kg P eq.
Ecotoxicity	6.67E-04	6.29E-04 (9.43E-04)	5.12E-04	5.07E-04 (9.46E-04)	5.09E-04 (9.00E-04)	1,4-DCB eq.
Human toxicity	1.78E-04	2.37E-04 (3.01E-04)	1.83E-04	1.36E-04 (4.12E-04)	1.67E-04 (3.69E-04)	1,4-DCB eq.
Water use	1062.58	998.57 (998.68)	818.74	802.21 (802.45)	807.78 (807.98)	m^3 water consumed
Land use	124.75	115.41 (115.88)	98.32	91.42 (92.40)	93.75 (94.57)	m^2 x annual crop eq.
Mineral resource scarcity	0.844	0.789 (0.794)	0.654	0.632 (0.642)	0.640 (0.648)	kg Cu eq.
Fossil resource scarcity	47.00	44.15 (44.32)	36.29	35.49 (35.83)	35.78 (36.07)	kg oil eq.

Note: Emissions shown are for production of 1 m^3 of mortar or concrete.
Values not within brackets are results for economically allocated palm oil clinker (POC).
Values enclosed within brackets are results for mass allocated POC.

TABLE 7. PERCENT CHANGE IN RELATION TO REFERENCE MIXES

Impact category	Economic allocation			Mass allocation		
	M-40[a] (%)	C-100C[b] (%)	C-100F[b] (%)	M-40[a] (%)	C-100C[b] (%)	C-100F[b] (%)
Climate change	-6	-1	-1	-6	-1	0
Ozone depletion	-6	-2	-1	-6	-1	-1
Ionising radiation	-14	-26	-17	-14	-26	-17
Fine particulate matter formation	-6	-1	-1	-6	-1	-1
Photochemical oxidant formation	-6	-2	-2	-6	-2	-1
Terrestrial acidification	-6	-1	-1	-6	-1	-1
Freshwater eutrophication	-10	-15	-10	15	47	42
Ecotoxicity	-6	-1	-1	12	46	39
Human toxicity	-6	-2	-1	-6	-2	-1
Water use	-7	-7	-5	-7	-6	-4
Land use	-6	-3	-2	-6	-2	-1
Mineral resource scarcity	-6	-2	-1	-6	-1	-1
Fossil resource scarcity	-6	-1	-1	-6	-1	0

Note: Underlined values show increased impacts compared to reference mixes.
[a]Percent change in relation to M-0.
[b]Percent change in relation to C-0.

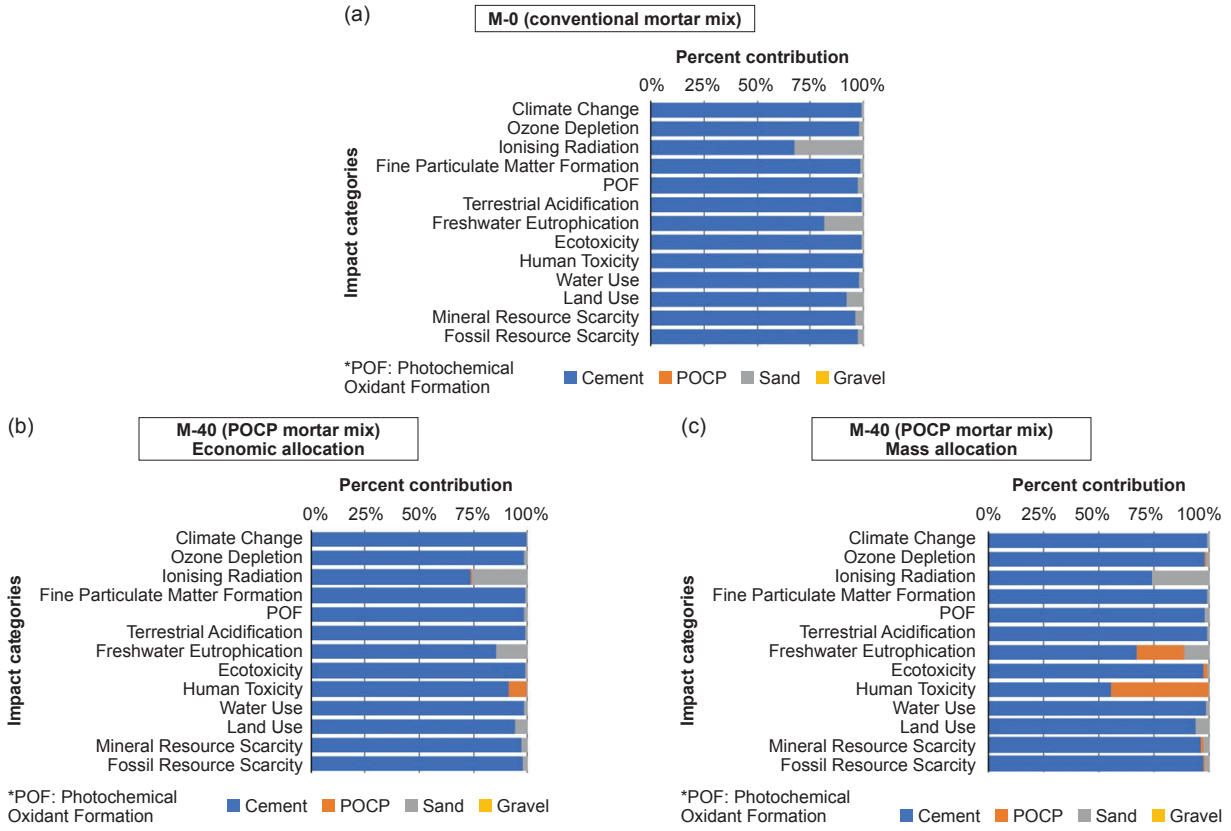


Figure 7. Contribution analysis for (a) cement mortar, (b) economically allocated palm oil clinker powder (POCP) mortar and (c) mass allocated (POCP) mortar.

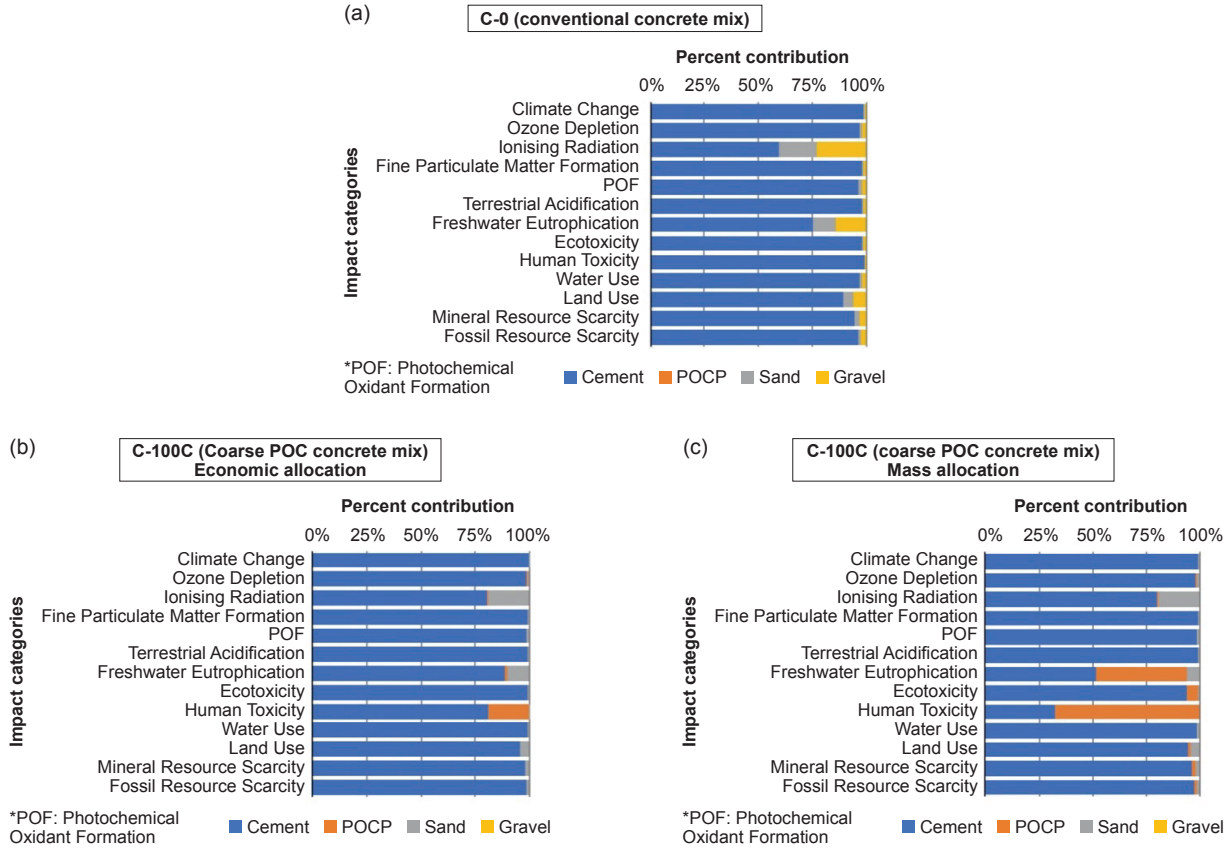


Figure 8. Contribution analysis for (a) conventional concrete, (b) economically allocated coarse palm oil clinker (POC) concrete and (c) mass allocated coarse POC concrete.

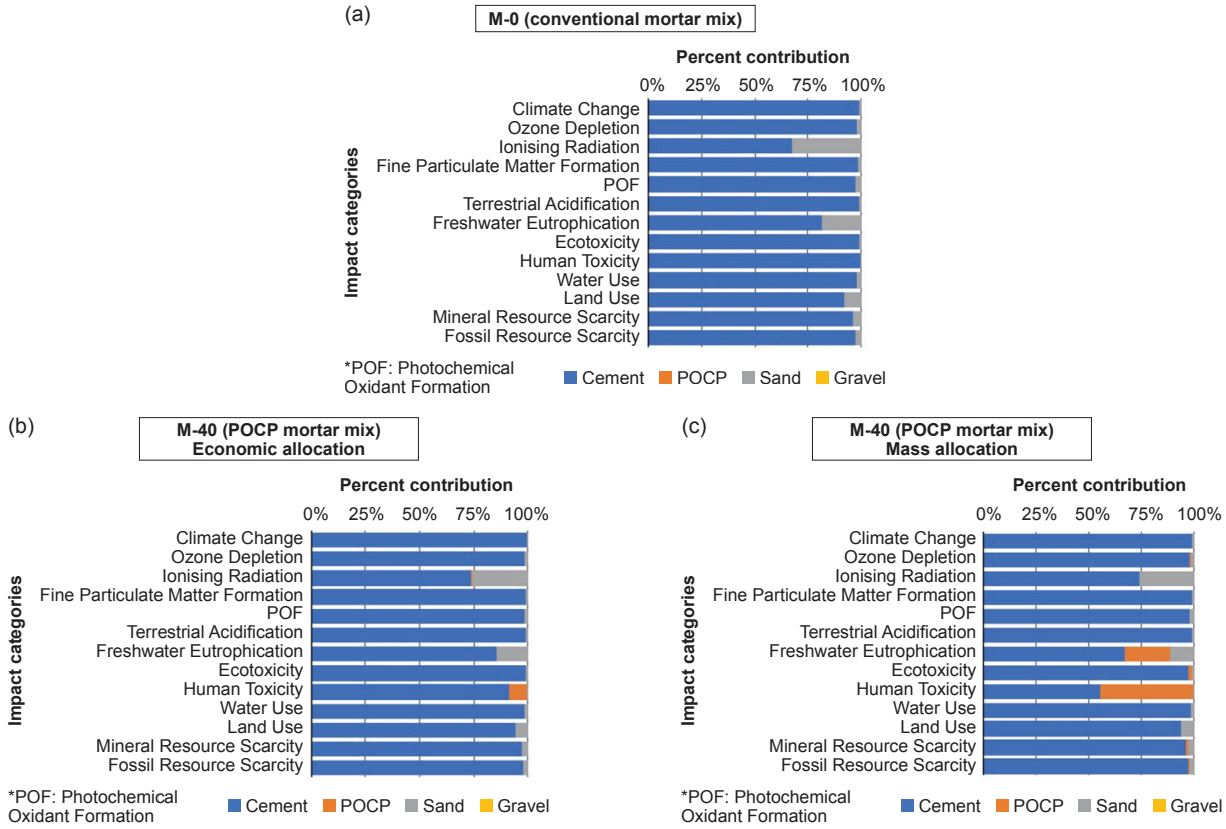


Figure 9. Contribution analysis for (a) conventional concrete, (b) economically allocated fine palm oil clinker (POC) concrete and (c) mass allocated fine POC concrete.

Transport Emissions

Table 8 shows the percent change in emissions of POC mixes compared to reference mixes. The percent change for a particular mix was equal across all impact categories. In Scenario 1, transportation emissions of POC mixes were higher compared to reference mixes. Emissions for M-40 were approximately 9% higher compared to M-0 in all impact categories. For concrete mixes, transportation emissions for C-100C and C-100F were higher than C-0 in all impact categories by approximately 19% and 25% respectively. The increased emissions for POC mixes in this scenario may be explained by the increased average transport distance from the palm oil mill to the concrete construction site.

On the other hand, in Scenario 2, transportation emissions of POC mixes were lower than those of reference mixes. Emissions for M-40 were approximately 8% lower compared to M-0 in all impact categories. For concrete mixes, transportation emissions for C-100C and C-100F were lower than C-0 in all impact categories by approximately 19% and 6% respectively. This reduction may be attributed to the reduced combined weight of all materials required for POC mixes. For example, the combined weight of cement and sand (per unit volume of concrete) for M-0 is 2200 kg m^{-3} while that of cement, sand and POC for M-40 is 2010 kg m^{-3}. Since the transport distances were equal for all materials in this scenario, then the emissions values were influenced only by the combined weight of all materials of the mix. In this case, the reduced total weight of POC mixes was an advantage since it reduced transportation emissions. This was a similar advantage reported for lightweight concrete (Shafigh *et al*., 2014).

Comparison with Other Studies

It was useful to compare the results of normal concrete in this study with the literature. The impact values from the climate change impact category for normal concrete mixes were used as the main point of comparison. Specifically, the GWP of which is expressed in the unit kilograms of carbon dioxide equivalent (kg CO_2 eq.). The GWP for various normal concrete mixes in the literature and their corresponding compressive strength are shown in *Table 9* (Marinkovic *et al*., 2010; Tosic *et al*., 2015; Kim *et al*., 2016; Tait and Cheung; 2016, Braga *et al*., 2017; Mohammadi and South, 2017; Ahmad *et al*., 2017; Kurda *et al*., 2018). The Z-score was calculated for the GWP value of mix C-0 in this study (GWP = 436 kg CO_2 eq.) and compared to the GWP values of similar concrete mixes in the literature (mean = 366.38, standard deviation = 52.87). Based on Shiffler (1988), this Z-score (Z = 1.32) was well below the threshold (Z = 2.47) and was not considered as an outlier. The

results show that GWP for C-0 was 1.03 standard deviations away from the mean and was not an abnormal data point. This is an evidence to suggest that the results of this study are consistent with the literature.

Figure 10 shows a correlation plot between cement content and GWP value for which the correlation coefficient was calculated. The results show that cement content was strongly positively correlated to GWP value (R^2 = 0.72). Cohen (1992) suggested the following levels for measuring effect size of correlations: R^2 = 0.01 is a small effect size; R^2 = 0.09 is a medium effect size; and R^2 = 0.25 is a large effect size. This further supported findings from previous literature that cement was the main governing material for environmental impact of concrete (Braga *et al.*, 2017).

Kanadasan and Razak (2015a) reported that the carbon emissions for concrete where aggregate was replaced with 100% POC as 420 kg CO_2 eq. The GWP for mixes C-100C and C-100F are within the range of 432-434 kg CO_2 eq. for both allocation methods. Therefore, this further indicates evidence that the results of this study are consistent with those obtained from the literature.

Implications and Recommendations

The results of the study provided evidence to support the claim that POC is an environmental-friendly building material. The results for mass allocated POC represented a sensitivity analysis on the significant effect of POC when its environmental impacts were scaled up. For example, the increased impacts in FEP and HTP should not be ignored.

Apart from that, the predicted increased future yield of oil palm would mean a proportionately increased amount of oil-palm-related waste which would need to be properly managed or utilised (Murphy, 2014). The use of this waste in the construction industry would avoid the negative environmental impacts arising from their disposal.

In addition, the use of POC to replace gravel and sand as aggregates in the construction industry may decrease demand for extraction of mineral-based materials such as cement, gravel and sand from earth's lithosphere (Estanqueiro *et al.*, 2016). Thus, the local construction industry's reliance on these materials are gradually diminished which leads to reduced damage to the surrounding landscape as well as subsequent problems that arise from it (Veraart, 2018). However, care must be taken to expand oil palm plantations in a sustainable manner. For example, careful management of fertiliser application should be practised to reduce damage to the ecosystem and, subsequently, human health (Darras *et al.*, 2019). This is to prevent further increase of the environmental impacts of POC to the point where it is no longer a viable alternative to natural aggregates. Increasing yield of oil palm should go beyond simply increasing fertiliser inputs. Methods such as gene modification may produce higher yields with minimal negative environmental impact (Barcelos *et al.*, 2015).

TABLE 8. PERCENT CHANGE IN TRANSPORTATION EMISSIONS

Impact category	Scenario 1			Scenario 2		
	M-40[a] (%)	C-100C[b] (%)	C-100F[b] (%)	M-40[a] (%)	C-100C[b] (%)	C-100F[b] (%)
All impact categories	9.01	19.44	25.09	-8.64	-19.07	-6.68

Note: The percent change in emissions is equal for all impact categories.
[a]Percent change in relation to M-0.
[b]Percent change in relation to C-0.

TABLE 9. SUMMARY OF NORMAL CONCRETE RESULTS FROM THE LITERATURE

Reference	Cement content (kg m^{-3})	Compressive strength (MPa)	GWP value per m^3 concrete (kg CO_2 eq.)
Marinkovic *et al.* (2010)	315	39.2	307
Tosic *et al.* (2015)	384	41.5	340
Kim *et al.* (2016)	400	40	450
Tait and Cheung (2016)	380	32-40	339
Braga *et al.* (2017)	350	30-37	317
Mohammadi and South (2017)	380	50	379
Ahmad *et al.* (2017)	420	35	437
Kurda *et al.* (2018)	350	55.8	362
Mean	372.38	43.58	366.38
Standard deviation	32.90	7.75	52.87

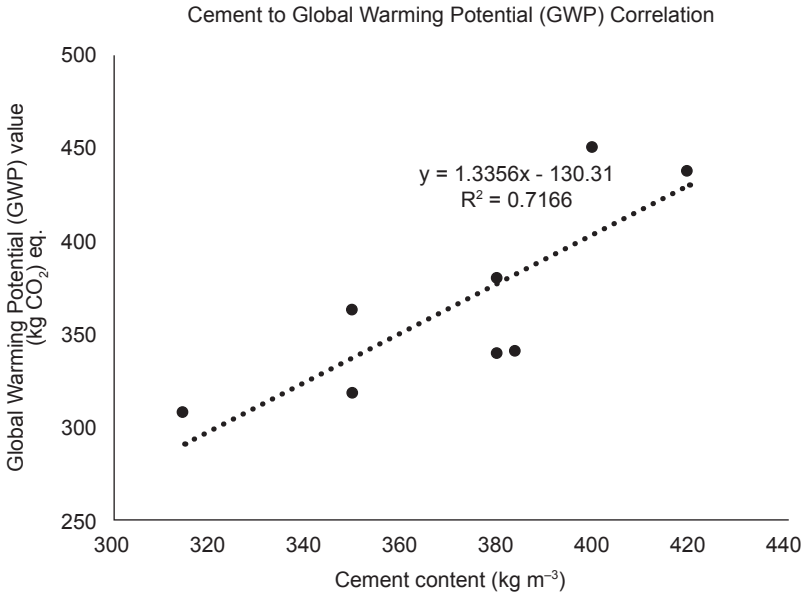


Figure 10. Correlation plot for cement content and Global Warming Potential (GWP) value.

Other than that, Braga *et al.* (2017) found that increasing the strength of the concrete does not necessarily increase environmental impacts. Cement represents approximately 6%-7% of all anthropogenic carbon dioxide (Akan *et al.*, 2017). Thus, it is important to reduce cement content by substitution or use less of it to achieve similar engineering properties. The reduced cement may be substituted with suitable alternatives such as POCP. The reduction in compressive strength was not directly proportional to POCP content. Therefore, it may be possible to increase the proportion of POCP without significant decrease in compressive strength for mortar mixes.

Furthermore, some palm oil mills were reported to capture the emissions from palm oil mill effluent digestion (Subramaniam *et al.*, 2008). This could further reduce the impact of POC in the Climate Change impact category. However, the capture of methane from palm oil mill effluent was not reflected in the Ecoinvent or MY-LCID databases. Future studies could consider this technique in their LCA to further reduce values of the climate change impact category.

Besides that, the mortar and concrete mixes in the study did not contain any fly ash which were reported to increase strength and durability of concrete containing POC (Ahmad *et al.*, 2007). Thus, future studies could attempt to study the environmental impact of fly ash in concrete incorporating POC. Inclusion of fly ash into POC concrete could further decrease environmental impacts as reported by Marinkovic *et al.* (2016) for recycled aggregate concrete.

Finally, the FU selected for this study did not include durability characteristics of the concrete. This could be included in the FU if more studies on the durability of POC concrete were available such as chloride penetration and carbonation coefficient. Thus, a future study could be undertaken to establish an equivalent functional unit for POC concrete similar to work by Dobbelaere *et al.* (2016) for recycled aggregate concrete. Such a study would improve the accuracy of future LCA on POC concrete.

CONCLUSION

When economically allocated, the use of POC as a powder, coarse aggregate and fine aggregate in mortar or concrete had reduced impacts in all categories. Contribution analysis showed that POC contributed less than 1% of the total environmental impact in all impact categories when economically allocated. Cement was shown to be the material which contributed the most towards the total environmental impacts in all mixes.

When mass allocated, only the FEP and HTP impact categories showed significant increase in impact values. Reductions were observed in the majority of impact categories for mass allocation. Despite this, POC contributed minimally to the total impact in most categories.

For transport emissions, when transport distances were estimated based on GIS software, transport of POC showed increased impacts. This was due to the higher transport distance for POC compared to cement, sand or gravel. On the other hand, when transport distances of all materials were equal, then impacts of mixes with POC showed decreased impacts. This was explained by the lower total weight per unit volume of mixes containing POC.

The GWP of normal concrete in this study was compared with similar mixes in the literature. Results show that the GWP of normal concrete for this study did not exceed the threshold to classify it as an outlier, indicating the results of this study were consistent with the literature. In addition, there was a strong positive correlation between cement content versus GWP value for the compared mixes in the literature.

Overall, the use of POC in concrete was shown to be beneficial for directly reducing the embodied environmental impacts of mortar and concrete. However, the allocation method strongly influenced the magnitude of the results. Thus, care must be taken to select a proper allocation method, preferably one which does not rely on physical properties.

In addition, the indirect benefits of using POC extend towards both the construction and agricultural industries. For example, the use of POC as a building material would avoid the negative environmental impacts arising from its disposal. It may also spur growth in the agricultural industry due to demand for its many useful co-products. In addition, it could reduce the demand on sand and gravel for use as aggregates in concrete, alleviating the exploitation of these materials from the natural system. Therefore, the use of POC as a construction material is recommended especially for countries with high CPO production such as Malaysia, Indonesia and Thailand. However, the long-term durability properties of structural members made with POC should be thoroughly explored.

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REFERENCES

Abd Rashid, A F and Yusoff, S (2015). A review of life cycle assessment method for building industry. *Renewable and Sustainable Energy Reviews, 45*: 244-248.

Abdullah, N and Sulaiman, F (2013). The oil palm wastes in Malaysia. *Biomass Now - Sustainable Growth and Use.* IntechOpen. p. 75-100. https://www.intechopen.com/books/biomass-now-sustainable-growth-and-use/the-oil-palm-wastes-in-malaysia, accessed on 6 February 2020.

Abutaha, F; Abdul Razak, H and Kanadasan, J (2016). Effect of palm oil clinker (POC) aggregates on fresh and hardened properties of concrete. *Construction and Building Materials, 112*: 416-423.

Aghamohammadi, N; Reginald, S; Shamiri, A; Zinatizadeh, A; Wong, L and Nik Sulaiman, N (2016). An investigation of sustainable power generation from oil palm biomass: A case study in Sarawak. *Sustainability, 8*(5): 416.

Ahmad, M H and Nurazuwa, M N (2007). Physical properties of local palm oil clinker and fly ash. Paper presented at the 1st Engineering Conference on Energy and Environment. 27-28 December 2007, Kuching, Sarawak.

Ahmad, M H; Sofian, M and Nurazuwa, M N (2007). Mechanical properties of palm oil clinker concrete. Paper presented at the 1st Engineering Conference on Energy and Environment. 27-28 December 2007, Kuching, Sarawak.

Ahmmad, R; Alengaram, U J; Jumaat, M Z; Sulong, N H R; Yusuf, M O and Rehman, M A (2017). Feasibility study on the use of high volume palm oil clinker waste in environmental friendly lightweight concrete. *Construction and Building Materials, 135*: 94-103.

Akan, M O A; Dhavale, D G and Sarkis, J (2017). Greenhouse gas emissions in the construction industry: An analysis and evaluation of a concrete supply chain. *J. Cleaner Production, 167*: 1195-1207.

Alengaram, U J; Mahmud, H and Jumaat, M Z (2011). Enhancement and prediction of modulus of elasticity of palm kernel shell concrete. *Materials and Design, 32*: 2143-2148.

Alengaram, U J; Mohottige, N H W; Wu, C; Jumaat, M Z; Poh, Y S and Wang, Z (2016). Response of oil palm shell concrete slabs subjected to quasi-static and blast loads. *Construction and Building Materials, 116*: 391-402.

Allacker, K; De Souza, D M and Sala, S (2014). Land use impact assessment in the construction sector: An analysis of LCIA models and case study application. *The Int. J. Life Cycle Assessment, 19*: 1799-1809.

Anton, L A and Diaz, J (2014). Integration of LCA and BIM for sustainable construction. *Int. J. Social, Education, Economics and Management Engineering, 8*: 1356-1360.

Aslam, M; Shafigh, P and Jumaat, M Z (2016). Oil-palm by-products as lightweight aggregate in

concrete mixture: A review. *J. Cleaner Production, 126*: 56-73.

Barcelos, E; Rios Sde, A; Cunha, R N; Lopes, R; Motoike, S Y; Babiychuk, E; Skirycz, A and Kushnir, S (2015). Oil palm natural diversity and the potential for yield improvement. *Frontiers in Plant Science, 6*: 190.

Black, L (2016). Low clinker cement as a sustainable construction material. *Sustainability of Construction Materials* (Khatib, J M ed.). Woodhouse Publishing, Duxford. p. 415-457.

Braga, A M; Silvestre, J D and De Brito, J (2017). Compared environmental and economic impact from cradle to gate of concrete with natural and recycled coarse aggregates. *J. Cleaner Production, 162*: 529-543.

Chen, C; Habert, G; Bouzidi, Y; Jullien, A and Ventura, A (2010). LCA allocation procedure used as an incitative method for waste recycling: An application to mineral additions in concrete. *Resources, Conservation and Recycling, 54*: 1231-1240.

Chiew, Y L and Shimada, S (2013). Current state and environmental impact assessment for utilizing oil palm empty fruit bunches for fuel, fiber and fertilizer - A case study of Malaysia. *Biomass and Bioenergy, 51*: 109-124.

Cohen, J (1992). A power primer. *Psychological Bulletin, 112*: 155-159.

Dalil, M; Amodu, I O; Abbas, Y A and Husaini, A (2017). Effect of cement factory on land use-land cover in Obajana Lokoja local government area, Kogi State, Nigeria. *African J. Environmental Science and Technology, 11*: 384-392.

Darras, K F A; Corre, M D; Formaglio, G; Tjoa, A; Potapov, A; Brambach, F; Sibhatu, K T; Grass, I; Rubiano, A A; Buchori, D; Drescher, J; Fardiansah, R; Hölscher, D; Irawan, B; Kneib, T; Krashevska, V; Krause, A; Kreft, H; Li, K; Maraun, M; Polle, A; Ryadin, A R; Rembold, K; Stiegler, C; Scheu, S; Tarigan, S; Valdés-Uribe, A; Yadi, S; Tscharntke, T and Veldkamp, E (2019). Reducing fertilizer and avoiding herbicides in oil palm plantations - Ecological and economic valuations. *Frontiers in Forests and Global Change, 2*: 65.

De Vries, S C; Van De Ven, G W; Van Ittersum, M K and Giller, K E (2010). Resource use efficiency and environmental performance of nine major biofuel crops, processed by first-generation conversion techniques. *Biomass Bioenergy, 34*: 588-601.

Dobbelaere, G; De Brito, J and Evangelista, L (2016). Definition of an equivalent functional unit for structural concrete incorporating recycled aggregates. *Engineering Structures, 122*: 196-208.

Estanqueiro, B; Silvestre, J D; De Brito, J and Pinheiro, M D (2016). Environmental life cycle assessment of coarse natural and recycled aggregates for concrete. *European J. Environmental and Civil Engineering, 22*: 429-449.

European Union (2008). Directive 2008/98/EC of the European Parliament and of the council on waste and repealing certain directives. *Official J. European Union, L312*: 3-30.

Germer, J and Sauerborn, J (2008). Estimation of the impact of oil palm plantation establishment on greenhouse gas balance. *Environment, Development and Sustainability, 10*: 697-716.

Grbeš, A (2015). A life cycle assessment of silica sand: Comparing the beneficiation processes. *Sustainability, 8*: 11.

Hamada, H M; Jokhio, G A; Yahaya, F M; Humada, A M and Gul, Y (2018). The present state of the use of palm oil fuel ash (POFA) in concrete. *Construction and Building Materials, 175*: 26-40.

Hamid, W A and Rahman, S B W A (2016). Comparison results of forest cover mapping of Peninsular Malaysia using geospatial technology. *IOP Conference Series: Earth and Environmental Science, 37*: 012027.

Hansen, S B; Olsen, S I and Ujang, Z (2014). Carbon balance impacts of land use changes related to the life cycle of Malaysian palm oil-derived biodiesel. *Int. J. Life Cycle Assessment, 19*: 558-566.

Hansen, S B; Padfield, R; Syayuti, K; Evers, S; Zakariah, Z and Mastura, S (2015). Trends in global palm oil sustainability research. *J. Cleaner Production, 100*: 140-149.

Hashim, Z; Subramaniam, V; Harun, M H and Kamarudin, N (2018). Carbon footprint of oil palm planted on peat in Malaysia. *Int. J. Life Cycle Assessment, 23*: 1201-1217.

Huijbregts, M A J; Steinmann, Z J N; Elshout, P M F; Stam, G; Verones, F; Vieira, M D M; Hollander, A; Zijp, M and Van Zelm, R (2016). *ReCiPe 2016: A Harmonized Life Cycle Impact Assessment Method at Midpoint and Endpoint Level*. National Institute for Public Health and the Environment. 194 pp.

Hosseinian, S M and Nezamoleslami, R (2018). Water footprint and virtual water assessment in cement industry: A case study in Iran. *J. Cleaner Production, 172*: 2454-2463.

ISO (2006a). Environmental management - Life cycle assessment - Principles and framework (ISO Standard No. 14040:2006). https://www.iso.org/standard/37456.html, accessed on 1 December 2019.

ISO (2006b). Environmental management - Life cycle assessment - Requirements and guidelines (ISO Standard No. 14044:2006). https://www.iso.org/standard/38498.html, accessed on 1 December 2019.

Kanadasan, J and Abdul Razak, H (2015a). Engineering and sustainability performance of self-compacting palm oil mill incinerated waste concrete. *J. Cleaner Production, 89*: 78-86.

Kanadasan, J and Abdul Razak, H (2015b). Utilization of palm oil clinker as cement replacement material. *Materials, 8*: 8817-8838.

Kanadasan, J; Ahmad Fauzi, A F; Abdul Razak, H; Selliah, P; Subramaniam, V and Yusoff, S (2015). Feasibility studies of palm oil mill waste aggregates for the construction industry. *Materials, 8*: 6508-6530.

Karim, M R; Khandaker, M U; Asaduzzaman, K; Razak, H A and Yusoff, S B (2018). Radiological risks assessment of building materials ingredients: Palm oil clinker and fuel ash. *Indoor and Built Environment, 28*: 479-491.

Kim, T; Tae, S and Chae, C (2016). Analysis of environmental impact for concrete using LCA by varying the recycling components, the compressive strength and the admixture material mixing. *Sustainability, 8*: 389.

Kongsager, R and Reenberg, A (2012). *Contemporary Land-use Transitions: The Global Oil Palm Expansion*. Copenhagen, GLP International Project Office. 45 pp.

Kurda, R; Silvestre, J D and De Brito, J (2018). Life cycle assessment of concrete made with high volume of recycled concrete aggregates and fly ash. *Resources, Conservation and Recycling, 139*: 407-417.

Landfield, A H and Karra, V (2000). Life cycle assessment of a rock crusher. *Resources, Conservation and Recycling, 28*: 207-217.

Marinkovic, S; Dragas, J; Ignjatovic, I and Tosic, N (2017). Environmental assessment of green concretes for structural use. *J. Cleaner Production, 154*: 633-649.

Marinkovic, S; Habert, G; Ignjatovic, I; Dragas, J; Tosic, N and Brumaud, C (2016). Life cycle analysis of recycled aggregate concrete with fly ash as partial cement replacement. Paper presented at the Sustainable Built Environment (SBE) Regional Conference. 15-17 June 2016.

Marinkovic, S; Radonjanin, V; Malesev, M and Ignjatovic, I (2010). Comparative environmental assessment of natural and recycled aggregate concrete. *Waste Management, 30*: 2255-2264.

Martínez-Rocamora, A; Solís-Guzmán, J and Marrero, M (2016). LCA databases focused on construction materials - A review. *Renewable and Sustainable Energy Reviews, 58*: 565-573.

Mazlan, D; Krishnan, S; Din, M F M; Tokoro, C; Khalid, N H A; Ibrahim, I S and Komori, D (2020). Effect of cellulose nanocrystals extracted from oil palm empty fruit bunch as green admixture for mortar. *Scientific Reports, 10*: 6412.

Mo, K H; Visintin, P; Alengaram, U J and Jumaat, M Z (2016). Prediction of the structural behaviour of oil palm shell lightweight concrete beams. *Construction and Building Materials, 102*: 722-732.

Mohammadi, J and South, W (2017). Life cycle assessment (LCA) of benchmark concrete products in Australia. *The Int. J. Life Cycle Assessment, 22*: 1588-1608.

Mohd Hilton, A; Lee, Y L and Nurazuwa, M N (2008). Water permeability of Malaysian palm oil clinker concrete. Paper presented at the Malaysian Technical Universities Conference on Engineering and Technology. 8-10 March 2008.

MPOB (2015). *Malaysian Oil Palm Statistics 2015.* MPOB, Bangi. 210 pp.

MPOB (2020). Overview of the Malaysian palm oil industry 2019. http://bepi.mpob.gov.my/images/overview/Overview_of_Industry_2019.pdf, accessed on 3 June 2020.

Muhammad-Muaz, A and Marlia, M H (2014). Water footprint assessment of oil palm in Malaysia: A preliminary study. *AIP Conference Proceedings, 1614*: 803-807.

Murphy, D J (2014). The future of oil palm as a major global crop: Opportunities and challenges. *J. Oil Palm Res. Vol. 26*: 1-24.

Mutert, E; Fairhurst, T H and von Uexküll, H R (1999). Agronomic management of oil palms on deep peat. *Better Crops International, 13*: 22-27.

MY-LCID (2020). *Malaysia Life Cycle Inventory Database*. http://mylcid.sirim.my/sirimlca/, accessed on 20 May 2020.

Nambiappan, B; Ismail, A; Hashim, N; Ismail, N; Shahari, D N; Idris, N A N; Omar, N; Salleh, K M; Hassan, N A M and Kushairi, A (2018). Malaysia: 100 years of resilient palm oil economic performance. *J. Oil Palm Res. Vol. 30(2)*: 13-25.

Nemecek, T; Knuchel, R F; Alig, M and Gaillard, G (2010). The advantages of generic LCA tools for agriculture: Examples SALSAcrop and SALCAfarm. Paper presented at the 7th International Conference on Life Cycle Assessment in the Agri-Food Sector. 22-24 September 2010. Bari, Italy.

Onn, C C; Mo, K H; Radwan, M K H; Liew, W H; Ng, C G and Yusoff, S (2019). Strength, carbon footprint and cost considerations of mortar blends with high volume ground granulated blast furnace slag. *Sustainability, 11*: 7194.

Ooi, Z X; Ismail, H; Bakar, A A and Teoh, Y P (2014). A review on recycling ash derived from *Elaeis guineensis* by-product. *Bioresources, 9*: 7926-7940.

Quadry, M O; Mohamad, A and Yusof, Y (2017). On the Malaysian Ringgit exchange rate determination and recent depreciation. *Int. J. Economics, Management and Accounting, 25*: 1-26.

Sayer, J; Ghazoul, J; Nelson, P and Boedhihartono, A K (2012). Oil palm expansion transforms tropical landscapes and livelihoods. *Global Food Security, 1*: 114-119.

Rahman, Z A; Gikonyo, E W; Silek, B; Goh, K J and Soltangheisi, A (2014). Evaluation of phosphate rock sources and rate of application on oil palm yield grown on peat soils of Sarawak, Malaysia. *J. Agronomy, 13*: 12-22.

Safitri, L; Hermantoro, H; Purboseno, S; Kautsar, V; Saptomo, S and Kurniawan, A (2018). Water footprint and crop water usage of oil palm (*Elaeis guineensis*) in Central Kalimantan: Environmental sustainability indicators for different crop age and soil conditions. *Water, 11*: 35.

Said, N; Ahmad, S M S; Malar, V S; Syafiqah, A K; Hajar, A M S and Aina, A R (2016). Precious nickel recovery from palm oil mill fuel ash waste (Ni-POMFAW) using acidic leaching extraction (ALE). *Key Engineering Materials, 705*: 374-379.

Salleh, S F; Abd Rahman, A and Tuan Abdullah, T A R (2018). Potential of deploying empty fruit bunch (EFB) for biomass cofiring in Malaysia's largest coal power plant. Paper presented at the 7th International Conference on Power and Energy. 3-4 December 2018.

Shafigh, P; Mahmud, H B; Jumaat, M Z and Zargar, M (2014). Agricultural wastes as aggregate in concrete mixtures - A review. *Construction and Building Materials, 53*: 110-117.

Shiffler, R E (1988). Maximum Z scores and outliers. *The American Statistician, 42*: 79-80.

Shin, Y-S and Cho, K (2015). BIM application to select appropriate design alternative with consideration of LCA and LCCA. *Mathematical Problems in Engineering, 2015*: 281640.

Sims and Brown (1998). Concrete aggregates. *Lea's Chemistry of Cement and Concrete* (Hewlet, P C ed.). Butterworth-Heinemann, Oxford. p. 907-1015.

Subramaniam, V; Choo, Y M; Muhammad, H; Hashim, Z; Yew, A T and Puah, C W (2010). Life cycle assessment of the production of crude palm kernel oil (Part 3a). *J. Oil Palm Res. Vol. 22*: 904-912.

Subramaniam, V and Hashim, Z (2018). Charting the water footprint for Malaysian crude palm oil. *J. Cleaner Production, 178*: 675-687.

Subramaniam, V; Ma, A N; Choo, Y M and Nik Meriam, N S (2008). Life cycle inventory of the production of crude palm oil - A gate to gate case study of 12 palm oil mills. *J. Oil Palm Res. Vol. 20*: 484-494.

Sumesh, M; Alengaram, U J and Nayaka, R R (2018). Effect of binder content and water-binder ratio in mortar developed using partial replacement of cement with palm oil clinker powder. *IOP Conference Series: Materials Science and Engineering, 431*: 082007.

Suttayakul, P; Aran, H K; Suksaroj, C; Mungkalasiri, J; Wisansuwannakorn, R and Musikavong, C (2016). Water footprints of products of oil palm plantations and palm oil mills in Thailand. *Science of the Total Environment, 542*: 521-529.

Tait, M W and Cheung, W M (2016). A comparative cradle-to-gate life cycle assessment of three concrete mix designs. *The Int. J. Life Cycle Assessment, 21*: 847-860.

Tay, J H (1990). Ash from oil-palm waste as concrete material. *J. Materials in Civil Engineering, 2*: 94-105.

Tosic, N; Marinkovic, S; Dasic, T and Stanic, M (2015). Multicriteria optimization of natural and recycled aggregate concrete for structural use. *J. Cleaner Production, 87*: 766-776.

Van den Heede, P and De Belie, N (2012). Environmental impact and life cycle assessment (LCA) of traditional and 'green' concretes: Literature review and theoretical calculations. *Cement and Concrete Composites, 34*: 431-442.

Van den Heede, P and De Belie, N (2014). A service life based global warming potential for high-volume fly ash concrete exposed to carbonation. *Construction and Building Materials, 55*: 183-193.

Veraart, F (2018). Building materials and construction: Sustainability, dependency and foreign suppliers. *Well-being, Sustainability and Social Development: The Netherlands 1850-2050* (Lintsen, H; Veraart, F; Smits, J-P and Grin, J eds.). Springer, Cham. p. 417-434.

Zambelli, B and Ciurli, S (2013). Nickel and human health. *Metal Ions in Life Sciences, 13*: 321-357.

Zarcinas, B A; Ishak, C F; McLaughlin, M J and Cozens, G (2004). Heavy metals in soils and crops in southeast Asia. 1. Peninsular Malaysia. *Environmental Geochemistry and Health, 26*: 343-357.

SHORT COMMUNICATION

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A REVIEW ON THE MALAYSIAN SUSTAINABLE PALM OIL CERTIFICATION PROCESS AMONG INDEPENDENT OIL PALM SMALLHOLDERS

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ABSTRACT

In recent years, palm oil faces various issues on the global market. Therefore, Malaysia launched the Malaysian Sustainable Palm Oil (MSPO) certification as the national scheme to systematically certify palm oil industry in Malaysia as an effort towards sustainable production as well to address some of the issues raised on the global market such as the requirement by importing countries for a completely certified sustainable palm oil supply chain. Various strategies have been developed to effectively certify independent smallholders such as the establishment of the Sustainable Palm Oil Clusters (SPOC). The aim of this article is to extend knowledge and experience gained towards MSPO certification approach among independent oil palm smallholders in Malaysia. It also provides a basis for operation frameworks towards certification approach for smallholders especially in developing countries. Apart from that, this article highlights the progress and national initiatives on the establishment of MSPO certification in Malaysia. It gives insight on the challenges and way forward of MSPO certification approach in Malaysia.

Keywords: Malaysian Sustainable Palm Oil (MSPO), independent oil palm smallholders, Sustainable Palm Oil Clusters (SPOC), certification.

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INTRODUCTION

Palm oil has been one of the major sources of oils and fats in the world (Kushairi *et al.,* 2018). Oil palm crop is known to be highly productive compared to other competing crops due to its high yield per hectare as well as the low requirement of land area (Rival and Levang, 2014; Morley, 2015). Palm oil is well-known for its versatility which have been used in various sectors including non-food sector and well-known as a renewable energy feedstock other than its major role in providing food security to the world. Despite being the most efficient oil crop, palm oil has been suffering from various anti-palm oil sentiments and increasing scrutiny over the years (Murphy, 2014). Some of the issues on the global market are on the allegations and misunderstandings about oil palm industry which are linked to intensive deforestation and destruction of biodiversity. There is a concern on the trend of increasing food standards which might act as a new trade barrier for developing countries (Augier *et al.*, 2005; Brenton and Manchin, 2002; Ferrantino, 2006; Garcia and Poole, 2004). Increasing complexity of standards imposed on small farmers by global market creates some advantages as well as disadvantages among small farmers especially for export market (Humphrey, 2006). The palm oil industry faces various issues especially in Europe which started in 2015 with the Amsterdam Declaration which calls for a complete sustainable palm oil supply chain in Europe by 2020. Following that, the European Parliament Environmental (ENVI) report stated few recommendations on palm oil and deforestation with one of them is to eliminate

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the use of vegetable oils that cause deforestation as a component of biofuels in which it included palm oil. Then ENVI voted to ban palm oil biofuels from Europe from 2021, as part of the European Union (EU)'s Renewable Energy Directive (RED). Malaysia takes continuous effort to improve and elevate the palm oil industry to the global standard by addressing various requirement imposed by stakeholders as well as to cater for the growing global demands for vegetable oils. In 2018, the largest Malaysian palm oil exports markets were India with a total of 2.51 million tonnes followed by EU with a total of 1.91 million tonnes (Kushairi *et al.*, 2019). Markets for certified sustainable palm oil (CSPO) in developed countries especially the EU have been on the increasing trends. In near future, these trends are expected to influence other traditional markets such as China, India and Pakistan to demand for CSPO. Oil palm which was initially brought in as an ornamental plant has become a major industry for Malaysia. After more than a century of commercial cultivation of oil palm, Malaysia currently has 5.85 million hectares of oil palm area (MPOB, 2018). Palm oil production is vital for the socio-economic development of Malaysia especially the independent oil palm smallholders which account for 16.8% of the total planted area in Malaysia. Palm oil in Indonesia has been a powerful tool for poverty alleviation especially for smallholders by creating job opportunities and creates spin-off economic activities (Edwards, 2015).

METHOD

Desk research was conducted to review certification approach for smallholders in Malaysia. Primary data on readiness assessment among smallholders prior to joining the Malaysian Sustainable Palm Oil (MSPO) certification were gathered through survey questionnaire using an information and communications technology (ICT) tool. Apart from that, data on cost of certification as well as the frameworks on the MSPO certification and other relevant data were gathered from internal sources available at the Malaysian Palm Oil Board (MPOB).

MSPO Certification

Although the word sustainability can be traced back much earlier in history, it becomes widely used after the Report of the World Commission on Environment and Development (1987) which describes it as development that meets the needs of the present without compromising the ability of future generations to meet their own needs. Three fundamentals of sustainable development were highlighted which consist of environmental protection, social responsibility and economic practices. Since then, various certification standards have been developed all over the world using sustainability as their fundamentals (DeFries *et al.*, 2017; Potts *et al.*, 2014).

In Malaysia, there are several palm oil certification standards currently being used. However, the most common standards are MSPO, Roundtable on Sustainable Palm Oil (RSPO), and International Sustainability and Carbon Certification (ISCC). RSPO is an international oil palm certification scheme developed in 2004 which consists of members from all stages of oil palm supply chain. ISCC was introduced in 2010 mainly for certification of palm oil used as a feedstock for biofuels. Other than that, Indonesia has introduced their own certification scheme known as the Indonesian Sustainable Palm Oil (ISPO) in 2011 to certify palm oil industry in Indonesia.

The report on United Nations Conference on Environment and Development (1992) on Agenda 21 consists of a comprehensive plan of action, recommended by UN summit to be taken globally. In Agenda 21, article 8.6 states that, countries could develop systems for monitoring and evaluation of progress towards achieving sustainable development by adopting indicators that measure changes across economic, social and environmental dimensions. Standards developed in different parts of country or geographical area might not be effective to other countries if the standard does not cater for local conditions. Local requirement and crop specific standards can be further incorporated into a generic standard to make sure the criteria are not superfluous (Dankers and Liu, 2003). Therefore, in 2013, MSPO certification scheme was launched as the national scheme for Malaysia to systematically certify palm oil industry in Malaysia. Since then, MSPO implementation has been on a voluntary basis until the government announced its mandatory implementation in 2017. Initially, the mandatory timeline for companies that have been certified under RSPO need to obtain MSPO certification by 31 December 2018 while companies without RSPO certification were given extended time until 30 June 2019. Independent and organised smallholders were given until 31 December 2019 to obtain MSPO. However, recently the timeline were revised to a single timeline where the implementation of MSPO certification for all categories would be made mandatory by 31 December 2019. In 2014, an independent non-profit organisation known as the Malaysian Palm Oil Certification Council (MPOCC) was established as the scheme owner to implement and operate the MSPO certification scheme in Malaysia.

Initially, MSPO was initiated by MPOB in 2010 where MPOB is the authorised Standards-Writing Organisation (SWO) appointed by SIRIM Bhd which

is the national standards development agency to develop the standards. Prior to MSPO, MPOB has been implementing the MPOB Codes of Practice (CoP) since 2007 to assist the industry on the best practices throughout the palm oil supply chain. The MSPO standards was developed by MPOB through a standards development process under the purview of the Department of Standard Malaysia (DSM) which is the National Standards Body and the National Accreditation Body with inputs from relevant stakeholders of the oil palm industry. MSPO scheme contains four parts such as MSPO 2530-1:2013 Part 1: General principles, MSPO 2530-2:2013 Part 2: General principles for independent smallholders, MSPO 2530-3:2013 Part 3: General principles for oil palm plantations and organised smallholders, and MSPO 2530-4:2013 Part four: General principles for palm oil mills. In addition, MSPO Supply Chain Certification Standard (SCCS) was launched by MPOCC to extend the standard to the downstream industry. MSPO is seen as the Malaysia's answer to the Amsterdam Declaration which wants a complete certified sustainable palm oil supply chain in Europe by 2020 with the hope that MSPO is accepted as a certification system in Europe. The Amsterdam Declaration is currently signed by European countries of Denmark, Germany, Norway, Netherlands, the United Kingdom, France and Italy to show their commitment to support a fully sustainable palm oil supply chain by 2020.

According to the International Organisation for Standardisation (ISO), certification means the provision by an independent body of written assurance (a certificate) that the product, service or system in question meets specific requirements. To achieve this, the auditing and certification process are carried out by independent certification bodies (CB) which are subject to accreditation by DSM in accordance to the international standard of MS ISO/IEC 17011 (Ainie *et al.,* 2015). Currently, there are 13 CB consisting of both international and local companies involved in certifying independent smallholders in Malaysia. Application of MSPO part 2 for independent smallholders are currently under the purview of MPOB. Meanwhile, application of MSPO part 3 for oil palm plantations and organised smallholders and MSPO part 4 for palm oil mills are currently under the responsibility of MPOCC.

Concept of Sustainable Palm Oil Clusters (SPOC)

As of April 2019, there are 262 724 independent smallholders owning 1 015 524 ha of oil palm in Malaysia. Independent smallholding are defined as those who own oil palm land less than 40.46 ha or in aggregate amount of less than 40.46 ha. These smallholders are scattered all over the country with various age profile and practices while managing their oil palm land on their own. The average land holding of independent oil palm smallholders in Peninsular Malaysia is 2.3 ha (Parthiban *et al.,* 2017b). In this case, certification cost might be high if smallholders were to be certified individually (AgroEco and Grolink, 2008). In 2002, MPOB has established extension services known as *Tunjuk Ajar dan Nasihat Sawit* (TUNAS) to provide extension services on oil palm to independent smallholders. Current ratio of TUNAS extension agent to smallholders is 1:1500 which poses some challenges to provide extension services to all smallholders especially for MSPO certification which has been made mandatory by 31 December 2019. One of the strategies of MPOB to prepare smallholders for MSPO certification is by establishing SPOC all over the country to group smallholders within a small group of between 1000-2000 smallholders for each group (*Table 1*). This grouping enables them to be certified together under a single certificate. Each SPOC will be managed by one TUNAS officer who acts as the Group Manager (GM) to ensure that all individual members comply with the standard (*Figure 1*). Every four to five SPOC will be further managed by one head of TUNAS officer who acts as an Internal Control System (ICS) officer who will oversee and establish the general control system for every SPOC. In addition, external groups such as dealers, mills and cooperatives also can be formed and they can act as a GM for smallholders to be certified under MSPO. Eventually, it is up to the decision of individual smallholders to join any one of the groups in order to be certified under MSPO.

Several countries have successfully implemented group certification to enable farmers to be certified under a standard. Thailand has established Good Aquaculture Practice standard for shrimp production via group certification to enable shrimp farmers to access the EU market (Laila *et al.,* 2011). In India, better management practices (BMP) for shrimp production were successfully adopted by farmers via the formation of clusters (Philips *et al.,* 2008).

TABLE 1. DISTRIBUTION OF SMALLHOLDERS AND SPOC IN MALAYSIA (data as of April 2019)

No.	State	No. of SPOC	No. of smallholders	Hectarage (ha)
1	Johor	43	80 869	231 621
2	Kedah and Perlis	3	6 485	28 315
3	Pulau Pinang	1	1 918	9 443
4	Perak	23	46 689	127 763
5	Selangor	11	21 429	45 464
6	Negeri Sembilan	4	6 051	27 347
7	Melaka	2	3 179	13 400
8	Pahang	9	13 489	55 426
9	Terengganu	3	3 702	13 523
10	Kelantan	2	2 157	7 065
11	Sabah	29	35 338	220 911
12	Sarawak	32	41 418	235 246
	Total	162	262 724	1 015 524

Note: SPOC - Sustainable Palm Oil Clusters.

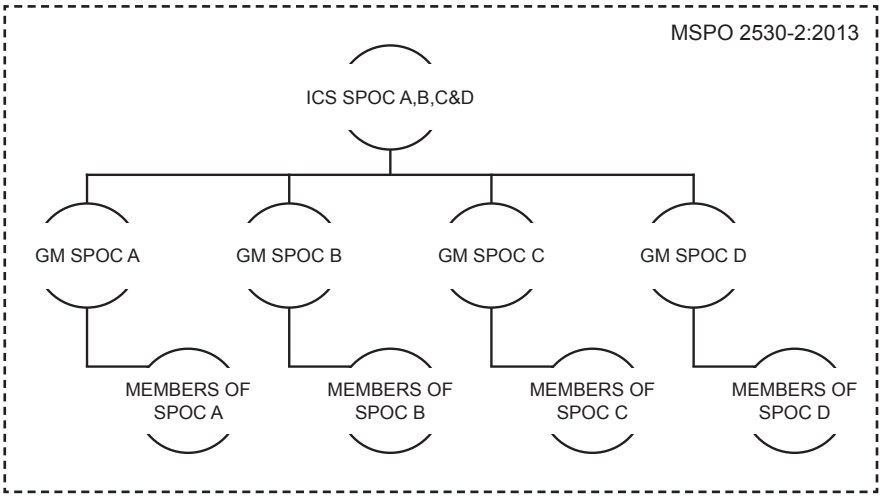


Figure 1. Concept of Sustainable Palm Oil Clusters (SPOC) under MPOB.

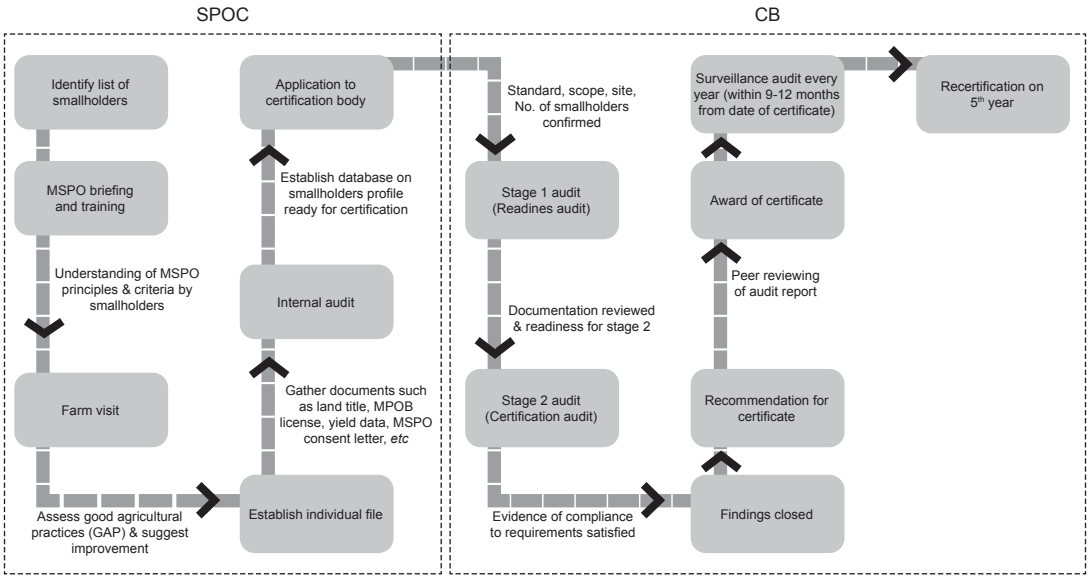


Figure 2. Frameworks of the Malaysian Sustainable Palm Oil (MSPO) certification for independent smallholders.

Certification Process

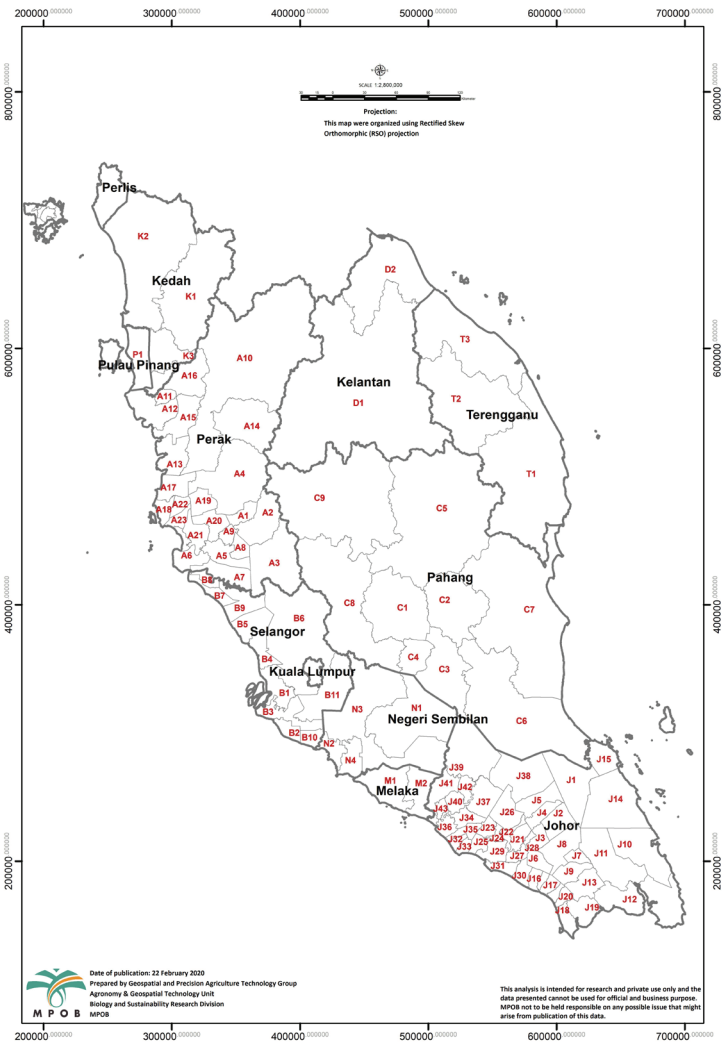
The process of certification starts when the GM identifies list of smallholders and invites them to join MSPO briefing and training (*Figure 2*). Once the GM gets smallholders consent to join MSPO, further visit to their farms are organised to assess the extent of Good Agricultural Practice (GAP) at their farms. Suggestion of any improvement on their existing GAP is explained and time is given for them to implement all the suggestions. At the same time, various training and extension activities would be conducted to expose smallholders to the principles and criteria of MSPO with the involvement of other agencies such as the Department of Occupational Safety and Health, the Department of Environment, the Department of Wildlife and National Parks and others. Individual file of each smallholders would be created containing basic documents such as land title, MPOB license, yield data, MSPO consent letter and others. A database containing detailed information of all the smallholders who are ready to be certified for each SPOC would be created by the GM. An internal audit would be conducted to further assess the level of readiness before inviting external certification body to carry out the auditing process. SPOC would undergo phase 1 auditing process to assess the level of readiness before proceeding to phase 2 audit. Random sampling would be used to select members from the SPOC based on risk factor. The CB would conduct the audit based on requirements stated in part two of the MSPO for independent smallholders which contains 7 principles, 22 criteria and 33 indicators.

Once completed, the CB would undergo various steps before awarding MSPO certificate which is valid for five years. From there on, surveillance audit would be conducted each year before recertification audit on the fifth year. The GM would continue all the processes above to add new smallholders to the clusters in preparation for audit each year.

Progress and National Initiatives of MSPO Certification

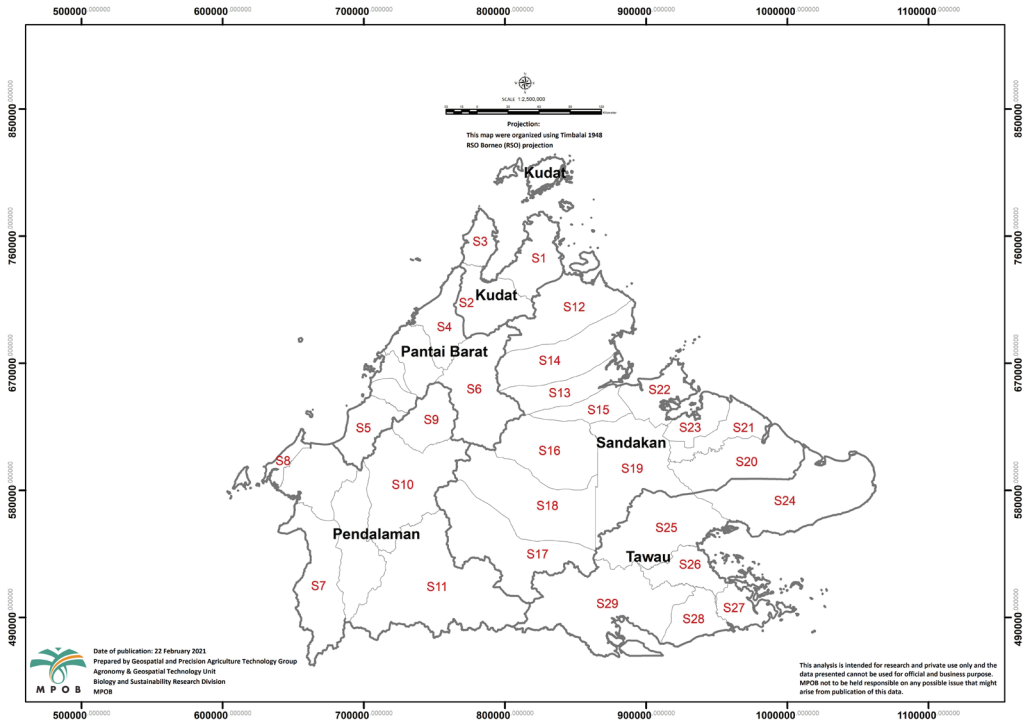
To date, 162 SPOC have been established all over the country to cover all the 262 724 of smallholders in Malaysia (*Figures 3* to *5*). As of July 2020, 30.28% of the total smallholders have been certified under 162 SPOC covering 75 890 smallholders with 305 348 ha. Certifying all the smallholders has proven to be a great challenge compared to bigger plantations as these smallholders are independent in managing their land without any structured management. However, the government of Malaysia is intensifying the effort to certify all the smallholders in Malaysia.

Certification often resulted in high cost as demonstrated by Basiron *et al.* (2016) which reported the high cost of pursuing RSPO certification in Malaysia. Similarly, Brandi *et al.* (2015) reported that one of the challenges in certifying small producers under RSPO is the financial barrier. Smallholders can be further left out if they are not able to afford the high cost of certification (Defries *et al.*, 2017). Therefore, the Malaysian government has allocated RM 30 million which was announced in the 2019 budget to fully fund expenses for MSPO audit including training for independent smallholders. Total cost per smallholder to be certified under MSPO is approximately between RM 1000 to RM 1200 calculated based on 500 smallholders for each SPOC which include the cost of auditing, training and to provide Personal Protective Equipment (PPE) as well as shelves to keep herbicides or pesticides.



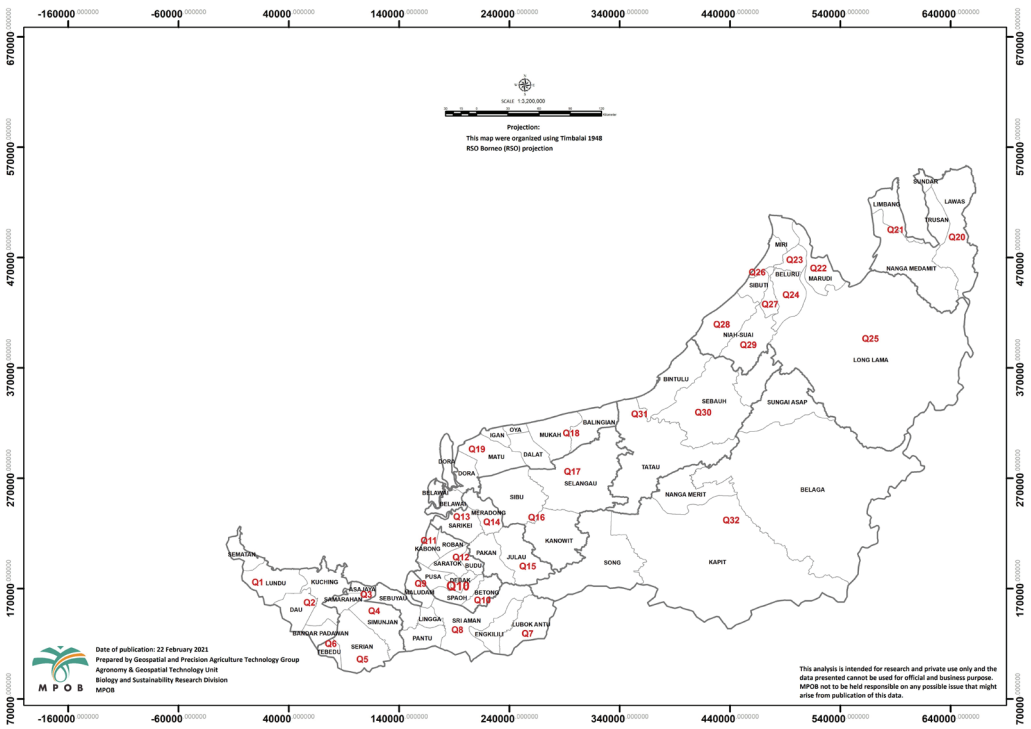
Note: The indication of alphabets in the map represent different states in Malaysia while the numbering indicates numbers of SPOC in each state.

Figure 3. Distribution of Sustainable Palm Oil Clusters (SPOC) in Peninsular Malaysia.



Note: The indication of alphabets in the map represent different states in Malaysia while the numbering indicates numbers of SPOC in each state.

Figure 4. Distribution of Sustainable Palm Oil Clusters (SPOC) in Sabah.



Note: The indication of alphabets in the map represent different states in Malaysia while the numbering indicates numbers of SPOC in each state.

Figure 5. Distribution of Sustainable Palm Oil Clusters (SPOC) in Sarawak.

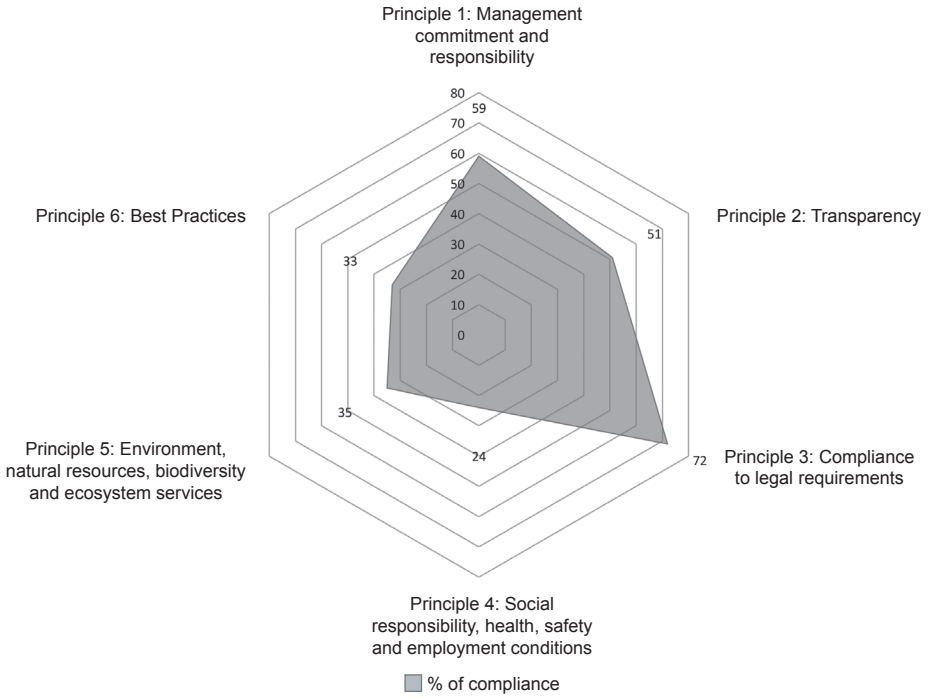


Figure 6. Graph on the level of readiness compliance on Malaysian Sustainable Palm Oil (MSPO) principles among smallholders who achieved high level compliance (80%-100%).

Other than that, MPOB together with Solidaridad which is an international network organisation have collaborated to develop an ICT tool to assess the level of compliance among smallholders in terms of readiness with MSPO prior to certification. Based on early readiness assessment conducted on 100 smallholders prior to joining MSPO certification in one of the SPOC found that majority (52%) of the smallholders have moderate level of compliance towards MSPO. This was followed by 46% of the smallholders in the category of high level compliance while the rest (2%) belongs to the category of low level compliance towards MSPO. Principle 7 on development of new planting was not applicable as all the respondents have already cultivated oil palm. Further analysis among the respondents who achieved high level compliance (80%-100%) found that majority of the smallholders recorded high level of compliance (72%) on Principle 3 regarding compliance to legal requirements while Principle 4 on social responsibility, health, safety and employment conditions recorded low level of compliance at only 24% (*Figure 6*). This might be due to the fact that most of the respondents have valid land title and MPOB licenses therefore they were able to comply on most of the criteria under Principle 3. However, low level compliance on Principle 4 might be due to the fact that most of the oil palm smallholders in Malaysia were hiring part-time labour to conduct their farm works (Parthiban *et al.*, 2017b). Therefore, they might not be aware on the requirement on employee's condition as well as the safety and health requirement.

Challenges and Future Strategies

Based on early assessment on all the audit reports prepared by CB in 82 SPOC covering 5586 smallholders with 20 441 ha as of December 2018, it was found out that majority of the SPOC have common non-compliance on MSPO clause 4.2.1 on traceability. Earlier study reported that most of the oil palm smallholders in Malaysia are not practicing proper record keeping (Ayat *et al.*, 2008; Parthiban *et al.*, 2017a). For the purpose of auditing, normally SPOC GM has to rely on dealers to provide records of fresh fruit bunches (FFB) transaction of smallholders for each audit; this is not efficient. There should be a real-time computerised system to record FFB transaction among smallholders in real time for efficient data gathering (Parthiban *et al.*, 2017a). The second most common non-compliance on MSPO was raised for clause 4.5.3 on waste management and disposal. Smallholders often lack the awareness on the proper way to dispose empty chemical containers. Awareness training on the proper disposal should be intensified and more affordable collecting agents should be identified for each SPOC.

Smallholders are facing a lot of challenges, especially to meet the global and national certification standards in line with the global demand for sustainable palm oil. Therefore, it is necessary to have a harmonisation between different sustainability certification systems with a mutually accepted standard criteria. Other than that, land issues especially requirement on land use for different crops have to be addressed effectively

at a higher administrative levels perhaps via inter-ministerial and state government cooperation to find an effective solution. Low voluntary participation for MSPO certification among smallholders presents a real challenge for extension agent to convince them on the importance of MSPO. A more comprehensive license act to include MSPO requirement during every license application or renewal will be more effective to increase the MSPO awareness among smallholders.

A better assessment system needs to be in place to evaluate the performance of smallholders. Extension agent needs to identify the training needs on MSPO and assess their progress after each training via score assessment to better evaluate their performance. Introduction of any solution in the name of certification must be sustainable and be profitable in an effort to make sustainable palm oil to become the new norm among smallholders. Certification process requires significant costs, time and effort especially for smallholders to be successfully certified. As a long-term initiative, certification should outweigh the costs so that producers especially smallholders find it worthwhile to get certified. Amekawa (2013) reported that one of the factors that limit producers from adopting certification is because of the lack of economic benefits associated with getting the certificate. Smallholders are giving less attention to non-economic benefits of certification such as social and environmental aspect rather than the economic benefits (Nia *et al.*, 2015).

Palm oil share is on the rise in the oils and fats global market, however, the premium price on certified palm oil is still largely very low (Byerlee and Rueda, 2015). The current mechanism of assistance from the government for MSPO certification might not be sustainable in the long run. Therefore, it needs to have a long-term mechanism in place to finance the cost of certification without government fund such as through cooperative establishment among smallholders themselves. Other suggestion is to give more focus to implement MSPO certification through existing clusters such as dealers, mills, associations and contractors since these clusters have already established relationship with smallholders.

Other than that, acceptance to change among smallholders is low as most of the oil the palm smallholders in Malaysia are older farmers with the average age of 54 years old (Parthiban *et al*., 2017b). Sometimes, they found it hard to accept any changes and recommendations by extension agents based on principle and criteria of MSPO. Apart from that, they also normally hire workers or outsource work to contractors or dealers. In order to be effective in increasing awareness on MSPO, all the stakeholders especially dealers and contractors need to be involved in MSPO initiative as they are part of the supply chain.

Although Malaysia is moving fast to be certified under MSPO, however there should be no compromise on the quality of process leading towards getting the MSPO certificate especially the auditing process by the CB. Fontaine *et al.* (2008) also suggested that increased competition between certification agencies might result in lower certification cost especially in developing countries where it is generally expensive. There is no harm to encourage the development of more competent CB to conduct MSPO certification. However, strict requirement for competency and accreditation must be imposed to make sure the quality is not compromised. There should be a reliable control procedure to provide check and balance in maintaining higher standard in MSPO certification.

CONCLUSION

The MSPO standards were developed through a standards development process with inputs from relevant stakeholders of the oil palm industry to better suit the local requirements. Low ratio of extension officer to smallholders poses some challenges to provide extension services to all smallholders especially for MSPO certification. Therefore, MPOB has established 162 SPOC all over the country to group smallholders within a small group of between 1000-2000 smallholders to enable them to be certified together under a single certificate. As of July 2020, 30.28% of the total smallholders have been certified. The process of certification starts with the GM identifying list of smallholders, conduct training, visit to farm to assess the extent of GAP, establish individual file and finally invite the CB to conduct the audit before awarding the certificate. Based on early readiness assessment prior to joining MSPO, it was found out that majority of the smallholders have moderate level of compliance towards MSPO with most of them having common non-compliance on MSPO clauses related to traceability due to lack of proper record keeping. Some of the recommendation is to have a harmonisation between different sustainability certification systems with a mutually accepted standard criteria, establish a comprehensive license act to include MSPO requirement, address land use requirement via inter-ministerial and state government cooperation and to have an assessment system to evaluate the performance of smallholders. Other than that, the use of information technology to gather and report data of FFB transaction among smallholders will help in efficient data gathering as well as to address the issues on traceability. Malaysia is in the right track towards sustainable production of palm oil via MSPO certification from a broader

perspective. However, there are several challenges and constraints ahead that need to be addressed effectively especially concerning independent oil palm smallholders. Collaboration between all the stakeholders is important to embrace and adopt MSPO certification and sustainability as the new normal of oil palm management. Continuous effort towards higher standard and quality are necessary to develop MSPO certification to be internationally recognised and to remain relevant globally.

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REFERENCES

Agro Eco and Grolink (2008). *Organic Exports, A Way to a Better Life? Export Promotion of Organic Products from Africa*. Report publication. http://www.grolink.se, accessed on 30 March 2019. 105 pp.

Ainie, K; Kushairi, A and Choo, Y M (2015). Malaysian sustainable palm oil. *Oil Palm Bulletin No. 71*: 1-7.

Amekawa, Y (2013). Can a public GAP approach ensure safety and fairness? A comparative study of Q-GAP in Thailand. *The J. Peasant Studies, 40(1)*: 189-217.

Augier, P; Gasiorek, M and Lai, T C (2005). The impact of rules of origin on trade flows. *Economic Policy, 20(43)*: 567-623.

Ayat, K A R; Ramli, A; Faizah, S and Arif, S (2008). The Malaysian palm oil supply chain: The role of the independent smallholders. *Oil Palm Industry Economic J. Vol. 8(2)*: 17-27.

Basiron, Y and Foong, K Y (2016). The burden of RSPO certification cost on Malaysian palm oil industry and national economy. *J. Oil Palm, Environment and Health, 7*: 19-27.

Brandi, C; Cabani, T; Hosang, C; Schirmbeck, S; Westermann, L and Wiese, H (2015). Sustainability standards for palm oil: Challenges for smallholder certification under the RSPO. *J. Environment and Development, 24*: 292-314.

Brenton, P and Manchin, M (2002). *Making the EU Trade Agreements Work: The Role of Rules of Origin. Working Document 183*. Centre for European Policy Studies, Brussels. 26 pp.

Byerlee, D and Rueda, X (2015). From public to private standards for tropical commodities: A century of global discourse on land governance on the forest frontier. *Forests, 6(4)*: 1301-1324.

Dankers, C and Liu, P (2003). *Environmental and Social Standards, Certification and Labelling for Cash Crops. FAO Commodity and Trade Policy Research Working Paper No. 2*. Food and Agriculture Org, Rome. 111 pp.

Defries, R S; Fanzo, J; Mondal, P; Remans, R and Wood, S A (2017). Is voluntary certification of tropical agricultural commodities achieving sustainability goals for small-scale producers? A review of the evidence. *Environmental Research Letters, 12(3)*: 033001.

Edwards, R (2015). *Is Plantation Agriculture Good for the Poor? Evidence from Indonesia's Palm Oil Expansion. Working Paper No. 2015/12*. Crawford School of Public Policy, Australian National University. 61 pp.

Ferrantino, M (2006). *Quantifying the Trade and Economic Effects of Non-tariff Measures. OECD Trade Policy Papers No. 28*. OECD Publishing, Paris, DOI: https://doi.org/10.1787/837654407568 70 pp.

Fontaine, D; Gaspart, F and De Frahan, B H (2008). Modelling the impact of private quality standards on the fresh fruit and vegetable supply chains in developing countries. Paper presented at the 12th EAAE Congress - People, Food and Environments: Global Trends and European Strategies. Gent, Belgium. 26-29 August 2008.

Garcia, M and Poole, N (2004). The development of private fresh produce safety standards: Implications for developing Mediterranean exporting countries. *Food Policy, 29(3)*: 229-255.

Humphrey, J (2006). Policy implications of trends in agribusiness value chains. *The European J. Development Research, 18(4)*: 572-592.

Kushairi, A; Meilina, O A; Balu, N; Elina, H; Mohd Nor Izuddin, Z B; Razmah, G; Shamala, S and Ghulam, K A P (2019). Oil palm economic performance in Malaysia and R&D progress in 2018. *J. Oil Palm Res. Vol. 31(2)*: 165-194.

Kushairi, A; Soh, K L; Azman, I; Elina, H; Meilina, O A; Zanal, B M N I; Razmah, G; Shamala, S and Parveez, G K A (2018). Oil palm economic performance in Malaysia and R&D progress in 2017. *J. Oil Palm Res. Vol. 30(2)*: 163-195.

Laila, K; Subasingae, R and Philips, M (2011). *Aquaculture Farmer Organizations and Cluster Management: Concepts and Experiences, FAO Fisheries and Aquaculture Technical Paper No. 563*. FAO, Rome. 104 pp.

Morley, D (2015). RSPO, the global standard for sustainable palm oil. *Agro Food Industry Hi-tech, 26(6)*: 29-30.

Murphy, D J (2014). The future of oil palm as a major global crop: Opportunities and challenges. *J. Oil Palm Res. Vol. 26(1)*: 1-24.

Malaysian Palm Oil Board (2018). Overview of the Malaysian oil palm industry 2018. http://bepi.mpob.gov.my/images/overview/Overview_of_Industry_2018.pdf, accessed on 1 December 2019.

Nia, K H; Pieter, G and Astrid, O (2015). Sustainability certification and palm oil smallholders' livelihood: A comparison between scheme smallholders and independent smallholders in Indonesia. *International Food and Agribusiness Management Review, 18(3)*: 25-48.

Parthiban, K; Nurhana, B; Khairuman, H; Hamdan, A B; Wahid, O; Shahrun, N K; Zamri, M S and Nurhanani, M (2017a). Monitoring and reporting of oil palm fresh fruit bunch (FFB) transactions among independent smallholders and dealers: An analysis of a case study in Selangor, Malaysia. *Oil Palm Industry Economic J. Vol. 17(2)*: 68-81.

Parthiban, K; Tan, S P; Siti, M A; Idris, A S; Ayatollah, K; Khairuman, H; Hamdan, A B and Wahid, O (2017b). Knowledge assessment of basal stem rot disease of oil palm and its control practices among recipients of replanting assistance scheme in Malaysia. *International J. Agricultural Research, 12(2)*: 73-81.

Phillips, M; Subasinghe, R; Clausen, J; Yamamoto, K; Mohan, C V; Padiyar, A and Funge-Smith, S (2008). Aquaculture production, certification and trade: Challenges and opportunities for the small-scale farmer in Asia. *Aquaculture Asia Magazine, 13(1)*: 5-8.

Potts, J; Lynch, M; Wilkings, A; Huppe, G; Cunningham, M and Voora, V (2014). *The State of Sustainability Initiatives Review 2014: Standards and the Green Economy.* International Institute for Sustainable Development (IISD) and the International Institute for Environment and Development (IIED). Manitoba, Canada and London, United Kingdom. 354 pp.

Rival, A and Levang, P (2014). *Palms of Controversies: Oil Palm and Development Challenges*. Center for International Forestry Research (CIFOR), Bogor, Indonesia. 59 pp.

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- The reference list should be in alphabetical order by first authors. There is no numbering.

Tables

- Tables should be on separate sheets and properly numbered. The same applies to figures, diagrams and other illustrations.
- Tables must not duplicate material in text or figures.
- The word `TABLE´ and the remainder of the table heading should be in capitals.
- Each table should have a brief title: explanatory notes should be in the legend, not in the title.
- Tables should only have three horizontal lines.
- Non-significant decimal places in tabular data should be omitted.
- Short or abbreviated column heads should be used and explained if necessary in the legend.
- Table footnotes should be listed in order of their appearance and identified by standards symbols: *, ‡, § for three or fewer: for four or more, consecutive superscript lower case letters should be used (*e.g.* [a], [b], [c], *etc*.).
- Authors are encouraged to view a recent issue of the Journal for table layouts.

Illustrations

- All illustrations should be cited consecutively and referred to in the text.
- Original artworks or other images must be submitted with the original copy of the manuscript. Photocopies are not acceptable.
- All photomicrographs should be supplied with a suitable scale.
- All illustrations should be numbered in sequence with Arabic numerals, in order of appearance in the text, as *Figure 1*, *Figure 2*, *etc.* Terms such as ‘Chart’ or ‘Plate’ should be avoided.
- The word ‘Figure’ and the caption should be italicised.
- High resolution digital photographs (>300 dpi) are acceptable.

Units of Measurements

- Metric units or the International System of Units should be used. Leave space between numbers and units, *e.g.* 20 ha, 5-8 ml, use kg m^{-1} and not kg/m.

Review

- All manuscripts will be referred to appropriate reviewers. Authors are encouraged to nominate possible reviewers, but the final say would be with the Publisher.

Proofs

- One set of proofs will be sent to the corresponding author to be checked for printer’s errors, and it is the responsibility of the author(s) to submit corrections to the editor within the stipulated time.

Manuscript Submission

- Manuscripts should be submitted via: https://mc04.manuscriptcentral.com/jopres
- Kindly ensure that the names of authors are not written on the manuscript but are indicated in the electronic submission form provided.
- For a guide on tables/figures and references, please refer to the latest edition of JOPR at: http://jopr.mpob.gov.my

Contents of the Coming Issue
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Note: * Subject to change.