AFOB MALAYSIA CHAPTER
Asian Federation of Biotechnology

# Biotechnology Innovations for Sustainable Future

Asian Federation of Biotechnology
Malaysia Chapter International
Symposium 2022

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# *Biotechnology Innovations for Sustainable Future*

Asian Federation of Biotechnology Malaysia Chapter
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1. Multidisciplinary Research
2. Biotechnology
3. Bieconomy

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# Table of Contents

# WELCOME ADDRESS

**Prof Ts Dr Suraini Abd-Aziz**
**President, AFOB Malaysia Chapter**
**Advisor, AFOB Malaysia Chapter International Symposium 2022**

It is my great pleasure to welcome all the participants to the 4th Asian Federation of Biotechnology Malaysia Chapter International Symposium (AFOBMCIS 2022) from 18th – 21st September 2022 which will be held in hybrid format i.e., online and physical at Pelangi Beach Resort & Spa, Langkawi, Malaysia. This symposium is jointly organised by AFOB-Malaysia Chapter (AFOB-MC), Universiti Putra Malaysia (UPM) and Universiti Kuala Lumpur (UniKL). Also, several universities and research institutions in Malaysia as collaborators included Universiti Teknologi Malaysia (UTM), Universiti Teknologi MARA (UiTM), Universiti Malaya (UM), Universiti Malaysia Sarawak (UNIMAS), Universiti Malaysia Sabah (UMS), Universiti Malaysia Perlis (UniMAP), Universiti Malaysia Pahang (UMP), Malaysia Palm Oil Board (MPOB) and Malaysian Agricultural Research and Development Institute (MARDI). The 4th AFOBMCIS 2022 was Chaired by Assoc Prof Ts Dr Mohamad Faizal Ibrahim (UPM) and co-chaired by Ts Dr Sharifah Soplah Syed Abdullah (UniKL).

AFOB Malaysia Chapter (AFOB-MC) is a regional branch of AFOB with headquarters at Incheon, Korea is a non-profit organization, established and registered with the Registrar of Society in 2013. In line with the function of the Asian Federation of Biotechnology (AFOB), AFOB-MC also aims to promote cooperation on scientific grounds, between the scientists from academia and industry in the Asian region, for the general advancement of biotechnology as an interdisciplinary field of research and as a means of bringing the scientific development to the industrial level. Also, as an NGO that brings biotechnology knowledge and application to the community. As a record, the AFOB-MC had organised the AFOB Regional Symposium 2014 (ARS2014) in Seri Pacific Hotel, Kuala Lumpur, Asian Congress on Biotechnology 2015 (ACB2015) in Hotel Istana, Kuala Lumpur, AFOBMCIS 2018 in Pullman Kuching, Sarawak, AFOBMCIS 2019 at The Everly Hotel, Putrajaya and AFOBMCIS 2021 successfully held online due to COVID-19 pandemic.

The conference theme "Biotechnology Innovations for Sustainable Future" will contribute to the current biotechnology area with 12 technical sessions that cover a vast area of biotechnology. Alhamdulillah, after a lot of struggles to bring the symposium to success, this year with the hard work of the AFOBMCIS 2022 Organising Committee, the symposium will be held in a hybrid format.

I hope that this symposium will be a successful event with the enthusiastic participation of locals and worldwide biotechnologists for creative innovation toward our sustainable future. It also will contribute to the collaborative research programs, hence strengthening research relations and networking between universities, industries and government. I would like to express appreciation to the AFOBMCIS 2022 Organising Committee members for their effort and hard work to ensure a successful and meaningful symposium for all of us.

# WELCOMING REMARKS

**Assoc Prof Ts Dr Mohamad Faizal Ibrahim**
**Chairperson,**
**AFOB Malaysia Chapter International Symposium 2022**

It is my great pleasure to welcome all of you to the 4th Asian Federation of Biotechnology Malaysia Chapter International Symposium 2022 (AFOBMCIS 2022). AFOBMCIS is one of the annual events of the AFOB Malaysia Chapter. The 1st AFOBMCIS was held in 2018 in Kuching, Sarawak, followed by AFOBMCIS 2019 in Putrajaya and AFOBMCIS 2021 was held virtually. This year, AFOBMCIS 2022 is organised physically and virtually (hybrid) to meet the current pandemic situation, which also allows participants to join this event online. AFOBMCIS 2022 aims to provide a platform for local and international scientists, academicians, and industries to share their knowledge and expertise, ideas and opinions and showcase research outcomes in biotechnology.

This year AFOBMCIS highlights the multidisciplinary focus, and emerging scientific and technological developments areas related to biotechnology. The theme of the symposium is “Biotechnology Innovations for Sustainable Future” which covers various biotechnological fields, which are Agricultural and Food Biotechnology; Applied Microbiology; Biopharmaceutical and Medical Biotechnology; Biocatalysis and Protein Engineering; Bioprocess and Bioseparation Engineering; Bioenergy and Biorefinery; Environmental Biotechnology; Marine Biotechnology; Nanobiotechnology, Biosensors and Biochips; Systems and Synthetic Biotechnology; Tissue Engineering and Biomaterials and Bioindustry Promotion and Bioindustry.

To share recent innovations in biotechnology, 3 plenary speakers have been invited together with 8 keynote speakers and 22 invited speakers from various countries to share their knowledge and expertise. This symposium also organises young researcher sessions, and physical and online poster presentations that will be evaluated by our appointed judges. Besides, suitable topics presented in this symposium will be invited for publication in Special Issues by Frontiers in Bioengineering and Biotechnology, Food Research Journal, Energies by MDPI and Journal of Oil Palm Research.

I would like to extend my gratitude to all the participants of this AFOBMCIS 2022. I also would like to express my appreciation to all the sponsors. Thank you also for all the commitments and hard work given by all the committee members of AFOBMCIS 2022. I hope that this symposium provides the opportunity for participants to share and discuss current progress in biotechnology, and establish collaborative research programs, hence strengthening research relations and networking among universities, industries, and government, bringing back a memorable and fruitful meeting experience.

Thank you.

# Oxidative Stress Status of Curcuminoid Analogue, BHMC on Human Liver Cancer, HepG2 Cells

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## ABSTRACT

Natural bioactive compounds are increasingly being recognised for their various pharmacological properties. Curcumin is one of the natural bioactive compounds derived from the rhizomes of Curcuma longa known to possess various pharmacological properties, specifically anti-proliferation, antioxidant and induces apoptosis (Shanmugam *et al.,* 2015). Due to poor bioavailability, a curcuminoid analogue called 2,6-bis(4-hydroxy-3-methoxybenzylidene)cyclohexanone (BHMC) was developed by keeping the phenolic hydroxyl group while getting rid of the unstable β-diketone moiety and transforming it into a conjugated double bond (Syed Alwi *et al.,* 2019). Curcumin has been shown to act as a pro-oxidant, antioxidant, and chemoprotective agent by either reducing or elevating ROS over the threshold level to cause cell death in malignant cells while having minimal cytotoxicity in normal cells. Therefore, it would be beneficial to look at the oxidative stress induced by BHMC on HepG2 cells.

The total antioxidant activity of BHMC was determined using biochemical FRAP assay. FRAP assay in the presence of HepG2 cells was also performed to determine the total antioxidant activity of BHMC on HepG2 cells (Noorfaiz *et al.,* 2022). To further confirm the effect of BHMC on the ROS, intracellular ROS level was measured via DCFDA assay to determine the oxidative stress level induced by BHMC on HepG2 cells (Alexander *et al.,* 2019). Further analysis, glutathione measurement and Keap1/Nrf2 expression via immunocytochemistry (ICC) were determined to understand the molecular mechanism triggered in HepG2 treated BHMC (Liang *et al.,* 2019).

The results demonstrated that BHMC significantly exhibited lower antioxidant activity in the absence of HepG2 cells compared to curcumin in a concentration-dependent manner. However, both BHMC and curcumin significantly exert total antioxidant activity in concentration-dependent manner compared to control in the absence of HepG2 cells. In the presence of HepG2 cells, BHMC shows higher total antioxidant activity at 6.25µM, 12.5µM and 25µM while lower total antioxidant activity at 50µM compared to curcumin. BHMC and curcumin in the presence of HepG2 cells exert total antioxidant activity in concentration-dependent manner compared to untreated HepG2 cells. Further intracellular ROS measurement demonstrated BHMC and curcumin significantly lower the ROS levels in HepG2 compared to the untreated group at both concentrations of 15µM and 25µM after 18 hours of treatment as well as 10µM and 25µM after 24 hours of treatment, respectively. BHMC and curcumin show to act in concentration-dependent manners in both timepoint. This has been confirmed with the

glutathione analysis which suggested that BHMC significantly triggered the production of total glutathione upon treatment with 15µM after 18 hours of treatment and 10µM after 24 hours treatment in HepG2 cells which led to the reduction in the level of oxidative stress. However, the ratio of GSH/GSSG indicated that curcumin led to oxidative stress damage compared to BHMC. Further molecular analysis on antioxidant defence mechanism, it demonstrated that BHMC induced the expression of NRF2 at 20µM after 24 hours of treatment while curcumin induced the NRF2 expression at 50µM in both timepoint.

In conclusion, BHMC may exhibits both pro-oxidant and antioxidant properties depending on the concentration, incubation time and type of cell lines that been used. Hence, it is suggested that BHMC may have a direct effect on the glutathione activity in modulating the redox reaction.

*Key words: BHMC; curcuminoid derivative; oxidative stress; glutathione; HepG2*

## ACKNOWLEDGEMENT

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# A Preliminary Convolutional Neural Network Model for Locally Isolated Microalgae Identification

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**ABSTRACT**

Microalgae are widely known to produce biofuel and health supplements, but they are also associated with a harmful algal bloom, whereby the occurrence can harm the ecosystem. Despite that, the method to identify them is still obsolete and requires many resources and professionals, such as chemicals, technology, time, and researchers with microalgae laboratory knowledge. Recently, researchers have suggested using a convolutional neural network for image classification. The convolutional neural network can reduce high dimensionality without losing information and automatically extract features. Therefore, this research aims to build a preliminary convolutional neural network model to identify microalgae and further understand the architecture for improvements. The microalgae images' datasets are self-collected from the AlBio laboratory at the Malaysia-Japan International Institute of Technology, Universiti Teknologi Malaysia, using a light microscope (Olympus, BH2-RFCA, Japan). Also, the strains are locally isolated in Malaysia; *Acutodesmus obliquus*, *Monoraphidium sp*, *Spirullina sp*, *Tetradesmus deserticola*, *Desmodesmus perforatus*. The architecture used to identify the microalgae in this research is the convolutional neural network. The preliminary trained model achieved an accuracy of 79% with a loss of 0.623. This study signifies the improvement of the microalgae identification system by producing a new automated method. Besides understanding the basic concept of deep learning, CNN architecture may help to build an even better version for an extensive scale species identification system.

Microalgae is one of the many eukaryotic microorganisms available in this world that has yet to be used to its full potential. It is estimated to be more than millions of species worldwide (Guiry, 2012), with only 167 381 species recorded in the AlgaeBase (http://www.algaebase.org) database as of August 2022. Microalgae are widely known for producing biofuel and health supplements (Chen *et al.,* 2018). However, uncontrollable amounts of microalgae in the sea and freshwater could cause harmful algal blooms (HAB). HAB is an annual occurrence in Malaysia whereby the toxins produced by microalgae infect most citizens and sea creatures (Hii *et al.,* 2021).

The conventional method is still outdated in identifying microalgae species. The traditional process requires a lot of budget, time, technologies, and human resources. However, parallel to the fourth industrial revolution, the growth of the implementation of artificial intelligence (AI) in the industry increased, hence the beginning of using AI to identify microalgae in 2017 (Corrêa, Drews-Jr, Botelho, Souza, & Garcia, 2017). The new method is expected to help cut most of the disadvantages of the conventional way.

This research focused on building deep learning architecture, mainly CNN. The CNN was chosen to perform the identification task as it automatically extracts the features of images

(Pedraza *et al.,* 2017). In addition, the ability of the architecture to uphold large-scale data also contributed to producing better results and higher accuracy in correctly identifying each microalgae species (Chollet, 2018). The preliminary CNN model built in this research comprises six convolutional layers and two fully connected layers resulting in an accuracy of 79% and a loss of 0.623 utilizing 1000 self-collected microalgae images. Improvements to the model's architecture can be implied to increase the accuracy. Also, increasing the sample size could help to improve the results. The results gained proved that the use of deep learning can indeed differentiate between each microalgae species with further architectural and dataset improvements.

The samples are locally isolated in Malaysia; *Acutodesmus obliquus*, *Monoraphidium sp*, *Spirullina sp*, *Tetradesmus deserticola*, *Desmodesmus perforates*. The testing and validation dataset are self-collected at the Algae-Biomass laboratory in the Malaysia-Japan International Institute (MJIIT) faculty at Universiti Teknologi Malaysia (UTM). The photos are taken from the light microscope, BH2-RFCA, attached to a camera brand AmScope. The light microscope, along with the camera, is connected to a laptop installed with AmScope software. It is used to view and capture images before saving them in a .bmp format with 2048 x 1536 dimensions. The total number of pictures taken is 1,000 before augmentation.

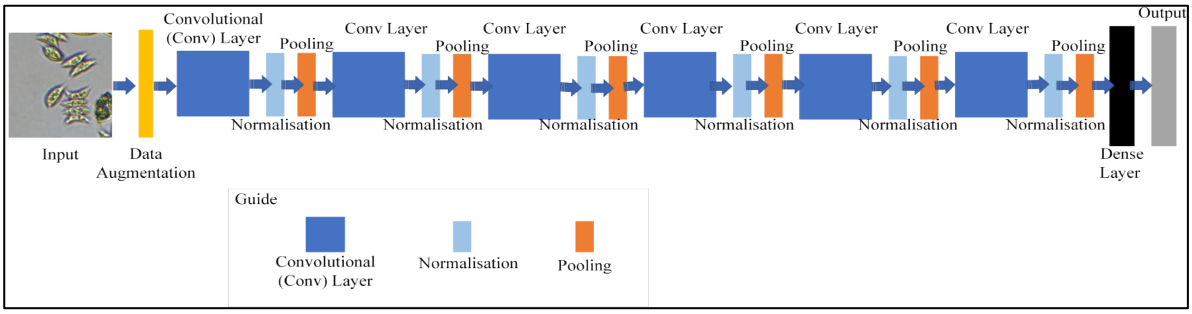


FIGURE 1: Preliminary Convolutional Neural Network architecture

The CNN model usually consists of several layers that act as filters before passing down more information. The layers are often composed of dozens to hundreds of successive representational layers, which it automatically extracts by receiving training data (Ongsulee, 2017). The architecture of the preliminary CNN model consists of the input layer, where the microalgae images are resized from 2048 x 1536 to 256 x 256. Then, the photos are augmented before being passed down to the first layer, the Convolutional 2D (Conv2D) layer. The augmentation of the images is done to increase the number of datasets whereby the photos are flipped horizontally, contrast controlled, randomly rotated, and zoomed.

All the Conv2D layers used ReLU as the activation function. Each Conv2D kernel size is three, and padding is valid. Next, the data will pass through to the normalization layer. The implementation of Batch Normalisation as a normalization layer helps to standardize the data, which helps to increase the learning speed. When the images go through the pooling layer, it is expected that the size of the photos to shrink. This process is significant in reducing the network's computational time. In this research, the pooling layer implemented is the MaxPooling2D. The input images must be passed on each layer successfully before going through the dense layer. The final dense layer uses Softmax activation as we have five species; this activation function is designed for multi-class. The preliminary model was evaluated based on the accuracy and loss of the training and validation of the model. The training and validation dataset are separated by 20% validation and 80% training.

The preliminary network was trained using the open source TensorFlow and Keras from the open library via Google Colaboratory notebook. The Google Colaboratory utilized NVIDIA K80 and GPU memory of 12GB. The preliminary model trained for a number of 50 epochs, with a batch size of 64. The dataset is self-collected from the light microscope, BH2-RFCA, with 2048 x 1536 dimensions. All the hyperparameters are tuned and retrained to obtain the optimal accuracy hence a learning rate of 0.001 was used. After running through 50 epochs, the preliminary CNN model obtained an accuracy of 79% and a loss of 0.623. Figure 2 shows the preliminary model's accuracy and loss, respectively.

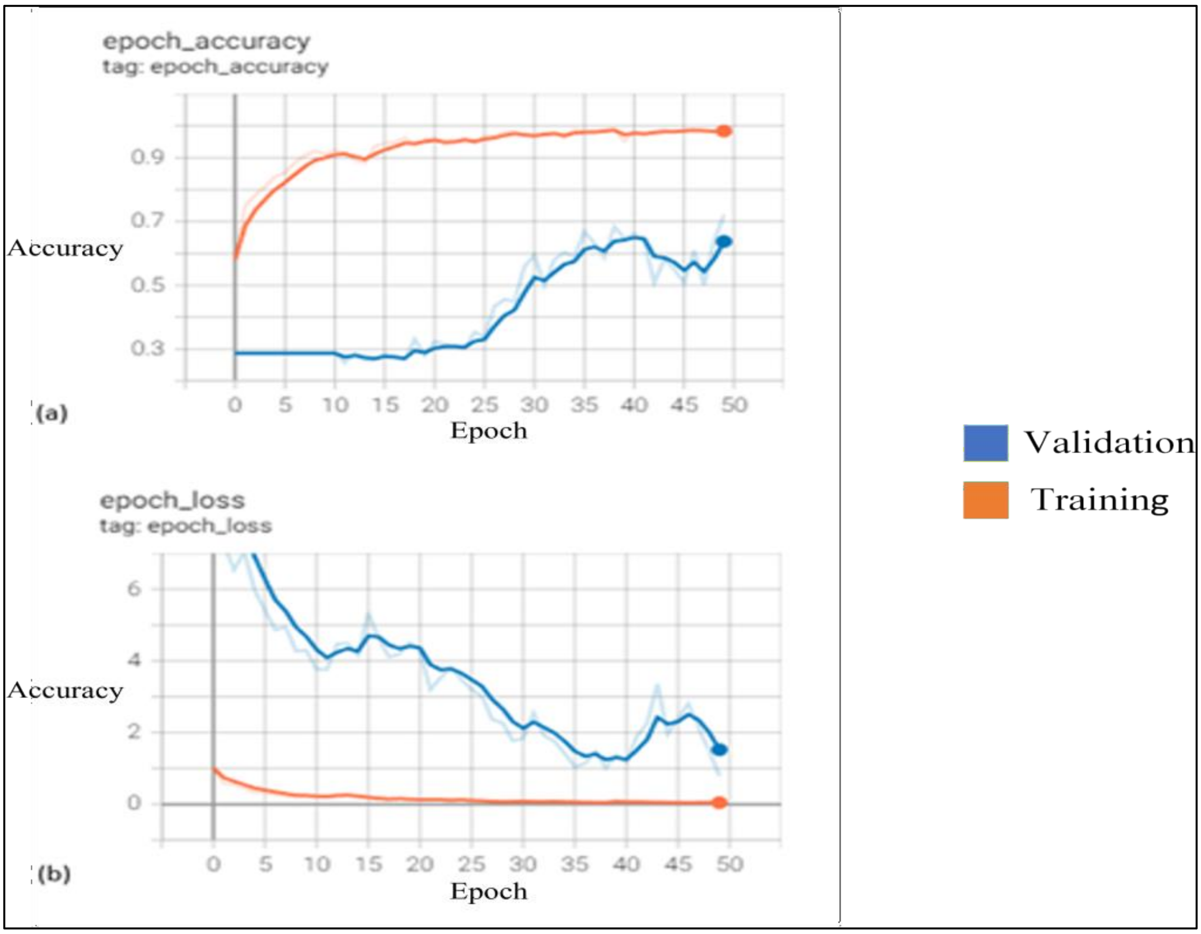


FIGURE 2: The figure labeled (a) accuracy of the preliminary model while (b) loss of the preliminary model

Figure 2 (a) shows that the model's training accuracy has reached a linear trend, whereas the validation accuracy is still increasing. The increasing trend is because the learning process of the model is still ongoing (Khan, Zhang, Alazab, & Kumar, 2019). Therefore, the validation results have not yet reached an end-point, as the graph shows a significant gap between the validation and training accuracy (Hang, Zhang, Chen, & Wang, 2019). Whereas Figure 2 (b) shows that the loss of the model for both training and validation has signification differences. The loss value indicates how poorly or well a model behaves after each iteration (Zhu, Zhang, Wang, & Ye, 2019). Therefore, from the graph, a more significant loss for validation demonstrates that the model is underfitted (Emmert-Streib & Dehmer, 2019). As a preliminary model to identify microalgae species, much room for improvements can be implemented in the model. For example, adding or hanging the parameters, layers, and tuning the hyperparameter. Thus, a better result can be achieved by improving the model parameters (Pan, Lu, Xu, & Gao, 2019) and increasing the number of datasets (Kukreja, Kumar, Kaur, Geetanjali, & Sakshi, 2020).

The preliminary CNN model provided evidence that deep learning can be used to identify microalgae species, with an accuracy of 79%. However, a better architecture can be built with further improvements, which may help improve the model's performance.

*Keywords: deep learning; microalgae; identification; convolutional neural network; artificial intelligence*

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# Rheological Assessment on Sodium Alginate Beads Under Different pH Environment

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## ABSTRACT

Probiotics are recognised as good bacteria that provides health benefits to its host. Depending on the species and strains, it has the ability to improve our immune, metabolic and digestive system. These beneficial bacteria need to be transported to our gut or intestines. Thus, delivery vehicle is needed to deliver probiotic to reach our gut, maintaining its viability along the gastrointestinal path. Various concentration of alginate solution was prepared and dripped in 1% (w/v) calcium chloride solution. After 15 minutes, they were rinsed and dried on filter papers. The beads were subjected to physicochemical and rheological tests to assess its strength. An average diameter of 4.32±0.68 mm was obtained from beads' formulations with clear, round shape. Immersions of the produced beads in acidic environment (pH 2) which simulates the stomach was executed, and the beads tend to shrink – up to 30% from its original water capacity. Shrinking of beads indicates the beads' pore size reduction thus act as protection for probiotics. While in simulation of intestine (pH 7) which is our destination, these beads absorbed water, expand in size and started to degrade. It could promote the release of internal compound, probiotic for example. In addition, beads produced possess the shear-thinning properties based on rheological test, evolution of apparent viscosity against shear stress. This trait provides favourable insight upon the oral processing condition.

Probiotics, living microorganisms either bacteria or yeast, play a huge role in human's body to provide health benefits on the host. Proof from different studies to date suggests that probiotics may beneficially affect human health (Anselmo *et al.,*2016) through modulating immunity, metabolic activities, and functions of the digestive system, thus reducing incidences of certain diseases such as diabetes and Clostridium difficile. To obtain potential benefits of probiotics, they should be transferred to the gastrointestinal tract before reaching its destination. Alginate is a highly versatile polysaccharides, widely investigated and used in different industries as food thickener, wound dressing and others (Lee & Mooney, 2012). Its properties such as its biocompatibility with human, high biodegradability, low toxicity and others are suitable candidate for encapsulation material. Also known as hydrogel, it has a unique volume transition of water when exposed to physical or chemical stimuli such as pH, temperature and pressure which is a good candidate for encapsulation material.

Various of alginate concentration are selected to produce beads ranging from 0.5% to 2 % (w/v) by modified extrusion method. To begin with, sodium alginate was weighted using a balance and transferred into 50 ml of two different solvents: phosphate buffer saline (PBS) solution and distilled water (DW) at different pH. Different pH values were chosen: pH 4, pH 7 and pH 10. On the other part, 1% (w/v) of calcium chloride solution, $CaCl_2$, was prepared by dissolving granular calcium chloride, Nacalai Tesque (Japan) into distilled water. Calcium chloride played a role as the gelling agent for alginate solution. The hydrogel solution produced is then poured into a container where it is aspirated into Vaccu pette 96-well pipettor with the aid of 35ml disposable sterile syringe. Then, it is dropped into a bowl of $CaCl_2$ solution where the gelation is occurred without stirring. Three random beads from each sample were chosen and measured by using a vernier calliper with precision 0.02mm (Brennet, China). Images of the beads were captured by using a smartphone camera. The dried beads then were kept in disposable petri dish and stored in 4 °C refrigerator.

The immersions of various alginate beads formulations were carried out to gain insight on swelling or shrinking of beads in different pH environment. In some cases of immersion, degradation can be observed too thus post treatment of filtering and drying were performed delicately to ensure samples were kept intact. 1g of the beads from each sample were, where initial diameter and image of 3 random beads were recorded and then immersed into phosphate buffer saline solution (pH 2, 4 and 7) at room temperature (25 °C) without stirring. At each 15 minutes and 30 minutes of immersion, the beads were collected, filtered, and dried. Collection of data from each sample was executed on the final weight of the immersed sample, final diameter and images of 3 random immersed beads. Triplicate of test were performed. The changes of sample weight can be deduced as the percentage of water intake or loss (as in the formula below) due to the ability of alginate beads as hydrogel to perform volume transition of water.

$$\%Water\ intake/loss = \frac{Wf - Wi}{Wi} \times 100\% \quad (1)$$

Wi : Initial weight of alginate beads sample (~1g)
Wf : Final weight of alginate (g)

One bead from each sample was put on the plate of Haake Mars rotational rheometer to be tested. The rotational rheometer has been setup with the cone and plate cartridge (CP2/60: PL65). Apparent viscosity is the desired parameter to study in order to assess the rheological behaviour of samples. Thus, investigation on the evolution of apparent viscosity against shear rate on the beads was performed. The range of shear rate to determine the apparent viscosity was fixed from 0.1 to 10 s-1. The samples were tested under two operating temperature values: 25°C for room temperature and 37°C for human body temperature. Triplicates of beads from a sample were measured. Data is collected and graph of evolution of apparent viscosities against shear rate were constructed and extrapolated to determine the rheological behaviour of alginate beads.

The formulations of beads produced different size, texture, appearance when alginate concentration, alteration of pH and type of solvent used is employed. Overall, the range of alginate beads diameter is in millimetre. The biggest beads recorded had diameter of 6.36 mm while the lowest was 3.48 mm. Gelation of alginate beads is highly influenced by the polymer concentration (Chan *et al.,*2011). Higher concentration of alginate contains higher G residues thus increase the number of crosslinking sites with divalent cations.

Rheological behaviour of alginate beads is a very important characteristic to be investigated as it allows to determine the desirability for oral condition processing (Jun Chen *et al.,*2017). Figure 1 showed that as shear rate was increasing, apparent viscosities of all alginate beads had a decreasing trend. Thus, shear thinning properties is adapted for all sample. The pseudoplastic fluids are characterized by the decrease of apparent viscosity as a function

a) b)

of the deformation rate applied (Coutinho *et al.,* 2019). Currently, we have the alginate beads which exhibit the same pseudoplastic behaviour. These pseudoplastic and shear thinning traits are huge upper hand of alginate beads for food processing conditions. It allows a smooth process for consumption. In addition, higher alginate concentration contributes to higher apparent viscosity value of alginate beads which can be observed at each shear rate point.

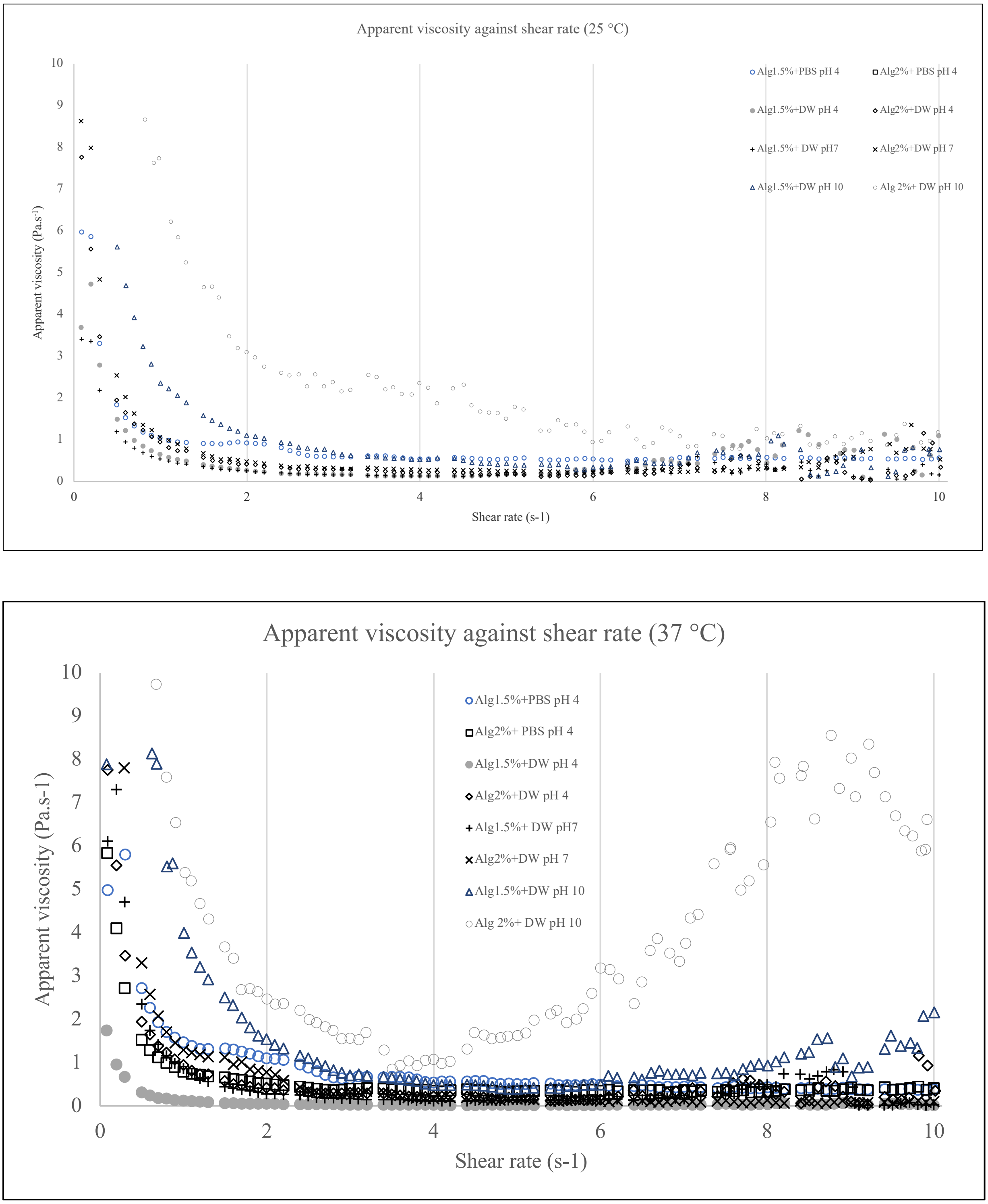


FIGURE 1: a) Apparent viscosities of different alginate formulations against shear rate at 25°C b) Apparent viscosities of different alginate formulations against shear rate at 25°C

Figure 2 shows the progression on the water uptake across related interval time of immersions. Immersion of alginate beads in pH 2 showed a significant different from immersion in pH 4 and pH 7. This immersion of alginate beads in acidic environment, pH 2 caused their size to decrease for each sample. This situation happened due to the presence of carboxylate acid (in form of-COOH) which can be found in the polymer-polymer chain. They are dominant over the polymer-water interaction force (Yin *et al.,*2018) which lead to the rejection of water. Data collected from table 6.2 shows that water loss for alginate beads are greater as we compare the initial, 15 minutes immersion and 30 minutes immersion. This confirms that water was being rejected from the alginate beads due to chemical stimulus response: acidic pH in the system. Hence, further deswelling behaviour could be observed on the beads.

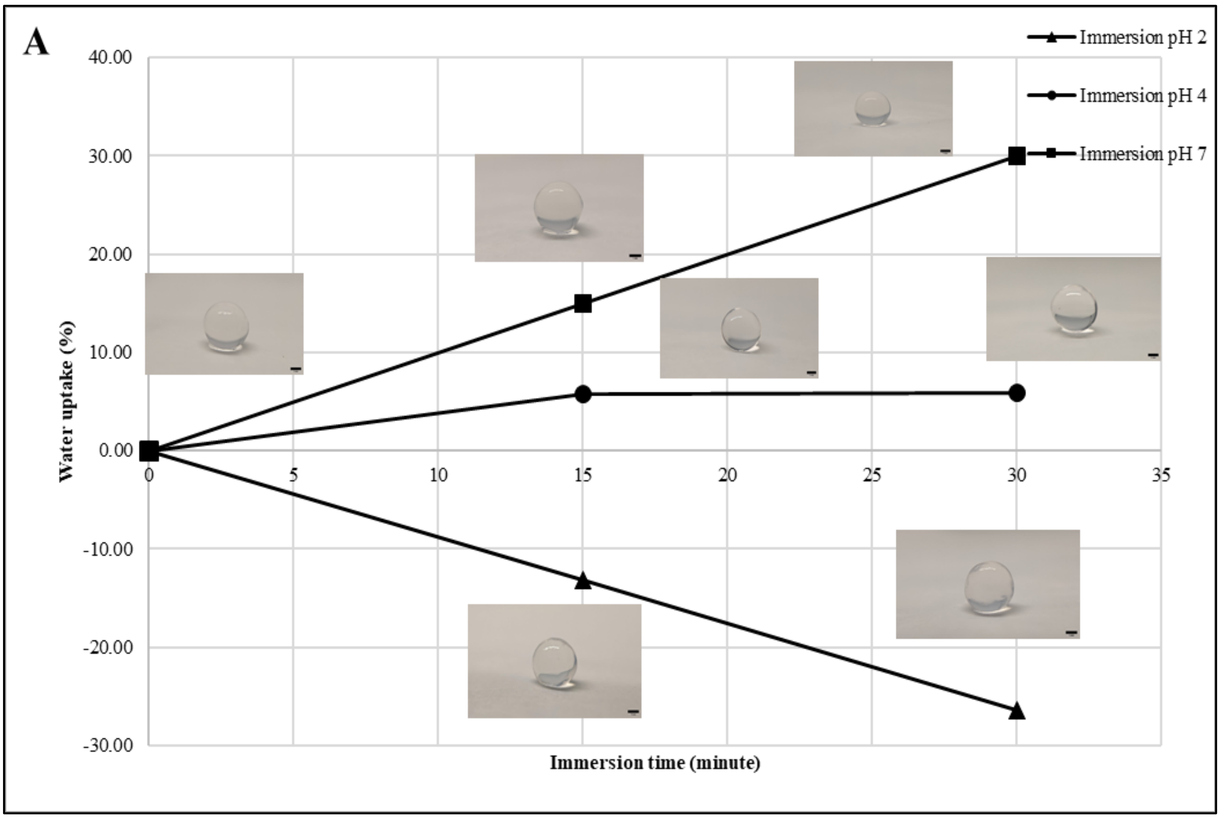


FIGURE 2: Water uptake performance against immersion time

The ability of alginate gels to possess clear and strong, firm gel is the main attractive points that can be used in many ways. Alginate beads also can be tailored through pH and type solvent used, to produce desired visual and mechanical properties. Furthermore, the shear thinning properties that exhibited by alginate beads is very favourable for oral processing condition. It can be referred as a prediction on the beads' reaction towards the pressure or force exerted during swallowing. 2% (w/v) alginate concentration is chosen as the best formulation due to its good flexibility to shrink and swell in different pH conditions.

*Keywords: alginate; hydrogel ; probiotic encapsulation*

## ACKNOWLEDGEMENT

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# Development of Aptamer-based Biosensor for the Detection of Permethrin

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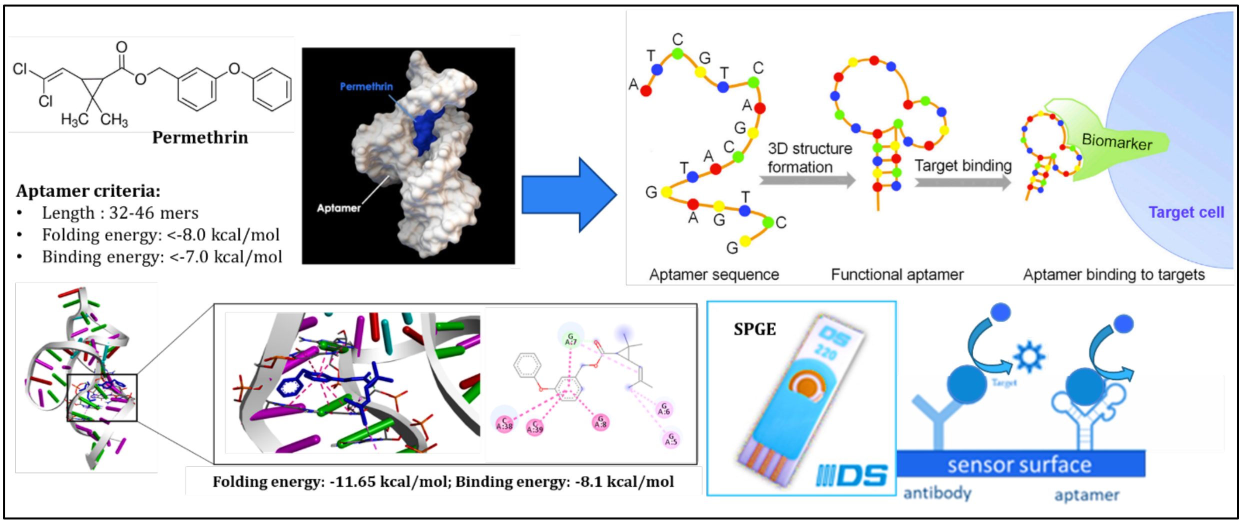
**ABSTRACT**

Synthetic pyrethroids are used widely in domestic, public health, agriculture, forestry, and veterinary applications. Although pyrethroids are thought to be safe for humans, reversible symptoms of poisoning and suppressive effects on the immune system have been reported after exposure. Depending on their chemical structures, synthetic pyrethroids can be divided into two groups; Type 1 with no cyano group, and Type 2 that contains cyano group. We reported here the detection of permethrin, a Type 1 synthetic pyrethroid, by means of aptamer sensor via electrochemical detection. Aptamer against permethrin were designed and synthesized via in-silico approach. The best aptamer candidate chosen has length of 44 mers, folding energy of -11.65 kcal/mol and binding energy of -8.1 kcal/mol. The thiolated aptamer was immobilized on gold screen printed electrode via passive adsorption for 1 hour. The binding between aptamer and its targeted permethrin was measured electrochemically using differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) techniques. Good linear regressions of permethrin aptasensor were achieved with $R^2$ value of 0.9626 and 0.9844 via DPV and EIS respectively for standard curves of 0.0 - 3.0 ppm. A reproducible and sensitive electrochemical impedance spectroscopy (EIS) on modified gold electrode is developed, reaching a limit of detection of 0.06 ppm ($n$ = 3) compared to the maximum residue limit (MRL) for permethrin of 5 ppm.

Aptamer, a new tool in biosensor recognition molecule, has been regarded as next generation for smart biosensing. Aptamer has the ability to imitate antibody structure without the need to perform tedious in-vivo immunization in small animals that may require animal ethic consideration. Until today, no aptamer has been designed for synthetic pyrethroids detection. As reviewed, the only electrochemical and photoelectrochemical aptasensor that has been reported were for the detection of Acetamiprid, Chlorpyrifos and Malathion (Li *et al.,* 2019).

Biosensor technique lends itself well as a potential tool for simple, rapid, sensitive and cost-effective monitoring that allow sample testing to be performed on-site with minimal sample preparation (Reynoso *et al*, 2019; Zamora-Sequeira, 2019). Until today, biosensor approach in detecting pesticides merely rely on immunosensor and enzyme approach. For instance, immunoassay and amperometric biosensor were employed in the detection of deltamethrin in seawater (Fruhmann. *et al.,* 2018). Screen-printed acetylcholinesterase-based biosensors for inhibitive determination of permethrin was also reported in 2021 (Domínguez-Renedo, *et al.,* 2012).

Herein, we first design and synthesis the aptamer via *in-silico* approach. During this first step, the binding energy and length of aptamer produced will be studied. Next, we investigate the direct detection between the synthesized aptamer and for synthetic pyrethroids via electrochemical measurements namely differential pulse voltammetry (DPV) and electrochemical impedance spectroscopy (EIS) on screen-printed gold electrode (SPGE) (see Schematic 1). These two techniques are able to probe detection at molecular level, requiring no label and thus offer direct detection for the target analyte. Initially used for the determination of the double layer, EIS is now a powerful tool in electroanalysis as it provides information regarding electrode interface, its structure and the reactions i.e. modulation of blocking ability on the sensor surface. Theoretically, the binding between aptamer and its target analyte will result in higher binding force and stronger charge that will affect surface resistance for the redox-active substance.




SCHEMATIC 1: Illustration of the steps of aptamer design and synthesis via an *in-silico* approach and aptamer immobilization on a screen-printed gold electrode (SPGE).

The preparation of the synthesized aptamer and its characterization has been monitored by Different Pulse Voltammetry (DPV) and Electrochemical Impedance Spectroscopy (EIS) respectively. A Dropsens ceramic-based screen-printed gold electrode (SPGE) (250BT) was used as a transducer where the aptamer solution was immobilized on the surface of working electrode via physical adsorption technique for 30 minutes incubation time. Permethrin (PM) immobilized aptamer was characterized and compared with the bare SPGE. All electrochemical studies were performed in redox solution containing 5.0 mM K3[Fe(CN)6] and 5.0 mM K4[Fe(CN)6].

The results of electrochemical analysis by using different pulse voltammetry (DPV) technique of the bare SPGE and immobilized SPGE with 25 nM Permethrin's (PM's) aptamer were showed in the voltammograms (see Figure 1a). It shows that the peak of gold electrode that has been immobilized with PM's aptamer was decreased proportionately with increasing of target concentration.

Linear regression of PM's aptamer by using different pulse voltammetry (DPV) technique was $y = -7.8908x + 48.744$, $R^2 = 0.9628$ as shown in Figure 1b. Which magnitude of the current response decreases linearly with increasing permethrin (PM) concentration.

According to three times of standard deviation of blank, 3*Sa*/*b* ($n$ = 3), the SPGE/PM's bioelectrode shows low detection limit, 0.75 ppm with linear range of 0.0 ppm to 3.0 ppm for PM concentration.

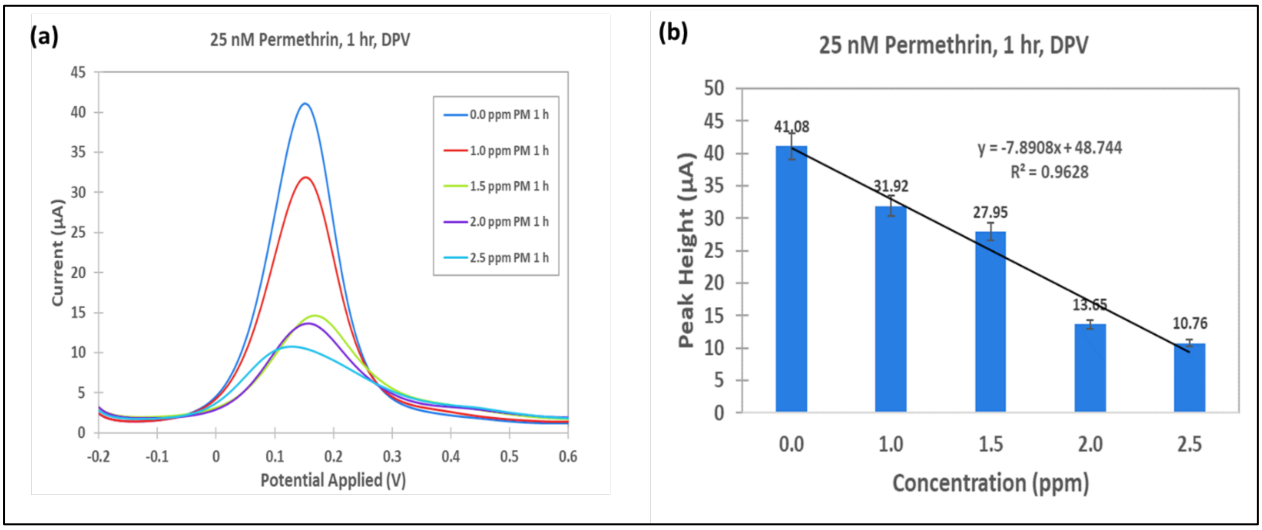



FIGURE 1: The (a) voltammograms and (b) linear regression of Permethrin's (PM's) aptamer by using different pulse voltammetry (DPV) technique.

The EIS spectrum consists of semicircular and linear parts. The diameter of the semicircle is related to the electron transfer resistance at higher frequencies, and the linear part corresponds to the diffusion process at the lower frequencies. The results of electrochemical analysis by using electrochemical impedance spectroscopy (EIS) technique of bare SPGE and immobilized SPGE with 25 nM PM's aptamer were showed in the Nyquist Plots (see Figure 2a). The semicircle of bare gold electrode was smaller because as the unmodified surface has no resistance that allows the redox solution to reach the surface. However, when immobilised with 25 nM PM's aptamer, the semicircles are increasing indicating the increment of resistance barrier on the gold surface due to the presence of the adsorbed aptamers.

Good linear regressions of permethrin aptasensor were achieved with $y = 199.1x + 98.733$, $R^2$ value of 0.9844 via electrochemical impedance spectroscopy (EIS) technique for standard curves of 0.0 ppm to 3.0 ppm (Figure 2b). A reproducible and sensitive EIS on modified gold electrode is developed, reaching a limit of detection of 0.06 ppm ($n$ = 3) compared to the maximum residue limit (MRL) for permethrin of 5 ppm.

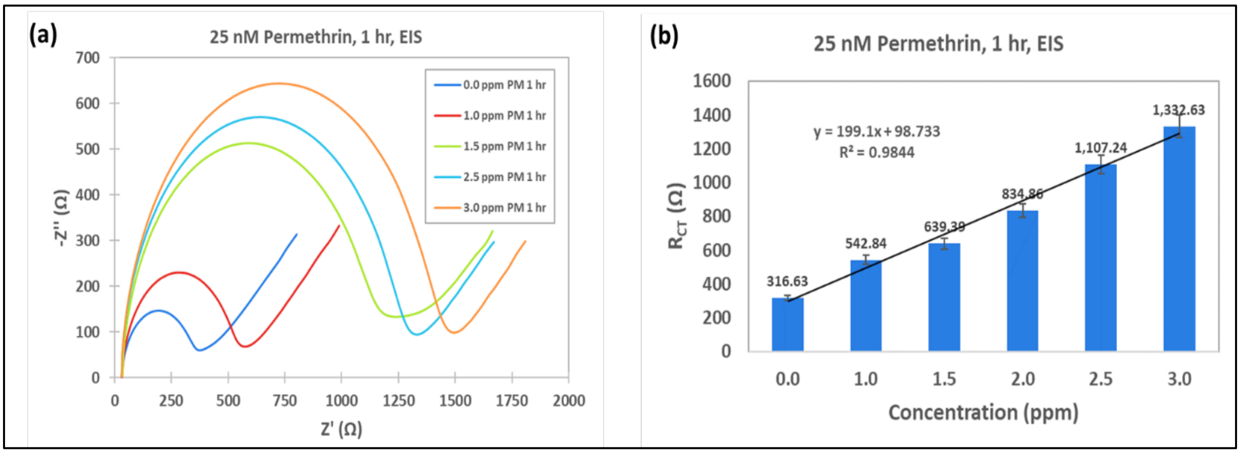



FIGURE 2: The (a) Nyquist plots and (b) linear regression of Permethrin's (PM's) aptamer via electrochemical impedance spectroscopy (EIS) technique.

Generally, any interaction between biological components will elicit signals in terms of current or mass changes. This signal could be amplified and transmitted into biosensor system using either electrochemical, acoustic wave or optical approach. Electrochemical biosensor is favoured over other fore mentioned approaches as it is more sensitive, specific, stable and feasible. By utilizing the designed aptamer, we are keen to study the aptamer interaction with its targeted synthetic pyrethroids in terms of electrochemical sensor.

*Keywords: aptamer; electrochemical biosensor; molecular docking; permethrin; synthetic pyrethroid*

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# Development of Agricultural Waste Rice Husk Fiber for Recycled Plastic-based Composites

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**ABSTRACT**

Throughout recent years, environmental impacts related to the production and disposal of industrial wastes materials including agricultural by-products and petroleum-based polymeric materials have aroused global attention. The new trend of the circular economy instigates the preservation of the planet's resources. In line with the circular economy, biodegradable composite materials, especially those obtained from are abundantly available plastic and agro-industrial wastes, are considered an interesting and critical strategy to replace non-degradable composites. Bio-based composites provide benefits of being economical and environmentally friendly at the same time.

Rice husk is one of the agricultural by-products produced in a huge amount worldwide (Chen *et al.,* 2021). Present short review attempts to discuss an up-to-date recent works, which conducted between 2018 and 2022, on the rice husk-reinforced biocomposites focally made from recycled polymers, as shown in Table 1. It highlights on the structure, interaction between the matrix and filler, and their effect on the performance. Literature revealed that rice husk has been used as reinforcing filler in various types of recycled polymers such as polyethylene (Sadik *et al.,* 2021; Susanto *et al.,* 2021; Flach *et al.,* 2022; Rigail-Cedeño *et al.,* 2022), polypropylene (Morales *et al.,* 2021) and expanded polystyrene (Akinterinwa *et al.,* 2020). Recently, our research has prepared rice husk reinforced composites based on recycled polymer blend of HDPE/PET (Chen *et al.,* 2018; Chen *et al.,* 2021; Chen & Ahmad 2021) which abled to use the rice husk content up to 80 wt%. Blending material is mostly reported to be beneficial as compared to its respective counterparts. According to Nor Arman and co-researchers (Nor Arman *et al.,* 2022), recycled HDPE/PET is less brittle than the neat PET but the blend is stiffer and faster cooling when compared to the neat HDPE.

TABLE 1. Current development of Rice Husk/Recycled Plastics Composites

| Plastic Wastes | Rice Husk (wt%) | Preparation method | Performance | Modification | Ref. |
|---|---|---|---|---|---|
| HDPE | 3 | Extrusion | Not able to process without coupling agent. | Coupling agent enhance the filler-matrix and lower specific energy consumption. | Rigail-Cedeño *et al.,* (2022) |
| | 10-15 | Extrusion | 12 wt% exhibited the best tensile properties. | Better tensile strength if better distribution or by using coupling agent. | Susanto *et al.,* (2021) |
| LDPE | 20-40 | Injection molding | Tensile strength reduced with increasing filler.<br>Water absorption was lower than 1.1%. | Rice husk ash with smaller particle size was better than rice husk filler. | Flach *et al.,* (2022) |
| LLDPE | 65 | Two roll machine | For uncompatibilized system, tensile strength reduced and highly increased water absorption. | With MAPE coupling agent, good interfacial interaction via hydrogen linkages offered better tensile strength and water resistance.<br>Inclusion of 6 phc nanosilica or 4 phc nanoclay improved tensile and flexural properties and lowered water absorption. | Sadik *et al.,* (2021) |
| PP | 5-10 | Extrusion | With filler, mechanical properties decreased (but sufficient).<br>Lower warping than recycled PP.<br>More fiber, lighter and more cost-effective filament. | The limited interfacial interaction can be improved by physical and mechanical treatment or reactive coupling agent. | Morales *et al.,* (2021) |

| | | | | | |
|---|---|---|---|---|---|
| EPS | 33-50 | Casting | Water absorption increased and flammability reduced with addition of filler. | Finer rice husk particles lead to higher density and poorer water resistance but better strength, flammability and durability. | Akinterinwa *et al.,* (2020) |
| HDPE/ PET | 40-80 | Extrusion | Optimum tensile strength at 60 wt%, whereas optimum tensile modulus at 80 wt%. | Gamma radiation at low dose of 25-50 kGy improved tensile strength and water resistance but decreased thermal stability. | Chen *et al.,* (2021) |

Note: HDPE denotes high-density polyethylene; LDPE denotes low-density polyethylene; LLDPE denotes linear low-density polyethylene; MAPE denotes maleic anhydride polyethylene; EPS denotes expanded polystyrene

The quality of fiber-matrix interface interaction that principally governed by the intrinsic characteristics of the components is a major factor to affect the performance of rice husk incorporated biocomposites. Several approaches of surface modification (Halip *et al.,* 2021) such as chemical treatment on rice husk fiber like sodium hydroxide (NaOH) (Chen & Ahmad 2021), were implemented to improve the interfacial interaction which led to subsequent properties enhancement. From the aspect of material designs, the inclusion of inorganic filler, nanoparticles or additives (Awang *et al.,* 2021; Sadik *et al.,* 2021; Dada *et al.,* 2022) with high end performance or functional properties was deployed as secondary filler to produce hybrid composite system.

Literature revealed the comparable performance in comparison many conventional composites and the existing wood-based products. Therefore, it shows good potentiality in the field of applications of such as automotive, construction, packaging and so on.

*Keywords: Polymer composite; recycled thermoplastic; natural fiber; interfacial; performance*

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# Antibacterial of Lemon Myrtle Essential Oil Nanoemulsion Towards *Xanthomonas oryzae* pv. *oryzicola* Without Harming the Soil Bacterial Communities

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**ABSTRACT**

Rice bacterial leaf streak (BLS), caused by *Xanthomonas oryzae* pv. *oryzicola* (*Xoc*) is one of the serious rice bacterial diseases. The disease is widely spread in Asia, and an increase in BLS cases has been reported recently (Afolabi *et al.,* 2014; Diallo *et al.,* 2021; Onaga *et al.,* 2018).. Chemical control measures have been used to prevent BLS disease spread for decades and are considered an effective and rapid disease management strategy (Nasir *et al.,* 2019; Zhang *et al.,* 2012). Control of chemically induced bacterial plant diseases, which pose a significant risk to human, animal, and environmental health, remains a major issue due to public awareness. (Abdullahi *et al.,* 2020). An interesting option is using essential oils (EOs), the naturally biologically active plant products, as an alternative to synthetic chemical pesticides in controlling BLS pathogens (Sergeeva, 2016).Our previous study showed that lemon myrtle EO has an antibacterial effect against *Xoc* and has potential as an antibacterial agent for BLS control. It is necessary to ensure that the antibacterial agent has fno negative effect on other good bacterial communities. Therefore, this research objective is to study the effect of lemon myrtle nanoemulsion on soil bacterial communities.

The study started with the preparation of lemon myrtle EO as a nanoemulsion. Lemon myrtle EO and surfactant were mixed together by vortex and constituted the organic phase. The aqueous phase consists of only double distilled water. The composition of the formulation was set at 1 % of lemon myrtle EO, 5 % of surfactant mixture (Tween 80 mixed with Span 80) and 94 % of water. An analytical balance was used to measure the quantities of each constituent in the emulsion (w/w). Nanoemulsion sample was vortexed at 2000 rpm for 2 min to form a coarse emulsion. A fine emulsion was then prepared by sonicating the coarse emulsion using a high intensity ultrasonic processor (Q125 sonicator, QSONICA) for 5 min. In order to avoid heating of the sample, the sonication process was done with pulses of 5 s ON and 7 s OFF (Nirmal *et al.,* 2018).

To ensure that lemon myrtle essential oil does not have a negative effect on soil bacteria, antibacterial tests have been carried out on more than 10 different species of soil bacteria. The disc diffusion method was used for the antibacterial assay on 90 mm of a petri dish containing potato sucrose agar (PSA) for *Xoc* bacteria and Luria-Bertani (LB) agar for soil bacteria. The soil bacteria were grown in LB broth, and the cultures were standardised at O.D. 0.5. The standard bacterial inoculum (100 µL) was uniformly spread using a sterile spreader on the agar plate (Bajpai *et al.,* 2010). The inoculums were allowed to dry for 5 min. A sterile filter paper disc (6 mm in diameter) was individually impregnated with 5 µL of lemon myrtle

nanoemulsion (at 1 % concentration) and was then put in the middle of the inoculated agar plate. Sterile distilled water was used as a control. The plates were then incubated at 30 °C for the *Xoc* and at 36 °C for the soil bacteria. The antibacterial activities were observed after 24 hours for the soil bacteria and 48 hours for the *Xoc*, by measuring the discs' clear zone formation. All tests were conducted in three replicates.

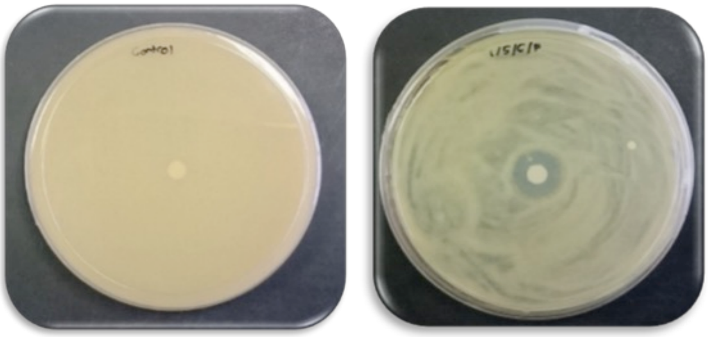


FIGURE 1: Antibacterial effect (clear zone formation) of lemon myrtle nanoemulsion disc on *Xoc* after 48 hours incubation at 30 °C.

TABLE 1: List of soil bacterial communities tested and their antibacterial effect

| No | Soil Bacteria | Antibacterial effect |
|---|---|---|
| 1 | *Bacillus amyloliquefaciens* subsp. plantarum UCMB5036 | X |
| 2 | Bacillus cereus | X |
| 3 | *Bacillus cereus* F837/76 | X |
| 4 | Bacillus megaterium strain CCMM B583 | X |
| 5 | *Bacillus megaterium* strain LAMA 262 | X |
| 6 | *Bacillus pumilus* strain AUCAB16 | X |
| 7 | *Bacillus* sp. 4035 | X |
| 8 | Bacillus sp. KZ_AaeF_Ma1 | X |
| 9 | *Bacillus* sp. LS29 | X |
| 10 | *Bacillus thuringiensis* serovar kurstaki str. BMB171 | X |
| 11 | *Bacillus thuringiensis* serovar kurstaki str. HD73 | X |
| 12 | *Chryseobacterium indologenes* strain WZE87 | X |
| 13 | *Deltia acidovorans* SPH-1 | X |
| 14 | *Lysinibacillus sphaericus* C3-41 | X |
| 15 | *Paenibacillus barcinonensis* strain : BP-23 | X |
| 16 | *Paenibacillus* sp. 2104 | X |

X: No antibacterial effect

From the results, we found out that the antibacterial activity of lemon myrtle was specific for *Xoc* based on the clear zone formation (14 mm) around the paper disc on the agar plate (FIGURE 1). Citral is a major constituent in lemon myrtle essential oil. More than 90% of lemon myrtle EO is citral, an isomeric combination of geranial and neral (E-isomer and Z-isomer). The rich citral concentration of lemon myrtle EO contributes to its antibacterial activity. It has also been reported that a minor constituent of lemon myrtle, such as linalool, myrcene, and citronellal, in combination with citral, might improve antimicrobial action, making lemon myrtle essential oil an efficient antibacterial agent (Nirmal *et al.,* 2018; Sultanbawa, 2016).

Meanwhile, there was no antibacterial effect of lemon myrtle nanoemulsion on all the bacterial communities tested (TABLE 1). The soil bacterial communities consist of Gram-positive and Gram-negative bacteria. Gram positive bacteria are more easily affected by EO and antibacterial agents compared to Gram negative bacteria due to their differing cell wall structures (Li *et al.,* 2019). Several studies have shown that the outer membrane of Gram-negative bacteria is an effective and evolving barrier. EOs, on the other hand, offer a promising alternative because they are not restricted to a specific class of bacteria and have been shown to have potent effects on both Gram-negative and Gram-positive bacteria (Yap *et al.,* 2021). Since the lemon myrtle EO nanoemulsion antibacterial effect is specific against *Xoc* and has no effect on the soil bacteria, these results give an advantage to lemon myrtle as a potential alternative anti-bactericide agent for protecting rice plants from the BLS disease. At the same time, it also ensures environmental safety without harming soil bacterial communities. Soil bacteria perform many important ecosystem services in the soil, including improving soil structure and recycling soil nutrients.

*Key words: rice bacterial leaf streak, Xanthomonas oryzae pv. oryzicola, lemon myrtle essential oil, antibacterial activity, soil consortium bacteria.*

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# Versatile Applications and Potential Valorization of Oil Palm Fiber in 2022

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**ABSTRACT**

The growing population and demand for food, livestock feed and biofuels have led to more than 40% of the Earth's land are covered by crops and pastures. Main current issue in 2021 is that the agricultural activities have changed our world more than other human activities. The main vegetable tropical oils in the world are palm oil and coconut oil while sunflower oil, rapeseed oil, olive oil, soybean oil, and groundnut oil are categorized as non-tropical vegetable oils. As the main vegetable tropical oils globally, palm oil has been used as major sources of dietary fats for developing countries like Ghana due to their significant functional and nutritional properties (Boateng *et al.,* 2016). Palm oil has been used as major sources of dietary fats for developing countries due to their significant functional and nutritional properties. Being the most consumed edible oil worldwide and dynamic biofuel feedstocks, the demand on palm oil will continue to boost as a leading driver in global vegetable oil markets. Nevertheless, along with the rising demand and interest on oil palm as bioresource, the utilization of oil palm by-products in versatile applications is vital for environmental sustainability (Gan *et al.,* 2018). The main contributors in oil palm plantation worldwide, which generates enormous amount of biomass annually with only a small portion been transformed into value-added products whereas the remaining parts are unused. Huge number of by-products are generated by palm oil mills every year, which is about 5 times of the palm oil production.

Lignocellulosic biomass such as oil palm trunk (OPT), oil palm frond (OPF), oil palm empty fruit bunch (OPEFB), oil palm kernel shell (OPKS) and oil palm mesocarp fiber (OPMF), would the major residue from the oil palm millings (Baharin *et al.,* 2016). Although there are detrimental impacts on biodiversity allied with the waste disposal ways, the oil palm fiber by-products could be an economically useful alternative as several potential applications have been developed in these 10 years back. Elbersen *et al.,* (2005) revealed that the present palm oil production system is considered as unsustainable because of the by-products could not add value to the palm oil production chain and disposal issues on oil palm wastes. Although there are detrimental impacts on biodiversity allied with the waste disposal ways, the oil palm fiber by-products could be an economically useful alternative as several potential applications have been developed in these 10 years back. These includes animal feed, water treatment,

renewable energy, architecture, automobile, pulp and paper, agriculture as well as polymer and composite sectors.

Although there is still great amount of oil palm by-products are left underutilised, several technologies were progressively developed to convert the oil palm biomass fiber into feasible and useful bioproducts such as composites, paper, renewable energy source, architecture, adsorbents, etc. This review focuses on a brief overview of existing technologies for utilising the oil palm fiber to circumvent the challenges posed by improper disposal of by-products, as well as their versatile applications and potential as a sustainable alternative. There are various applications that can be obtained from oil palm fiber and the bibliometric analysis on the literature either it is research or review publications were recorded in Scopus database (www.scopus.com). In the past decade, many researches related to the oil palm fiber can be found in various applications such as pulp and paper, automotive, agriculture, animal feed, renewable energy, water treatment, polymer, architecture, and composite. However, not all sectors using oil palm fiber are still blooming. As shown from the listed database in Table 1, the top three sectors that have been focused as review articles in the year of 2022 are composite, architecture and renewable energy. Review article is mainly the compilation or comprehensive review on the findings related to the certain topics from researchers.

TABLE 1 Reviews on the numerous applications of oil palm fiber published in 2022

| No. | Pulp & Paper | Water treatment | Automotive | Renewable energy | Architecture | Composite | References |
|---|---|---|---|---|---|---|---|
| 1 | | | | ■ | | | (Ng *et al.*, 2022) |
| 2 | | | | | ■ | | (Rama Rao and Ramakrishna 2022) |
| 3 | | | | ■ | | | (Ayodele *et al.*, 2022) |
| 4 | ■ | | | | | | (Simanjuntak, Julian, and Kresnowati 2022) |
| 5 | | | | | | ■ | (Nuryawan *et al.*, 2022) |
| 6 | | | | | | ■ | (Asyraf *et al.*, 2022) |
| 7 | | | | | | ■ | (Alfatah *et al.*, 2022) |
| 8 | | | | | ■ | | (Anitha and Senthilselvan 2022) |
| 9 | | | | ■ | | | (Suhartini *et al.*, 2022) |
| 10 | | | | | ■ | | (Zaimi *et al.*, 2022) |
| 11 | | | | ■ | | | (Rosyidah *et al.*, 2022) |
| 12 | | | | | ■ | | (Al-Sabaeei *et al.*, 2022) |
| 13 | | | | | | ■ | (Kuram 2021) |
| 14 | | | | ■ | | | (Bakar *et al.*, 2022) |
| 15 | | | | | ■ | | (Kilani *et al.*, 2022) |
| 16 | | | ■ | ■ | | | (Jahan *et al.*, 2022) |
| 17 | | | | | ■ | | (Omar *et al.*, 2022) |
| 18 | | | | | ■ | | (Elias and Othman 2022) |
| 19 | | | | | | ■ | (Putro *et al.*, 2022) |
| 20 | ■ | | | | | | (Padzil *et al.*, 2022) |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 21 | | | | | | | (Ilyas *et al.*, 2022) |
| 22 | | | | | | | (Chin *et al.*, 2022) |
| 23 | | | | | | | (Windiastuti *et al.*, 2022) |
| 24 | | | | | | | (Paul Nayagam and Prasanna 2022) |
| 25 | | | | | | | (Ahmad *et al.*, 2022) |
| 26 | | | | | | | (Bakar *et al.*, 2022) |
| 27 | | | | | | | (Kumar *et al.*, 2022) |

From the findings, the global production of palm oil is increasing steadily throughout the years to fulfill the increasing demand of the consumers, and remain the highest production among the vegetable oils. Thus, more oil palm by-products need to be utilized. In composite sector, oil palm fibers have been reinforced in many thermoplastic and thermoset polymer matrices and their mechanical performance were well established. Nonetheless, the detail studies on oil palm fiber biocomposites such as moisture absorption resistance, weather resistance, dimensional stabiltiy as well as interfacial adhesion properties seem to be lacking.

*Key words: oil palm fiber; biomass; renewable.*

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# Evaluation on Microalgae Biorefinery Performance for Bioproduction of High Value Products

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## ABSTRACT

Microalgae is a promising future cell factory for the production of biobased products such as biofuel, bio-fertilizers, pharmaceuticals, and recently, electricity. However, its adoption in commercial settings is largely hindered by high production costs, especially for low-value products such as biofuels. To reduce overall production costs and increase commercial viability of biological processes, biorefinery is a promising approach. Nevertheless, less effort has been dedicated to study the requirement and characteristics of a suitable microalgae biorefinery. The hypothesis of this study is specific cell characteristics and optimum cultural conditions would significantly enhance microalgae's suitability as biorefinery for bio-based product production. Therefore, the objectives of this research are to investigate the characteristics of microalgae species for high-level production of bio-electricity, bio-diesel and carotenoids and to determine the effect of cultural conditions on biorefinery performance.

The first part of this study is to construct a biophotovoltaic (BPV) system that would allow microalgae to grow and produce electricity and bioproducts. Unlike common photo-bioreactors, bio-photovoltaic system requires cathode and anode system capture electrons (produced by microalgae) and generate electricity and allow microalgae to grow. For this study, the design of BPV system created by Bateson *et al.,* (2018) was referred as the blueprint for the system. The system is consisted of a bottle device and microalgae (refer to Figure 1 for BPV design). The bottle lid, which consist of three multiports, will host the anode (aluminium foils), the cathode (pencil lead) and one port for sampling purposes. Finally, cathode-anode steel wire will be used to connect the BPV system to a digital multimeter for electric current measurement. Microalgae *Chlorella vulgaris* (*C. vulgaris*) and Tris-Acetate-Phosphate (TAP) media will be used in this part of research to evaluate and benchmark the constructed BPV system. The voltages generated across a fixed external load (65 Ω) by the BPV systems and the abiotic BBV (i.e., BBV systems containing all the abiotic components, including the algal medium and operated without algal cells) were monitored every day using a digital multimeter. The bottles were manually agitated once per day and opened in a sterile condition every day to permit gas exchange and cell sampling. Microalgae fermentation was done at 25°C with 12 hours dark and 12 hours light cycle for 25 days.

The second part of the research involved analysing of microalgae biomass for carotenoid and biodiesel compounds. Analysis of carotenoids will be conduct using a High Perfomance Liquid Chromatography (HPLC) system whereas a Gas Chromatography Mass Spectrometry (GC-MS) system will be used to analyse biodiesel compounds extracted from the microalgae.

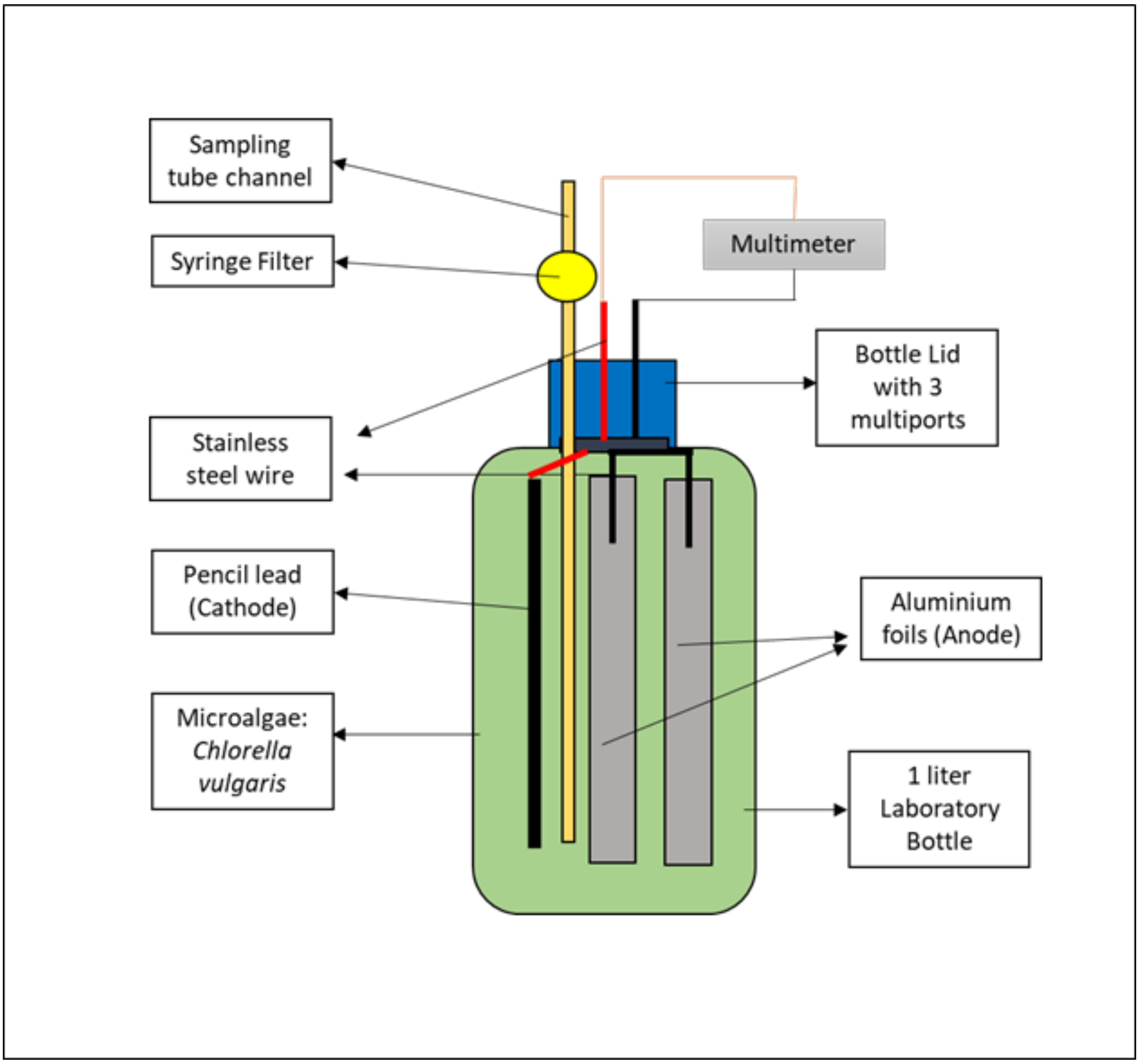


FIGURE 1: A Biophotovoltaic (BPV) prototype that would allow microalgae to grow and produce electricity and bioproducts. The system consists of 1L Laboratory Bottle containing microalge *Chlorella vulgaris*, aluminium foil as anode and a pencil lead that represent the cathode. Multimeter was used to measure the current generated from the system.

The current output was calculated for the BPV systems and the abiotic BPV systems from Ohm's law (Bateson *et al.,* 2018), as shown in the Equation (1).

$$Current\ (Ampere) = potential\ (Volt)/Resistance\ external\ (Ohm) \quad (1)$$

The amount of chlorophyll was calculated by subtracting the 750 nm OD value from the 680 nm OD value and multiplying the total by 44.609 (Bateson *et al.,* 2018). There was a strong correlation (r2 = 0.949) in determining chlorophyll concentration between this method and the well-established chlorophyll quantification protocol, as described by Porra *et al.,* (1989).

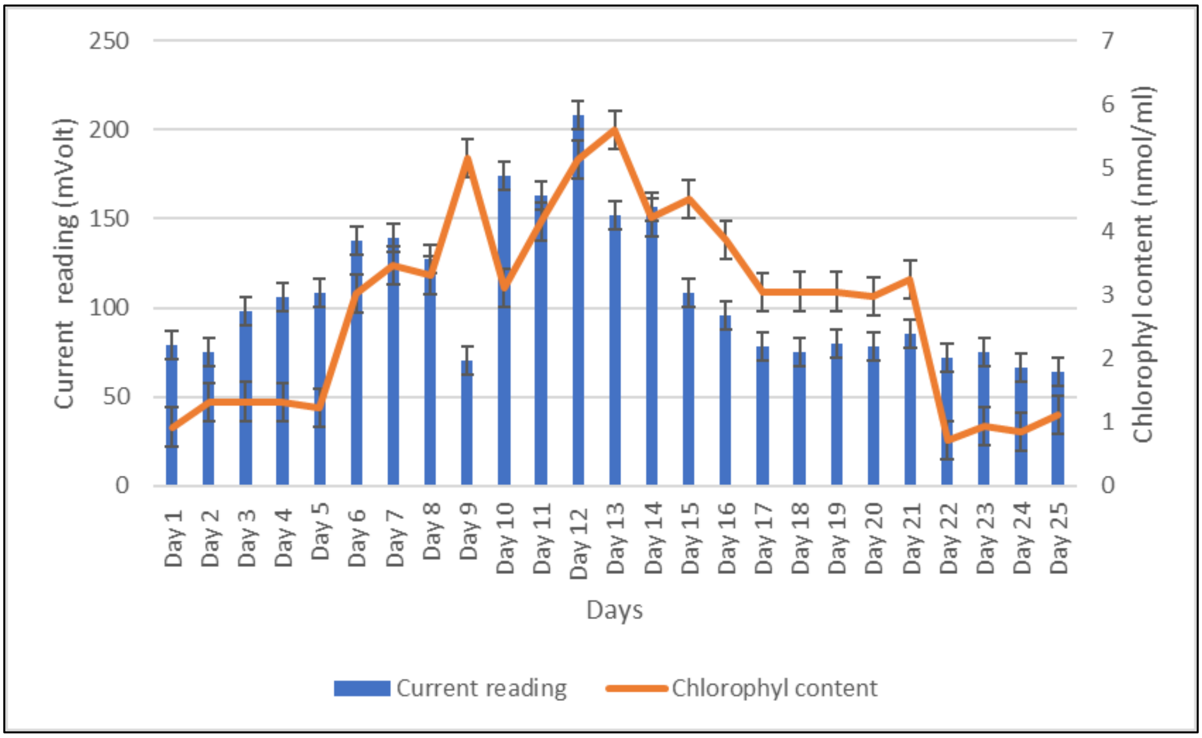


FIGURE 2: Current reading by digital multimeter and chlorophyl content measured for *C. vulgaris* in the BPV system for 25 days.

In this study, *Chlorella vulgaris* was studied for its performance in biorefinery pipeline to determine cell characteristics with bio-based product production in a fabricated BPV system for 25 days. Experimental results showed *C. vulgaris* were able to maintain their growth and gained significant electric generation up to 21 days in the BPV system (Figure 2). The electric current generated was proportionate to the amount of chlorophyl content, which means there was strong correlation between microalgae biomass and current generated from the system.

For bioelectric production, the current output from BPV system was at the average of ~6.3µA per bottle. HPLC analysis for carotenoid extracts from microalgae showed four major carotenoid compounds found in *C. vulgaris* which were Neoxanthin, Violaxanthin, Astaxanthin and 9-cis antheraxanthin. For biodiesel study, GC-MS analysis was able to detect 22 compounds mostly long chain alkanes that are potential to become lipid-based biofuel. Evaluation of *C. vulgaris* data showed that it has enormous potential for biorefinery performance for the production of carotenoid, biodiesel and bioelectric. Expected outputs from this project include novel findings on engineering microalgae biorefinery for high-level bioproduction.

*Key words: microalgae; biorefinery; carotenoid; biodiesel; bioelectricity*

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# Green Synthesis and Characterization of Silver Nanoparticle using *Citrus aurantifolia* Fruit Peel Extract

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**ABSTRACT**

Silver nanoparticles are commonly synthesized using chemical and physical methods. However, these methods required the used of toxic chemicals (N, N-dimethyl formamide (DMF) or trisodium citrate), high pressure, high energy, and temperature. Recently a green technique has been suggested to substitute the nature-unfriendly chemical and physical methods. The aim of this study was to synthesized silver nanoparticle using the peel extract of *Citrus aurantifolia* fruit and characterized the nanoparticles. The synthesis was conducted through bio-reduction of silver ions (Ag+) to silver nanoparticles (AgNP) using peel extract of *Citrus aurantifolia* fruit and characterized using UV-Vis, HR-TEM, FESEM, EDX, and FTIR. The synthesized silver nanoparticle (AgNP) has displayed the characteristics surface plasmon resonance (SPR) band peak from 360 nm-415nm which indicate the formation of silver nanoparticle. Effect of reaction time on the silver nanoparticle synthesis was also studied with UV-Vis absorption spectra, and it is observed that with an increase in time, the peaks become more intense. The HR-TEM analysis of synthesized nanoparticle showed a polydisperse, spherical in shaped with an average size of 24.023 (nm). EDX analysis further revealed that silver is the most abundant element found in the micrograph of the nanoparticle. Moreover, Fourier transform infrared spectrometer (FTIR) further confirmed the presence of different functional group in the surface of nanoparticle that are involve in reduction of silver ions. Based on the finding of this study it is understood that the variety of natural compounds that are present in plant extracts of *Citrus aurantifolia* fruit peel can act as both reducing and stabilizing agents for synthesis of silver nanoparticles. It is therefore, concluded that *Citrus aurantifolia* peel extract can be potentially used for the large production of silver nanoparticle for various of applications.

Silver nanoparticles are one of the most extensively studied nanomaterials, due to their high stability and low chemical reactivity in comparison to other metals. They are commonly synthesized using chemical and physical methods. However, these methods required the used of toxic chemicals (N, N-dimethyl formamide (DMF) or trisodium citrate), high pressure, high energy, and temperature. Hence, the need developed to new eco-friendly method. Recently a green technique has been suggested to substitute the nature-unfriendly chemical and physical methods. The aim of this study was to synthesized silver nanoparticle using the peel extract of *Citrus aurantifolia* fruit and characterized the nanoparticle using different techniques.

The dried peel powder of *Citrus aurantifolia* fruit was purchased from Chemical Engineering Pilot Plant, University Teknologi Malaysia (UTM), Johor, Johor Bahru, Malaysia. For the synthesis of silver nanoparticles silver nitrate (AgNO3) were purchased from a certified supply (Biotek Abadi Sdn Bhd, Malaysia).

The plant extraction was conducted using distilled water. One-hundred-grams (100g) of the peel powder of *Citrus aurantifolia* fruit was weighed using a digital weighed and macerated in 500 mL of distilled water at the ratio of 1:5 W/V. The mixture was maintained by constant shaking at 167 rpm for 24 hrs. The crude extract was then filtered using a muslin cloth and later filtered using a Whatman No 1. filter paper. The aqueous extract was then kept in refrigerator at 4°C until further use (Daskum, Godly, & Qadeer, 2019).

The synthesis of silver nanoparticles (AgNPs) was conducted following the method previously described by Nur Syazana *et al.,* (2018) with some modifications. Fifty milliliters (50 mL) of plant extract were added into 450 mL solution of 1mM of silver nitrate (AgNO3). The mixture was then kept in a water bath at 70 ºC and allowed to incubate for 2 hrs and during the 2hrs of the reaction time, the reduction of silver ions and synthesis of silver nanoparticle was monitored by UV-visible spectroscopy at different time interval of 30 min, 1hr, 1hr 30 min, and 2hrs. The formation of silver nanoparticles was determined by the color change of the mixture from yellow to brown. The mixture containing the colloidal nanoparticles was centrifuged at 4,000 rpm for 20 min using centrifuge machine (Hettich Rotofix 32 A, U.S.A) and the pellets obtained were lyophilized (Ayodele *et al.,* 2020).

The UV-vis spectrophotometer was used to determine the absorption spectra of the synthesized silver nanoparticle. the absorbance was recorded after diluting the aliquot (0.5mL) with 2 mL of the deionized water. The sample was scanned over the wavelength range from 200 nm to 800 nm at room temperature.

The sizes and morphology of the synthesized silver nanoparticles (AgNPs) was analyzed using HR-TEM. A dried powder of silver nanoparticle was prepared by dispersing a desired amount of the powder in acetone and ultrasonicated for 5 minutes. The sample was placed on a copper grid coated with carbon film and dried at room temperature for 15 minutes. Average diameters and shapes of silver nanoparticles were determined from the HR-TEM micrographs, while the distribution of the nanoparticles was determined by counting the approximate number of nanoparticles on the micrograph (Ahmad & Sharma (2012). Thereafter, the EDX analysis of the silver nanoparticles was also conducted using the HR-TEM in order to determine the elemental composition of sample.

The Fourier-transform infrared spectroscopy (FTIR) of the sample was carried out according to the method previously described by Awwad, Salem, & Abdeen, (2013), with some modifications. The small portion of the sample of silver nanoparticle powder was collected using micro spatula and mixed with 0.1 mg of KBr to form pellets in a disc. The pellet was then placed in the FTIR sample holder and FTIR measurements of the absorption bands were observed in the regions of 650cm-1 to 4000cm-1 at 4cm-1 resolution. The fingerprint of the chemical compounds generated were presented as FTIR spectrum.

The biosynthesis of silver nanoparticle in this study was confirmed by visual observation of colour change of the solution from slightly yellow to brown after the addition of *Citrus aurantifolia* peel extract into 1 mM silver nitrate solution. The colour change was rapidly, and it was observed virtually after 15 min of reaction time. Previous studies have also reported similar finding and it was suggested that the colour change was linked to the reduction of silver ion to silver nanoparticle by different bioactive compounds present in the plant extract that serve as reducing and stabilizing agents (Ahmed & Mustafa, 2020). The synthesized silver nanoparticle (AgNP) was further confirm using UV-vis spectroscopy and the results showed UV-vis absorption spectra between 360-415nm (Figure 1). This is in line with previously reported findings that reported UV-vis absorption spectra of 360 nm-470 nm (Awwad *et al.,* 2013; Reenaa & Menon, 2017). The particle size, shape, and distribution of the synthesized silver nanoparticle was analysed using HR-TEM and the results showed that the nanoparticles were polydisperse, and spherical in shaped, whereas some few are elliptical in shaped, highly distributed with the average particle size of 24.023 (nm) (Figure 2). Moreover, the EDX analysis of the synthesized silver nanoparticle revealed that silver had the highest peak which indicate that silver nanoparticle had been successfully synthesized and silver is the main element element present (Figure 3). The results in figure 4 show the FTIR spectrum of the synthesized silver nanoparticle and the absorption band appear at 3898.14, 3856.04, 3741.90, 3360.04, 2305.27, 1845.36, 1642.55, 1540.50, 1058.28, and 773.77 in the region of 4000 cm-1-650 cm-1. However, the absorption band appear at 3360.04 shows the presences of O-H stretching vibration of hydroxyl group and this indicates the presence of phenols and alcohols (Pirtarighat *et al.,* 20019; Vanaja *et al.,* 2013), while the peak at 1642.55 cm-1 shows C=C stretching vibration for alkene and C=N amine stretching, and the band appears at 1540.50 $cm^{-1}$ was identified as amide (Mayasari, Lestari, & Saraswatic, 2020; Nur Syazana Jalani, *et al.,* 2018).

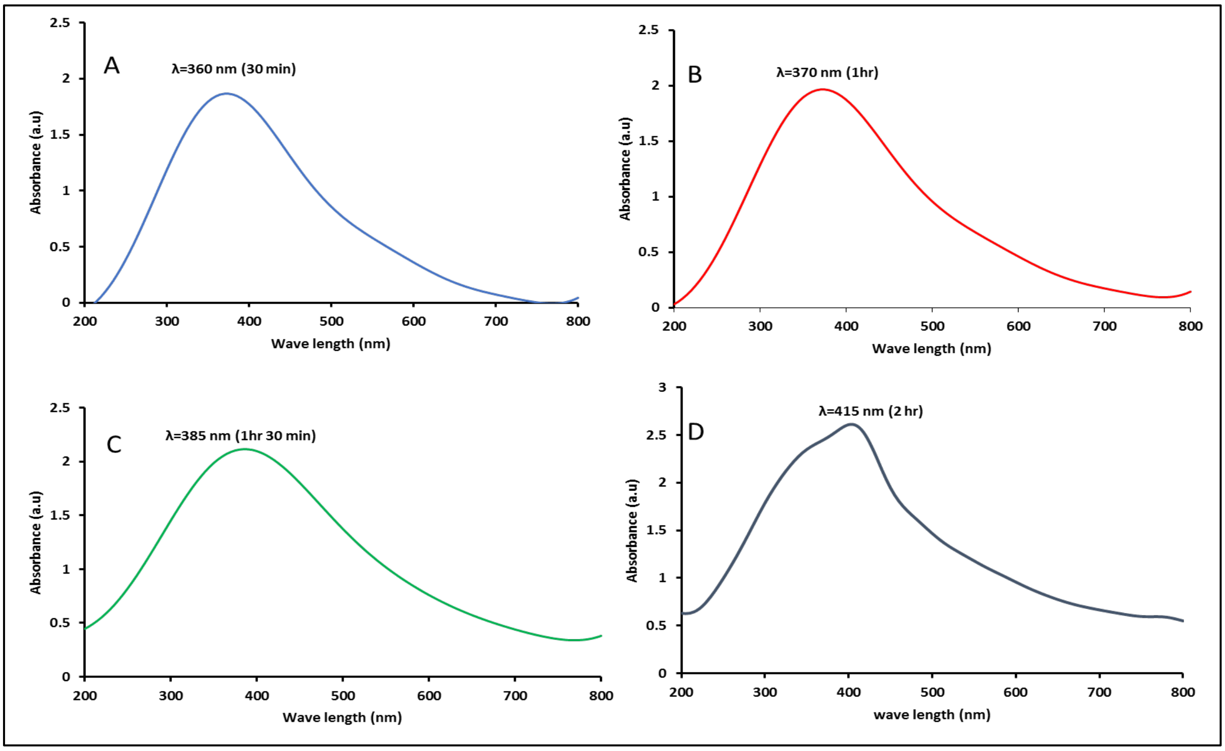


FIGURE 1: The UV–visible absorption spectra of AgNPs synthesized after 30 min, (A) 1hr (B), 1hr, 30 min (C) and 2hr (D) of incubation.

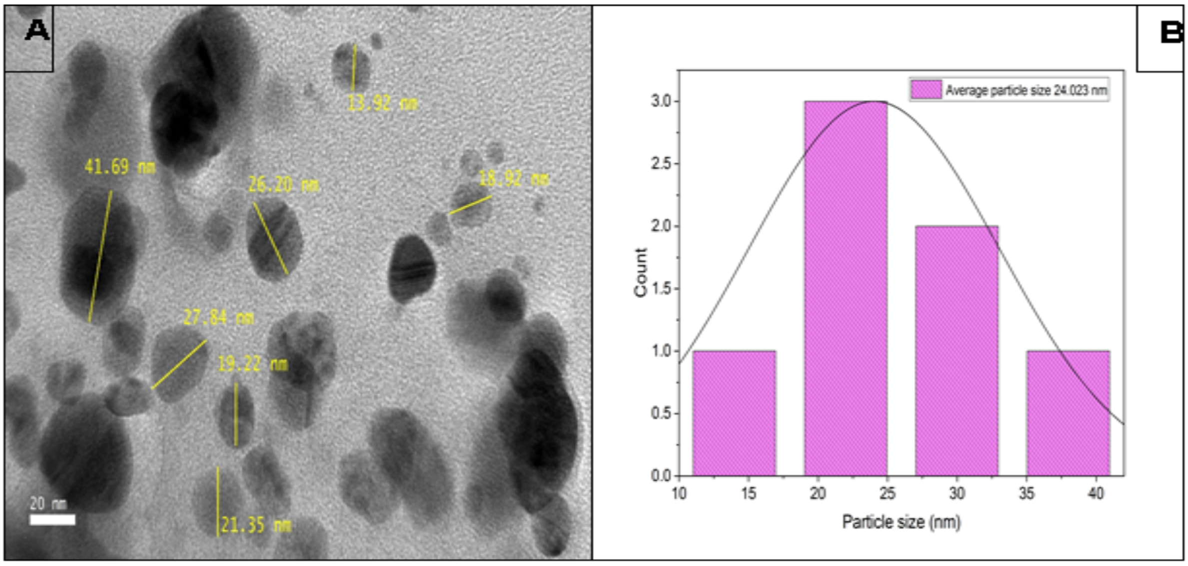


FIGURE 2: (A) HR-TEM micrograph of silver nanoparticles showing scattered, polydisperse and spherical shaped nanoparticles (B) Histogram of average particles size of silver nanoparticles (x100,000)

Based on the finding of this study it is understood that the variety of natural compounds that are present in plant extracts of *Citrus aurantifolia* fruit peel can act as both reducing and stabilizing agents for synthesis of silver nanoparticles. It is therefore, concluded that *Citrus aurantifolia* peel extract can be potentially used for the large production of silver nanoparticle for various of applications. Further studies are also recommended in order to explore and clarify the exert mechanism of synthesis of silver nanoparticle using the plants extract.

*Key words: silver nanoparticle; green synthesis; Citrus aurantifiolia*

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# Performance of Oil Palm *Dura* × *Pisifera* Progenies Based on Different Deli *Dura* Populations

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**ABSTRACT**

Majority commercial oil palm *dura* × *pisifera* (D×P) seed production programmes in Malaysia are based on Deli × AVROS. Various populations of the Deli have evolved due to different selection criteria by various research organisations. Thus, the study was conducted to evaluate performance of D×P progenies based on different Deli *dura* populations for bunch yield, bunch quality components and vegetative measurements. Some 15 D×P progenies were derived from crosses among four populations of Deli *dura*, namely Banting (D1), Johor Labis (D2), Ulu Remis (D3) and Ulu Remis×Elmina (D4) with four AVROS *pisifera* palms. Analysis of variance (ANOVA) showed highly significant differences for all traits between Deli *dura* populations, indicating the presence of genetic variability which is essential for breeding programme. In term of bunch yield, progenies of D3 recorded the highest mean of fresh fruit bunch yield at 191.12 kg palm$^{-1}$ year$^{-1}$ due to its high bunch number (12.09 bunches palm$^{-1}$ year$^{-1}$) which was significantly different with other Deli *dura* populations. Progenies of D3 also had the highest means of oil to bunch (27.13%), oil yield (53.72 kg palm$^{-1}$ year$^{-1}$) and total economic product (59.66 kg palm$^{-1}$ year$^{-1}$), with no significant variation than progenies of D4 for those traits. Meanwhile, progenies of D4 showed the highest means for other traits of bunch quality components such as mesocarp to fruit (82.19%) and oil to dry mesocarp (79.66%). For vegetative measurements, progenies of D3 had the lowest petiole cross-section (27.60 cm$^2$) and trunk height (2.30 m) means with significant variation than the other Deli *dura* populations. The low petiole cross-section and trunk height would increase ease of fresh fruit bunch harvesting. Therefore, D3 and D4 can be selected as parents for *dura* improvement programme to ensure continuous production of improved D×P planting materials in the future.

The *dura* fruit form from the Deli breeding stock was the first oil palm commercial planting material in Malaysia. Since late 1959, the *tenera* fruit form from the hybrid of Deli *dura* × AVROS *pisifera* have been used as commercial planting material (Kushairi *et al.,* 1999), after the discovery of monogenic inheritance of shell thickness by Beirnaert and Vanderweyen (1941). Since then, the breeding programmes were focused on separate selection of *duras* and *pisiferas* using the reciprocal recurrent selection (RRS) or modified recurrent selection (MRS) schemes (Kushairi & Rajanaidu 2000). Breeding populations of Serdang Avenue, Elmina, Ulu Remis and Johor Labis of *duras* were developed from programmes by the Department of Agriculture (DOA) at Serdang and Elmina Estate, Kumpulan Guthrie Berhad at Ulu Remis and Elaeis Estate as well as Société Financiére de Caoutchouces (Socfin) at Johor Labis Estate. Meanwhile, Banting *dura* was from breeding programme by Harrisons & Crosfield at Oil Palm Research Station (OPRS) in Banting. Malaysian Agricultural Research and Development Institute (MARDI) later managed DOA oil palm breedingmaterials. The management was then transferred to Palm Oil Research Institute of

Malaysia (PORIM) (currently known as Malaysian Palm Oil Board, MPOB) when it was formed in 1979.

Continuous production of improved *dura* × *pisifera* (D×P) commercial planting materials would require the improvement of both parental *dura* and *pisifera*. Furthermore, increased oil yield has always been the main breeding objective for seed production. Besides, better oil quality, slow height increment and tolerance to pest and disease are a few traits of interest in selection. Therefore, the objective of this study is to evaluate performance of different Deli *dura* populations as female parents in the D×P progenies.

Some 15 D×P progenies derived from crosses among four populations of Deli *dura*, namely Banting (D1), Johor Labis (D2), Ulu Remis (D3) and Ulu Remis×Elmina (D4) with four AVROS *pisifera* palms were planted at MPOB Research Station Hulu Paka, Terengganu using a Randomized Complete Block Design (RCBD) in three replications in 2007. Bunch yield recording was initiated in 2010 by recording the bunch weight (BWT) and bunch number (BNO) on individual palm basis. The sum of BWT was fresh fruit bunch (FFB) yield, the total bunch count was BNO, while the quotient between FFB and BNO was average bunch weight (ABW). The best average of four consecutive recording years (2014-2017) was used for data analysis.

In the meantime, bunch quality components were estimated using bunch analysis method developed by Blaak *et al.,* (1963) and modified by Rao *et al.,* (1983) from 2011 until 2018. The bunch quality components include mesocarp to fruit (M/F), oil to dry mesocarp (O/DM), oil to bunch (O/B), oil yield (OY) and total economic product (TEP). Meanwhile, one round of non-destructive method of vegetative measurements (VM) such as petiole cross-section (PCS) and trunk height (HT) was carried out in 2015 as proposed by Corley and Breure (1981). All data of bunch yield, bunch quality components and vegetative measurements was then analysed using analysis of variance (ANOVA), while comparison between different Deli *dura* populations was by Fisher's Least Significant Difference (LSD) at 5% level of probability.

Among the 15 D×P progenies, ANOVA showed highly significant differences for all traits of bunch yield, bunch quality components and vegetative measurements. It indicates the present of ample genetic variation for selection of these traits. Contrastingly, non-significant effect of progeny × replication interaction was observed for all traits except PCS and HT, which showed consistency in performance of these traits over the three replicates.

Table 1 showed performance for bunch yield, bunch quality components and vegetative measurements in 15 D×P progenies based on Deli *dura* populations. Progenies of D3 recorded the highest mean of FFB yield at 191.12 kg palm$^{-1}$ year$^{-1}$ and BNO at 12.09 bunches palm$^{-1}$ year$^{-1}$, where its BNO mean was significantly different with progenies of the other Deli *dura* populations based on Fisher's LSD. Marhalil *et al.,* (2016) evaluated two generations of Deli *dura* and found that there was an increase in FFB yield of 12% for Banting *dura* and 16% for Elmina *dura* in the later generation compared to the previous generation. However, in this study, progenies of Banting *dura* or D1 had the lowest means of FFB yield and BNO at 169.61 kg palm$^{-1}$ year$^{-1}$ and 9.19 bunches palm$^{-1}$ year$^{-1}$, respectively. Both its FFB yield and BNO means showed significant variation than progenies of the other Deli *dura* populations. Progenies of D1 also recorded the highest mean of ABW at 18.99 kg, but showed no significant different with progenies of D2 (18.15 kg).

For bunch quality component, progenies of D3 showed the best performance with the highest means of O/B (27.13%), OY (53.72 kg palm$^{-1}$ year$^{-1}$) and TEP (59.66 kg palm$^{-1}$ year$^{-1}$). However, it showed no significant different with progenies of D4 for those traits based on Fisher's LSD. The excellent performance of Ulu Remis *dura* was also reported by Swaray *et al.,* (2020) in their study on progenies from various *dura* and *pisifera* crosses. Based on their evaluation, progeny of Ulu Remis × Nigeria (ECP HP 500) recorded the highest mean of FFB yield at 184.62 kg palm$^{-1}$ year$^{-1}$, while progeny of Ulu Remis × AVROS (PK 4674) showed good performance in oil-related traits such as OY (52.66 kg palm$^{-1}$ year$^{-1}$), TEP (57.36 kg palm$^{-1}$ year$^{-1}$) and total oil (TOT) (56.57 kg palm$^{-1}$ year$^{-1}$). Meanwhile, progenies of D4 had the highest means of M/F at 82.19% and O/DM at 79.66%. Its M/F mean showed significant variation than progenies of the other Deli *dura* populations, while its O/DM was not significantly different with progenies of D3. On the other hand, progenies of D1 recorded the lowest means of M/F (76.29%), O/DM (77.81%), O/B (23.63%), OY (41.80 kg palm$^{-1}$ year$^{-1}$) and TEP (47.71 kg palm$^{-1}$ year$^{-1}$), where its means of all traits except O/DM were significantly different with progenies of the other Deli *dura* populations.

TABLE 1. Means for bunch yield, bunch quality components and vegetative measurements in 15 D×P progenies based on Deli *dura* populations

| Deli *Dura* Populations | N | Bunch Yield | | | N | Bunch Quality Components | | |
|---|---|---|---|---|---|---|---|---|
| | | FFB | BNO | ABW | | M/F | O/DM | O/B |
| Banting (D1) | 131 | **169.61$^{b}$** | **9.19$^{c}$** | **18.99$^{a}$** | 66 | **76.29$^{c}$** | **77.81$^{b}$** | **23.63$^{c}$** |
| Johor Labis (D2) | 89 | 182.65$^{a}$ | 10.25$^{b}$ | 18.15$^{a}$ | **62** | 78.77$^{b}$ | 77.83$^{b}$ | 25.36$^{b}$ |
| Ulu Remis (D3) | 301 | **191.12$^{a}$** | **12.09$^{a}$** | **16.06$^{b}$** | **175** | 78.93$^{b}$ | 79.55$^{a}$ | **27.13$^{a}$** |
| Ulu Remis × Elmina (D4) | 65 | 182.89$^{a}$ | 10.92$^{b}$ | 16.95$^{b}$ | 30 | **82.19$^{a}$** | **79.66$^{a}$** | 25.98$^{ab}$ |
| Mean | 586 | 184.11 | 11.03 | 17.13 | 333 | 78.67 | 78.90 | 26.00 |

Continue…

| Deli *Dura* Populations | Bunch Quality Components | | N | Vegetative Measurements | |
|---|---|---|---|---|---|
| | OY | TEP | | PCS | HT |
| Banting (D1) | **41.80$^{c}$** | **47.71$^{c}$** | 137 | 33.03$^{ab}$ | 2.35$^{bc}$ |
| Johor Labis (D2) | 47.58$^{b}$ | 53.39$^{b}$ | **91** | **34.19$^{a}$** | 2.46$^{ab}$ |
| Ulu Remis (D3) | **53.72$^{a}$** | **59.66$^{a}$** | **307** | **27.60$^{c}$** | **2.30$^{c}$** |
| Ulu Remis × Elmina (D4) | 50.94$^{ab}$ | 56.15$^{ab}$ | 66 | 32.37$^{b}$ | **2.57$^{a}$** |
| Mean | 49.97 | 55.81 | 601 | 30.36 | 2.37 |

Means with the same letter are not significantly different at $p \leq 0.05$ based on Fisher's Least Significant Difference (LSD). Figures in bold within the mean column are minimum and maximum values. FFB = fresh fruit bunch yield (kg palm$^{-1}$ year$^{-1}$), BNO = bunch number (no. palm$^{-1}$ year$^{-1}$), ABW = average bunch weight (kg), M/F = mesocarp to fruit (%), O/DM = oil to dry mesocarp (%), O/B = oil to bunch (%), OY = oil yield (kg palm$^{-1}$ year$^{-1}$), TEP = total economic product (kg palm$^{-1}$ year$^{-1}$), PCS = petiole cross-section (cm$^{2}$), HT = trunk height (m).

In term of vegetative measurements, progenies of D2 had the highest mean of PCS at 34.19 $cm^2$ with no significant variation than progenies of D1. In the meantime, the highest mean of HT was recorded by progenies of D4 at 2.57 m and showed no significant different with progenies of D2. Progenies of D3 which significantly showed the lowest means of PCS (27.60 $cm^2$) and HT (2.30 m) would have an advantage as small PCS and low HT would increase ease of fresh fruit bunch harvesting. The low HT also may increase economic lifespan due to slow height increment.

Based on results of ANOVA, high genetic variability is present in the D×P progenies. Progenies of two Deli *dura* populations, namely D3 and D4, were found to have high yielding. Thus, palms from both populations could be selected as parents for *dura* improvement programme to ensure continuous production of improved D×P planting materials in the future.

*Key words: Oil palm; Deli* dura*; AVROS* pisifera

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