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Editors

Muhammad Helmi Nadri, A Rafidah A Mohd Yunos, Muhammad Hazim Yusof

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Plant Growth Performance of Maize (*Zea Mays* L.) Cultivars Influence by Different Fertilizer Application Rate and Method Under Biotic Farming Condition

Mohd Farid Ismail[1], Muhammad Helmi Nadri[1,2], Kian-Kai Cheng[1,2] and HongYeng L.[1,2*]

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ABSTRACT
Food security has always been a key issue in agriculture sector and it has become even more crucial in recent decades due the decreasing of available resources and climate change. Maize is one of the important and popular grain in the world. It is a major source of carbohydrates not just for human but also livestock. Therefore, it is important to identify planting practice that will improve the maize yield and quality. A field experiment was carried out to identify ideal fertilization application method and rate for optimal maize yield under biotic farming condition (chemical free). Bio-fertilizer (N:P:K 5:5:5) was applied with broadcasting and furrow method at application rate: 1, 2, 3 and 4 tons ha^{-1} at three replications (n=48). The plant growth performance of maize was evaluated based on plant height, number of leaves, stem diameter, corn weight, cob length and thousand-kernel mass. Results suggested no significant difference between broadcast and furrow methods on plant height, number of leaves, stem diameter, corn weight, cob length and thousand-kernel mass. In terms of fertilizer application rate, no significant difference observed on plant height, number of leaves and stem diameter, except for cob length, corn weight and thousand-kernel mass. The overall results showed fertilizer method using furrow application at 3 tons ha^{-1} is the ideal practice for optimal yield under biotic farming condition. In summary, application rate has a significant impact on cob quality disregards whether is broadcast or furrow method.

Keywords: Fertilization method, fertilization application rates, corn yield component

1. Introduction
After wheat and rice, maize (*Zea mays L.*) is third most vital cereal crop in the world [1]. Maize is grown at varies region with different range of climates. Maize is an important food for not just human but also animals. In addition, maize is also being used in varies industries such as pharmaceutical, medicine, herbal, and biofuel. The crop production and costs are highly relying on soil condition, climate, maize variety and fertilizer regime. Appropriate fertilizer management could increase the yield and production efficiency. Precision fertilizer management with optimal application method, timing and rate is particularly crucial to ensure crop yield and reduce nutrient wastage that impact the environment [2],[3]. Fertilizer plays a key role in ensuring crop yield, this has leads to excessive fertilizer application by farmers with the aims to boost crop yield. Unfortunately, this has resulted in severe environment problems such as soil deterioration, reduce soil productivity and pollution [4].

Fertilizer application method is vital in good agricultural practices. Selection of optimal fertilization method and rate ensure adequate amount of nutrient being supply to plants. This able to increase maize yield in the same time minimize nutrient wastage. Manual broadcasting method is common practiced in majority of commercial farms in Malaysia. The disadvantage of broadcast fertilizing method is the higher loss of fertilizer due to precipitation, irrigation and evaporation [5].

Few researches have reported the localize fertilizer application method has better nutrient use efficiency than broadcast method [6],[7]. Research by Welch [8] reported banding of phosphorus fertilizer gave higher corn yield as compared to broadcast method. Majority of the researches were conducted under conventional farming method. This study aims to determine optimal fertilizer application method and rate for optimal plant growth and yield of maize grown under biotic farming method (pesticide and herbicide free).

2. Materials and methods

The fieldwork was initiated in Mac 2019 and the data were collected for single cropping cycle. This study was carried out at biotic research farm located at Innovation Centre in Agritechnology, Universiti Teknologi Malaysia, Pagoh, Johor, Malaysia. Biotic farming is a planting concept whereby, plants are grown under natural conditions without any pesticides and herbicides input. This site is located at 2°09'18.8"N 102°43'57.8"E. The average height of the site is 134 m above sea level. The climate is tropical with average temperature of 27.5 °C and average rainfall of 183 mm/year. The experimental field was characterized by the sandy soil of palm oil plantation reclamation land.

2.1 Planting and cultural practice

The study area size was about 135 m^2 (4.5 m × 30 m) and was weed free during the study. A total of 10 soil bed with each having 36 ft in length and 2 ft in width with 2 ft in between were established. Maize seeds (Leckat Seed 516, Sugar King F1 Hybrid) were directly sown on March 3rd, 2019. Two weeks after sowing, a total 40 treatment sections were set up with randomized complete block design (RCBD) with each section consists of 10 seedlings. Mechanical weed control was done every four weeks to keep the plot weed-free. The treatment plot was watered five days a week for first five weeks after seeding.

2.2 Fertilizer application method

Agricode Bio-Organic compound fertilizer 555 (N:P:K 5:5:5) fertilizer was applied at fifth weeks after seeding. Four fertilizer rates (1,2,3, and 4 tons ha^{-1}) were applied with two application technique, furrow application method (FAM) and broadcasting application method (BAM) with four replication each (n=40). FAM is an application method which a 10 cm deep furrow was dug at each side of the maize plant and the fertilizer was then applied into the furrow which later cover with soil. BAM was carried out by uniformly spread the fertilizer over the soil along the rigdes around the basal of each maize plant.

2.3 Plant growth performance

Plant growth performance was assessed during harvesting stage. A total of 12 plants were randomly selected and measured for the following parameters; plant height (cm), stem diameter (mm), number of leaves (n), cob length (cm), corn weight (g) and thousand kernel mass (g). Tukey's HSD (THSD) multiple comparison test was used to assess significant difference in all the treatments at $p<0.05$.

3. Results and Discussion

3.1 Effect of fertilization method on plant growth and yield

Table 1 shows BAM method resulted in the highest thousand kernel mass and mean biological weight. In addition, BAM is the most ideal method from the economic view point as BAM is less laborious during fertilizer application as compared to FAM which required additional digging work for the furrow. The results also suggested the BAM resulted in better overall plant growth performance. Bakhtiari [6] and Adiaha [9], reported similar results in which the application methods had significant effect on yield and thousand-kernel mass.

Table 1: Comparison of maize growth and yield for different fertilization method

Treatment	Plant height (cm)	Leaves number (n)	Cob length (cm)	Stem diameter (mm)	Biological weight (g)	Thousand kernal mass (g)
BAM	130.38^{a}	10.20^{a}	18.10^{a}	16.35^{a}	237.08^{a}	344.50^{a}
FAM	134.72^{a}	10.19^{a}	18.43^{a}	16.10^{a}	233.12^{a}	325.75^{a}
Control	130.38^{a}	9.580^{a}	17.08^{a}	16.00^{a}	172.92^{b}	312.00^{a}

Means in every column with similar capital letters were not significantly different at 5% level by THSD

3.2 *Effect of fertilizer rates on plant growth and yield*

Results in Table 2 shows no significant difference observed for the different application rate on plant height and leaves number ($p>0.05$). On the other hand, a significant difference was reported for cob length and biological weight. At fertilization rate of 3 tons ha^{-1}, highest biological weight and thousand kernal mass were observed although the plant height and number of leaves suggested otherwise. With reduction of 25% of fertilizer use compared to 4 tons ha^{-1}, it will contribute to the reduction of production cost [7]. Greater stem diameter and plant height were observed with application of fertilizer at 3 tons ha^{-1} as compared to control (without fertilizer) and 4 tons ha^{-1} (excessive fertilizer). Study by Yu suggested appropriate application fertilizer rate can reduce 50% fertilizer application rates, while retaining optimum growth performance in terms of plant height and stem diameter [10].

Table 2: Comparison of plant growth and yield for different fertilizer at different levels of fertilizer application rate

Fertilization rate (tons ha^{-1})	Plant height (cm)	Leaves number (n)	Cob length (cm)	Stem diameter (mm)	Biological weight (g)	Thousand kernal mass (g)
0	130.38^{a}	9.58^{a}	17.08^{abe}	16.00^{a}	172.92^{ad}	312.00^{a}
1	136.52^{a}	10.21^{a}	16.83^{ab}	15.29^{a}	187.29^{ad}	308.50^{a}
2	128.57^{a}	9.71^{a}	17.37^{ab}	16.20^{a}	211.87^{acd}	328.50^{b}
3	133.65^{a}	10.25^{a}	19.37^{cde}	17.25^{a}	288.54^{b}	361.50^{c}
4	131.44^{a}	10.63^{a}	19.50^{cdf}	16.17^{a}	252.71^{bc}	342.00^{bd}

Means in every column with similar capital letters were not significantly different at 5% level by THSD

Analysis of maize yield performance at different fertilization rate using BAM shows only cob length and biological weight were significantly different (Table 3). Similar pattern was seen in Table 2 with treatment 3 produce better yield in term of mean cob length, biological weight and thousand kernel mass. Thus, it supports the ideal rate of fertilization in maize at 3 tons ha^{-1} in producing better maize yield.

Table 3: Comparison of plant growth and yield for different fertilizer application rate using broadcasting application method (BAM)

Fertilization rate (tons ha^{-1})	Plant height (cm)	Leaves number (n)	Cob length (cm)	Stem diameter (mm)	Biological weight (g)	Thousand kernal mass (g)
0	130.38^{a}	9.58^{a}	17.08^{a}	16.00^{a}	172.92^{a}	312.00^{a}
1	133.55^{a}	10.17^{a}	16.75^{a}	15.41^{a}	191.67^{a}	295.00^{a}
2	130.17^{a}	10.08^{a}	16.83^{a}	16.75^{a}	193.33^{a}	340.00^{a}
3	127.43^{a}	10.50^{a}	19.00^{ab}	17.58^{a}	283.75^{b}	383.00^{a}
4	130.38^{a}	10.08^{a}	19.83^{b}	15.67^{a}	279.58^{c}	360.00^{a}

Means in every column with similar capital letters were not significantly different at 5% level by THSD

Table 4 shows that maize yield for FAM with treatment of 3 tons ha^{-1} application rate produced the highest biological weight, cob length and thousand kernel mass. It further supports the statement made previously.

Table 4: Comparison of plant growth and yield for different fertilizer application rate using furrow application method (FAM)

Fertilization rate (ton ha^{-1})	Plant height (cm)	Leaves number (n)	Cob length (cm)	Stem diameter (mm)	Biological weight (g)	Thousand kernal mass (g)
0	130.38^{a}	9.58^{a}	17.08^{ab}	16.00^{a}	172.92^{a}	312.00^{a}
1	139.47^{a}	10.25^{a}	16.92^{a}	15.17^{a}	182.92^{ac}	322.00^{a}
2	127.00^{a}	9.33^{a}	17.92^{ab}	15.67^{a}	230.42^{a}	317.00^{a}
3	139.90^{a}	10.00^{a}	19.75^{b}	16.92^{a}	293.33^{b}	340.00^{a}
4	132.51^{a}	11.17^{b}	19.17^{ab}	16.67^{a}	225.83^{a}	324.00^{a}

Means in every column with similar capital letters were not significantly different at 5% level by THSD

Although many study concluded increasing of fertilizer level (nitrogen) improve plant growth of maize, study by Dawadi stated excessive amount of nitrogen will result in delaying of plant maturity and harvesting time [11]. For commercial maize production, fertilizing rate is very important as it will eventually translate into the increasing production cost. Thus, optimum level of fertilizer rate is essential to ensure optimal yield, good profit return, and reduces planting period.

4. Conclusions

Overall, the analysis of maize growth and yield performance shows the BAM was out performing FAM. Results indicated optimal yield can be obtained at 3 tons/ hectare (N:P:K 5:5:5) under biotic farming condition. The study showed the fertilizer application rate had significant impact the cob quality.

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Antioxidant Properties of *Hylocereus polyrhizus* Aqueous Extract and Its Effect on Lipid Stability in Bakery Product on Muffin

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Abstract
Baked goods contained high amount of fat and are prone to oxidative rancidity that can decrease its shelf life to less than a week. Synthetic preservatives have commonly been used in commercial bakery products. The use of synthetic preservatives has long been a concerned for its safety and long-term health effects. Due to this, safe and natural preservatives is highly demanded. The bright red colour of red pitaya fruit (*Hylocereus polyrhizus*) has gained increasing interest for its antioxidative potential to be used as natural preservatives. In this study, aqueous extract of skin and flesh of pitaya fruit were analysed for its antioxidant activity and its effect on the oxidative stability of fat in muffin was investigated. Firstly, the antioxidant activity of the red pitaya flesh and peel aqueous extracts were determined. The total phenolic contents (TPC) and the antioxidant activity were analyzed using 1,1-diphenyl-2-picrylhydrazyl free radical scavenging (DPPH) assay and its IC_{50} were compared to synthetic antioxidant; butylated hydroxyanisole (BHA). Then, the extract with the highest properties of antioxidant was added into muffin and its effect on the quality of muffin were determined by using peroxide value (PV) assay. The TPC of the flesh and peel were 0.21 mg g^{-1} and 0.25mg g^{-1} gallic acid equivalent respectively. The IC_{50} DPPH scavenging power of peel and flesh extract is 2900 ppm and 3270 ppm respectively. The peel aqueous extract was then chosen to be added into muffins and the PV analysis showed that the extract was able to delay the oxidative rancidity of fat in the muffin and extend its shelf life for more than eight days and were comparable to BHT. The study indicated that the aqueous peel extract from pitaya has strong antioxidant properties and can be used as a potential natural antioxidant in food to prolong the shelf life.

Keywords: *Hylocereus polyrhizus*, antioxidants, lipids, muffin, peroxide value

1. Introduction
Interest for natural antioxidants from fruits and vegetables have been increasing among consumer and scientific community as a lot of studies have indicated that frequent consumption of these antioxidant is associated with a lower risk of cardiovascular disease (CVD) and cancer [1]. Antioxidant has the ability to scavenge and inhibit the formation of free radicals in the human body by giving up hydrogen and thereby reducing the damage done on the biological molecules such as lipids and proteins [2]. The major groups that was found to contribute to the antioxidant activity in fruits and vegetables; phenolic (tocopherols, flavonoids, and phenolic acids), nitrogen compound (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids and ascorbic acids [3]-[5].

Commercial baked goods such as cake, bread, pastries, muffin and others usually contained high percentage of fat. The ratio of flour and fat could be 1:1 or more. Due to this, baked goods usually has short shelf life storage which is between 2-7 days due to oxidation which lead to fat rancidity in the product. Rancidity is caused by a slow lipid autoxidation involving free radical mechanism during storage. Currently, synthetic antioxidant such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), tertiary butylhydroquinone (TBHQ) and propyl galate and others synthetic antioxidants has been widely used to prolong the baked goods shelf life by delaying lipid peroxidation. However, the toxicological safety of synthetic antioxidants and colorants and the banning of several red dyes cause health concern among consumer. This has encouraged the development and application of natural antioxidants from natural colorants such as betalains and anthocyanin. Looking at the potential of waste material from fruits and vegetables from food industry, research and development for natural antioxidant are being made extensively [6],[7].

Red pitaya (RP) or Dragon fruit (*Hylocereus polyrhizus*) is a cactus fruit and native in tropical country [5]. Recently, RP has become a popular fruit consumed in Malaysia. It has mildly sweet taste and aroma. Its bright color is due to betalains pigment consisting of bright red-violet betacyanins [8]. Betacyanins has been found to have good functional food properties, anti-carcinogenic effect and good for maintaining body health [8],[9].

2. Materials and Method

2.1 Materials

Red pitaya fruit was obtained from local market in Shah Alam, Selangor, Malaysia. Folin-Ciocalteu's phenol reagent, sodium carbonate, gallic acid, trichloroacetic acid, potassium ferric cyanide, ferric chloride, 0.2 M phosphate buffer, ethanol, 1,1-diphenyl-2-picrylhydrazyl (DPPH), methanol, potassium iodide, sodium thiosulphate, glacial acetic acid and chloroform was bought from Sigma Aldrich, Germany.

2.2 Sample preparation

Red pitaya fruit was washed and cut. The flesh and skin were separated. The flesh and skin were ground with a food processor (Panasonic) until homogenous prior to water extraction. The sample was extracted according to the method of [10]. Briefly, 80 g of sample was mixed with 2.4 L of water and was boiled for 10 minutes. It was then filtered and concentrated at 40 °C using a rotary evaporator (IKA, Malaysia). The concentrated filtrates were finally freeze dried and kept in the amber tight bottles in -20 °C for further analysis.

2.3 Determination of the total phenolic content (TPC)

The total phenolic content was determined according to method by Duh and Yen [11] with some modifications. A total of 100 µl of extract solution (0.10 – 0.50 mg/ml) was added into a 10 ml volumetric flask with 0.5 ml of Folin-Ciocalteu reagent and the mixture was left for 8 minutes at room temperature. Then, 1.5 ml of sodium carbonate (20%) was added and was made up to 10 ml with distilled water. The mixture was left for 90 minutes at room temperature. The absorbance was measured at 725 nm using a UV-Vis spectrophotometer (Perkin Elmer, US). Dilutions were done if the absorbance reading exceeds 0.7. The concentration of total phenolic compounds in the extracts was determined as µg of gallic acid equivalent (GAE) obtained from the standard gallic acid graph.

2.4 Free radical scavenging activity (DPPH)

The effect of fruit and peel extract on DPPH radical was estimated according to the method by Duh and Yen [11] with some modifications. Briefly, 30 µl of the extracts (1mg/ml) was added to 30 µl of DPPH (1mM in methanolic solution). The mixture was shaken for 10 s and left for 20 minutes in dark room at room temperature. The absorbance of the resulting solution was measured with UV-Vis spectrophotometer (Perkin Elmer Lambda 35, US) at 517 nm. The radical scavenging activity was measured as a decreasing in the absorbance of DPPH in two minutes interval and was calculated by following formula [12]:

$$\text{DPPH scavenging effect (\%)} = 100 - [(A_0 - A_1/A_0) \times 100]$$

Where, A_0 was the absorbance of the control reaction and A_1 was the absorbance in the presence of the sample.

2.5 Preparation of muffin

Table 1 shows the formulation of muffin prepared based on the total flour weight of 1200 g. The dry ingredients were mixed together homogenously and the liquid ingredients were added slowly and was mixed. The mixture was weighed into a paper cup with 200 g for each cup. The mixture was baked for 30 minutes at 180 °C until cook or turn to golden brown. The muffin was kept in a controlled environment chamber at relative humidity 75% and temperature of 25 °C for 10 days. In every two days' interval, three muffins were taken for oil extraction using method described by Othman et al [12].

Table 1: Formulations of muffin

Material	**Formulation (g)**		
	Control	1	2
White flour	1200	1200	1200
White sugar	900	900	900
Salt	10	10	10
Baking powder	19	19	19
Margarine	1000	1000	1000
Milk	250	250	250
Egg	400	400	400
BHA	0	0.02	0
Pitaya extract	0	0	0.02

2.6 Peroxide value

Peroxide value was analysed using method from [14]. Briefly, 5 grams of oil extracted from a muffin was weighed into a 250 ml conical flask with stopper and 30 ml of solvent (glacial acetic acid and chloroform (2:3 v/v)) was added. The mixture was shaken until all fat was dissolved in the solvent. Then, 1.5 ml of saturated potassium iodide was added. It was shaken for another 1 minute and then 30 ml of distilled water was added. The mixture was titrated with 0.01 M sodium thiosulfate solution by adding the titrant slowly with continuous vigorous shaking, until the yellow colour was almost discharged. 5 ml of starch solution was added and titration was continued until the colour was discharged. Blank analysis was done under the same condition. Peroxide value was calculated as follows:

$$\text{Peroxide value} = (V_s - V_b)/W \times N \times 1000$$

Where, V_s was the volume of titre for sample, V_b was the volume of titre for blank, N was the Normality of Na thiosulfate and W was the weight of sample.

3. Results and Discussions

3.1 Extract yield and total phenolic content

The flesh and peel of pitaya was extracted with aqueous and the total phenolic content was measured accordingly to the Folin Ciocalteu method. The total extract yield was 2.7% and 1.6% for flesh and peel respectively while the TPC of the flesh and peel were 0.21 mg g^{-1} and 0.25mg g^{-1} gallic acid equivalent respectively. Aqueous was used to extract phenolic for this study because it is safe for human consumption [16] and can easily be incorporated into food products without compromising its sensory properties and palatability. Previous studies reported that the property of polyphenols in pitaya fruit is most likely a hydrophilic as it dissolves well in polar solvent such as methanol, ethanol and acetone [1],[6],[11],[16]. The Folin-Ciocalteu reagent determines total phenols (and other easily oxidized substances) by producing a blue colour through reducing yellow hetero polyphosphomolybdate-tungstate anions. The method gives an overall measurement of phenolics content in the extract, although not all phenolics compounds exhibit the same level of activity to the assay [18]. The observation during the extraction process, the colour of water extract is dark purple indicating the presence of colour pigments. Some researchers showed that betacyanins is the major pigments found in *H. polyrhizus* which give its deep purple colour [4],[8],[11],[16],[18],[20],[29]. Betacyanins was found to contained phenol structure which contributes to its phenolic content [23]. A study was also conducted to profiled the betacyanins in pitaya fruit and found that it contained 10.3±0.22 and 13.8±0.85 mg/ 100 g of flesh and peel respectively expressed as betanins equivalents [17].

3.2 Free radical scavenging activity

The DPPH radical scavenging assay is used to measure the ability of plant or fruit extract's antioxidant properties to scavenge free radical in the assay by donating hydrogen to it [24]. Figure 1 showed the scavenging percentage of RP extract of peel and flesh. Recently, IC_{50} was frequently used to express the amount or concentration of extracts needed to inhibit 50% of the free radicals and it was inversely proportional to the scavenging activity of the extracts [24]. Calculated from graph's equation, The IC_{50} DPPH scavenging power of peel and flesh extract were 2900 ppm and 3270 ppm respectively. The scavenging power of the peel was higher than peel due to the polyphenols type. Studies showed that the biological properties of these phytochemical were profoundly affected by any changes in the structure and usually linearly correlated between betalains and anti-proliferative activity of the compound [1]. Likewise, the deep red purple colour in the flesh of red pitaya indicates abundance amount of betalains because it is found to be water soluble [6].

Phenolics also influence antioxidant-activity measurement. They interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and scavenging oxygen. The effect of flesh and peel on antioxidant activity could be a result of the types of polyphenolics they contained. Phenolics influence antioxidant-activity measurement by interfering with the oxidative process by reacting with free radicals, chelating catalytic metals and scavenging oxygen. The effect of flesh and peel on antioxidant activity could be a result of the polyphenols they contained. An increase number of hydroxyl groups (-OH) or other hydrogen-donating groups (=NH, -SH) in the molecular structure led to higher antioxidant activity. Betalains contain imino groups and hydroxyl groups and would contribute antioxidant activity, which could explain, in part is a better antioxidant due to a higher level of betalains in the flesh.

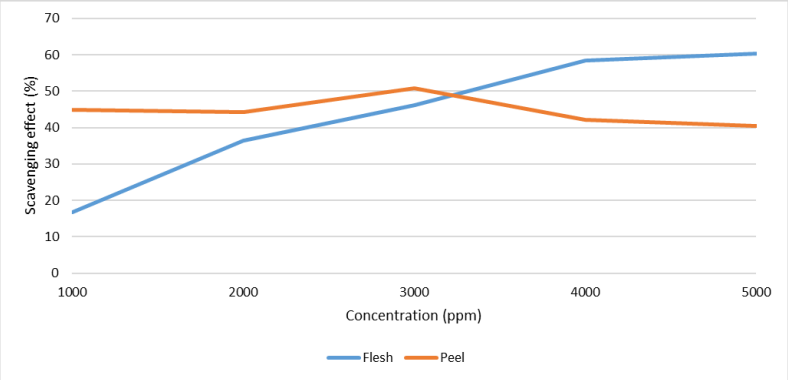

Figure 1: The scavenging activity (%) of Red pitaya peel and flesh extract.

The scavenging power of fruit extract is also due to the phenolic compound [4],[5]. Because of its stability and antioxidant properties, peel extract was chosen to be incorporated into the muffins formulations. The amount used was 200 mg per 1000 g of fat to match the amount of synthetic antioxidant (BHA) used in the formulation.

3.3 Oxidative stability of muffin incorporated with pitaya peel extract

The unsaturated fatty acid that reacts with oxygen forms hydroperoxide and chain reaction. Although not responsible for off-flavours and odours, the hydroperoxide composes further to yield the odorous aldehydes, ketones, acids, and alcohols which cause rancidity. The quantity of hydro-peroxide in a fat sample can be measured and it is called the peroxide value (PV). PV is usually used as an indicator to determine the freshness of food. As muffin contained high amount of fat, it is very susceptible to oxidative rancidity because it is usually kept at room temperature and are exposed to oxygen and light. Muffin usually has the shelf life from three to five days.

Figure 2 showed the oil extracted from the control muffin started to became rancid significantly after day six. Meanwhile, muffins added with BHA and pitaya flesh extract shows similar trends which is delayed in rancidity activity up to eight days. Fat with the peroxide value under 20 mEq/kg were considered fresh [24].

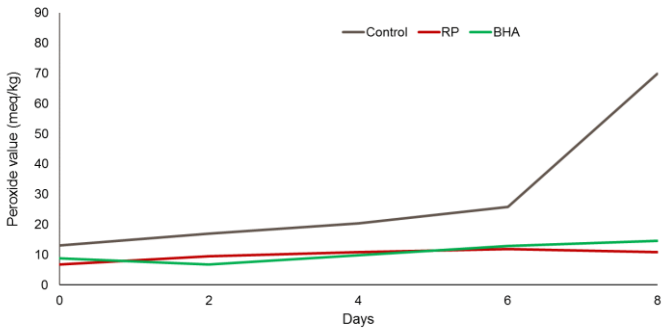

Figure 2: The peroxide value of control, added peel extract and BHA antioxidant muffin and tested in 8 days.

4. Conclusions

This study concluded that aqueous extract of red pitaya flesh and peel have high total phenolic content. Peel extracts showed high antioxidative activity in DPPH assay. Both natural and

synthetic additives conferred similar antioxidant activity to the muffins. The peroxide value of muffin with the peel extract is significantly lower than the control muffin due to the high antioxidant properties of the phenolic content in the extract. Hence pitaya peel extract has the potential as natural antioxidant that can extend the shelf life of baked food.

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Effect of Organic and Inorganic Fertilization on Soil Organic Matter, Carbon and Nitrogen Accumulation in a Newly Cultivated Farmland

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ABSTRACT
Good soil management is needed for improvement of soil fertility and maintaining optimum productivity of the crop production through the appropriate fertilizer application. However, excessive application of fertilizer may result in wastage or even pollution. Therefore, optimization of fertilization management is required to achieve sustainable crop production. An experiment was conducted to assess the effects of organic and inorganic fertilizer on the soil organic matter (SOM), carbon (C) and nitrogen (N) accumulation. The polyculture planting method was used in this study. Three types of plants - water spinach, okra and yard long bean were poly-cultured. The study consists of five treatments with three replicates each; control with no fertilizer (T1), organic fertilizer (T2), inorganic fertilizer (T3), organic-inorganic compound fertilizer (T4), organic fertilizer + inorganic fertilizer (T5). The highest organic matter content was found in soil treated with T5 (4.01%) whereas T4 (3.04%) had the lowest SOM. The soil organic matters level for T1, T2 and T3 were 3.28%, 3.34% and 3.51%, respectively. However, no significant differences between the treatments. The results suggested the soil applied with mixture of organic and inorganic fertilizer has higher soil C (3.70%) as compared to the separate application of the organic (3.34%) and inorganic fertilizer (3.02%). The soil treated with combination of organic and inorganic fertilizer showed improvement in terms of SOM and C content. In conclusion, the integration of the organic and inorganic into fertilization regime has benefit for the soil organic matter and carbon accumulation.

Keywords: Inorganic fertilizer, organic fertilizer, polyculture, carbon, nitrogen

1. Introduction

Inorganic fertilizer is the most commonly used fertilizer in the agricultural sector because it is cheap and high nutrient content. Besides, nutrient from inorganic fertilizer is readily available when applied on the soil and can be absorbed easily by plants. Intercropping and companion plant are common polyculture technique that has been used in agriculture sector. Polyculture technique involved planting more than one types of plant at the same time at same land area [1]. Soil organic matter is a key for the soil to maintain the good physical properties and increase the total nutrient in the agriculture land. The decomposition of the organic matter influenced by the rainfall rate, land usage and the biological activity [2]. Carbon and nitrogen are some of the most important nutrient that can be used as an indicator for the nutrient uptake in plants. It also associated to the soil water holding capacity and the soil erosion [3]. However, over use of the fertilizer can lead to the toxication on the soil [4]. An experiment was conducted to assess the effects of organic and inorganic fertilizer on the soil organic matter (SOM), carbon (C) and nitrogen (N) accumulation.

2. Materials and methods

The experiment was conducted between Jun until October 2018. The location of the study was at ICA UTM Pagoh. ICA UTM Pagoh is situated in area with an average rainfall 2125 mm and temperature 27 ^{o}C. Polyculture systems was applied in this experiment by using three types of plants; water spinach, yardlong bean and okra. There were five treatments: no fertilizer (control) (T1), organic fertilizer (T2), inorganic fertilizer (T3), organic inorganic compound fertilizer (T4), organic + inorganic fertilizer (T5). The fertilization was applied for five time during the experiments. Table 1 showed the fertilizer application rate at 1st, 2nd, 6th, 10th and 14th week after planting. The fertilization regime for all treatments were standardized to supply 24 g/m^2 of N, P and K to crop.

Table 1: The application rate of fertilizer

Treatments (g/row)						
Weeks	**T1**	**T2**	**T3**	**T4**	**T5**	
					Organic	**Inorganic**
1	0	533	100	267	267	50
2	0	667	125	333	333	63
6	0	667	125	333	333	63
10	0	667	125	333	333	63
14	0	667	125	333	333	63

The collected soils samples were analyzed for the soil organic matter (SOM), carbon (C) and nitrogen (N) content. The organic matter was estimated by heating samples in furnace at 440˚C for 24 hours.The analysis of the C and N was done by the CHNS analyzer (BS EN ISO 16948:2015). The results were analyzed using Statistical Packages for Social Sciences (SPSS). One-way of variance (ANOVA) and Duncan's multiple comparisons were performed to determine the differences among the fertilizer treatments in terms of the soil properties. Means comparisons between treatments were performed at $p<0.05$.

3. Result & Discussion

3.1 Effect of different types of fertilizer treatments on soil carbon and nitrogen

It was observed that the application of T2 resulted higher C (3.34 g/mg) as compared to the T1 (3.23 g/mg) and T3 (3.02 g/mg). T3 has the lowest C as compared with all other treatments. Application of the organic and inorganic fertilizer combination (T5) significantly increased C by 34% over the sole application of the organic (T2) and the inorganic fertilizer (T3). The sole application of the organic fertilizer (T2) increased C by 21% as compared to the inorganic fertilizer (T3) which was only 9%. On the other hand, the organic and inorganic compound fertilizer (T4) increased SOC by 24% which is slightly higher than the T2. The use of chemical fertilizer contributed to least amount of C increment which was about 9%. No significant difference was observed for all treatment over the C and N concentration. Soil treated with T1, T2, T3, T4 and T5 showed an increase of 17%, 21%, 9%, 24% and 34% of SOC, respectively. It is observed the soil C was higher in soils that received organic fertilizer alone or in combination with inorganic fertilizers than control (no fertilizer or manure) and inorganic fertilizer treated soil. This is supported by Gilani & Bahmanyar [5] and Abebe & Deressa [6] who reported that the application of organic fertilizer improved the carbon content in soil as compared to the inorganic fertilizer. These results are consistent with the previous research done by Šimon & Czakó [7] found that the combination of organic and inorganic fertilizer increased the soil C levels. On the other hand, T1, T2, T3, T4 and T5 resulted increased of soil N stock of 47%, 61%, 64%, 55% and 97%. However, too much of N stock in soil has negative

impact to environment because N is highly mobile which can be easily leached to the underground water and causes water pollution [8].

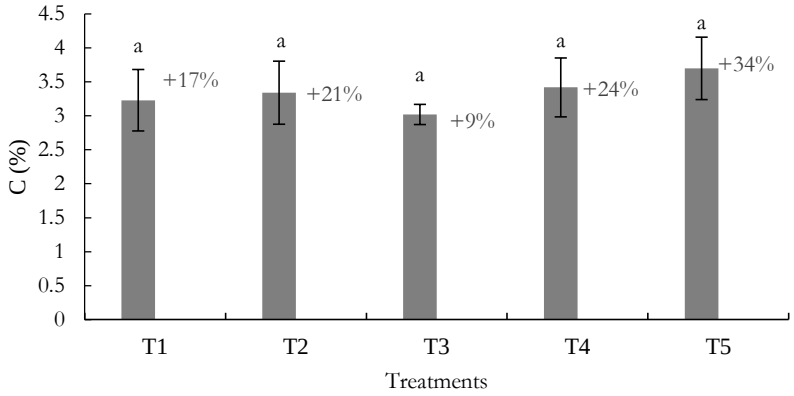

Figure 1: Effects of treatments on soil carbon. T1: No fertilizer, T2: Organic fertilizer, T3: Inorganic fertilizer, T4: Organic inorganic compound fertilizer, T5: Organic + inorganic fertilizer. Means for treatment followed by the same letter was not significantly different (Duncan, P≤0.05).

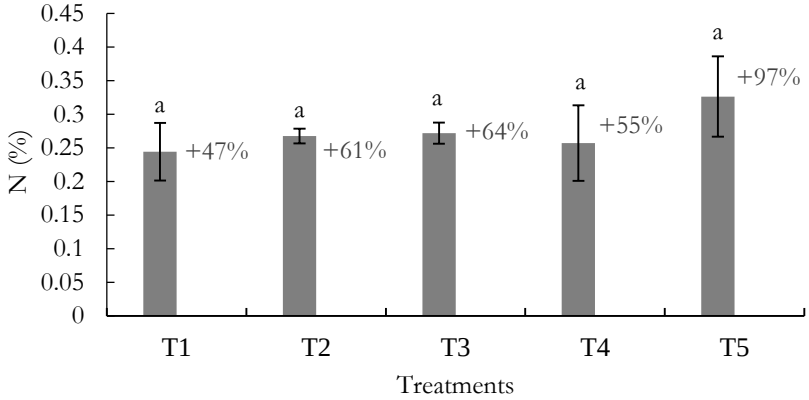

Figure 2: Effects of treatments on soil nitrogen. T1: No fertilizer, T2: Organic fertilizer, T3: Inorganic fertilizer, T4: Organic inorganic compound fertilizer, T5: Organic + inorganic fertilizer. Means for treatment followed by the same letter was not significantly different (Duncan, P≤0.05).

3.2 Effect of different types of fertilizer treatments on soil organic matter

The soil treated with T1, T2, T3 and T5 reported increased of SOM between 3% to 25%, in contrast the SOM for T4 decreased by 5%. The average organic matter for all treatment ranged from 3% to 4%. The combined application of organic and inorganic fertilizer able to increase the SOM content significantly by 25% as compared to T1. The SOM content in the soil treated with organic (T2) and inorganic fertilizer (T3) showed a positive effect in which the SOM increased by 4% and 10%, respectively. Overall, the treatment of T2, T3, and T5 were significantly higher than the control treatment (T1). The increased in SOM might contributed by the low microbial decomposition of organic matter. A study suggested N fertilization decreases SOM decomposition [9]. This was also supported by one study in paddy soils who also reported intensive fertilization reduces SOM decomposition due to increase microbial turnover, which might positively affect C sequestration [10]. The decreased of SOM decomposition might increases the soil C sequestration efficiency because of higher percentage of undecomposed organic residues. However, application of T4 showed a loss of SOM content

with a decreased of 5%. This can be due to the rapid mineralization or soil erosion and nutrient leaching which reduced the organic matter content [11],[12].

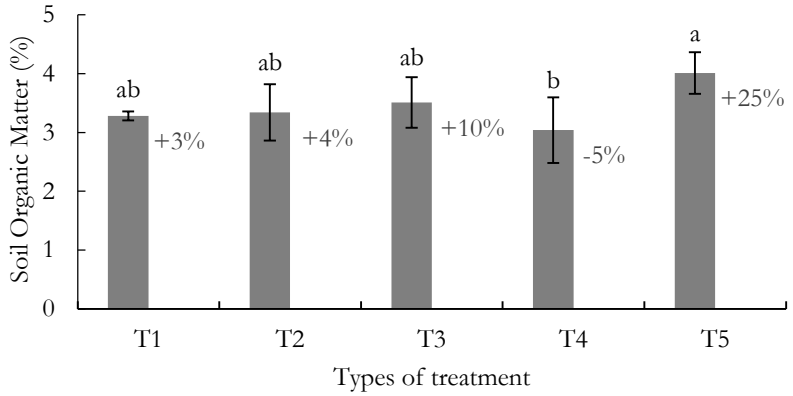

Figure 3: Effects of treatments on soil organic matter. T1: No fertilizer, T2: Organic fertilizer, T3: Inorganic fertilizer, T4: Organic inorganic compound fertilizer, T5: Organic + inorganic fertilizer. Means for treatment followed by the same letter was not significantly different (Duncan, $P \leq 0.05$).

4. Conclusion

The soil treated with combination of organic and inorganic fertilizer showed improvement in terms of soil organic matter and carbon content. In conclusion, the integration of the organic and inorganic into fertilization regime has the benefit of enhancing soil organic matter and carbon accumulation.

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Carbon and Nitrogen Accumulation in *Abelmoschus esculentus* and *Vigna unguiculata* subsp. *sesquipedalis* Treated with Different Fertilizer Regime Under Polyculture System

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ABSTRACT
The metabolism of cellular carbon (C) and nitrogen (N) is crucial in sustaining optimal growth and development for plant to perform routine cellular activities. In addition, soil C:N ratio is critical indicator for soil fertility and decomposition rate. Yield and quality of crops including the carbon and nitrogen accumulation in plant could be improved through proper soil fertility management. In this study, *Abelmoschus esculentus,* and *Vigna unguiculata* subsp. *sesquipedalis* were grown under polyculture system and treated with five different types of fertilizer regime (T1: without fertilizer, T2: organic fertilizer, T3: inorganic fertilizer, T4: biofertilizer and T5: organic + inorganic fertilizer). The results suggested under polyculture condition, no significant difference ($p>0.05$) of okra and yardlong bean on carbon content (C%) for all treatments. Okra showed significant difference ($p<0.05$) on nitrogen content (N%) when treated with inorganic fertilizer (T3: 2.83±0.24%) and organic fertilizer mix inorganic fertilizer (T5: 2.90±0.25%). Yardlong bean showed no significant difference ($p>0.05$) on nitrogen content (N%) for all treatments. For C:N ratio, okra treated with biofertilizer (T4: 16.73±1.29%) resulted the highest significant difference ($p<0.05$). In conclusion, no significant effect of C accumulation rate in plant resulted from the variation in fertilizer regime. In contrast, the variation of fertilizer types has noticeable effect on N accumulation on plants.

Keywords: Organic fertilizer, inorganic fertilizer, carbon, nitrogen, polyculture crop

1. Introduction
At the beginning of second half of the twentieth century, development of conventional agriculture escalates rapidly to intensify the crop production. Conventional agriculture practices in monoculture crop production rises proportionally with agrochemical industrial products, uniform high-yield hybrid crops and modern technology for land cultivation [1]. Conventional agriculture with single plant using intensive chemical fertilizers might lead to unstable agroecosystem primarily, nitrate and phosphate in synthetic fertilizer often pollute water and soil [2]. Moreover, frequent usage of pesticides might enter the food chain which affects the health of higher consumer trophic level. Sustainable agriculture has been developed to minimize the adverse effects of conventional agriculture. Approach of sustainable agriculture including the connection of environment quality, economic profitability and social equity [3]. Integration of sustainable agriculture with conventional agriculture minimized the negative impacts of conventional agriculture by combining biological, technical and chemical measures. Organic agriculture has been introduced as one of sustainable agriculture practices that crops are plant without synthetic fertilizer and pesticides input. Organic agriculture focussing on

maintenance and improvement of soil fertility and biodiversity. The crop production was designed in a way to create complete nutrient cycle. In contrast with one-crop system, intercropping involves at least two crop species grown simultaneously in the same field and grown in alternate rows or wide strips. In commercial industry, one-crop system predominant, yet intercropping continuous to be important today [4]. Example of intercropping include mix crop of leafy vegetable, fruit vegetable and legumes. Intercropping enable a better utilization of nutrient and water from the soil by growing different shallow root and deep-rooted crop together. In addition, intercropping system can contribute to the suppression of weed growth and to reduce pest incident. Cellular carbon (C) and nitrogen (N) is strongly related and are crucial coordinators to sustain optimal growth and development for plant to perform the routine and cellular activities. C:N ratio is also critical for the ecosystem response to produce a fertile soil and as indicator of decomposition rate. Yield and quality of polyculture crops could be achieved through proper soil fertility management which including the carbon and nitrogen content of plant. A lot of farmers reluctant to incorporate polyculture system into their existing farm due to the potential risk nutrient competition between the crops. Thus, this study was developed to determine the carbon and nitrogen accumulation in polyculture cropping system with different fertilizer regime.

2. Materials and methods

Field experiment was conducted at research farm (2°09'22'N, 102°44'00'E) of Universiti Teknologi Malaysia, Pagoh, Muar, Johor for one crop cycle with the intercropping of okra (*Abelmoschus esculentus*), and yardlong bean (*Vigna unguiculata* subsp. *Sesquipedalis*) for three months. The weather of the site study was classified as tropical. The area of experiment was oil palm reclamation with sandy loam soil. Seeds of plants were sown in seed trays (5 cm-diameter) containing peatmoss. After five days, the plants seedlings were transplanted to 15 rows of planting beds (3 × 6 ft). The seedlings are polycultured alternately in the same bed. The plants were treated with three types of commercial fertilizer: organic certified fertilizer (N:P:K, 3:3:3), inorganic fertilizer (N:P:K, 16:16:16), and biofertilizer (N: P: K, 6:6:6). (T1: control treatment without fertilizer input; T2: organic fertilizer, T3: inorganic fertilizer, T4: biofertilizer, T5: organic + inorganic fertilizer). A total of 24 g m^{-2} of nitrogen, phosphorus and potassium were applied for each treatment. Fertilizer were applied once for two weeks after planting. Experiment was in completely randomized block design with three replications per treatments. Plants were watered twice a day with 20 L water/ bed**.** The fruits were harvested once matured.

Soil sample (0-30 cm below ground) were collected for each treatment. Gravels and roots mat near the surface were removed before the sample was collected. The composite soil samples (n=5) were collected by using 2.5 cm-diameter auger. Soil samples of the same planting beds were homogenized prior to analysis. The carbon and nitrogen content of dried plant and soil samples were analysed by using Elementor Vario Micro Cube CHNS analyser (BS EN ISO 16948:2015). Variance analysis of data (one-way ANOVA) were performed using Statistical Packages for Social Sciences (SPSS) 16.0 program. Mean were analysed according to least significant different (LSD) test at $p<0.05$.

3. Results

Table 1 shows the carbon content (C%), nitrogen content (N%) and C:N ratio of okra and yardlong bean in polyculture condition with different fertilizer input.

Table 1: Carbon content, nitrogen content and C:N ratio of okra and yardlong bean in polyculture condition with different fertilizer input.

Plant Type	Treatment	C%	N%	C:N
Okra	1	38.31±0.16	2.33±0.22	16.52±1.67
	2	37.96±0.43	2.46±0.27	15.59±1.78
	3	38.67±0.56	2.83±0.24*	13.71±1.11
	4	38.96±1.02	2.34±0.25	16.73±1.29*
	5	38.51±1.07	2.90±0.25*	13.36±1.57
Yardlong bean	1	40.19±0.53	4.05±0.40	9.97±0.93
	2	40.65±0.08	4.39±0.34	9.29±0.69
	3	40.94 ±0.40	4.51±0.18	9.09±0.32
	4	41.08±0.14	4.42±0.54	9.38±1.20
	5	40.61±0.75	4.74±0.46	8.62±0.92

* Indicates statistical significant at $p<0.05$ (LDS)

The results suggested under polyculture condition, no significant difference ($p>0.05$) between okra and yardlong bean on carbon content (C%) for all treatments. The overall reading of carbon content (C%) in yardlong bean was higher than okra for all treatments. Under polyculture condition okra showed high significant difference ($p<0.05$) on nitrogen content (N%) when treated with inorganic fertilizer (T3: 2.83±0.24%) and organic fertilizer mix inorganic fertilizer (T5:2.90±0.25%). Yardlong bean showed no significant difference ($p>0.05$) on nitrogen content (N%) for all treatments. Overall, nitrogen content (N%) of yardlong bean was higher than okra for all treatments. For C:N ratio, okra treated with biofertilizer (T4: 16.73±1.29%) resulted the highest significant difference ($p<0.05$) as compared to other treatments. Meanwhile, yardlong bean showed no significant difference ($p>0.05$) for all treatments. Overall results showed C:N ratio of okra was higher than yardlong bean.

Table 2 shows the carbon content (C%), nitrogen content (N%) and C:N ratio of soil before and after treated with different types of fertilizer input.

Table 2: Carbon content, nitrogen content and C:N ratio of soil with different types of fertilizer input

Treatment	C (%)	N (%)	C:N (%)
Initial	3.03±0.30	0.19±0.03	16.24±0.77
1	3.18±0.44	0.23±0.02	13.71±1.71
2	3.51±0.55	0.26±0.01	13.60±2.50
3	3.23±0.33	0.27±0.04	12.10±0.70
4	3.56±0.63	0.25±0.03	14.68±1.99
5	3.75±0.15	0.32±0.03*	11.98±1.19

* Indicates statistical significant at $p<0.05$ (LDS)

Initial reading of soil carbon content (C%), nitrogen content (N%) and C:N ratio were taken before the soil treatment. Initial reading of carbon content (C%) and nitrogen content (N%) showed lower value as compared to after fertilizer treatments. There was no significant difference ($p>0.05$) of carbon content (C%) between all treatments. Meanwhile, nitrogen content (N%) of soil has significant higher ($p<0.05$) when treated with combination of organic fertilizer and inorganic fertilizer (T5: 0.32±0.03%).

4. Discussion

In this study, polycultures plant contained carbon content at the range of 37.96±0.43% (T2, okra) to 41.08±0.14% (T4, yardlong bean). Similar study by Juan et al, revealed carbon content were similar across various vegetation from~36% to~42% [5]. In polyculture system, both plant have no competition in absorbing nitrogen from different types fertilizer. Every plant has different metabolism toward N uptake. A study conducted by Xu et al suggested that the nitrogen content of plant tissue from~2.2 % to~2.8% [6]. Similarly, the nitrogen content of okra plants in this study were reported between 2.34±0.22% (T1) to 2.90±0.25% (T5). In contrast, content of nitrogen in yardlong bean were higher which ranged from 4.05±0.40% (T1) to 4.74±0.46% (T5). Yardlong bean, a leguminous family known as nitrogen-fixing plant that has higher N uptake. Lower C:N ratio of plant indicate higher decomposition rate. Plant with lower C:N ratio would be much easier to be decomposed by soil microorganism. Cellular C and N must be tightly coordinated. This coordination occurs at different level. Plant need carbon from the atmospheric carbon dioxide to produce sucrose. Assimilation of sucrose synthesized glutamate which incorporated with cellular N to produce amino acid that essential for almost cellular activities [7]. When plant was deficient in N, photosynthetic output was negatively affected [8].

5. Conclusions

In conclusion, plant in polyculture system have similar carbon content but variable in nitrogen content. Variation in fertilizer regime has no effect on nitrogen intake of plant when applied at same amount of nitrogen.

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Nutritional Requirements of *Azotobacter chroococcum* for Growth and Their Use as Biofertilizer

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ABSTRACT
Azotobacters sp have generated a good deal of interest in the agritechnology because they can fix nitrogen aerobically. *Azotobacter sp* play a vital role in every ecosystem, working to make nitrogen available to all organisms. In this study, *Azotobacter chroococcum* is known as genus that synthesizes of phytohormones are the primary substances regulating the enhanced growth. It stimulates rhizospheric microbes, protects the plants from microbial pathogens, and improves nutrient uptake and ultimately improvement of biological nitrogen fixation. In order to guarantee the high effectiveness of inoculants and microbiological fertilizers it is necessary to understand the growth requirement of *A.chroococcum* for high cell mass cultivation for application of this strains as additive in biofertilizer. Therefore, the aim of this study was to screen nutrition factors that effects high cell mass of *A.chroococcum* with low EPS production in a semi-defined medium. The one factor at one time (OFAT) tests were employed to obtain the favorable conditions for high cell mass yield in a semi-defined medium and found that glucose as the main carbon sources for the maximum cell mass production of 1.35 g/L with very low exopolysaccharide (EPS) yield. Furthermore, growth of *A.chroococcum* when yeast extract at 5 g/L supplemented in the medium cultivation medium increased up to 60% of cell mass when compared with growth in tri-ammonium citrate medium. In this study, no significant influences of KH_2PO_4 and Mg^{2+} as source of minerals to the cell growth and EPS production. The most significant factors that influences the growth and EPS production of *A.chroococcum* were CN ratio of 6 after 20 hours cultivation.

Keywords: *Azotobacter chroococcum*, nitrogen fixing, biofertilizer, optimization, cell mass

1. Introduction
Azotobacter sp. is a group of beneficial bacteria play an important role in plant growth by colonizing in a plant rhizosphere to fix the nitrogen. Biological fixing nitrogen is an important microbial activity to enhance the plant development, produces plant growth regulators and increase mineral phosphate solubility [1]. They grow in aerobic condition and known as diazotroph microorganism which is a free-living nitrogen-fixing that can convert atmospheric nitrogen into ammonia in the presence of atmospheric oxygen. Application of *Azotobacte*r sp. biomass in biofertilizer has potential as a nitrogenous fertilizer to increase plant growth and crop yield [2]. In this study, *Azotobacter chroococcum* was used to produce a high cell mass by using the OFAT (one factor at one time) media to guarantee the high effectiveness of inoculants for application of microbial in fertilizers. Therefore, the aims of this study to screen nutrition factors that effects high biomass at low cost of *A.chroococcum* with low EPS production in semi-defined medium.

2. Materials and methods

2.1 Microorganism

A.chroococcum ICAB003 was used throughout this study and was activated in ATCC 14 broth medium then incubated for 24 hours at 30 °C and 180 rpm.

2.2 Medium screening

Strain was grown in eight different broth media to screen the most suitable medium composition. The carbon sources were autoclaved separately and added to medium before the inoculation.

Table 1: Eight different media were used in this study for primary evaluation for primary selection of the highly productive medium

Label	Composition	References
M1	Glucose, 20.0; KH_2PO_4, 2.4; $MgSO_4.7H_2O$ 0.6; $FeSO_4.7H_2O$, 0.12; $Na_2MoO_4.2H_2O$ 0.03; $CaCl_2$ (anhydrous), 0.45	[3]
M2	Glucose 20.0; Yeast extract, 0.3; $(NH_4)_2SO_4$ 0.6; Na_2HPO_4, 2.0; $MgSO_4.7H_2O$, 0.3	[4]
M3	Sucrose, 20.0; Yeast extract, 3.0; KH_2PO_4, 0.66; K_2HPO_4, 0.16; MOPS, 1.42; $CaSO_4$, 0.05; NaCl, 0.2; $MgSO_4.7H_2O$, 0.2; $Na_2MoO_4.2H_2O$, 0.0029; $FeSO_4.7H2O$, 0.027	[5]
M4	Glucose, 10.0; $MgSO_4.7H_2O$, 0.4; $FeSO_4.7H_2O$, 0.012; $Na_2MoO_4.2H_2O$, 0.01; K_2HPO_4, 2.0; NaCl, 0.4; $(NH_4)_2SO_4$, 0.1	[6]
M5	Glucose, 30.0; Yeast Extract, 5.0; triammonium citrate, 0.052; $(NH_4)_2SO_4$, 1.9; KH_2PO_4, 0.8; K_2HPO_4, 0.2, $MgSO_4.7H_2O$, 0.2; $CaSO_4.2H_2O$, 0.1; $FeSO_4.7H_2O$, 0.005; $Na_2MoO_4.2H_2O$, 0.0002	[7]
M6	Sucrose, 35.0; Yeast extract, 5.0; KH_2PO_4, 2.45; K_2HPO_4, 3.13; $CaCl_2$, 0.005; Trace elements, 1 ml **Trace elements (in g/L):** $MgSO_4.7H_2O$, 71.2; $ZnSO_4.7H_2O$, 0.44; $MnSO_4.4H_2O$, 0.812; $CuSO_4$, 0.05, $Na_2Mo_4.2H_2O$, 0.252; $FeSO_4.7H_2O$, 4.98; H_3BO_3, 1.02	[8]
M7	Sucrose, 20.0; ammonium acetate, 1.0; KH_2PO_4, 0.16; K_2HPO_4, 0.66; $CaSO_4$, 0.05; NaCl, 0.2; $MgSO_4.7H_2O$, 0.2; $Na_2MoO_4.2H_2O$, 0.0029; $FeSO_4$, 0.027	[9]
M8	Sucrose, 20.0; Yeast extract, 3.0; KH_2PO_4, 0.16; K_2HPO_4, 0.66; $CaSO_4$, 0.05; NaCl, 0.2; $MgSO_4.7H_2O$, 0.2; $Na_2MoO_4.2H_2O$, 0.0029; $FeSO_4$, 0.027	[10]

2.3 Medium optimization by OFAT

The first stage of the optimization process was carried out using the one factor at one time (OFAT) medium to obtain the high yield of cell biomass. after 20 hours incubation at 30 °C in the incubator shaker, the culture broth was centrifuged at 8000 rpm for 20 minutes to precipitate the cells [11].

2.4 Analysis

The supernatant was collected and used for EPS analysis by precipitation with cold ethanol (95%) at ratio of 1:3. The cells were dried at 60 °C in oven. The data were analyzed by using Origin 1.6 and the results were given as mean ± SD.

3. Results and Discussion

3.1 Medium screening

Medium optimization (OFAT) was used to optimize four different components of M5 which showing high biomass production with low EPS production (Figure 1). The main components of semi-defined medium namely carbon sources, nitrogen sources, yeast extract and phosphate were optimized in this study.

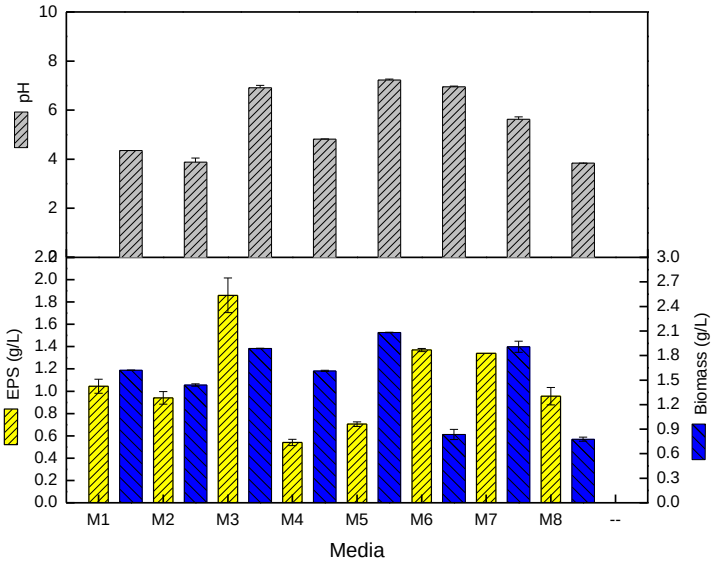

Figure 1: Cell growth and EPS production by *A.chroococcum ICAB003* in different industrial media.

3.2 Medium optimization by OFAT

Medium optimization (OFAT) was used to optimize four different components namely carbon sources, nitrogen sources, yeast extract and phosphate. The growth of *A.chroococcum* was maximum by using glucose as the main carbon sources with very low EPS yield (Figure 2). 60% increase of cell biomass when yeast extract supplemented in medium cultivation (Figure 3). CN ratio 6 is the most significant factors that influence the growth and EPS production after 20 hours cultivation (Figure 4).

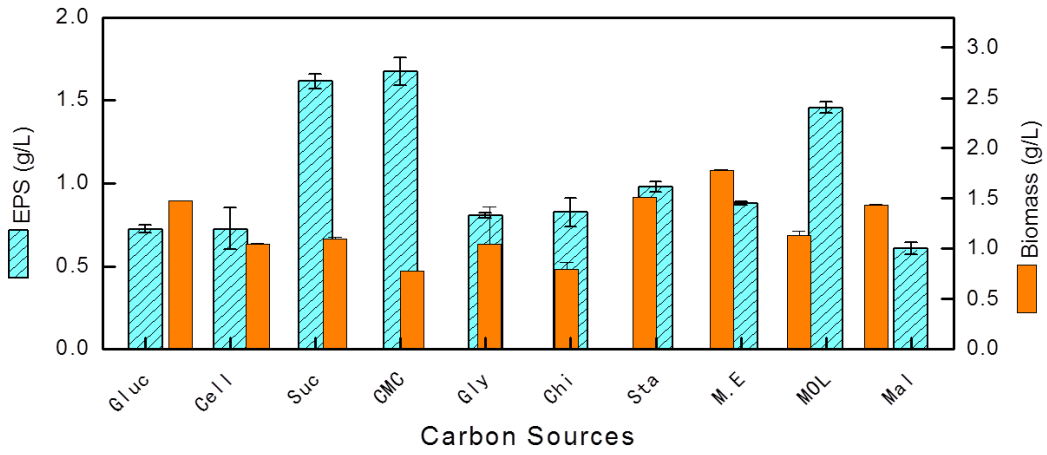

Figure 2: Effect of different types of carbon sources on biomass and EPS production by *A.chroococcum* ICAB003 during cultivation in shake flask culture

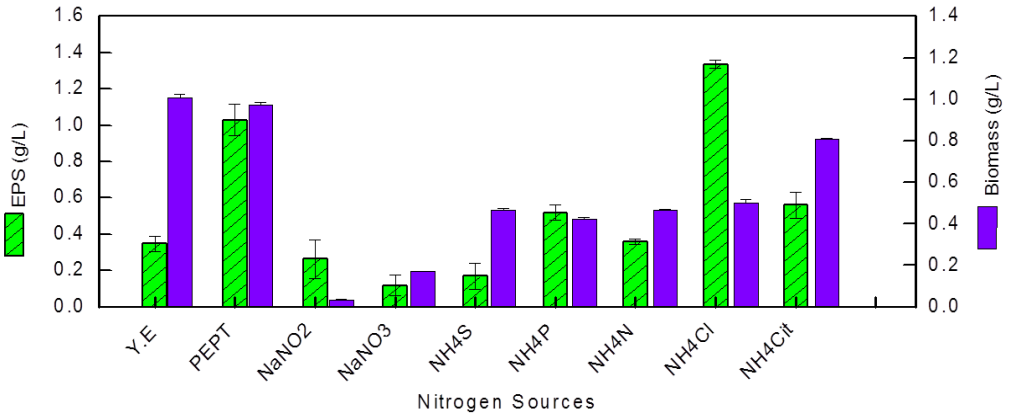

Figure 3: Effect of different types of organic and inorganic nitrogen sources on biomass and EPS production *A.chroococcum* ICAB003 during cultivation in shake flask culture

Figure 3 showed the growth of *A.chroococcum* increased up to 60% of cell biomass when yeast extract at 5 g/L supplemented in the medium cultivation compared to the growth in tri-ammonium citrate medium. Even though *A.chroococcum* was able to fix atmospheric nitrogen by adding nitrogen from another source will give positive effect on biomass production [12]-[13].

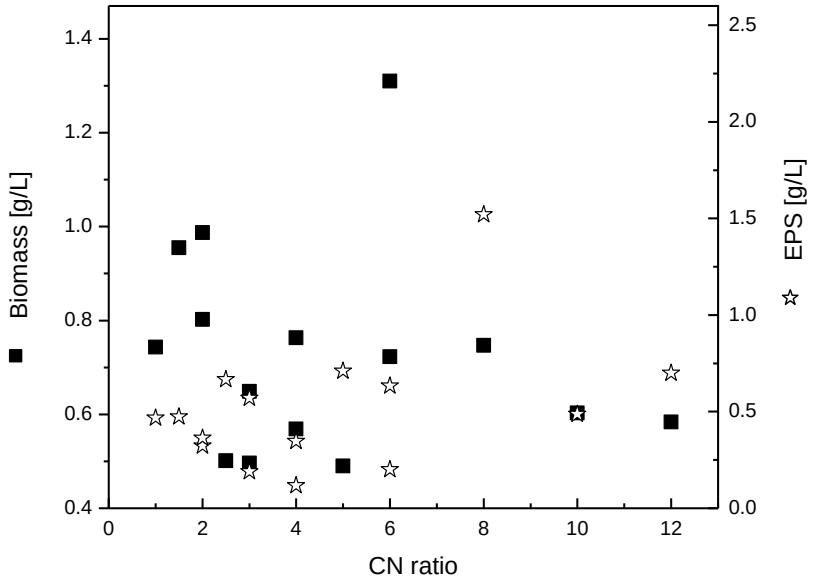

Figure 4: Effect of CN ratio on biomass and EPS production *A.chroococcum ICAB003* during cultivation in shake flask culture

Phosphate is an essential element for biofertilizer to deal with dual function in improving the growth and yield of crop production [14],[15]. Based on the obtained results, KH_2PO_4 no significant differences when increase KH_2PO_4 concentration of on biomass of *A. chroococcum*. It gave a big impact on EPS production (Figure 5). The specific growth rate of *A.chroococcum* in the optimized medium was 0.04 hr^{-1} with maximum biomass production 2.1 g/L after more than 30 hours cultivation with the maximum yield coeeficient of EPS about 0.53 g/g (Figure 6).

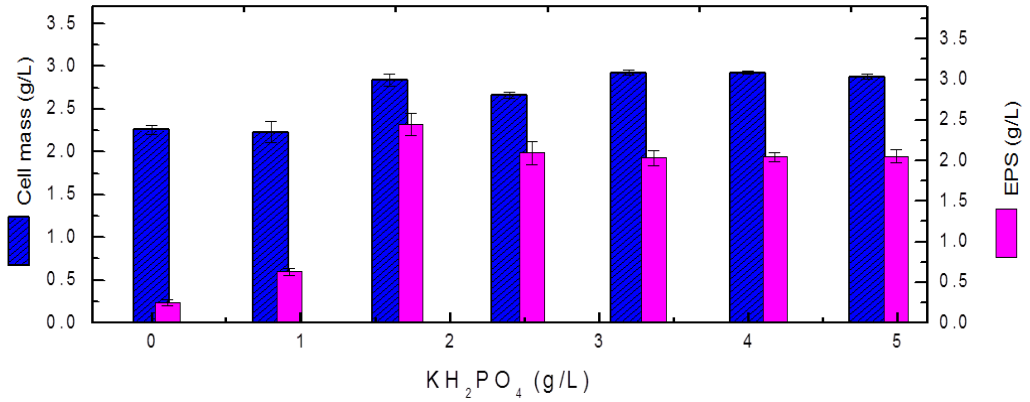

Figure 5: Effect of KH_2PO_4 on biomass and EPS production *A.chroococcum* ICAB003 during cultivation in shake flask culture

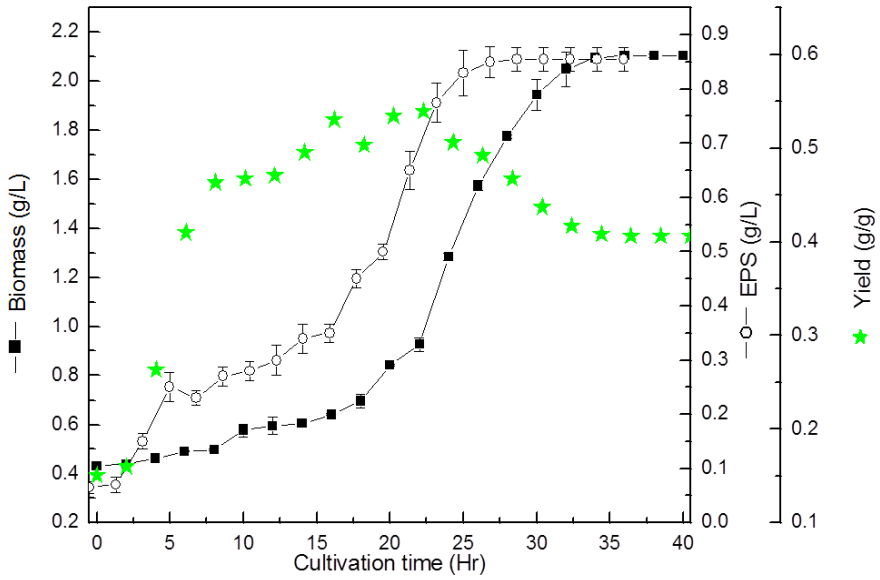

Figure 6: The growth kinetic of *A.chroococcum* ICAB003 showing changes in cell growth, EPS production and pH during cultivations in shake flask culture

4. Conclusion

An increasing of cell biomass productivity *A.chroococcum* from the OFAT medium when glucose as the main carbon sources and supplemented with yeast extract. The interaction effects between medium components may attribute to the effectiveness of OFAT medium in determining the optimum nutrient to obtain the high cell biomass of *A.chroococcum* ICAB003.

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The Response of Extracellular Soil Enzyme Activities under Different Treatment of Fertilizer in Polycropping System

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ABSTRACT
Polyculture cropping system has emerged as one of the high promising cropping system for better utilization of the land, enhanced the nutrients uptake and sustains yields at higher level. Integrating fertilizer not only enables the farming system to better adjust to the effects of climate change but also offers a chance for restoring soil fertility on a sustained basis. However, little is known about persistence influences of inorganic, organic fertilizer or combination both of them especially in on-field polyculture cropping system (e.g: okra-dwarf bean-water spinach (*kangkung*)). Soil enzymatic activities are considered as well-known soil indicator for investigation of soil nutrient cycling and soil fertility. Hence, this study is aimed to determine the influences of different treatment of fertilizer on physiochemical attributes and extracellular enzyme activities in polycropping system. The performance and interaction response between the polyculture cropping system, soil enzymatic activities and nutrients uptake of plant and soil that have give rise to soil fertility are studied. Highest enzymatic activities of dehydrogenase, arylsuphatase and catalase were achieved at 2.443 g/mg, 5.178 g/mg, 9.25 g/mg with the application of combined organic and inorganic fertilizer, respectively; whereas, the analyzed enzymatic activities shown significant inhibition effects during the used of inorganic fertilizer. Their respectively soil physiochemical characterization before and after the used of different fertilizers are also reported.

Keywords: Soil enzymatic activities, organic and inorganic, fertilizer, polyculture cropping system, soil physiochemical

1. Introduction
Polyculture cropping system is a basis practice of growing three or more mixed crops simultaneously in a field [1]. Many agricultural systems worldwide have been developed to productive high-input and specialized cropping systems with limited options for versatile rotations, perennials, and polycultures (mixed crops). Polycropping system are beneficial to each other creating a balance in the soil and environment concurrent use of multiple crops [2]. The excessive use of various types of fertilizer nowadays making the soil health deteriorate and the good strategy is to use polycropping planting system that will enhance the nutrient development on the soil [3]. Extensive fertilizer or soil tillage may reduce in polycultures system. Many studied reported that the application of chemicals fertilizer causes significant environmental impact, deteriorates the soil health, and leads to poor profitability in farm. Organic fertilizer differs from chemicals fertilizer in such a way of using natural resource to provide essential nutrient to the plants and soil. Fertilizer regime is an important agricultural practice to enhance the nutrition in plants resulting producing high yield of crop [4]. However, little is known about persistence response of inorganic, organic fertilizer or combination both

of them especially in on-field polyculture cropping system (e.g: okra-groundnut-water spinach (*kangkung*). Enzymatic activity basically reflects an essential part of soil functional diversity, which is controlled by genetic diversity of soil microorganisms, plants and soil animals in close relation to environmental effects and ecological interactions [5]. Hence, this study was aimed to determine the influences of different treatment of fertilizer on physiochemical attributes and extracellular enzyme activities in the polycropped with mixed crops system are investigated.

2. Material and Methods

2.1 Description of the study area

An open-field experiment was conducted at Universiti Teknologi Malaysia, Pagoh, Muar, Johor research farm which to study the response of chemical, organic or combination of both chemical and organic fertilizers on the physiochemical properties and enzymatic activities of soil in on-field polyculture cropping system (e.g: okra-groundnut-water spinach (kangkung). The fertilizer regime treatments were weighted and applied every two weeks. For each plot, seed were sown and were planting with uniform seedling while transplanted.

2.2 Soil physiochemical properties

Sample of soil were taken every 10 cm soil depth from the surface to 30 cm deep randomly from each of treatment plots using mini hand soil auger. Soil sample were collected before planting crops and three times after planting the mixture of crops from each plot. All samples of soil physico-chemical properties including soil pH, electrical conductivity (EC), moisture content, C/N ratio, macro and micro nutrient analysis namely nitrogen (N), phosphorus (P) and potassium (K) using different types of fertilizer were investigated. The measurement of soil physico-chemical followed the method by Hou et al [6]. Soil pH and electrical conductivity (EC) were determined by pH meter in a 1:2.5 and 1:5 soil: water suspension respectively, while the soil organic matter and soil moisture were determined by furnace.

2.3 Enzymatic activities

Enzyme activities in different fractions of the soil treatment including soil surface and root surface were studied caused by the polyculture cropping system. The dehydrogenase and arylsulphatase activities in soil were assayed following procedure presented by Sarathchandra & Perrott [7] and Sandrin et al [8]. Catalase activity was measured via potassium permanganate titration as 2 g soil were mixed with 5 ml 0.3% of H_2O_2 and 40 ml distilled water and then vibrated for 20 minutes. The mixture should be filtered immediately and added with 5 ml of 3N of H_2SO_4. After that, 25 ml filtrate was taken to titration by using 0.1 N $KMnO_4$ [9].

3. Results and Discussion

3.1 Soil physico-chemical properties

The physico-chemical characteristics of the soil in open-field were summarized in Table 1. The application of the fertilizers shown the decreased of the soil pH and increased of the soil EC. These results were agreed by the previous studies. Fertilizer had increased the electrical conductivity to the soil. For the organic matter shows that decreased of amount of organic matter when using of inorganic fertilizer. Significant increment of the total P, K, Mg and Ca at 1,564.7 mg/kg, 1,056.2 mg/kg, 41.8 mg/kg and 32.7 mg/kg were observed when application of both organic and chemical fertilizer, respectively.

Table 1: Effect of different treatment of fertilizer on some selected chemical properties of the soil in polycropping system

Group	Parameter							
	pH	**EC dS M^{-1}**	**Organic matter (%)**	**Total N (%) w/w**	**Total P (mg/kg)**	**Total K (mg/kg)**	**Mg (mg/kg)**	**Ca (mg/kg)**
Control	5.99	62.23	2.78	0.1	725.6	686.3	12.7	6.3
OF	5.66	100.63	3.07	0.1	916.2	558.4	65.6	68.2
CF	5.23	147.23	2.59	0.1	1431.6	627.1	9.5	7.8
OF+CF	5.42	127.03	3.37	0.0	1564.7	1056.2	41.8	32.7

OF stated for Organic fertilizer; CF stated for chemical fertilizer.

3.2 Soil enzymes activities

The response of analyzed soil enzymatic activities using different types of fertilizer is shows in Table 2. The soil catalase significantly higher at 2.13 g/mg in the soil samples with organic fertilizer (OF) treatment, while lowest concentration of catalase at 1.93 g/mg when applying chemical fertilizer (CF). The analyzed result indicated that combination of organic and chemical fertilizer significantly increased the dehydrogenase activity and arylsulphatase activity at 5.18 g/mg and 9.25 g/mg, respectively. The soil enzymatic activities of catalase, dehydrogenase and arylsulphatase were significant increased by 44.13%, 45.75% and 51.17% with the treatment of OF and CF, while shown significant inhibition effects during the used of chemical fertilizer.

Table 2: Soil enzymatic activities in polycropping system before treatment of different types of fertilizer

Treatment	Soil Enzymes Activities		
	Catalase (g/mg)	**Dehydrogenase (g/mg)**	**Arylsulphatase (g/mg)**
Control	2.13	3.83	7.07
OF	2.22	3.55	7.86
CF	1.93	2.12	4.17
OF + CF	2.41	5.18	9.25

OF stated for Organic fertilizer; CF stated for chemical fertilizer.

4. Conclusion

The treatment of combination organic and chemical fertilizers shows significant result to improve the soil quality from the aspect of physiochemical properties of soil (pH, EC, OM), enzymatic activities (catalase, arylsulphatase, dehydrogenase) in polyculture cropping system. Chemical, organic or combination of both fertilizer has its pros and cons in terms of nutrient supply, soil quality and crop growth. Develop the most suitable and optimum plant nutrient uptake that integrated uses of these soil enhancer is highly recommend.

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The Effects of Weeping willow (*Salix babylonica*) Grounds in Soil Mix as Growing Media for Choy Sum (*Brassica chinensis* var. *parachinensis*)

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ABSTRACT
Weeping willow (*Salix babylonica*) from the family Salicaceae, contains high concentration of natural indole-3-butyric acid (IBA). Liquid willow extract has a relatively short shelf life and less stable than powder form. This study was conducted to evaluate the growth response of choy sum (*Brassica chinensis* var. *parachinensis*) to weeping willow grounds in soil mix formula containing top soil, rice husk, coco peat, effective microorganisms and organic matter, and watering frequencies. A factorial design was performed with two factors in a randomized complete block design (RCBD) and replicated three times. The factors were (i) proportion of weeping willow grounds in soil mix (0%, 25%, 50% and 75%) and (ii) watering frequencies (once a day and once a week). Sampling were performed at 0, 7, 14, 21 and 28 days after transplanting (DAT) and analysed for total dry mass, leaf number, plant height and root length. There were significant differences in total dry mass, plant height and root length at 25% proportion in once a week watering frequency. Leaf number were not impacted negatively except at 50% and 75% proportions at once a week watering frequency. There was no significant difference in parameters at 25% proportion at once a day watering frequency except for total dry mass. These results were attributed to IBA which were favourable at low concentration but detrimental to root growth at high concentration in addition to watering frequencies. This study showed the potential of incorporating a proportion of weeping willow grounds to soil mix formula to improve crop yield.

Keywords: *Salix babylonica*, IBA, root growth, growing media, crop yield

1. Introduction
Plant cuttings are often subjected to rooting hormones before planting to encourage faster onset of new adventitious roots (AR), which forms from nonroot tissues. AR are also developed after seed germination and naturally induced by auxins, specifically indole-3-acetic acid (IAA). The more AR inductive auxin is indole-3-butyric acid (IBA), which is the precursor of IAA [1]. Most commercially available rooting hormones contain synthetic IBA which can be found in various forms of liquid, powder or gels. It was later found out that willows (Salix spp.) which belong to the family Salicaceae, contains high concentration of natural IBA at the growing tips of its branches. Additionally, willow extract also contains another auxin, salicylic acid (SA) which involved in plant natural defense against diseases [2]. Willow extract in the form of ready to use liquid has a relatively short shelf life and less stable than powder form. Moreover, too high concentration of IBA from inconsistent mix might inhibit the root length development and root number [3]. The objectives of this study were to evaluate the growth response of choy sum (*Brassica chinensis* var. *parachinensis*) to weeping willow (*Salix babylonica*) grounds

proportions in soil mix formula and the effects of watering frequencies on the vegetative parameters.

2. Materials and methods

2.1 Seedlings preparation
The seeds of Choy Sum were sown in 104 hole seeding trays containing only peat moss as growing media. The trays were covered for the first three days to promote germination before being exposed to sunlight for further germination. The seedlings were water twice daily (9 a.m. and 3 p.m.) to keep the peat moss from drying up.

2.2 Soil mix preparation
A soil mix containing top soil, rice husk, coco peat, effective microorganisms and organic matter were passed through 2 mm sieve before use and mixed well to ensure homogeneity.

2.3 Weeping willow grounds preparation
Young and flexible stems of weeping willow were cut from the much older stems. The leaves were then stripped from the stems. The bare stems were cut into short pieces and grounded in a blender. The resulted weeping willow grounds were stored in a sealed plastic bag before being used for the experiment the next day.

2.4 Experimental preparation
The soil mix and weeping willow grounds ratios were prepared based on volume. A control treatment was established with only soil mix, while another three treatments with varying proportions of weeping willow grounds (25%, 50% and 75% v/v) completed the experimental treatments. The second factor was watering frequencies, once a day or once a week. All watering was done at 9 a.m.

2.5 Transplanting
12 days old seedlings were transplanted from seeding trays into polyethylene bags. Each polyethylene bag was filled with 5 kg of soil mix treatments and contained four seedlings spaced evenly. The polyethylene bags were arranged in a randomized complete block design (RCBD) and replicated three times. N:P:K 15:15:15 were applied once at this time at a rate of 50 g per polyethylene bag.

2.6 Sampling and data analysis
Sampling were performed at 0, 7, 14, 21 and 28 days after transplanting (DAT) and analysed for total dry mass, leaf number, plant height and root length. Statistical data from the parameters observed were analysed using IBM SPSS Statistics version 22. Two-way analysis of variance (ANOVA) was performed to assess the effects of weeping willow proportion in soil mix and watering frequencies on the vegetative growth of choy sum. Treatment means were compared at 5% significance level with Tukey's HSD.

3. Results and Discussion
Significant interaction between weeping willow grounds proportions and watering frequencies were observed on total dry mass. Treatments at once a week watering frequency showed no significant interaction except on plant height.

3.1 Total dry mass

Total dry mass was significantly affected by different treatments at once a week watering frequency. As much as 30% (0.28 g) reduced total dry mass was observed compared to treatments at once a day watering frequency. This was due to the combined lower leaves and roots dry mass. The water stress condition was amplified by the addition of too high concentrations of IBA at 50% and 75% proportions. Total dry mass at 0% proportion was significantly higher than 25%, 50% and 75% proportions in once a day watering frequency at 7 and 14 DAT. This was due to the constant leaching of IBA into the soil mix even at 25% proportion that affected roots dry mass. Figure 1 shows the effects of weeping willow and watering frequencies on total dry mass.

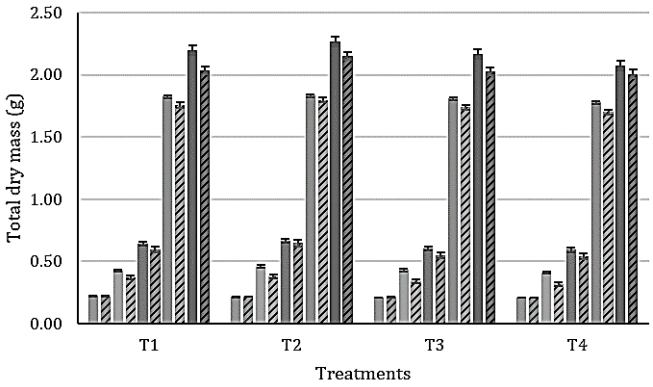

Figure 1: Effects of weeping willow proportions and watering frequencies on total dry mass. Every two adjacent columns represent values at 0, 7, 14, 21 and 28 DAT, respectively. Striped columns represent once a week watering frequency.

3.2 Leaf number

There was no significant difference in leaf number between treatments at once a day watering frequency. However, leaf number was significantly reduced due to leaves died off at 50% and 75% proportions at once a week watering frequency from an average of 15 to 14 and 13, respectively at 28 DAT. This showed the effect of water stress condition in addition to too high concentration of IBA on leaf number. The effects of weeping willow and watering frequencies on leaf number is presented in Figure 2.

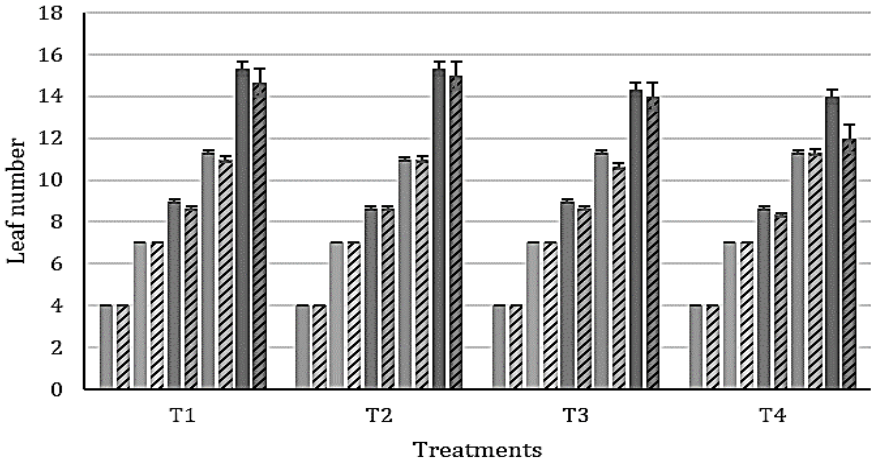

Figure 2: Effects of weeping willow proportions and watering frequencies on leaf number. Every two adjacent columns represent values at 0, 7, 14, 21 and 28 DAT, respectively. Striped columns represent once a week watering frequency.

3.3 Plant height

There was no significant difference in plant height between treatments at once a day watering frequency. However, there was a significant increase in plant height with 0.9 cm and 1.26 cm recorded at 25% proportion over 0% proportion at once a week watering frequency at 7 and 14 DAT, respectively. This observation was attributed to subsequent prolonged water stress condition which ultimately caused the reduction in plant height due to reduced plant cells [4]. The following Figure 3 shows the effects of weeping willow and watering frequencies on plant height.

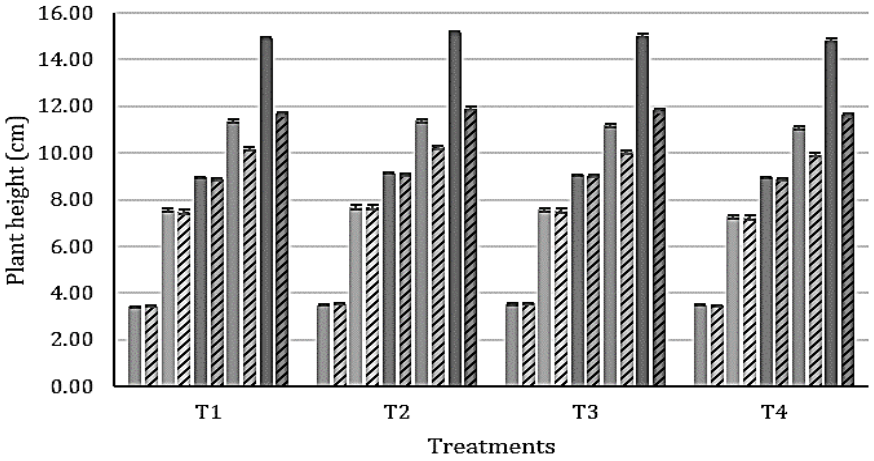

Figure 3: Effects of weeping willow proportions and watering frequencies on plant height. Every two adjacent columns represent values at 0, 7, 14, 21 and 28 DAT, respectively. Striped columns represent once a week watering frequency.

3.4 Root length

There was no significant difference in root length between treatments at once a day watering frequency. Significant reduction in root length was observed at 50% and 75% proportions at once a week watering frequency at 7, 14 and 21 DAT by between 0.26 cm to 1.1 cm. This was attributed to inhibitory effects of too high concentration of IBA coupled with water stress condition. Figure 4 summarized the effects of weeping willow and watering frequencies on root length.

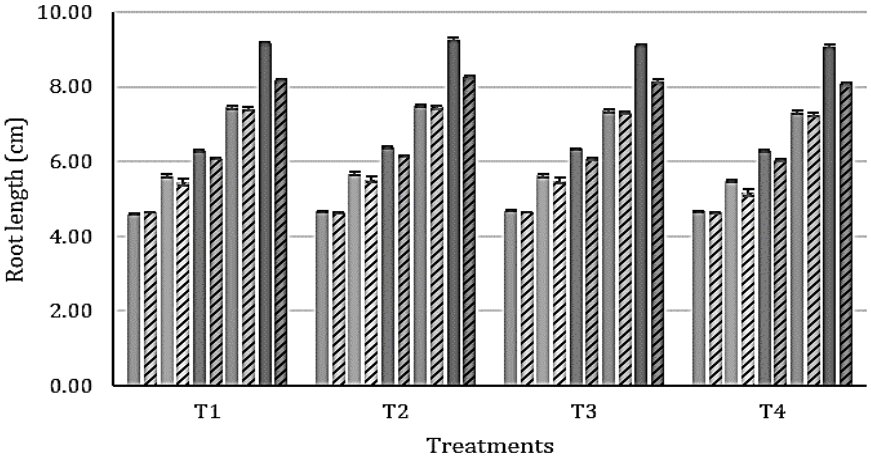

Figure 4: Effects of weeping willow proportions and watering frequencies on root length. Every two adjacent columns represent values at 0, 7, 14, 21 and 28 DAT, respectively. Striped columns represent once a week watering frequency.

4. Conclusions

This study showed that the incorporation of a proportion of weeping willow grounds to soil mix formula was advantageous in improving the ability of roots to adapt to various environmental cues which was crucial in ensuring maximum nutrient uptake, plant survival and crop yield. However, a careful calculation on the formula must be made to avoid detrimental effects on plant development that may lead to yield loss.

Acknowledgments

The author wishes to thank Kluang Municipal Council for providing the access to weeping willow trees needed for this study.

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Interaction of Rutin with Selected Polyphenol Affect Its Total Antioxidant Activity

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ABSTRACT
Antioxidant activity of dietary polyphenols is well established. However, little is known on polyphenol-polyphenol interaction and its effect on antioxidant activity. In this study, we investigated synergistic or antagonistic effects of rutin with other polyphenols in regards to antioxidant activity. To achieve this objective, we compared the antioxidant activity of rutin mixed resveratrol and quercetin. Antioxidant activity was measured using *in vitro* DPPH assay. Results indicated that combination of rutin and resveratrol promoted synergistic effects which reflect in increased in total antioxidant activity. Other combinations showed significant antagonistic effect. Findings from this study are useful in designing potent antioxidant agent. In conclusion, polyphenols tested in this study are rather interacting with each other and synergistically or antagonistically affect overall antioxidant capacity.

Keywords: Antioxidant, rutin, polyphenols, synergistic, antagonistic

1. Introduction
Polyphenols are secondary metabolites synthesised by plants that play important physiological function. These include protection against diseases, pathogenic microorganism, pest, ultraviolet radiation and oxidative stress [1]. Polyphenols also provide health benefits for human. In French paradox, polyphenols are thought to responsible in providing protection against cardiovascular disease [2]. A great number of research and review articles have shown health-benefits of polyphenols in preventing major chronic non-infectious diseases including cardiovascular disease, cancer, diabetes and obesity [3].

Antioxidant activity of polyphenols is well established. Most previous studies used single polyphenols to measure antioxidant with structure-activity relationship [4],[5]. Food contains mixture of polyphenols; therefore, it is difficult to predict total antioxidant activity of foods based on the sum of individual polyphenols. The antioxidant activity of particular foods is contributed by interaction between polyphenols [5]. The polyphenol-polyphenol interaction may display synergistic, antagonistic or additive which contributed to total antioxidant activity [6]. Rutin is a flavonol glycoside that can be found in variety of foods and vegetables. This compound exhibited several biological activities. Rutin showed direct free radical scavenging activity and increase intracellular antioxidant enzyme such as catalase and superoxide dismutase [7]. In the present study, we investigate interaction of rutin mixed with resveratrol and quercetin for total antioxidant activity with respect to synergistic or antagonistic effects.

2. Materials and methods

2.1 Chemicals

Reagent grade methanol (MeOH) and rutin were obtained from QRec. The DPPH (2,2-diphenyl-1-picrylhydrazyl) powder, resveratrol and quercetin were purchased from Sigma-Adrich. Water used in this study was obtained from Milli-Q Ultrapure Water Systems.

2.2 Antioxidant assay

Antioxidant activity was evaluated using DPPH assay in 96-well plate as previously reported [8]. Stock solution (100 ppm) of rutin, resveratrol and quercetin were prepared in methanol. Combinations were made and grouped in three experiment sets: i) individual polyphenol, ii) Combination of two (rutin+resveratrol and rutin+quercetin), iii) combination of three (rutin+resveratrol+quercetin). In order to maintain equal proportion, compounds in each combination were mixed on an equal 1 ml in the mixture. The final concentration for each compound is 0.725 ppm. A volume of 100 µl of individual or mixture compounds was mixed with 100 µl of DPPH solution. After 30 minutes of incubation, the absorbance was recorded at 517 nm. Antioxidant activity of compound was calculated using following equation:

$$\text{Antioxidant activity (\%)} = [(A_{control}-A_{sample})/A_{control}] \times 100$$

Where, $A_{control}$ is absorbance of DPPH solution alone, whereas A_{sample} is absorbance of DPPH solution in the presence of compound.

2.3 Determination of synergistic or antagonistic activity

Synergistic and antagonistic interaction was determined by comparing theoretical and experimental value [6]. Theoretical value is the sum of antioxidant activity of the individual compound in each combination [6]. The experimental value is the antioxidant activity of combination compound from experimental result [6]. Experimental value higher than theoretical value indicates synergistic interaction and vice versa [6].

2.4 Statistical analysis

Following assurance of Levene's test and Shapiro-Wilk test, data were analysed by one-way ANOVA using SPSS 23.

3. Results and Discussion

Antioxidant activity of selected compounds was tabulated in Table 1. Quercetin showed the highest antioxidant activity. There was no significant different between resveratrol and rutin in antioxidant activity. The antioxidant activity of these compounds also had been reported in previous studies. In accordance with previous study [9], rutin showed lower antioxidant activity than quercetin. The addition of glucoside group to quercetin reduces antioxidant activity.

Table 1: Antioxidant activity of rutin, resveratrol and quercetin

Compound	Experimental Value (%)
Rutin (Ru)	17.63 ± 1.78^{a}
Resveratrol (R)	18.69 ± 0.83^{a}
Quercetin (Q)	32.99 ± 1.65^{b}

Value is expressed as mean±standard deviation from quadruplicate data. Different superscript lower case letters in same column indicates significant different ($p<0.05$)

Table 2 showed antioxidant activity of rutin in combination with resveratrol and quercetin. Combination of rutin with resveratrol exhibits synergistic interaction, whereas other combination showed antagonistic interaction.

Table 2: Antioxidant activity of rutin mixed with resveratrol and quercetin

Combination	Experimental Value (%)	Theoretical Value (%)	Interaction
Ru + R	42.88[a]	36.54[b]	Synergistic
Ru + Q	42.08[a]	50.62[b]	Antagonistic
Ru + R + Q	42.48[a]	69.53[b]	Antagonistic

Means (n=4) with different superscript lower case letters at each row indicates significant different ($p<0.05$)

Previous study showed that mixture of rutin and quercetin exhibited very weak synergistic interaction as evaluated by Ferric Reducing Antioxidant Power (FRAP) method [10]. The contradiction of results between previous and present study is probably due to different in the nature of assay system.

The present results indicated that the combination of rutin and resveratrol can be used in designing antioxidant agent for functional food. Molar ratio should be determined for future study in order to define the best composition of each compound for maximum antioxidant activity. Molar ratio should be determined for future study in order to define the best composition of each compound for maximum antioxidant activity.

4. Conclusions

Rutin interact with resveratrol and quercetin that contributed to total antioxidant capacity. Antioxidant activity of rutin is significantly enhanced when mixing with resveratrol.

Acknowledgments

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Potential of *Ficus carica* Leaf Extract in Bacterial Disease Treatment of Tilapia (*Oreochromis niloticus*) *In Vivo*

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ABSTRACT
Fifteen days of study was conducted to observe the potential of *Ficus carica* leaf extract in the treatment of tilapia (*Oreochromis niloticus*) infected by *Aeromonas hydrophila*. *A.hydrophila* commonly found as pathogenic bacteria in aquaculture. This study was projected to the application of plant extracts in order to replace antibiotics used towards green aquaculture. 120 fish were injected intraperitoneally with 0.1 ml of *A.hydrophila* (10^6 cfu ml^{-1}) and stock in 12 aquaria (13 L). Three concentrations of *F.carica* leaf extract (0.3 g/L, 0.5 g/L and 0.7 g/L) were used as the treatment and the control was 0 g/L. Each treatment was done in triplicate. Water parameters such as dissolved oxygen, pH and temperature of the treatment tank were in the range of (4.05–4.20 ppm), (7.3–8.0) and (25.6–28.8 °C) respectively. The potential of the extract as a treatment agent in the infected fish was recorded in term of fish survival and physical condition such as the sign of bacterial infection and swimming behaviour. The results showed disease tilapia treated with *F.carica* leaf extract (0.7 g/L) possess the highest survival rate at day 9 (67%) and remain until day 15. Compared to the survival rate of 0%, 33% and 53% for the concentration *F.carica* leaf extract 0 g/L, 0.3 g/L, and 0.5 g/L respectively. Treatment with higher concentration of *F.carica* leaf extract showed improvement in physical condition and swimming behaviour of disease tilapia. It could be concluded that *F.carica* leaf extract has potential in treatment of *A.hydrophila* associate disease in tilapia fish culture.

Keywords: *Ficus carica*, *Aeromonas hydrophila*, bioassay, *Oreochromis niluticus*, *in vivo*

1. Introduction

Aquaculture sector is facing the challenge to increase the production in order to combat the protein hunger and to ensure livelihood and nutritional security in the future years. The rapid development of aquaculture system and growing demand of fish leads to the intensification of the culture practices, overdrawing stressors for fish and thus magnifying the risk of diseases. Chronic stress extremely affects fish health, causing in inhibition of specific immune responses and defence mechanisms which leads to the favourable condition of pathogen infections. Traditionally, synthetic chemicals and antibiotics have been used as preventive or prophylactic means of treating fish diseases. Until now, chemotherapy is the only option for prevention and treatment of aquaculture disease outbreaks. But the use of chemical drugs has several inherited negative impacts on the environment as well as human [1],[2]. *Ficus carica* leaf extract contains active substances such as flavonoid, tannin, and terpenoid which have been known for their antibacterial potency [3]. In view of reports on *F.carica* health benefits, hence this study was

conducted to observe the potential of *F.carica* leaf extract in treatment of tilapia infected by *A.hydrophila*.

2. Materials and Methods

2.1 Plant preparation and extraction

Ficus carica leaves were collected from fig farm at Greenhouse 4, Politeknik Sandakan, Sabah. Fresh plant materials were transported to the Food Technology Laboratory, Department of Agrotechnology and Bio-industry, Politeknik Sandakan, Sabah within one hour. The plant leaves were picked from the stems, cleaned and rinsed in distilled water. The leaves allowed to air dry. 100 g of leaves were weighed and ground into fine powder in a blender. 10 g of fine grounded *F.carica* leaf powder was measured using electronic balance (OHAUS: model NVL2101) and transferred into a conical flask containing 50 ml of distilled water. Then, the conical flask was corked with a cotton wool and foil, shaken gently and allowed to stand in room temperature for 24 hour. The content then was transferred to a funnel bearing a sterile muslin cloth and further filtered using a Whatman No. 1 filter paper. *F.carica* leaf extract was assumed as 100% concentration and stored in the refrigerator at 5 °C prior to use [4].

2.2 Bacterial strain and culture

Aeromonas hydrophila strain were obtained from Borneo Marine Research Institute, University Malaysia Sabah. The bacteria stock was transferred to Microbiology and Biotechnology Laboratory, Department of Agrotechnology and Bio-industry, Politeknik Sandakan. Prior to *in vivo* assay, the bacteria were recovered from glycerol stocks by streaking on indicated TSB agar medium and incubated at 30 ºC for 24 hours. Each bacterial pre-culture was prepared by inoculating 5 ml of the same broth medium with a single colony and incubated at 30 ºC while shaking for 16-32 hour. After incubation, bacterial suspension was then diluted with TSB broth at a cell density of 10^6 colony forming units/ml (CFU mL^{-1}) [5].

2.3 Challenge test

120 tilapia fish in average length of 3-4 inch were bought from aquaculture student at Politeknik Sandakan, Sabah. The fish was acclimatized in a polyethylene tank (500 L) for one week prior to the study. The fish were fed ad libitum twice per day and kept with proper aeration in dechlorinated tap water. Experiment was conducted with four treatment groups consist of T1, T2, T3 and TC in triplicate. Each replicate comprised of ten tilapia fish per 13 L aquarium and equipped with aeration system. Then, all four groups were injected with 10^6 cfu ml^{-1} of *A.hydrophila* suspension (0.1 ml $fish^{-1}$). Various concentrations of *F.carica* leaf extract (0.3, 0.5 and 0.7 g/L) were prepared in the aquarium treatment T1, T2, and T3 respectively. For the TC aquarium, fish did not treat with *F.carica* leaf extract. All the fish in all treatment were regularly monitored three times daily. Fish were fed ad libitum twice per day. Uneaten food was removed from the tank to maintain water quality. Water changed when necessary but the *F.carica* leaf extract concentration keep remain the same for each treatment until the end of the study.

2.4 Data collection and analysis

The survival rate (SR) was determined by using formula below:

$$SR\ (\%) = (\text{number of fish survived/number of fish injected}) \times 100.$$

The data were statistically analysed using one-way analysis of variance (ANOVA) and Duncan Multiple Range Test was applied to identify the significant differences formed at 95%

confidence level to show the differences means between the groups [6]. For clinical observation, fishes were examined externally for any injury, infections and diseases. The physical appearance and swimming activeness of tilapia fish were evaluated by using the number of scales that modified from Heiman-Carver colour rotor [7]. The scale consists of 5 numbers which are categorised as 1: very poor; 2: poor; 3: satisfied; 4: good; 5: excellent. The water temperature was taken daily during this study by using digital thermometer. The dissolved oxygen concentration was measured by using dissolve oxygen meter and water pH was measured using pH meter weekly [8].

3. Results

Water quality parameters measured throughout this study were summarized in Table 1. Only three basic water parameters measured in order to maintain the water quality. The dissolved oxygen was set in the range of 3.0–6.0 by aeration system with slow air bubble. The water pH was in the range of 7.2–8.2 where T3 (0.7 g/L) possess slightly alkaline water pH. Temperature for the treatment tank in this study was maintained in the range of 26–29 °C.

Table 1: Water parameters in challenged *Oreochromis niloticus* aquarium

Water paremeter	**Concentration of *F.carica* Leaf Extract**			
	TC (0 g/L)	**T1 (0.3 g/L)**	**T2 (0.5 g/L)**	**T3 (0.7 g/L)**
Dissolved Oxygen (ppm)	4.0-6.0	4.0-6.0	4.0-6.0	3.0-6.0
pH	7.2-7.8	7.2-8.0	7.4-8.2	7.3-8.0
Temperature (°C)	26.0-27.0	26.0-29.0	26.0-29.0	26.0-29.0

After injected by *A.hydrophila* and treated with *Ficus carica* extract, tilapia fish show mortality beginning at day 2 as shown in Figure 1. The mortality was recorded start from day two until day nine of experiment. For tilapia treated with *F.carica* leaf extract show survival in different rate while for the control treatment no fish survive after day seven of the study. Treatment T1 (0.3 g/L) show increasing mortality until day nine. Treatment T2 (0.5 g/L) showed increase mortality until day five of experiment as the same as T3 (0.7 g/L) but in different value of survival rate. Survival rate of T3 (0.7 g/L) and T2 (0.5 g/L) at day five and remain until day 15 were 67% and 53% respectively. Based on fish survival rate performance, we can see that increasing concentration of *F.carica* leaf extract influence the survival rate of challenge tilapia fish. For the highest survival rate was start from day five indicated that the diseased fish start fully recovered from *A.hydrophila* infection on day five and only fish treated with higher concentration of *F.carica* leaf extract showed higher survival rate. While for the treatment with lower concentration of *F.carica* leaf extract (0.3 g/L) still showed decreasing of survival rate after day five in the value of 60% and turn to 47% at day 6. For day seven and day eight the value of survival rate decreases to 40% and lastly at day 9 the survival rate become 33% and remain until day 15.

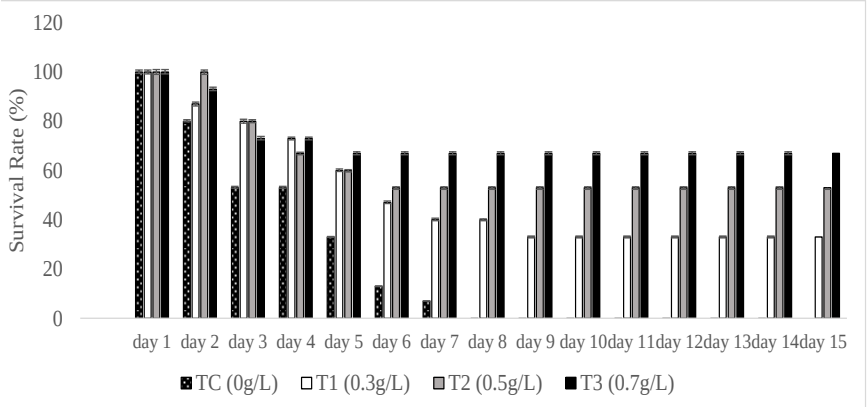

Figure 1: Survival rate of tilapia fish within the study period

At the end of the experiment, T3 (0.7 g/L) show significant different ($p<0.05$) of survival rate with value 67.00±0.12% compared to TC (0.0 g/L) and T1 (0.3 g/L) with the value 0.00±0.00% and 33.00±0.12% respectively as shown in Table 2. For the significant different ($p<0.05$) shown of survival rate among the fish treated and untreated with *F.carica* leaf extract indicate *F.carica* leaf extract have potential in treatment of *A.hydrophila* infection or *A.hydrophila* associated disease in tilapia.

Table 2: Final survival rate of challenge tilapia treated with *F.carica* extract

Treatment	Survival Rate (%)
TC (0g/L)	0.00 ± 0.00^{c}
T1 (0.3g/L)	33.00 ± 0.12^{b}
T2 (0.5g/L)	53.00 ± 0.12^{a}
T3 (0.7g/L)	67.00 ± 0.12^{a}

Values (Mean±SE) within the same column followed by different superscript were significantly different at $p<0.05$

Physical examination was done daily to observe any abnormality on the fish. The symptom of bacterial infection was appeared after second day of tilapia challenge by intraperitoneally injection using 0.1 ml of *A.hydrophila* per fish. The clinical sign of bacterial infection was shown in Figure 2. The most common clinical sign observed was redness or white areas on body and skin ulcers. The process usually started from skin erosion, ulceration when eventually the skin became shallow. Pop eyes and fin rot also were observed at most fish in all the treatment aquarium. The fish became inactive after four to five hours of *A.hydrophila* injection. They keep dormant at the aquarium bottom and show lethargic. After two days of injection, most of the fish show swimming abnormality where they were swirling slowly and rub their body to the aquarium wall. At the beginning of the challenge test, the off-feed was observed. The fish were ignored the feed given to them. The fish were fed on demand after five days of study and uneaten food was removed from the aquarium.

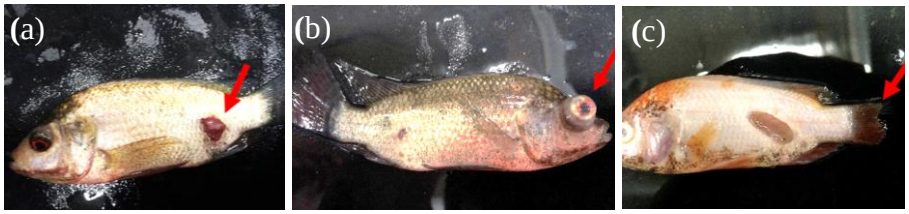

Figure 2: *A.hydrophila* associated disease appear after challenge test. (a) Haemorrhagic septicaemia clinical sign appears at the skin of fish; (b) Pop eyes (exophthalmia) appear where the eyes become big; (c) Sign of fin rot shown at the caudal fin

Physical appearance of tilapia evaluated in Table 3 referred to the physical condition of healthy fish. The challenge fish were compared to the healthy fish where the highest score of 5 indicate the physical of normal and health fish and the lower score of 1 refer to critical appearance of diseased fish. Swimming activeness of the fish refer to normal movement of fish where the score of 5 refer to normal movement of fish and lowest score of 1 refer to static fish. Treatment of *F.carica* leaf extract help in treating diseased tilapia fish where fish treated by *F.carica* leaf extract show better performance in physical appearance and swimming activeness with increasing score examined at day by day. Untreated fish in TC (0 g/L) show very bad performance where score of the physical performance was decreasing start from score of poor in physical appearance and swimming activeness after challenge test and become very poor until day 5. All the fish in TC (0 g/L) die at day 11 of challenge test. Only Fish in T3 (0.7 g/L)

was perform excellent score of physical appearance and swimming activeness at the end of the study which indicate the higher of *F.carica* leaf extract concentration faster the recovery of challenge fish to become normal back in appearance and activeness as health fish.

Table 3: Score of physical appearance and swimming activeness of *O.niloticus*

Day	Physical appearance				Swimming activeness			
	TC 0 g/L	T1 0.3 g/L	T2 0.5 g/L	T3 0.7 g/L	TC 0 g/L	T1 0.3 g/L	T2 0.5 g/L	T3 0.7 g/L
1	2	2	2	2	2	2	2	2
2	2	2	2	2	2	2	2	2
3	2	2	2	2	2	2	2	2
4	2	2	3	3	2	2	3	3
5	1	2	3	3	1	3	3	3
6	1	3	3	4	1	3	3	3
7	1	3	3	4	1	3	3	4
8	-	3	4	4	-	3	3	4
9	-	3	4	4	-	3	4	4
10	-	4	4	4	-	4	4	4
11	-	4	4	4	-	4	4	4
12	-	4	4	4	-	4	4	4
13	-	4	4	4	-	4	4	4
14	-	4	4	4	-	4	4	4
15	-	4	4	5	-	4	4	5

Values of score evaluated on scale of 1 to 5 representing very poor to excellent

Treatment of *F.carica* leaf extract showed the healing process in fish where the clinical sign of bacterial disease was reduced after few days of treatment. For example, tilapia in Figure 3 for the treatment of T3 (0.7 g/L), fish change in physical appearance where the eyes of the fish became big at day 3 of challenge test and the eyes back to normal size at day six of experiment. This mean *F.carica* leaf extract help in treating the pop eyes disease in tilapia infected by *A.hydrophila.* Another progress in bacterial disease treatment by *F.carica* leaf extract was observed in the healing process on diseases tilapia skin, fin and eye where the clinical sign of diseases was reduced during the treatment and absent at the end of the study. The *F.carica* leaf extract treatment revealed that the leaves have potential in bacterial disease treatment in fish.

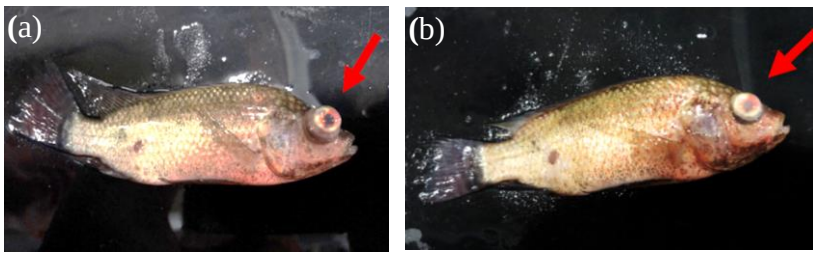

Figure 3: Changes in fish under treatment T3 (0.7 g/L). (a) Pop eye diseased appear at day 3 of challenge test; (b) Pop eye diseased absent at day 6 of experiment

4. Discussion

F.carica leaf extract contain antibacterial properties that can kills bacteria directly. After *A.hydrophila* injected into the fish, this *F.carica* leaf extract treatment may act as immunostimulant where the fish body will absorb the phytotherapy agent and leads to

increasing of immune factors. It can modulate the innate or non-specific immune response and can be used to control fish disease [9].

5. Conclusions

The effectiveness of *F.carica* leaf extract in *A.hydrophila* associate disease treatment were shown in this study. However, the efficacy of the treatment depends on the dosage applied and consuming time for recovery. The survival rate performance was not excellent where the highest rate was only 67% fish survive at the end of the experiment. Further investigation on improvement of the diseased fish survival rate is required in the future. Other factor such as extraction method and extract concentration are accountable.

Acknowledgments

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In Vitro Antioxidant and Antimicrobial Activities of *Garcinia cambogia*

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ABSTRACT
Garcinia cambogia is known locally as asam gelugor has been recognized in many Asian countries for its benefits to treat constipation, piles, rheumatism, irregular menstruation, and intestinal parasites. The previous study reported that the main extract of *G.cambogia*,(-)-hydroxycitric acid ((-)-HCA), an organic acid component of the fruit that exhibited anti-obesity activity. In the present, we have studied the antioxidant and antimicrobial activity of *G.cambogia* extract. Fruit rind was dried and extracted using water, methanol, acetone and ethanol. The highest yield of (-)-HCA in *G.cambogia* extract was analysed using HPLC-DAD and further screened for its activity against *Staphylococcus aureus* and *Escherichia coli* by agar well diffusion method. The *in vitro* antioxidant assay of *G.cambogia* extract was also evaluated. The water extract was justified to be as the best solvent system in extracting (-)-HCA and the biological activities tested for water extract of *G.cambogia* shows that it exhibited antioxidant activities for DPPH scavenging activity with IC_{50} value of 46.57±0.93. The antimicrobial activity showed the maximum inhibition zone was recorded against *S.aureus* (19.3±2.30 mm) and *E. coli* (15.3±2.30 mm) at concentration 0.5 g/ml crude extract of *G.cambogia*. The water extract of *G.cambogia* indicates that the fruit rind extract of the plant has excellent antimicrobial and antioxidant activities.

Keywords: *Garcinia cambogia*, antioxidant activity, antimicrobial activity, (-)-HCA

1. Introduction

Garcinia cambogia is popularly known as asam gelugur and it is one of the most medicinally important members of the Clusiaceae family. The fruit is normally used as flavouring agent, food preservative and as a traditional remedy to treat piles, constipation, intestinal parasites, irregular menstruation and rheumatism. The most abundant organic acid in *G.cambogia* is (-)-hydroxycitric acid ((-)-HCA) which has been reported have beneficial biological activities such as antimicrobial, anti-obesity, anti-inflammatory and anti-diabetic [1]. Apart from selecting the best method of extraction, selection of a suitable solvent is also important in the extraction process to obtain a high yield of target compounds. For the purpose of nutraceutical study using plant or herb extraction, the important character of extraction solvents must have a non-toxic effect or strong interfering efforts to living cells, animals, and human beings [2].

Antioxidant, the substance that can protect the cells and organ system against reactive oxygen species (ROS) by counteracting the free radicals. Before the free radicals attack the healthy cells, antioxidant plays a vital role in protecting membranes from oxidative damage. Plants are vital source of antioxidants in nature; they contain chemical compounds like flavonoids,

phenols, and other compounds which show high antioxidant activity. DPPH (2, 2-diphenyl-1-picrylhydrazyl) is widely used to quantify the free radical scavenging effect of natural antioxidant [3]. *G.cambogia* has been used traditionally for the treatment of diarrhoea and intestinal parasites that cause from the bacteria. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. Agar well diffusion method is widely used to evaluate the antimicrobial activity of plants extracts [4]. Thus, in the present study, the effect of solvent extraction of *G.cambogia* was studied. Furthermore, *in vitro* antioxidant by using DPPH radical scavenging activity and antimicrobial activity against human pathogens (*Escherichia coli* and *Staphylococcus aureus*) by agar well diffusion method was evaluated.

2. Materials and Methods

2.1 Materials and reagents

The fruits of *G.cambogia* were obtained from a local orchard in Taiping, Perak. All fruits were cleaned and checked to remove any damage, disease or pest infected fruits. The fruits were cut to small pieces and were dried in an oven (Memmert GmbH, Schwabach) at 45 °C. Then, the dried samples were ground into small particles powder. The samples were then stored under an aseptic condition in a container at 4 °C for further analysis.

(-)-hydroxycitric acid calcium salt standard was purchased from Wako Pure Chemical Industries, Ltd (Osaka, Japan). For the antioxidant assay, 2, 2-diphenyl- 1-picrylhydrazyl (DPPH) and ascorbic acid was used as the reference standard and was purchased from Sigma Aldrich (St Louis, MO, USA). The antimicrobial activity of *G.cambogia* extract was assessed against bacteria species; Gram-positive (*S. aureus,* ATCC 2592*)* and Gram-negative (*E. coli,* ATCC 35218). All the bacterial strains were grown on nutrient agar. All the other chemicals and solvents used for the analysis were of analytical grade

2.2 Ultrasonic-assisted extraction (UAE)

10 g of *G.cambogia* powder was placed in a 250 ml beaker and was added with 100 ml of the extraction solvents namely water, methanol, ethanol and acetone. The extraction was carried out by using ultrasonic with a probe sonicator 700 W Sonic Dismembrator, 220V (FB-705, Fisher Scientific, Loughborough, UK) with the amplitude of sonication at 22% for 21 minutes and the solid-liquid ratio at 6%. Afterwards, the treatment for the extraction of *G.cambogia* was slightly modified and done according to Jayaprakasha et al [6]. The content of (-)-hydroxycitric acid was determined by HPLC-PDA [6].

2.3 HPLC Analysis

Free (-)-HCA is not available commercially, therefore, salt of (-)-hydroxycitric calcium is needed to be converted to free HCA. The preparation of free (-)-HCA was done based on a study by Jena et al. [7]. Waters e2695 Alliance Separation Module liquid chromatography system comprising of vacuum degasser, quaternary pump, auto-sampler and Waters 2998 photodiode array detector (Milford, MA, USA) was used for the quantification of (-)-HCA. Empower software was used to control the HPLC system and data processing. Ascentis C18 (250 × 4.6 mm, 5 µm) was used as the stationary phase. The isocratic system was used for the separation was 6 mM sulphuric acid with the flow rate of 0.6 ml/min and the detection wavelength used was 210 nm. Sample injection volume was 20 µl [8]. The total running time for HPLC analysis for (-)-HCA was 10 minutes. The chromatographic peaks of the analytes were confirmed by comparing the retention times and UV spectra with the reference standards.

2.4 DPPH radical scavenging activity

100 µl of the *G.cambogia* extract with different concentration starting at 125 µg/L was mixed into 96-well microplate with 100 µl of 0.1 mM 2, 2-diphenyl-1-picrylhydrazyl (DPPH) solution in methanol. Then, it was incubated in ambient temperature for 30 minutes in the dark condition. The absorbance of the mixture was read using an ELISA (Versamax Microplate Reader, US) at 517 nm. The IC_{50} value was calculated and evaluated to obtain the concentration needed to inhibit 50% of the scavenging activity. The lower the IC_{50} value, the stronger the antioxidant activity. As a comparison, ascorbic acid was used as a standard. The corresponding blank reading was also taken, and the remaining DPPH was calculated. The percentage of radical scavenging activity by samples was determined by comparison with a 10 µl MeOH treated blank group. Inhibition of free radical DPPH in per cent was calculated as follows:

$$\text{Inhibition (\%)} = 100 - 100\ (A_s / A_o)$$

Where (A_o) is absorbance of the blank and (As) is absorbance of the sample at 517 nm.

2.5 Anti-microbial activity

The agar well diffusion method on Mueller-Hinton agar (MHA) was applied to determine the antibacterial activities of the *G.cambogia* extract against one Gram-positive, *S.aureus* and one Gram-negative, *E. coli*. The bacteria culture was diluted with sterile physiological saline solution with reference to the 0.5 McFarland standards to achieve an inoculum of approximately 1.5 x 100 CFU/ml. A 5 ml portion of this inoculum was placed onto the surface of nutrient agar plates and was allowed to remain in contact for one minute. The excess inoculum was removed using a sterile syringe and the plates were allowed to dry for 20 minutes at room temperature. Briefly, MHA agar plates were inoculated with bacterial strain under aseptic conditions and wells (diameter = 6 mm) were filled with 20 µl of the *G.cambogia* extract (0.5 g/ml). Then, the plates were incubated at 37 °C for 24 hours in an incubator (Memmert, Schwabach). The diameters of the inhibition zones were measured in millimetres. A zone of inhibition was compared with the standard antibiotic streptomycin (10 µg/disc), whilst a blank disc impregnated with the methanol was used as the negative control

3. Results and Discussion

3.1 Solvent properties

The influence of different solvents used for ultrasonic-assisted extraction on the extraction yields was investigated using water, methanol, ethanol and acetone. Figure 1 presents the extraction yields obtained using these solvent systems. The water extract was justified to be as the best solvent system in extracting (-)-HCA compared to methanol, ethanol and acetone. The highest yield for (-)-HCA was 26.67 g/100 g dw. Water has high polarity compared to other organic solvents and is suitable to solubilize many ionic species [6]. These results were in agreement with the previous studies by Jena et al [7] and Jayaprakasha et al [6], where they attained that the aqueous extract obtained the highest yield of (-)-HCA than acetone, methanol and ethanol.

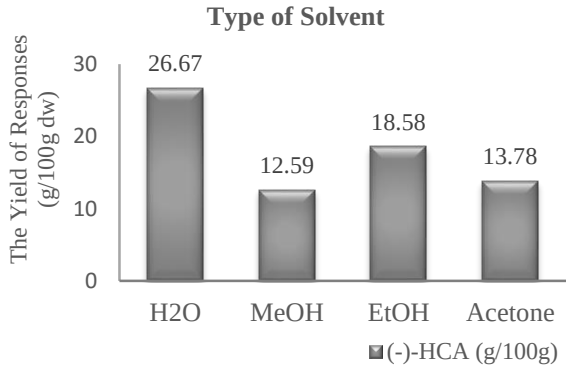

Figure 1: The yield of responses; (-)-HCA at different solvents extraction

3.2 Antioxidant activity

The highest yield of (-)-HCA in different solvent extraction was further analysed the free radical scavenging activity. The DPPH radical scavenging activity was determined for water extract *G.cambogia* at different concentrations ranging from and the calibration graph was developed. The value of IC_{50} for ascorbic acid was recorded in Table 1 was 2.000±0.001 μg/ml showed a greater ability to inhibit DPPH radical. Meanwhile, compared to *G.cambogia* extract presented lower antioxidant activity with an IC_{50} value of 46.57±0.93 μg/ml. However, DPPH radical scavenging activity of *G.cambogia* extract was significantly high and can be classified as a good antioxidant agent and it was similar to the finding of Subhashini et al [3]. They found that the IC_{50} of water extract of *G.cambogia* (WEGC) was 36.20±3.04 μg/ml and ascorbic acid as the reference standard. They concluded that the water extract of *G.cambogia* possessed strong antioxidant activity [3].

Table 1: The IC_{50} value for water extract of *G.cambogia*

Extract/Standard	Absorbance 517 nm Concentration at IC_{50} (μg/ml)
Water extract of *G.cambogia*	46.57±0.93
Ascorbic Acid	2.000±0.001

3.3 Antimicrobial Activity

The growth inhibition zones of *G.cambogia* extract against *E. coli* and *S.aureus* was measured by agar well diffusion method. The findings were presented in Table 2. After incubating at 37 ℃ for 24 hours, *G.cambogia* extract showed high antimicrobial activity against Gram-positive (*S.aureus*) and Gram-negative (*E. Coli*) bacteria compared to the positive control, streptomycin. The *G.cambogia* extract was tested at concentrations 0.5 g/ml which produced a zone of inhibition of *S.aureus* at value 19.3 mm. While for inhibition of *E. coli* gave the value of 15.3 mm. *E.coli* is the common bacteria in gastrointestinal tract in human [9]. Meanwhile *S.aureus* frequently found on the skin [10]. It is known that the life span of antibiotics is limited, hence new sources of antibiotic are required especially from plant source. *G.cambogia* can be one of the medicinal plants used in various traditional and alternative remedy to treat human disease [11]. It shows that *G.cambogia* extract against human bacteria pathogenic strains demonstrated the ability to inhibit the growth of the microorganism.

Table 2: Antibacterial properties of water extract of *G.cambogia* using agar-well diffusion method

Bacterial strain	Zone diameter inhibition (mm)		
	GC extract (0.5 g/ml)	Positive control	Negative control
S. aureus	19.3±2.3	18.0±1.0	0.0
E. coli	15.3±2.3	18.1±1.0	0.0

5. Conclusions

The water extraction shows the highest yield of (-)-HCA in *G.cambogia.* The water extract of *G.cambogia* can be classified as a good antioxidant agent and can provide potential therapeutic agents against bacterial infection. They showed antimicrobial activity against *E. coli* and *S.aureus* which were comparable to antibiotic streptomycin.

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Potential of Lemongrass (*Cymbopogon citratus*) Extract as Antiparasite in Tilapia (*Oreochromis niloticus*) Culture

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ABSTRACT
Fish parasites are composed of crustaceans, worms (trematodes, nematodes, cests) and protozoa. This parasite affects the fins, scales, operculum and gills of the fish and influence their survival rate. Prevention of infections using plant extracts is very desirable because of its low-cost and effectiveness. This study is projected to the application of plant extracts in order to replace chemotherapy used towards green aquaculture. The purpose of this study is to observe the efficacy of lemongrass extract in parasite management. Three concentrations of lemongrass extract (60 ppm, 80 ppm, 100 ppm) were used as the treatment and the control is 0 ppm. Each treatment is in triplicates. Fish survival, parasite occurrence and physical condition of fish were observed and recorded. From the results of this study, fish untreated by lemongrass showed a low survival rate of 0% due to parasite development in fish were uncontrolled. Whereas the concentrations lemongrass extract of 60 ppm, 80 ppm and 100 ppm showed high survival rates of 80%, 87% and 90% respectively and no parasites were found in fish at the end of the study. Fish are less responsive and off-feed in the treatment of 0 ppm compared to fish exposed lemongrass extract which show better condition and behaviour. The results show significant differences in survival rates and parasite existence between treated and untreated tilapia by lemongrass extract. Lemongrass extract has proven to treat parasite infections in tilapia fish culture.

Keywords: Antiparasite, survival rate, *Cymbopogon citratus*, aquaculture, tilapia

1. Introduction

Parasite is an important pathogen group that causes infection and diseases of both freshwater and marine fish. Systemic of parasites consist of eight groups. They are protozoa, helminths, monogenea, digenea, cestodes, acanthocephalan, nematode and crustacea. In developing aquaculture industry through intensification production, parasitic infestations are becoming threats for fish health management and aquatic crop production throughout the world. The infectious diseases account for 60% of the fish production loss in aquaculture ponds. Frequently occurring parasitic diseases are ichthyophthiriasis (white spot), agrulosis (fish louse), and myxoboliasis. Only few attempts were taken to their control measures using simple chemicals like salt, lime, formalin, dipterex and sumithion. Other than that, chemicals known to be used in parasite treatment are, salt+lime, potassium permangante, copper sulphate, malachite green, and dipterex+lime [1],[2].

Parasites commonly infects the gills of fish causing gill epithelial hyperplasia. For high intensity production of aquaculture, parasite infections reduced host growth and increased occurrence and severity of secondary infections. Current fish health management of parasite infection involves bath treatments of formalin or trichlorfon. Trichlorfon is widely used for the treatment of monogeneans in fish culture. However, there are reports of low efficacy for treatment using this synthetic chemical as the emergence of resistance in some monogeneans and host toxicity. This chemical also environmentally harmful. Formalin is toxic to phytoplankton, zooplankton and benthic organisms and becomes more toxic with increasing temperature. These chemical usages were unsafe for environment and application of an accurate dose is required to maintain efficacy while avoiding host toxicity. Alternate treatments for parasite that avoid the problems associated with formalin and trichlorfon use are therefore required [3].

The use of chemotherapeutic agent, pesticides and antibiotics in aquaculture farms and non-compliance to scientific management practices lead to adverse impact on aquaculture production, serious diseases outbreaks, development of drug resistance in microbes and pathogens, accumulation of antibiotics and pesticide residues in finfish and shellfish and environmental pollution. There is a strong encouragement to aquaculturists as well as aquatic animal health management professionals to find a suitable alternative therapy to replace antibiotics and chemotherapy usage in this aquaculture industry for sustainable aquaculture production. Phytotherapy has come to be recognized as a handy and viable alternative to chemotherapy, as it is economical, effective, non-resistance forming, renewable, eco-friendly and farmer-friendly. Although the use of medicinal plants is known to humanity since the beginning of human civilization for the treatment and control of human and animal diseases but its importance in combating finfish and shellfish diseases has been realized only recently [2]. Natural products were found to be less toxic and safer than chemical medicine for aquaculture industry. However, lack of information of *Cymbopogon citratus* usage for parasite treatment in aquaculture, thus the aim of this study is to evaluate the effect of *C.citratus* extraction on parasitic infection in tilapia fish.

2. Materials and Methods

2.1 Plant preparation and extraction

Lemongrass were collected from nursery at Politeknik Sandakan, Sabah. Fresh plant materials were transported to the Food Technology Laboratory, Department of Agrotechnology and Bio-industry, Politeknik Sandakan, Sabah within 1 hour. The plant part that usually used in cooking, approximately 7-inch part that grow from above ground were cut, picked, cleaned and rinsed in distilled water. The plant sample allowed to oven dried at 38 °C for 48 hours. 100 g of the lemongrass was weighed and ground into a fine powder in a blender. 10 g of fine grounded lemongrass powder was measured out using electronic balance (OHAUS: model NVL2101) and then transferred into a conical flask containing 50 ml of distilled water. It was corked with a cotton wool and foil, shaken gently and allowed to stand in room temperature for 24 hours. The content was then transferred to a funnel bearing a sterile muslin cloth and further filtered using a Whatman No. 1 filter paper. Lemongrass extract was assumed as 100% concentration and stored in the refrigerator at 5 °C prior to use [4].

2.2 Collection of parasite

The process of collecting parasites was done in fish hatchery, Politeknik Sandakan from fish that has parasitic infections. The way to extract the parasite is to extract the mucus from the infected fish body using the scraping method. The parasite of fish was observed first under microscope. The mucus was placed on a glass slide. Sodium chloride was added and covered

with a glass slip and observed under a microscope. After parasite was identified, then isolated parasites were concentrated with 70 µm mesh. The collected parasite was transferred into 1 L glass containing 1000 ml water [5],[6].

2.3 Experimental tank and protocol

A total number of 120 healthy tilapia fish in average length of 3-4 inch were bought from aquaculture student at Politeknik Sandakan, Sabah. The fish were exposed to the parasite using immersion method in a polyethylene tank (200 L) for two days prior to the study. Fish were not fed and kept with proper aeration to make sure them alive and infected by the parasite. After exposure to parasite, fish were transferred to treatment aquarium with aerated dechlorinated tap water. Experiment was conducted with four treatment groups consist of T1, T2, T3 and TC in triplicate. Each group comprised 10 tilapia fish per 13 L aquarium and equipped with aeration system. Various concentrations of lemongrass extract (60 ppm, 80 ppm and 100 ppm) were prepared in the aquarium treatment T1, T2, and T3 respectively. For the TC aquarium dis not treated with lemongrass extract. All the fish in all treatment were regularly monitored three times daily. Fish were fed ad libitum twice per day. Uneaten food was removed from the tank to maintain water quality. Water changed when necessary, but the lemongrass extract concentration keep remain the same until the end of the study [7].

2.4 Data collection and analysis

Observation and data collection of infected fish in treatment aquarium was done in seven days. The survival rate (SR) was calculated according to formula below:

$$SR\ (\%) = (\text{number of fish survived/number of fish injected}) \times 100$$

The data were statistically analysed using one-way analysis of variance (ANOVA) and Duncan multiple range test was applied to identify the significance difference formed at 95% confidence level to show the differences means between the groups. The water temperature were taken daily in this study by using digital thermometer. Dissolved oxygen concentration was measured by dissolve oxygen meter and water pH was measured using pH meter weekly [8].

3. Results

During the study fish were observed and monitored. The mucus on fish body and gills was sampled every day to examine the presence of parasite. The result and treatment effect were recorded which is presented in Table 1. The use of lemongrass extract treated “Ich” parasite after four days in T2 and T3. But “Ich” parasites were present in control group until all the fish dead. On day five, the parasite were observed only in treatment TC and T1 and no more parasite present after day five in T1.

Table 4: Parasiticidal activity of lemongrass extracts on parasite in tilapia during experiment period

Treatment	Experiment period						
	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**
TC (0 ppm)	+	+	+	+	+	+	+
T1 (60 ppm)	+	+	+	+	+	-	-
T2 (80 ppm)	+	+	+	+	-	-	-
T3 (90 ppm)	+	+	+	+	-	-	-

‘+’ show the presence of parasites, ‘- ‘show the absence of parasites

Final fish survival percentage is presented in Table 2. Treatment T1, T2 and T3 treated with showed significant high survival rate percentage ($p<0.05$) compared to the TC. The highest survival rate was related to group treatment TC (90ppm). There is no significant different ($p<0.05$) of survival rate percentage among T1, T2 and T3.

Table 5: Final survival rate of parasite infected tilapia treated with different concentration of lemongrass extract

Treatment	Survival rate (%)
TC (0 ppm)	0.00 ± 0.00^{b}
T1 (60 ppm)	80.00 ± 0.10^{a}
T2 (80 ppm)	87.00 ± 0.12^{a}
T3 (90 ppm)	90.00 ± 0.10^{a}

Values (mean±SE) within the same column followed by different superscript are significantly different at $p<0.05$

Unknown species of nematode and louse parasites were found on the body, gills and operculum of tilapia fishes were shown in Figure 2. The parasite affects the survival rate and habitat of the fish, which the fish do not respond to the food given. Parasites also cause changes to the tilapia fish, which is less active for swimming, isolated swimming, scraping their body and rub their body on the aquarium wall. Effectiveness of lemongrass extract as antiparasite was shown when no parasites observed in the mucus collected from tilapia immersed into lemongrass extract. Mucus observed under microscope were collected from the body, gills and operculum of tilapia fish. Apart from the absence of parasites in tilapia fish, observations on feeding response also showed significant changes compared to the beginning of the study. At the early stage of infection, fish show no feeding response in feed given to them. After the parasite reduce and absent, fish show positive response on the feeding activity. Fish swimming habits were also different between at the early of parasite infection and at the end of experiment. Fish show abnormal swimming behaviour at the early study and their swimming behaviour become normal at the end of the study. Furthermore, the survived tilapia fish are no longer rub their body on the aquarium walls at the end of study.

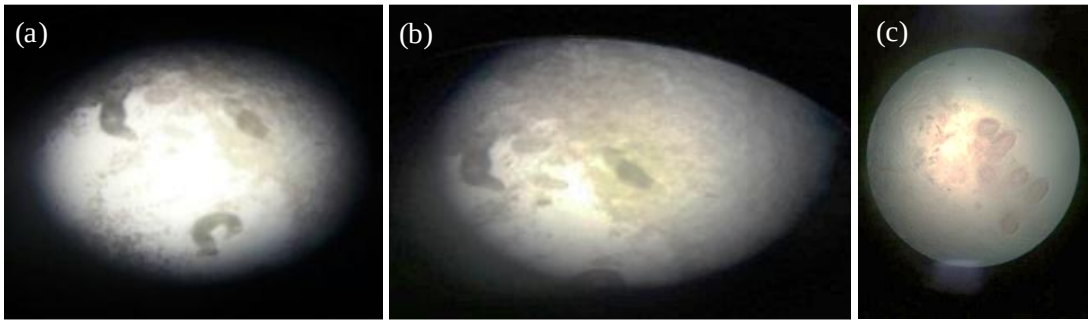

Figure 4 (a-c): Parasites from tilapia mucus observed under microscope

The water quality parameters measured throughout this study are summarized in Table 3. Three basic water parameters were measured to monitor water quality in this study. The dissolved oxygen was in the range of 3.0–6.0 where dissolve oxygen was maintained by aeration system with slow air bubble. The water pH was in the range of 5.8–8.2. Temperature for the treatment tank in this study was maintained in the range of 26–29 °C. All the water parameter recorded are favourable for fish culture.

Table 6: Water parameters in *O.niloticus* aquarium during the experiment

Water paremeter	Concentration of lemongrass extract			
	TC (0 ppm)	T1 (60 ppm)	T2 (80 ppm)	T3 (90 ppm)
Dissolved Oxygen (ppm)	3.0-6.0	4.0-6.0	4.0-6.0	4.0-6.0
pH	5.8-7.8	7.4-8.0	7.5-8.2	7.3-8.0
Temperature (°C)	26.0-29.0	26.0-29.0	26.0-29.0	26.0-29.0

4. Discussion

Lemongrass is one of the medicinal plants or herbs containing essential ingredients as a therapeutic agent. The advantage of lemongrass is that it is easy to obtain, cheap and can respond to a broad spectrum of pathogens. In addition, lemongrass has many nutrients that may treat various diseases and is able to kill parasites found in fish bodies. Lemongrass contains active ingredients such as saponin and mycrene which are effective antimicrobials, while some active components such as citronella, citronellol, geranilol and others act as disinfectants. Lemongrass that have been studied are found to have potential for use as anti-cancer (terpenoids), antifungal, antibiotics and insect repellents including mosquitoes. Lemongrass extract is also a potential stabilizer in fish farming. In the poultry industry, lemongrass is used as a supplement to guarantee high productivity indirectly guaranteeing double profits [9],[10].

This finding of lemongrass extract had potential in parasite treatment in fish supported by [11] who found that the essential oil extracted from *C. citrates* showed anti-protozoan activity against *Crithidia deanei*. *C.deanei* is a parasite species found in insect. Otherwise, Sherwani et al discovered that crude aqueous extract of *C.citratus* (lemon grass) showed anthelmintic activity [12]. The results indicated that the lemongrass crude extract possessed anthelmintic activity in dose dependent manner on earthworm (*Pheretima posthuma*). *P.posthuma* owing to its resemblance in terms of anatomy and physiology with the intestinal roundworm parasite of human beings. This treatment of *C.citratus* can be applied as an effective antiparasite agent in future after further exploration.

5. Conclusions

This study showed the effectiveness of lemongrass in parasitic disease treatment in fish. As lemongrass extract is very effective as a therapeutic agent in parasite infectious diseases, the product from lemongrass extract should be developed to be commercialize and practically used in aquaculture industry. This is significant towards aquaculture sustainability parallel to develop green aquaculture system in order to produce organic fish.

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Phenotypic and Genetic Variation of *Capsicum annuum* Germplasm Collection

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ABSTRACT
Capsicum annuum is widely cultivated in Malaysia. However, production of the local chili varieties is low and very prone to insects and diseases. Researches on diseases, agronomic practices and introduction of germplasm from abroad for local environment adaptability are very important for development of new superior varieties. This study aimed to estimate genetic diversity and heritability values of 30 genotypes of *C. annum* collected from Asian Vegetable Research and Development Center (AVRDC) and to identify potential superior accessions with high yield potential, and disease and insect resistant for future breeding program. The *Capsicum annuum* genotypes were characterized for days to flowering (DF), days to maturity (DM), plant height (PH), fruit yield (FY), fruit length (FL), number of fruit per plant (NFL), leaf area (LA) and disease score (DS). The data of these traits were subjected to analysis of variance (ANOVA), heritability component and correlation analysis, and multivariate analysis using cluster analysis based on percent similarity coefficient. Results showed significant differences for all the traits except on DM and PH at week 8, 10, and 12. High heritability values (> 60%) were observed for DF, FY, FL, DS, LA and, PH at weeks 2 and 4. Through cluster analysis, Cluster III (AVPP0012, AVPP0904, AVPP9905, and AVPP9813) was the best cluster as compared to others. In addition, cluster VII (AVPP 0804) was highly tolerance to mosaic disease. The genotypes in Cluster III had good performance in FY, NFL, DS and LA while Cluster VII for disease score. These potential genotypes can be recommended for future breeding program.

Keywords: Genotypes, genetic diversity, *Capsicum annuum*, heritability component analysis, cluster analysis

1. Introduction
Capsicum species known as pepper, is the second most important vegetable crop after tomato as it has high genetic diversity and great geographical distribution. This species which also known as chili, chile, chilli, aji, and paprika is belonging to Solanaceae family; a large family that includes tomato and potato [1]. There are about 27 species in chili, but only five species are majorly cultivated. Those are *C.annuum*, *C.frutescens*, *C.chinense*, *C.pendulum*, and *C.pubescens* [2]. Most of these species are originated from Central and South America and then spread throughout the world, including the tropics, subtropics and temperate regions. Chili is a very importance crop in Malaysia because it has a lot of uses and benefits in many aspects such as for foods, culinary uses, medicine and nutrition.

Chili with wide range of variability is grown all over Malaysia. As it was domesticated from their origins, their heritability may change due to the different environment factors. It may vary in form of their morphological performances such as shape, size and colour of fruit, colour and size of leaf, and agronomic performances such as fruit yield and number of fruits per plant [3]. The identification of genetic variability is important for breeding activity to produce better quality of chili that will have higher yield for domestication and large scale cultivation. Plant morphological characteristics or the quantitative traits are vital to calculate genetic parameters for initiating proper breeding procedures in crop improvement program. For example, the yield traits need to be observed and calculated to identify the heritability, for further selection purposes.

One of the major problem is low local production of chili. Besides, chili is exposed to insects and disease problems due to inconstant weather. The poor quality of planting materials was also aids in pest and disease problems, and result into lower yield. In addition, the genetic variability of the collected chili germplasm accessions is not known for example, which of the accession is hot, spicy or sweet [4]. Although chili can be used in many ways, its production is too low in Malaysia compared to other countries such as China, leading to higher import rate from neighbour countries for domestic uses. Furthermore, unstable weather in Malaysia has caused more problems of insects and diseases in chili production. Besides, the quality of planting materials is low and the genetic components diversity in chili is mainly unknown. Even though the number of literatures had been reported, information on *C. annuum* diversity is still lacking [5]. Therefore, this study was to estimate the genetic diversity of chili to assist future breeding improvement program. Evaluation of large number of accessions would assist in precise estimation genetic variability from phenotypic characteristics. Therefore, the study was conducted to evaluate the yield and yield components performance of 30 accessions of chili. From this research, the phenotypic and genetic variation of the accessions can be identified for the future breeding program.

2. Materials and methods

2.1 Plant materials

Thirty accessions of Capsicum annuum were collected from Asian Vegetable Research and Development Center (AVRDC). This experiment was carried out during April to August 2012, at the Rainshelter in Agrotech unit, Universiti Putra Malaysia, Serdang, Selangor by using the fertigation system.

2.2 Experimental design and planting

The study was conducted using randomized complete block design (RCBD) with 30 accessions replicated in three blocks. Each accession was represented with one plant in each block which made a total of 90 plants planted. The spacing used was 75 cm inter-row and 150 cm intra-row. The plants were planted by using the fertigation system which is injecting fertilizer into irrigation system. Firstly, the seeds were germinated in the petri dish and then were put into the germinator at laboratory for eight days. Then, the seeds that had germinated were sowed into seedling tray with one plant per hole for about two weeks, which until the seedlings were matured and healthy before they transplanted into the polybeg. The media used for germination in seedling tray was peat moss, while the media used for planting in polybag was coco peat. The fertigation system needs the stock solution of nutrients which should be given along with the water or irrigation system during the growth period of plants. In this study, the stock solution of calcium nitrate (solution A) and ferum ions (solution B) with KNO_3, KH_2PO_4, $MgSO_4$, $MnSO_4$, $CuSO_4$, $ZnSO_4$, boric acid, ammonium molibdat were used as the fertilizer

homogenously to all 90 plants. The EC of nutrient solution was kept from 1.0 µS to 2.5 µS, depends on the nutrients requirement by age of chili. The plants were frequently checked from insects and diseases, and also the timer and dripper whether those functioned normally or not. The pesticides were also applied to control insects and diseases.

2.3 Method of collecting data

The diversity parameters collected were days to flowering (DF), days to maturity (DM), plant height (PH), fruit yield (FY), fruit length (FL), number of fruits per plant (NF), leaf area (LA), disease score (DS)

2.4 Statistical analysis

At the end of experiment, the data were subjected to one-way analysis of variance (ANOVA) to test the different varieties on morphological traits by using Statistical Analysis Software (SAS) version 9.1 (SAS Institute Inc, 2005).

2.5 Clustering analysis

Clustering analysis was carried out using NTSYS-PC version 2.1 to assign the accessions into similar group. Throughout this analysis it formed the dendogram graph, two-dimensional principal component analysis figure and three-dimensional principal component analysis figure for easier observation and comparison of data.

3. Results and Discussions

From the experiment, results showed significant differences for all the traits except on DM and PH at week 8, 10, and 12. Effects of blocks were found to be significant only for plant height at week 4 and 6, fruit yield, number fruits per plant, and disease score (at $p \leq 0.05$). This indicates that variations among the blocks have affected these five traits. Effects of genotypes were highly significant (at $p \leq 0.01$) for plant height at week 4, fruit length, disease score, and leaf area. Results of ANOVA for each trait measured on the 30 chili accessions are shown in Table 1. High heritability values (> 60%) were observed in Table 2 for traits days of fruiting, fruit yield, fruit length, disease score, leaf area and plant height at week 2 and 4. This finding showed that the selection for these traits will be effective and less influenced by the environmental factors [6]. Hence, traits of these traits were recommended as selection criteria for the aim of improvement breeding. Through cluster analysis (Figure 1), Cluster III (AVPP0012, AVPP0904, AVPP9905, and AVPP9813) was the best cluster as compared to others. In addition, cluster VII (AVPP 0804) was highly tolerance to mosaic disease. This showed that both cluster was distinct and had wide distance relation with other genotypes. Both may be distinct due to characters of fruit yield, number of fruit per plant, disease core and leaf area compared with other genotypes [7],[8]. These potential genotypes can be recommended for future breeding program.

Table 1: Mean squares in ANOVA for all phenotypic traits on 30 accessions *C. annuum*

Source of variation	d.f	DF	DM	PH W2 (cm)	PH W4 (cm)	PH W6 (cm)	PH 8 (cm)
Blocks	2	1.98^{ns}	294.32^{ns}	0.65^{ns}	20.80*	75.25*	5.61^{ns}
Genotypes	29	10.7*	51.30^{ns}	8.66**	42.00**	2405.12*	101.90^{ns}
Error	54	3.63	106.76	2.48	6.03	45.66	68.88

Cont. Table 1: Mean squares in ANOVA for all phenotypic traits on 30 accessions *C. annuum.*

Source of variation	d.f	PH W10 (cm)	PH W12 (cm)	Yield (g)	NF	FL (cm)	DS (%)	LA (cm^2)
Blocks	2	83.58^{ns}	83.58^{ns}	102650.55*	6283.39*	2.60^{ns}	1359.95*	171001.51^{ns}
Genotypes	29	171.24^{ns}	171.24^{ns}	53649.96*	1295.66*	19.21**	870.34**	195746 3.49**
Error	54	116.13	116.13	18121.51	609.23	2.76	243.34	259541.09

DF = Days to flowering, PHW6 = Plant height at Week 6, FYLD = Yield per plant, LA = Leaf Area, DM = Days to maturity, PHW8 = Plant height at Week 8, NF = Number of fruits per plant, PHW2 = Plant height at Week 2, PHW10 = Plant height at Week 10, FL = Fruit Length, PHW4 = Plant height at Week 4, PHW12 = Plant height at Week 12, DS = Disease Score

Table 2: Heritability component and broad sense heritability

traits	mean	σ^2_g	σ^2_p	GCV	PCV	h^2_B (%)	GA	GG
DF	33.00	2.63	6.32	4.91	7.61	64.51	3.34	10.12
DM	77.50	0.00	87.38	0.00	12.07	0.00	0.00	0.00
PHW2	13.50	2.21	4.63	11.02	15.95	69.09	3.06	22.69
PHW4	29.80	12.96	18.86	12.07	14.56	82.90	7.42	24.87
PHW6	49.10	13.09	58.49	7.37	15.57	47.31	7.45	15.18
PHW8	54.90	12.33	78.93	6.39	16.17	39.52	7.23	13.16
PHW10	62.10	20.19	134.54	7.24	18.68	38.74	9.26	14.91
PHW12	62.10	20.19	134.54	7.24	18.68	38.74	9.26	14.91
Yield	207.20	12318.7	30273.4	53.57	83.97	63.79	228.64	110.34
NF	30.10	211.96	850.69	48.42	97.00	49.92	29.99	99.74
FL (cm)	12.50	5.77	8.48	19.16	23.23	82.49	4.95	39.47
DS (%)	66.60	209.51	459.33	21.72	32.17	67.54	29.82	44.75
LA	1917.10	584196	840114	39.87	47.81	83.39	1574.51	82.13

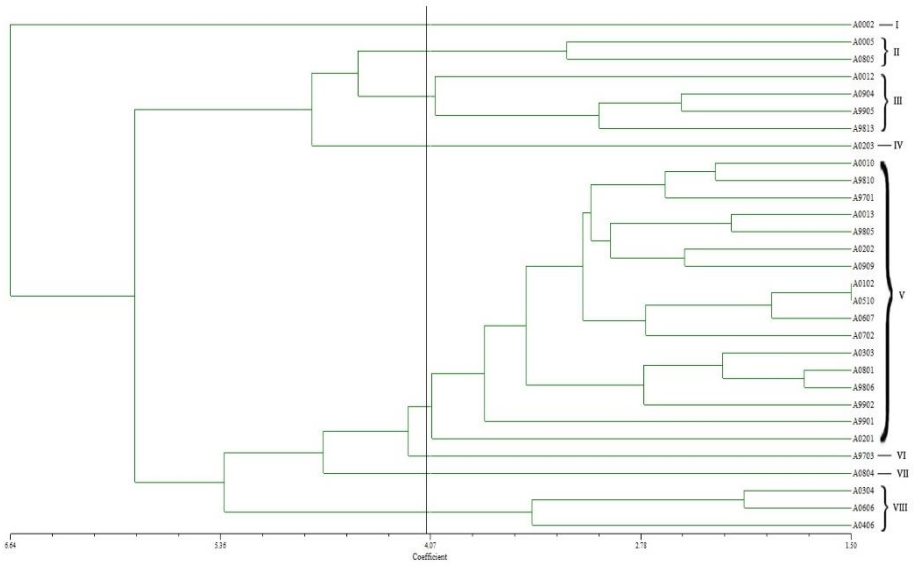

Figure 1: Relationship among the 30 chili genotypes based on phenotypic characteristics using SAHN clustering on UPGMA method

4. Conclusions

It is concluded that variation in terms of genetic content occurs within same species of *C.annuum*. The variability recorded in present studies reflects the possibility to improve the fruit yield and disease score traits initiation through hybridization in future breeding programme.

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Bioproduct from Chicken Waste Using Ultrasonic Extraction Method

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ABSTRACT
Chicken waste is an alternative source for biodiesel and bioplastic production. Chicken waste used in this study were chicken feather, chicken feather meal and chicken fat. Ultrasonic extraction process is a green extraction process using water as the solvent to extract the biodiesel and glycerin for bioplastic product. The analysis for biodiesel was done using gas chromatography (GC) and fourier-transform infrared spectroscopy (FTIR). Meanwhile, the properties of bioplastic were analysed physically such as odour, colour and strengths. Based on this study, the optimum solvent composition used in this experiment was 70% (KOH+MeOH) + 30% distilled water. The optimum temperature used was 50 ºC. The optimum time range used was 30 minutes. Biodiesel was not detected in chicken feather but was detected in chicken fat which component detected were from C14 to C20. Glycerin extracted from chicken waste was used to produce bioplastic and the results shows that sample contains 80% v/v of glycerine shows the highest tensile strength. The study shows that chicken waste is a good alternative source for bioplastic and biodiesel production and reduce the waste in the chicken farm industries.

Keywords: biodiesel, bioplastic, chicken waste, chicken feather, ultrasonic

1. Introduction
Biodiesel can be utilized as a fuel or mixed with petroleum based diesel. The upsides of biodiesel are available in its nontoxic nature, biodegradability, and insignificant substance discharges qualities. Biodiesel likewise benefits the earth by helping carbon dioxide reusing over short periods. Biodiesel can be produced by the transesterification process of triglycerides (from animal fats or vegetable oils) and alcohol with the presence of a proper catalyst. The raw materials employed in biodiesel production are usually categorized into animal fats, vegetable oils, and waste oils. Clean vegetable oils such as palm oil, rapeseed oil soybean oil, cottonseed oil and sunflower oil, are the most commonly used feedstock for biodiesel production. However, the work of edible vegetable oil sources serves to expand the generation cost as well as straightforwardly compete with the human food supply. It is estimated that the cost of raw materials represents to around 85% of the cost of biodiesel production. Therefore, the improvement of cheap, high quality, and excellent feedstock from waste by product is viewed as significant for the biodiesel business progress. The main raw material for biodiesel production are usually vegetable oils or animal fats, methanol and catalysers-are delivered and stored in the raw materials handling area. Different feed stocks produce biodiesel with well define qualities that must be considered when blending biodiesel with petroleum diesel for use in transportation and also in some other technologies [1].

Chicken waste is an alternative source or raw material for the process of producing biodiesel and bioplastic. Chicken feathers are waste products of the poultry industry. Expanded social process and increase in population has led an increase demand for biodiesel fuels. The outcome

is the costs of fuels are arriving at high consistently. Utilizing low cost feed stocks, for example, rendered animal fats in biodiesel production will decrease biodiesel uses. Poultry feathers is one of the low cost feed stock to use production of biodiesel. According to Mahidin (2018) from the Department of Statistic Malaysia, the demand for the livestock sub-sector chicken recorded the highest number of 308.3 million as compared to other livestock in 2017 which were followed by duck (10.4 million) swine (1.6 million) and goat (0.7 million) [2]. Thus, this big number of demand and supply shows that how much the waste of chicken feather or chicken waste would be if it not managed wisely.

This paper concludes another and environmentally friendly process for creating biodiesel production innovation from chicken feather meal generation in poultry industry. Chicken feather meal is handled at high temperatures with steam. This feather meal is utilized as animal feed and furthermore as fertilizers. Chicken feather meal has high percentage of protein and nitrogen. A lot of waste feathers in billions of tons were generated each year by poultry department and it was a great solid waste problem. Chicken feather meal has a 12% fat content, which could be used as non-food feedstock to make biodiesel [3]. The objectives of this study are to extract biodiesel and glycerin for bioplastic product from chicken waste using ultrasonic extraction and transesterification method and to analysis the properties of biodiesel and glycerin extracted from chicken waste.

2. Materials and methods

2.1 Preparation of raw material

The chicken feather which is rich in nitrogen and protein is suitable for the biodiesel extraction. Survey done among the types of chicken and suitable type of chicken feathers collected for the extraction process. Chemicals used such as potassium hydroxide and methanol where obtained from chemical store at Politeknik Tun Syed Nasir (PTSN) laboratory. The collected chicken feather was washed with distilled water a few times until it was clean and not smelly. After washed, chicken feather was then dried in the oven at 60 ºC for 24 hours. The dried chicken feather then grinds to produce smaller particle size of chicken feather.

2.2 Extraction of biodiesel by transesterification process

Extraction of biodiesel from chicken feather can be done manually by using few types chemicals such as potassium hydroxide and methanol by using some apparatus at appropriate temperature and pressure. Ultrasonic extraction process is an alternative green extraction methodology which use milder temperature and shorter time compare to other extraction methods. Heterogeneous catalysts can be used to separate more easily from reaction and less harsh reaction conditions than the supercritical methanol process. Ultrasonic process is interesting because the possibility to apply it for the transesterification of chicken feather to produce biodiesel. Procedure of ultrasonic extraction from chicken feather/ feather meal is shown in Figure 1 while procedure of ultrasonic extraction from chicken fat is shown in Figure 2 which includes the following steps:

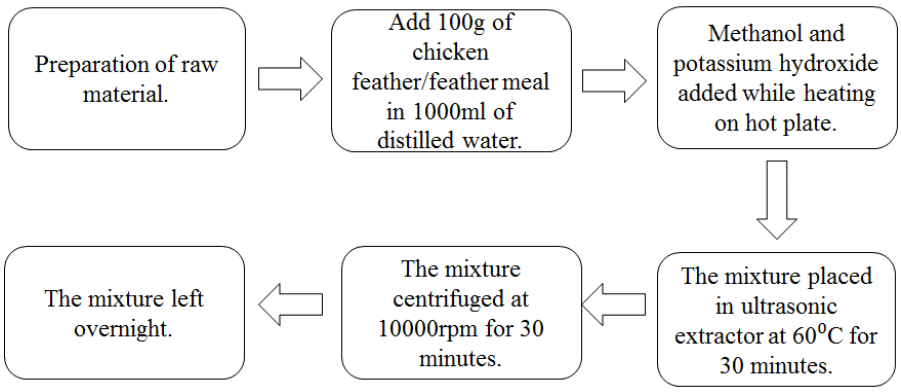

Figure 1: Procedure of ultrasonic extraction from chicken feather/ feather meal

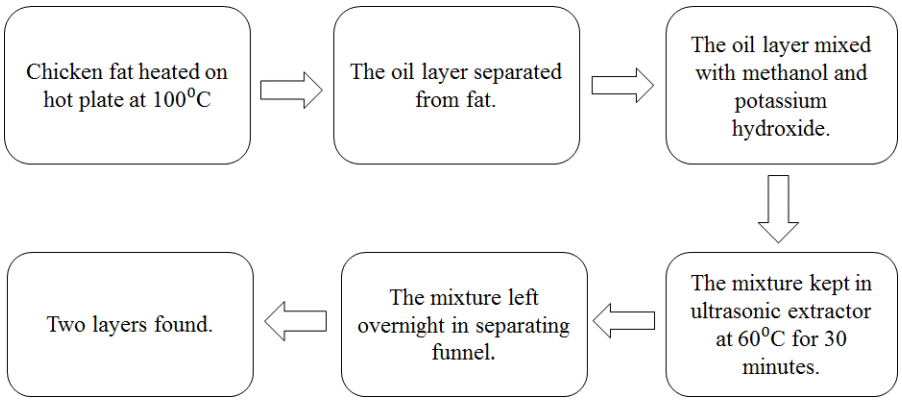

Figure 2: Procedure of ultrasonic extraction from chicken fat

2.3 Separation process

The sample was prepared and it was left in the separating funnel for overnight. The separating funnel was closed with the aluminium foil to prevent any impurities or other material entering inside sample. The next day, two layers was form which is glycerine and biodiesel. The glycerine and biodiesel was separated in a beaker. Moreover, the glycerine and biodiesel was sampled in falcon tube and labelling each one of the samples. After the separating process, the glycerine and biodiesel were collected separately for gas chromatography and fourier transform infrared spectroscopy analysis process.

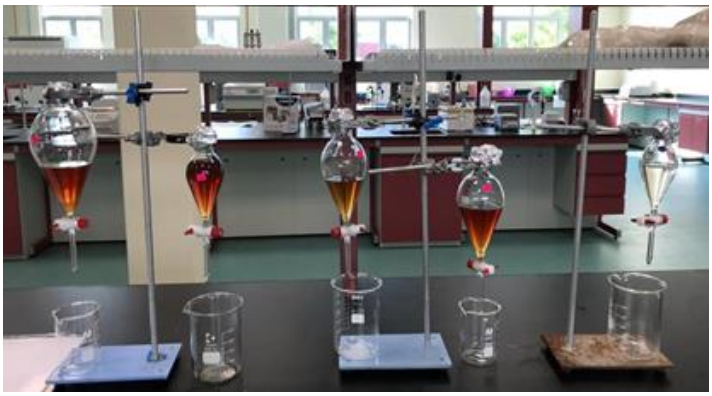

Figure 3: Biodiesel (up layer) and glycerine (bottom layer) were separated by using separating funnel.

2.3 Analysis

The data that resulted from gas chromatography (GC) reveals portion for fatty acid compositions is meristic acid, palmitic acid, stearic acid, oleic acid, linoleic acid and arachidonic acid [3]. GC is used to describe the group of analytical separation techniques that analyse volatile substances in the gas phase. 10 samples were sent to UTM Pagoh for analysis. The methods used was from Pinesi et al. [4]. The model used was Agilent Technologies (USA)7890b, test method was fatty acid methyl ester (FAME), the flow rate was 0.1 – 0.3 mL/min, the carrier gas was helium, hydrogen and nitrogen and the temperature used was -20 to 325 °C Meanwhile, for fourier transform infrared spectroscopy (FTIR), The model used was Brand Thermo Scientific Model 1sf, test method was Fatty Acid Methyl Ester (FAME), the flow rate was 50 mL/min, the carrier gas was nitrogen and the temperature used was 25 °C-175 °C.

3. Results

The results were obtained based on observation and analysis from different raw materials which were chicken feather, chicken feather meal and chicken fat. The paramaters studied were compositions of solvents and temperature.

3.1 Observation data

The tables show the physical observation of final product from chicken waste after ultrasonic extraction process and separation process, where Table 1 shows the observation from chicken feather, Table 2 shows the observation from chicken feather meal and Table 3 shows the observation from chicken fat.

Table 1: Result for chicken feather based on observation

Composition	Observation	Reason
30% (KOH+MetOH)	Two layer formed Light brown solution formed	Percentage of chemical used is less
70% (KOH+ MetOH)	Two layer formed Dark brown solution formed	Percentage of chemical used is more
100% distilled water	No layer formed White solution formed	No reaction occurred because no chemical were used.

Table 2: Result for chicken feather meal based on observation

Composition	Observation	Reason
30% (KOH+MetOH)	Dark brown Layers found	The speed of rate of reaction decreases when the concentration of potassium hydroxide and methanol decrease.
70% (KOH+ MetOH)	Dark brown Layers found	The more the concentration of potassium hydroxide and methanol, the more the rate of reaction.
100% distilled water	Dark brown Layers found	The reaction take place very poor because absence of concentration of potassium hydroxide and methanol in solution.

Table 3: Result for chicken fat based on observation

Temperature	Observation	Reason
50 °C	Clear biodiesel formed Yellow colour	Short time taken for oil to be formed.
60 °C	Very clear biodiesel formed Bright yellow	Shorter time taken for oil to be formed.
70 °C	Crystal clear biodiesel formed Clear and bright yellow	Shortest time taken for oil to be formed.

3.2 Bioplastic from glycerine

Glycerine that used in this experiment is our final product from the extraction of chicken feather. Glycerine is the bottom product of the experiment. Table 4 shows the results based on observation after glycerine is added to produce bioplastic. It shows that as the percentage of glycerine increase, the thickest of the sample also increase and the flexibility of bio product also increased. Meanwhile, the results of strength test are shown in Table 5. The trend of strength test also same, which as the percentage of glycerine increase, the tensile strength of the sample also increases.

Table 4: Result for chicken fat based on observation

Material	Sample 1	Sample 2	Sample 3	Sample 4
Distilled water	100 ml	100 ml	100 ml	100 ml
Corn starch	20 g	40 g	60 g	80 g
Glycerin	20 ml	40 ml	60 ml	80 ml
Vinegar	10 ml	20 ml	30 ml	40 ml
Observation	The sample is in a thick form. Clear colour.	The sample is in a thicker form. The product is flexible. Clear colour.	The sample is thicker. The product is flexible. Clear colour.	The sample is the thickest. Highest tensile strength. The product is more flexible. Clear colour.

Table 5: Strength test result for bioplastic

Volume of glycerine in bioplastic (%v/v)	Sample weight (g)	Sample area (h × w)	Tensile strength (N/m^2)
20.00	90.00	A= 22.50 cm^2	4.00 g/cm^2 392.266 N/m^2
40.00	200.50	A= 22.50 cm^2	8.90 g/cm^2 872.791 N/m^2
60.00	300.00	A= 22.55 cm^2	13.30 g/cm^2 1304.28 N/m^2
80.00	700.00	A= 22.55 cm^2	31.10 g/cm^2 3049.87 N/m^2

4. Discussion

4.1 *FTIR result of chicken feather meal*
From the result of water base sample, 79.46% of aliphatic ammonium carboxylic acid Salts was detected. This sample is fully based on water. No solvent was added in the sample. Usually the present of solvent will increase the reaction in the sample and make the sample to separate into two layers. But in this case, the solvent was not added and no reaction was occurred in this sample. So it could not reach the peak of biodiesel. From the result of second sample which is 30% KOH solvent, 79.01% aliphatic carboxylic acid salts were detected. This sample was prepared based on 30% KOH + 70% water. Solvent that added slightly increase the reaction of the sample. From the result of third sample which is 70% KOH solvent, 81.57% aliphatic carboxylic acid salts were detected. This sample was prepared based on 70% KOH + 30% water. Solvent that added increase the reaction of sample faster compare to other two sample. This is because the percentage of potassium hydroxide used in this sample is high compare to other sample. Potassium hydroxide was used as catalyst in the sample and its increased the reaction of the sample.

4.2 GC result for chicken feather meal.
Based on the gas chromatography result, it shows that biodiesel and glycerine was not detected. The test method that used in gas chromatography is fatty acid methyl ester (FAME). Biodiesel and glycerine could not detected because the yield percentage of biodiesel in chicken feather meal was less.

4.3 FTIR result of chicken fat
For the chicken fat sample, the solvent concentration was constant which was 70% (KOH + methanol). Three sample was prepared which were 50, 60 and 70 °C. The fat was heated on a hotplate in 250 ml distilled water. Then, the fat layer was separated from the mixture and the solvent was added in the separated fat layer. From the result of first sample which was biodiesel 50 °C, 87.94% aromatic fluorine, 79.92% aliphatic ammonium carboxylic acid salts and 73.27% inorganic phosphates was detected. This sample had slightly reach the peak of biodiesel.

From the result of second sample which is 60 °C, 89.94% aromatic fluorine, 83.16% aliphatic carboxylic acid salts and 71.17% primary aliphatic alcohol was detected. The primary aliphatic alcohol functional group shows that the percentage of biodiesel might be more compare to previous sample. From the result of third sample is 70 °C, 99.73% aromatic sulfoxides, 94.78% olefins and 92.52% inorganic phosphates were detected. This the best result that compare to other two temperature result. This was because the peak of the FTIR graph was almost reach the biodiesel peak.

From the first sample of glycerine 50 °C, 83.40% aliphatic hydrocarbon, 81.43% aliphatic ethers, 81.43% aromatic sulfonic acids were detected. For the second sample of glycerine 60 °C, 83.99% aliphatic hydrocarbon, 78.80% aliphatic ether and 78.80% aromatic sulfonic acids were detected. From the third sample which is glycerine 70 °C, 95.01% aromatic fluorine, 70.00% aliphatic ethers and 70.00% aliphatic nitro compounds were detected. For the first two sample, the third highest functional groups same and third sample had different highest functional group. The third sample shows the best glycerine. This was because the peak of the FTIR graph is almost reach the glycerine peak.

4.4 GC result for chicken fat

Based on the gas chromatography result in Table 6, all the component in biodiesel and glycerine sample were detected. The component that was detected are C14 to C20. The standard of biodiesel contained C12 to C20. From this result, biodiesel and glycerine that got as final product closely achieved the standard of the parameter which known as FAME. The top layer of the sample after separation process was closely matched the properties of FAME or biodiesel, while the bottom layer was for glycerine for bioplastic production.

Table 6: GC result for chicken fat

		Standard	**Top Layer**			**Bottom Layer**		
		Biodiesel	**50 °C**	**60 °C**	**70 °C**	**50 °C**	**60 °C**	**70 °C**
C14:0	Myristic acid	0.989	0.84	0.7	0.34	-	0.35	-
C16:0	Palmitic acid	35.36	40.73	31.35	11.55	2.33	15.6	9.56
C16:1	Palmetoleic acid	8.13	8.79	0.57	2.56	-	3.21	1.81
C17:0	Heptadecanoic acid	0.218	-	11.08	-	-	-	-
C18:0	Stearic acid	8.262	10.02	6.97	2.22	1.13	3.81	2.51
C18:1 CIS	Oleic acid	2.457	22.5	16.38	5.42	0.45	7.14	3.9
C18:2 CIS	Linoleic acid	35.38	42.18	2.09	0.68	0.94	12.16	7.87
C18:2 TRANS	Linoleic acid	35.38	2.65	27.96	11	-	-	-
C18:3n6 + C20	Gamma – linoleic acid arachidic acid	1.828	16.08	-	-	-	-	-
C18:3n3	Alpha – linoleic acid	0.504	0.86	1.94	0.8	-	0.86	0.59
C20:1	Cis – 11 elcosenoic acid	0.095	0.49	-	-	-	-	-

4.5 Bioplastic based on strength test

Glycerine that used in this experiment is our final product from the extraction of chicken feather. Glycerine is the bottom product of the experiment. The strength test result shows that, the 80 ml of glycerine produce the highest tensile strength bio plastic compare to other sample volume of glycerine.

5. Conclusions

This study concludes that chicken fats have enough potential to process them into biodiesel than chicken feather or poultry processing because of high fatty acid composition values and important properties of biodiesel. Chicken fats is very competitive to the chicken feather biodiesel by the presence of important fatty acids in chicken fats, it is suitable to produce more and potential biodiesel. The quality of obtained biodiesel samples was evaluated according to GC, FTIR and physical properties of biodiesel. Bioplastic was produced from glycerine. The quality of bio plastics was evaluated by strength test. The more the volume of glycerine added in a sample proved the better the strength and elasticity of bioplastic formed. Biodiesel was not detected in chicken feather but was detected in chicken fat. Glycerin extracted from chicken feather was used to produce bioplastic and the results shows that the sample of 80% v/v of glycerin gives the highest tensile strength. The study shows that chicken feather is a good alternative source for bioplastic and biodiesel production and reduce the waste in the chicken farm industries.

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Production of Corrosion Inhibitor by Using Piperine from Black Pepper (*Piper nigrum*)

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ABSTRACT
The objectives of this research were to produce anti-corrosion agent by using piperine extracted from black pepper and to compare the effectiveness of anti-corrosion coating based on different number of the coating layers. Corrosion is a general term used to describe various interactions between a material and its environment leading to degradation in the material properties. Interaction with oxygen can cause the formation of oxide layers via diffusion controlled growth. Corrosion inhibitors are defined by as 'a chemical substance that decreases the corrosion rate when present in the corrosion system at suitable concentration, without significantly changing the concentration of any other corrosion agent'. Most acid corrosion inhibitors are nitrogen, oxygen and sulphur containing organic compounds. Unfortunately, most of them are highly toxic to both human beings and environment. Hence, use of natural products which are eco-friendly are being used as corrosion inhibitors. In this research the piperine that was extracted from black pepper was used to coat the mild steel. The coated specimens with different number of coatings layers were tested by measuring weight loss after being immersed in the sulphuric acid for seven days. The results obtained from the test proved that the mild steel coated with most number of piperine anticorrosion coating had the least weight reduction after the immersion in the sulphuric acid. This showed that piperine was effective in protecting the mild steel from corrosion.

Key words: Piperine, corrosion, mild steel, black pepper

1. Introduction

Metal undergoes deterioration because it is exposed to acidic medium like sulphuric acid and hydrochloric acid which are normally used in industry for pickling and de-scaling of metals. Mild steel, a material of choice in fabrications of reaction vessels, storage tank and others, easily get corrode when exposed to acids. The reason behind this is because metals exist in unstable form and gain its stability by reacting with environment [1]. Mild steel industries encounter major problems in preventing corrosion that cost lots of money and resources. There are many ways for corrosion prevention such as by adding anti-corrosion chemicals [2]. The use of many inorganic inhibitors, particularly those containing phosphate, chromate, and other heavy metals, is now being gradually restricted or banned by various environmental regulations because of their toxicity and difficulties faced in their disposal especially in the marine industry, where aquatic life is at threat [3]. Synthetic organic inhibitors have also been extensively applied but their use is now being marred by their toxicity and high cost of manufacturing. According to a research, most of the anti- corrosion coatings that are found in market are expensive which approximately cost are more than RM50.00 per kilogram. The objectives of this study are to produce corrosion inhibitor by using piperine extracted from *Piper nigrum* and compare the effectiveness of corrosion inhibitor coating based on different number of the coating's layer. This research gives an overview on the inhibitive effect of natural extract which is black pepper

particularly for mild steel in acidic medium so as to provide industrialists with vital comparative literature for possible large scale use of natural inhibitors in their operations.

2. Materials and methods

There are many extraction methods to extract piperine such as accelerated solvent, solid-liquid extraction and solvent extraction method. Based on the observation, this study used the solvent extraction method by using soxhlet. This method is used because it is a simple technique and does not require large amount of solvent which is methanol. Moreover, soxhlet is also highly efficient as it extracts multiple times. The materials that are used in this study are black pepper, mild steel, distilled water, methanol and sulphuric acid.

2.1 Extraction of piperine

Black peppers were washed and dried. After it was dried completely, those peppers were grinded and crushed into powder form by using pestle and mortar. Those were kept sealed in a container. Then it was added into thimble inside soxhlet. The condenser was fixed on top of the thimble and an extractor was placed on top of condenser. The extractor's inlet was fixed with a tube that was connected to the pipe which allows water to flow in and the outlet with a tube that was placed in the laboratory sink. The boiling flask was filled with 500 ml of methanol that was measured using a measuring cylinder and was fixed at the bottom of condenser. The equipment was supported with retort stand. The boiling flask was heated using water bath. The piperine which was extracted after about 30 minutes flowed back into the boiling flask which contain methanol. The duration for the whole process to extract piperine was about 2 hours [4]. After two hours, the extracted yellowish green liquid which was filtered by pouring it through the filter funnel with cotton. Then, the piperine was washed with 10 ml of methanol though the funnel.

Figure 1: Extraction of piperine

2.2 Distillation of piperine

The mixture of piperine and methanol was poured into the distillation flask and magnetic flux was added into the mixture. The stopper was used to close on side of the distillation flask. The other side of the boiling flask was fixed with condenser. The condenser's inlet was fixed with a tube that was connected to the pipe which allows water to flow in and the outlet with a tube that was placed in the laboratory sink. The equipment was supported with retort stand. The distillation flask was heated using water bath. Once there was a small amount of piperine left

in the boiling flask, the process was stopped. Methanol was collected in a 100 ml beaker from the condenser.

2.3 Preparation of specimen and applying coating

Mild steel was prepared by cutting mild steel plate into pieces with dimension of 2 cm × 2 cm × 0.1 cm. The mild steels were divided into five pieces and initial weight were taken for each specimen. Mild steels were labeled with A, B, C, D and E respectively. All specimen was applied with coating of piperine except for specimen labeled A. Specimen B, C, D and E were applied with 5, 10, 15 and 20 layers of anti-corrosion coating respectively within two hours. All the specimens were immersed in beaker with 80 ml of H_2SO_4. After seven days, all mild steels were rinsed with distilled water. The weight of specimen was taken again.

3. Result and Discussion

After all the samples had been immmersed in the sulphuric acid for seven days, the reading of specimens were recorded and compared with the initial weight of specimens before being immersed in sulphuric acid. This was to compare the weight reduction percentage of the samples after corrosion process occured.

Table 1: Mass of specimen before and soaking in H_2SO_4

Number of coating	Before coating (g) (A)	After coating (g) (B)	After soaking in H_2SO_4 (g) (C)	Weight loss (g) (B – C)
0	4.9177	4.9177	4.8647	0.0530
5	4.9709	4.9709	4.9227	0.0482
10	4.6253	4.6253	4.5794	0.0459
15	4.8264	4.8265	4.7815	0.0449
20	4.7475	4.7476	4.7028	0.0448

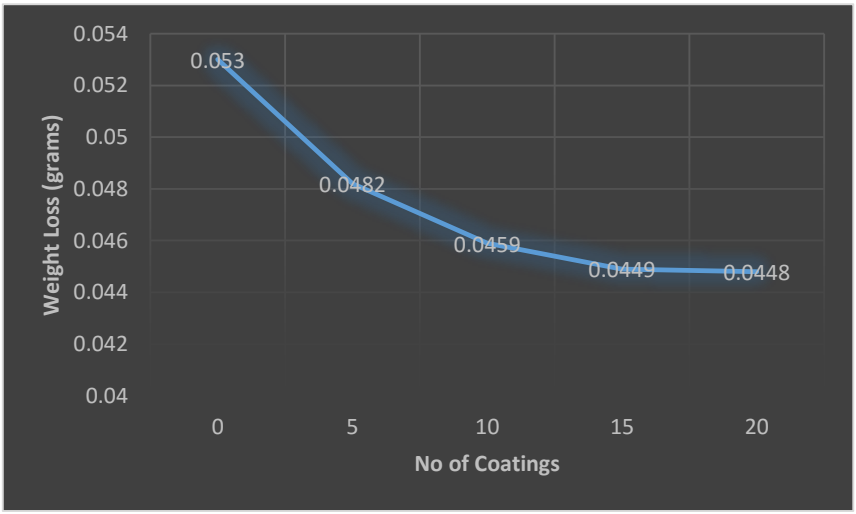

Figure 1: Weight loss difference against number of coatings

Figure 1 showed the weight loss difference of specimen A, specimen B, specimen C, specimen D and specimen E after being immersed in sulphuric acid. The horizontal axis represented the number of coatings and the vertical axis showed the weight loss difference of specimens. The specimen A without piperine coating has the highest weight loss which was 0.0530 g. Then, there was a decrease of weight loss in specimen B which had five coatings by 4.8×10^{-3} g. The weight loss of specimen C decreased by 2.3×10^{-3} g and specimen D's weight decreased by

1×10^{-3} g from the weight of specimen B and C respectively. The specimen E had the lowest weight loss which was 0.0447 g. The result showed that the most coating of piperine on a specimen had the lowest weight loss. The piperine coating was absorbed by the steel and formed layer on the surface of the specimen. The layer then prevented the contact of the acid to the surface of the steel.

3.1 Efficiency of inhibitor

Efficiency of inhibitor showed how good and effective the corrosion inhibitor. In general, the efficiency of an inhibitor increased with an increase in inhibitor concentration. The efficiency on an inhibitor can be calculated by using formula below:

$$\text{Efficiency of inhibitor} = \frac{(\text{weight loss without inhibitor} - \text{weight loss with inhibitor})}{\text{weight loss without inhibitor}} \times 100$$

Table 2: Efficiency of inhibitor for each specimens

Specimen	Before coating (g) (A)	After coating (g) (B)	After soaking in H_2SO_4 (g) (C)	Weight loss (g) (B – C)	Efficiency of inhibitor (%)
A	4.9177	4.9177	4.8647	0.0530	0
B	4.9709	4.9709	4.9227	0.0482	9
C	4.6253	4.6253	4.5794	0.0459	13
D	4.8264	4.8265	4.7815	0.0449	15
E	4.7475	4.7476	4.7028	0.0448	18

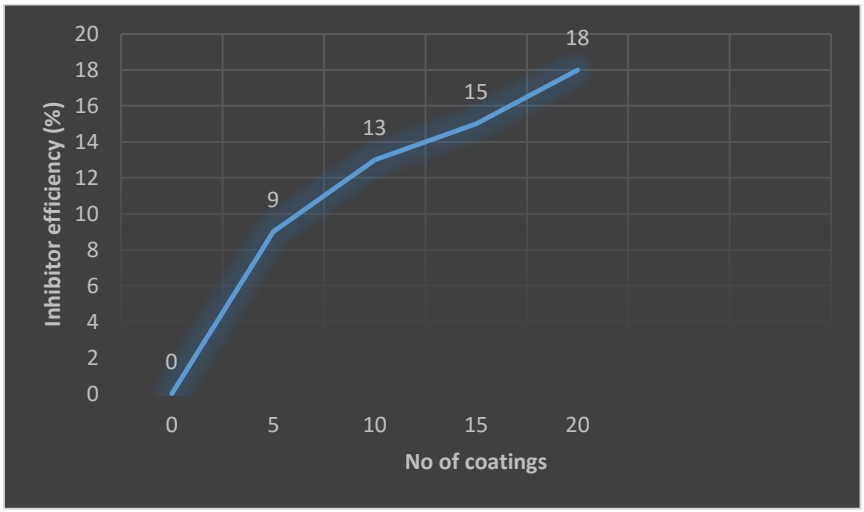

Figure 2: Inhibitor efficiency against number of coating

Figure 2 showed the inhibitor efficiency differences of specimen A, specimen B, specimen C, specimen D and specimen E after being soaked in sulphuric acid with, 5, 10, 15 and up to 20 coatings with increased of five coatings respectively. The horizontal axis represented the number of coatings and the vertical axis showed the inhibitor efficiency difference of specimens. The specimen A without piperine had no inhibitor efficiency. The specimen B with five layers of piperine had 9% percent of inhibitor efficiency. The specimen B with ten layers had 13% inhibitor efficiency and the specimen C with 15 layers had 15% inhibitor efficiency.

The specimen E with 20 layers of coating had the highest inhibitor efficiency which was 16%. The result showed the that weight loss of specimen E was the lowest compared to all other specimen in the experiment.

3.2 Corrosion rate

The rate of corrosion is the speed at which any given metal deteriorates in a specific environment. The rate, or speed, is dependent upon environmental conditions as well as the type, and condition, of the metal. To calculate the corrosion rate from metal loss, the given formula was used;

$$mm/y = 87.6 \times (W / DAT)$$

Where:
W = weight loss in milligrams
D = metal density in g/cm^3
A = area of specimen in cm^2
T = time of exposure of the metal specimen in hours

Table 3: Corrosion rate of each specimen

Specimen	Weight loss (mg)	Metal density (g/cm^3)	Area of metal (cm^2)	Time of exposure of metal (hours)	Corrosion rate (mm/y)
A	5.30×10^{-5}	7.85	6.25	168	5.633×10^{-7}
B	4.82×10^{-5}				5.123×10^{-7}
C	4.59×10^{-5}				4.878×10^{-7}
D	4.49×10^{-5}				4.772×10^{-7}
E	4.48×10^{-5}				4.761×10^{-7}

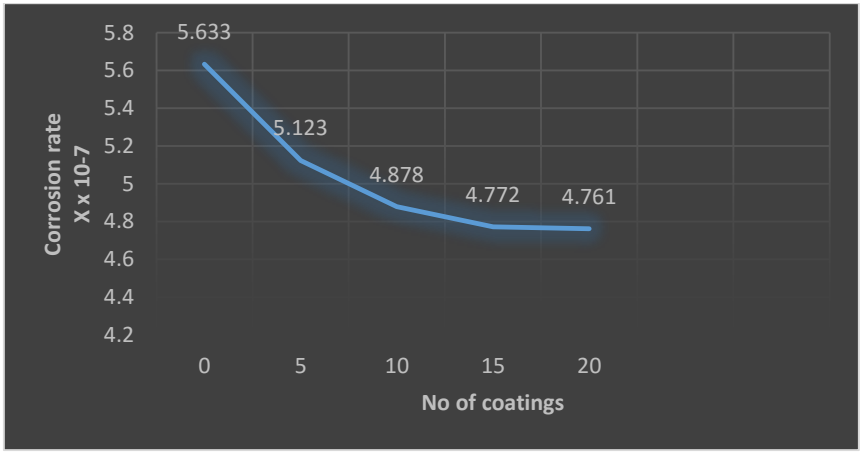

Figure 3: Corrosion rate against number of coatings

Figure 3 showed the rate of corrosion against number of coating for each specimen. Specimen A had no coating, specimen B had 5 coatings, specimen C had 10 coatings, specimen D had 15 coatings and specimen E had 20 coatings. The vertical axis represented the rate of corrosion and the horizontal axis represented the number of coatings applied on each specimen. According the graph specimen, A with no coating had the highest corrosion rate while specimen E had the lowest corrosion rate. Specimen B which had 5 coating hasdcorrosion rate of 5.123

$\times 10^{-7}$ mm/y. The difference between the corrosion rate of specimen C from specimen D would be 0.106×10^{-7} mm/y. The lowest corrosion rate would be 4.751×10^{-7} mm/y for specimen E which had 20 coatings on it. Thus, this graph showed that the more the layer of protection which was the coating of piperine, the less the corrosion rate occurred on the specimen.

4. Conclusions

Based on the result, the objectives of this project had been achieved. Corrosion inhibitor was produced by using piperine extracted from *P.nigrum*. The results showed that the more the number of coating on the mild steels, the less the weight loss and corrosion rate of mild steel plate. It had been proven as stated in results that the lowest weight loss and corrosion rate was specimen E compared to other specimens with less coatings. In term of inhibitor efficiency, specimen E showed the highest value of efficiency compared to other specimens. This was due to the adsorption of compounds elements in piperine on metals which forms a film that protected the surface and hinders corrosion. The protective layer protected from the contact of the acid to the surface of the steel [5]. In conclusion, piperine as a corrosion inhibitor successfully delayed the corrosion rate of mild steel.

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Synthesis of Natural Coagulant from Petai Belalang Peel

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ABSTRACT
Coagulation is the destabilization of colloids by neutralizing the forces that keep them apart. Cationic coagulants provide positive electric charges to reduce the negative charges of the colloids. The particles collide to form larger particles. Coagulation reagents are divided into two which are natural and chemical coagulants. Chemical coagulant such as aluminium chloride (Alum) widely used in industries and textile wastewater treatment plants but it produces negative impact onto human health and environment. Therefore, eco-friendly plant based coagulants been introduced as it is low cost and a safe method for textile wastewater treatment purposes. Petai belalang or scientifically known as *Leucaena leucocephala* is used to extract chitin which can be used as a natural coagulant in textile wastewater treatment field. The extraction of chitin is done by using various concentration of dilute hydrochloric acid (HCI) consisting of 1.0 M, 2.0 M and 3.0 M concentrations. The coagulation experiment using jar test were performed where the effect of coagulant dosage and the amount of color removal were examined. The sample will be sent into UV-Vis Spectrophotometer to analyze the absorbance and transmittance level of the sample thus finding the percentage of color removal through congo red (497 nm), methylene blue (609 nm) and pro indigo (602 nm) wavelength. From the observation, it was found that 30 ml of 3.0 M coagulant had the highest percentage of color removal from the sample under all three wavelengths. It can be concluded that 3.0 M solution was the most suitable for chitin extraction in synthesis of natural coagulant from petai belalang peel.

Keywords: Coagulant, textile wastewater, UV-Vis Spectrophotometer, petai belalang

1. Introduction

Waste water from textile industry contains non-gradable substances. Textile waste water causes a big environmental problem due to the huge amount of effluent generated from textile and dyeing processes. However, the main problematic pollutants from textile industries in aquatic environment are dyes mixtures. According to Yaseen & Scholz (2018) the direct discharge of dyes in concentration higher than 1 mg/l, treated or not could increase community complaints and concerns [1]. This is primarily due to the aesthetic problems linked to those dyes, especially for the non-acceptable colors of river such as purple and red compared to accepted colors such as green and blue. In addition, textile dyes in high concentration inhibits sunlight penetration, respiration activities and consequently upsetting the biological and photosynthesis processes in the aquatic environment. To sum up, waste water treatment and reuses of treated effluent in textile industry is a must.

The most vital element among natural sources is water. Natural organic matter is an important issue for water industries because it can cause the water to be colored, high chemical demand, lowers disinfectant residual and as a substrate for microbial growth in distribution system. The treatment of water most relies on coagulation, sedimentation, filtration and disinfection

process. Due to lack of proper water treatment system in rural or undeveloped communities, the best option is to use simple and relatively cost effective point of use technologies such as coagulant. According to Mengistie et. al (2008), number of treatment technologies has been used as water treatment such as filtration, adsorption, chemical precipitation, ion exchanges methods [2]. However due to its disadvantages for unable to removing heavy metal at low concentration and relatively high cost method. Therefore, coagulation which is simple and effective low cost method is desirable.

Natural coagulant also has been successfully used according to the past investigation where water clarification and reduction of microorganism organic matter removal by extraction of different plant material as natural coagulant [3],[4]. Polysaccharides behave as polyelectrolytes when charges are present and positively charged groups are ammonium groups while negatively charged groups are carboxylic groups or sulfate groups. The previous studies on nirmali seeds, *Moringa oleifera* and cactus show that plant based coagulant capable as natural coagulant [5],[6]. Chitin is second most abundant polysaccharide in nature and natural polymer that obtained by grinding the shells of shrimps and crabs. However, chitin also can be presence within the plant such as mushroom. Chitin and its derivatives are employed as chelating agent that used for waste water and water treatment by separating compounds and heavy metals and in sewage treatment isby precipitating certain anionic waste and trap pollutant. According to Rinaudo, chitin and its derivatives are applicable in many fields such as food, cosmetics, agriculture, textiles, waste water treatment [7]. The extraction of chitin has no effect to the ecosystem and contain all advantages provided by polysaccharides, considered that as the source of chitosan and both are bio-compatible bio-polymers for animal tissues with low toxicities.

Therefore, this study focuses on the extraction of chitin from petai belalang peel for development and natural coagulant. The main objectives of this study were to synthesis natural coagulant from petai belalang peel and to analyse the effectiveness of petai belalang coagulant in textile waste water treatment. Petai belalang or *Leucaena leucocephala* is a permanent non-climbing shrub tree which is wild and abundant in Malaysia. This plant is preferable since it is easy to find and grown naturally in Malaysia. This coagulant is used to analyze the effectiveness in removing color in the textile waste water sample. Sample waste water taken from textile company is tested to discover the percentage of color removal. Coagulation process which mainly about neutralization of colloid particles is been experimented through this research.

2. Materials and methods

2.1 Raw material collection

Petai belalang was collected at Educational Hub Pagoh area. Petai belalang was made sure dried before plucking it from the tree. It was dried naturally under sunlight for a week. The seed was removed from pods of petai belalang.

2.1.1 Grinding of petai belalang

Dried petai belalang was cut into small pieces and grinded using dry blender to mesh it. Then the meshed petai belalang was sent to the grinder mill to produce powder.

2.2 Preparation of dilute hydrochloric acid

The hydrochloric acid (HCl) was diluted in distilled water using $M_1V_1 = M_2V_2$ formula. The diluted acid consists of different concentration which are 1.0 M, 2.0 M and 3.0 M. The process

was conducted inside the fume hood which function to limit exposure to HCl fumes.

2.3 Sample textile wastewater
Textile wastewater samples was collected from an equalization tank of textile treatment plant of a dye mill in Batu Pahat, Johor.

2.4 Coagulant preparation
The petai belalang powder was added into three different concentrations of HCl solution consisting 1.0 M, 2.0 M and 3.0 M in three different beakers to determine the chitin. About 5 g of petai belalang powder was soaked and stirred until fully dissolved and become super saturated solution. The sample was filtered using filter paper to filter out the entire residue. The solution was dried using hotplate at 70 °C until all moisture evaporates. The chitin was form naturally as the moisture evaporated.

2.5 Coagulation process
In this experiment, jar test was conducted to determine the effects of different coagulant on color removal. A four-paddle stirrer with four beakers were used to conduct the jar test. Each beaker contains 150 ml of the textile wastewater sample. The initial pH of the sample was measured using a pH meter. Then, the coagulant was added to the beakers and the samples were mixed at 60-65 rpm for three minutes. When the formed flocs were allowed to settle, the respective settling time was recorded. The final pH for the solution was measured using a pH meter and the supernatant was taken for analysis.

The concentration of dye solutions was measured at a wavelength corresponding to the maximum absorbance by means of a UV-Vis Spectrophotometer. The percentage of color removal was calculated by comparing the absorbance value of supernatant to the initial value obtained before the experiment.

3. Results and Discussion

3.1 Observation of physical properties of three different sample from different concentration
As the different concentration of mixture was heated, only certain concentration of mixture managed to produce chitin. For 1.0 M, the chitin cannot be extracted from the mixture. Due to this, 1.0 M mixture was left behind from all the physical and chemical observation. 2.0 M and 3.0 M mixtures managed to produce chitin through the heating process. For 2.0 M concentration, all three samples managed to show the process of coagulation. Three samples consisting of 10 ml, 20 ml and 30 ml of extracted chitin and 50 ml of the textile waste water sample took an average of a day for the particles in the mixture to coagulated and settled down at the bottom of the container.

For 3.0 M concentration, the coagulation process occurred much more faster compared to 2.0 M sample concentrations. All three samples took an average of 3 hours only for the particles to coagulated and settled down at the bottom of the container. For odor properties, after mixing with different volumes of extracted chitin from each concentration, the bad odor of waste water sample had been removed during the coagulation process.

3.2 Absorbance level
Absorbance is a measure of the amount of light with a specified wavelength that a given material prevents from passing through it. 2.0 M and 3.0 M sample concentrations were used to determine the absorbance level of the mixture through different wavelengths.

Table 1: Absorbance value of the samples

Concentration of coagulant (M)	Absorbance value (Abs)			
	Amount of coagulant (ml)	Congo red (497 nm)	Methylene blue (609 nm)	Pro indigo (602 nm)
0	0	0.2784	0.1723	0.177
2	10	0.1136	0.0613	0.0645
	20	0.1037	0.0557	0.0571
	30	0.0955	0.0497	0.0520
3	10	0.0722	0.0307	0.0303
	20	0.1019	0.0551	0.0564

From Table 1, the 30 ml of extracted chitin recorded the lowest absorbance level for 3.0 M and 2.0 M sample concentrations. High volume of chitin affected the particles in the textile waste water sample to coagulated and the concentration of mixture decreased. This prove the Lambert-Beer law where the absorbance of the light absorbing material is proportional to its concentration in the solution.

3.3 Transmittance level

Transmittance is the ratio of the light passing through to the light incident of the specimens and the reflectance the ratio of the light reflected to the light incident. 2.0 M and 3.0 M sample concentration were used to determine the transmittance level of the mixture through different wavelengths.

Table 2: Transmittance value of the samples

Concentration of coagulant (M)	Transmittance value (T%)			
	Amount of coagulant (ml)	Congo red (497 nm)	Methylene blue (609 nm)	Pro indigo (602 nm)
0	0	53.726	69.108	68.633
2	10	78.916	89.176	88.400
	20	80.983	90.293	89.924
	30	84.634	93.629	93.371
3	10	84.725	93.768	93.670
	20	81.654	90.633	90.319
	30	85.952	94.377	94.022

From the Table 2, it had huge difference in transmittance level before and after mixing the coagulant with the textile waste water samples. As the amount of extracted chitin mixed with sample waste water increased, the transmittance level of the mixture increased.

3.4 The amount of color removal

The amount of color removal depends on the transmittance value of the coagulant before and after mixing with the textile waste water sample. High amount of color removal relates to the effectiveness of chitin on the textile waste water sample.

$$\frac{\text{Final transmittance value} - \text{Initial transmittance value}}{\text{Initial transmittance value}} \times 100$$

The formula above determined the calculation of the amount of color removal. Final transmittance value indicates the transmittance value after certain amount of coagulant added into the sample while initial transmittance value is the transmittance value without any

coagulant added to the sample waste water. Table 3 below shows the amount of color removal from samples.

Table 3: The amount of color removal from sample

Concentration of coagulant (M)	The amount of color removal (%)			
	Amount of coagulant (ml)	Congo red (497 nm)	Methylene blue (609 nm)	Pro indigo (602 nm)
0	0	0	0	0
2	10	46.89	29.04	28.8
	20	50.73	30.65	31.02
	30	57.53	35.48	36.04
3	10	57.70	35.68	36.48
	20	51.98	31.15	31.60
	30	59.98	36.56	36.99

3.5 Graph for amount of color removal according to wavelengths

3.5.1 The amount of color removal after treatment for congo red (497 nm)
The graph in Figure 1 displays the amount of color removal under congo red (497 nm) wavelength for 2.0 M and 3.0 M sample concentrations. The graph trend fluctuated as the amount of coagulant increased.

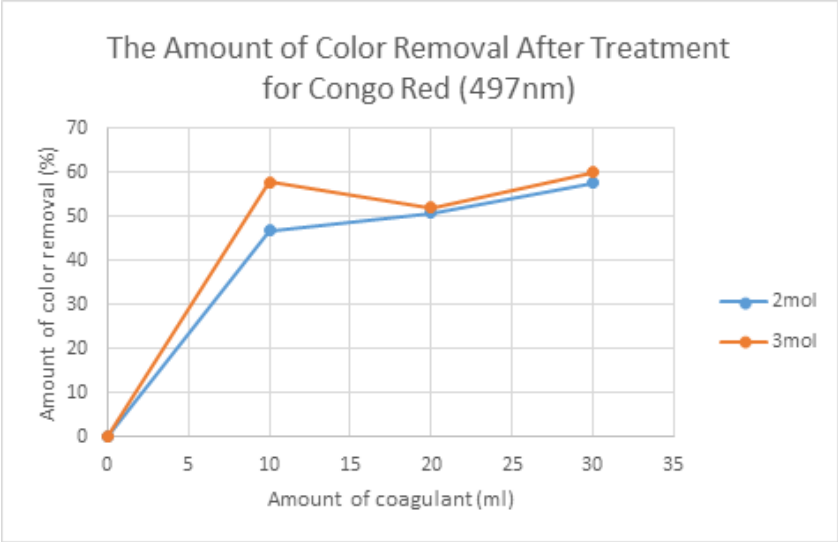

Figure 1: Graph of the amount of color removal after treatment for congo red (497nm)

For 2.0 M, the highest amount of color removal was 57.53% after 30 ml of coagulant was mixed with the textile waste water sample. The average of color removal for 2.0 M sample concentration was 51.71% under congo red (497 nm) wavelength. For 3.0 M, the highest amount of color removal was 59.98% after 30 ml of coagulant was mixed with the sample waste water. The average of color removal for 3.0 M sample concentrations were 56.55%. To sum up, the amount of color removal increased as the amount of coagulant mixed with the textile waste water sample increased.

3.5.2 The amount of color removal after treatment for methylene blue (609 nm)
The graph in Figure 2 displays the amount of color removal under methylene blue (609 nm)

wavelength for 2.0 M and 3.0 M sample concentrations. The graph trend fluctuated as the amount of coagulant increased.

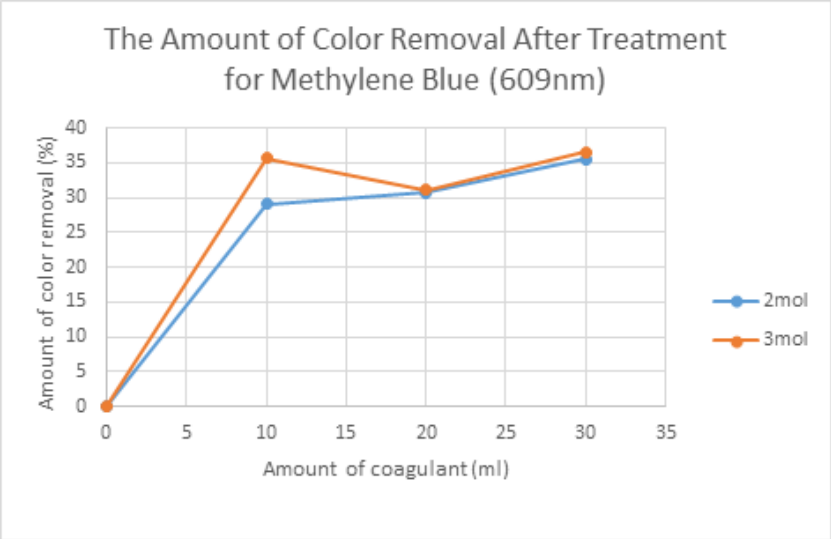

Figure 2: Graph of the amount of color removal after treatment for methylene blue (609 nm)

For 2.0 M, the highest amount of color removal was 35.48% after 30 ml of coagulant was mixed with the textile waste water sample. The average of color removal for 2.0 M sample concentrations was 31.72% under methylene blue (609 nm) wavelength. For 3.0 M, the highest amount of color removal was 36.56% after 30 ml of coagulant was mixed with the sample waste water. The average of color removal for 3.0 M sample concentrations were 34.46%. To sum up, the amount of color removal increased as the amount of coagulant mixed with the textile waste water sample increased.

3.5.3 The amount of color removal after treatment for pro indigo (602 nm)

The graph in Figure 3 displays the amount of color removal under pro indigo (602 nm) wavelength for 2.0 M and 3.0 M sample concentrations. The graph trend was fluctuated as the amount of coagulant increased.

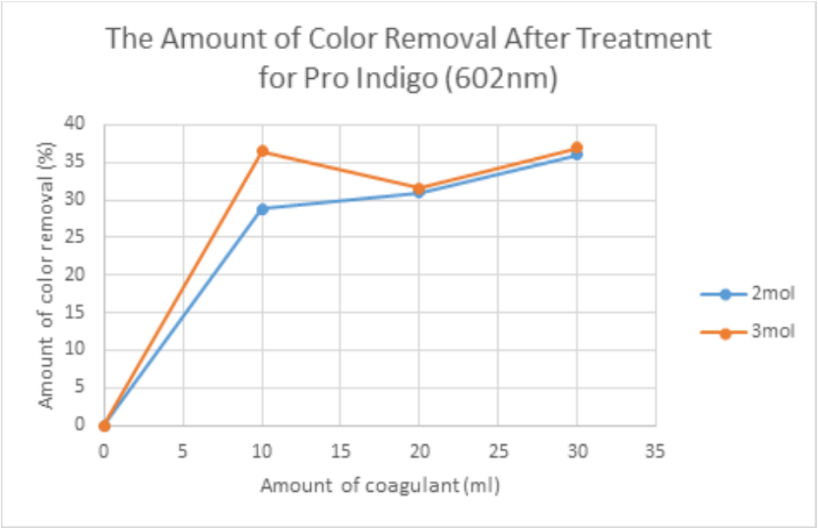

Figure 3: Graph of the amount of color removal after treatment for pro indigo (602 nm)

For 2.0 M, the highest amount of color removal was 36.04% after 30 ml of coagulant was mixed with the textile waste water sample. The average of color removal for 2.0 M sample concentrations was 31.95% under pro indigo (602 nm) wavelength. For 3.0 M, the highest amount of color removal was 36.99% after 30 ml of coagulant was mixed with the sample waste water. The average of color removal for 3.0 M sample concentrations was 35.02%. To sum up, the amount of color removal increased as the amount of coagulant mixed with the textile waste water sample increased.

5. Conclusions

In this study, it showed that among three wavelengths consisting congo red (497 nm), methylene blue (609 nm) and pro indigo (602 nm), the highest amount of color removal recorded in congo red (497 nm) wavelength at 59.98% from 3.0 M concentration sample of 30 ml of coagulant. For every wavelength, 30 ml of coagulant of 2.0 M and 3.0 M sample concentrations recorded higher amount of color removal compared to 10 ml and 20 ml of coagulant. In conclusion, the highest amount of color removal was achieved through congo red (497 nm) wavelength where high transmittance value when 30 ml of coagulant mixed with the textile waste water sample.

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Enhancing the Total Phenolic Content and Antioxidant in Dates Fruit by Applying UV-C Radiation

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ABSTRACT
UV-C radiation have promoted the antioxidant and bioactive compounds of fresh fruit and vegetables that are shown in several studies. The aim of this study was to enhance the total phenolic content and antioxidant by applying UV radiation in Medjool dates fruit, thus more bioactive compounds should be available for extraction. Small cube of dates was placed under a UV lamp and treated with different time while untreated was used as a control. The total phenolic content and antioxidant are significantly affected by UV-C radiation. The longer treatment time, the higher amount of total phenolic content and antioxidant activity. The UV radiation exposure are more effective at 180 seconds than other time limit to increase the antioxidant and total phenolic content in Medjool date fruits. The results of this study show that UV-C treatment has the potential to increase the extraction of bioactive compounds of dates fruits. Dates fruit is considered a source of antioxidant. Dates are important cause there are believe to be rich in nutrients and has many beneficial nutrients particularly polyphenols which have antioxidant properties.

Keywords: Medjool dates, UV-C radiation, antioxidant, total phenolic

1. Introduction
In arid and semiarid regions of the world palm date has been an important crop. Dates has always played an important role in the economic, social lives and nutritional of the people of these regions. The scientific name of Medjool dates is *Phoenix dactylifera* originates from its fruit which is phoenix from the Greek means purple or red and dactylifera refers to the finger like appearance of the fruit bunch. Medjool dates have a rich almost like caramel taste and a soft chewy texture. Dates have the highest concentration of polyphenols among the dried fruits. The most abundant secondary metabolites are phenolic compounds. Phenolic compounds are synthesized by plant as response to external stress such as wounding, parasites and predators, ultraviolet radiation and aggression by pathogens [2].

UV-C radiation has been applied to fruits and vegetables because it can prolong their storage shelf life cause UV-C is a non-ionizing radiation and electromagnetic. UV-C radiation also may promote the bioactive compounds and antioxidants apart from extending the shelf life of vegetables and fruits [3],[4]. The consumption of vegetables and fruit has many benefits like anti-inflammatory, anti-mutagenic, anticarcinogenic, antimicrobial, antibiotic activities and neuroprotective as well as reduction of cardiovascular diseases and cholesterol [5]-[9]. In addition, [10] showed that UV treatment has significantly increased the antioxidant capacity and total phenolic content of tomatoes compared to untreated sample.

The problem statement are diseases such as cancer, cardiovascular disease and Alzheimer's disease have increase over the year due to eating food that contain little amount of antioxidants.

Antioxidant which is synthetic are added into foods that don't normally contain to preserve the food. Next, phenolic and antioxidants is controlled by their volatility and decomposition at process temperatures. The objectives of this study are to evaluate the efficiency of using UV rays towards Medjool dates fruits. Besides that, to analyse possible correlation between the antioxidant activity and total phenolic content and to measure the value of antioxidant and total phenolic content increase when using UV radiation.

2. Materials and methods

2.1. Plant material

To prepare the Medjool dates sample, the dates were deseeded. The samples were cut into small cubes using sharp knife. Samples were placed on a tray to expose under UV radiation.

2.2. UV-C radiation exposure

The radiation of the UV light was 254 nm. The distance between the UV light and the dates on the tray were 22 cm. The time taken to exposed the dates were manipulated (0, 60, 120, 180, 240, 300, 360 seconds). For each second, 77gram of dates were exposed.

2.3. Extraction process

20 g of dates were mixed with 40 ml distilled water. The mixture was then blended. 1 g of samples was taken from the mixture and mixed with 5 ml of methanol and 100 ml of distilled water. The samples were then mixed in conical flask and were put on a hot plate to mix for one hour. Then, the samples were kept in a chiller for further analysis.

2.4. Total Phenolic Content (TPC)

2.0 ml of 10% Folin-Ciaocalteu reagent/ FCR and 1.0 ml of the sample extraction were mixed. The mixture was allowed to stand for five minutes followed by adding 2.0 ml of sodium carbonate (7.5% w/v) to the mixture. The mixture was then allowed to stand in the dark for one hour. The absorbance of the mixture against a blank was measured using spectrophotometer at 765 nm.

2.5. Antioxidant capacity

1.0 ml of sample was mixed with 2.0 ml of DPPH reagent and 1.0 ml of methanol. The mixtures then were mixed thoroughly. The mixtures were kept in a dark room for 30 minutes. The absorbance of the mixture was measured at 517 nm using spectrophotometer.

3. Results

3.1 Total phenolic content (TPC)

In the present study, six same samples which are Medjool dates fruit are exposed under UV light at different time were investigated and compare in the terms of their total phenolic content. The highest phenol content of Medjool dates fruit is at 180 seconds which was 83.0 mg GAE/ml extract at the concentration of 0.25 mg/ml. At 240 seconds, the phenolic content drop to 74.0 mg GAE/ml extract at the concentration of 0.25 mg/ml and increase back at 300 seconds which was 92.0 mg GAE/ml extract at the concentration of 0.25 mg/ml.

Table 1: Gallic acid equivalent

Concentration (mg/ml)	Gallic acid equivalent (mg GAE/ml)					
	0 seconds	60 seconds	120 seconds	180 seconds	240 seconds	300 seconds
0.05	21.0	27.0	31.0	35.0	30.0	37.0
0.10	27.0	37.0	43.0	49.0	46.0	52.0
0.15	33.0	38.0	51.0	56.0	53.0	64.0
0.20	46.0	52.0	58.0	65.0	57.0	74.0
0.25	47.0	58.0	76.0	83.0	74.0	92.0

The total phenolic content of the Medjool dates fruit has a significant effect when exposed under UV light. The Medjool dates fruit exposed for 180 seconds had the highest total phenolic content 0.192 absorbance while the control had the lowest 0.109 absorbance. The higher the absorbance shows the higher the amount of total phenolic content in the Medjool dates fruit. As stated by Kim. J et al. [11] had mention that electron beam radiation increases the total phenolic content. The increase of total phenolic content in treated Medjool dates fruit could be attributed to the increased activity of phenylalanine ammonium lyase. Phenylalanine ammonium lyase is an enzyme in a plant cell for phenolic compound. It will catalyses the conversion of L-phenylalanine to trans-cinnamic acid and ammonium which is an initial step for the synthesis of polyphenols. The Medjool dates fruit that are exposed too long under UV light which is more than 180 seconds will result in a reduction of total phenolic content. As overall, amount the different time of sates exposed under the UV light, at 180 seconds resulted the higher total phenolic content of the Medjool dates fruits compared to the control. The longer the time of Medjool dates fruit exposed under UV light the higher the amount of total phenolic content in the sample.

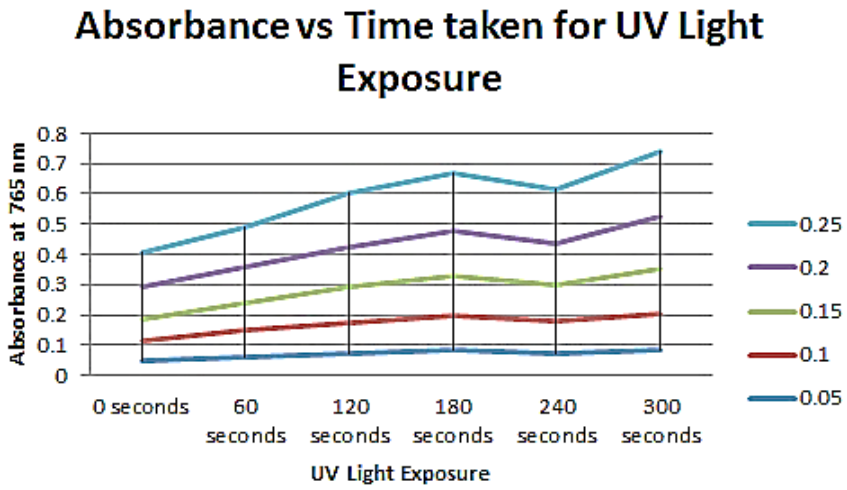

Figure 3.1: The effect of different UVC radiation on Total Phenolic Content.

3.2 DPPH radical scavenging activity

The effect of the UVC radiation on the antioxidant capacity of Medjool dates fruit was measured using DPPH since each antioxidant assay has its own advantages and limitations. Three types of antioxidants assay CUPRAC, FRAP and DPPH. The attributed that cause the increase of antioxidant enzyme, the compounds are flavonoids, phenols or other non-phenolic compounds such as enzymes. The antioxidant of the Medjool dates fruit has a significant effect when exposed under UV light. The Medjool dates fruit exposed for 180 seconds had the highest

amount of antioxidant 0.488 absorbance while the control had the lowest 0.354 absorbance at the concentration 1.0 mg/ml.

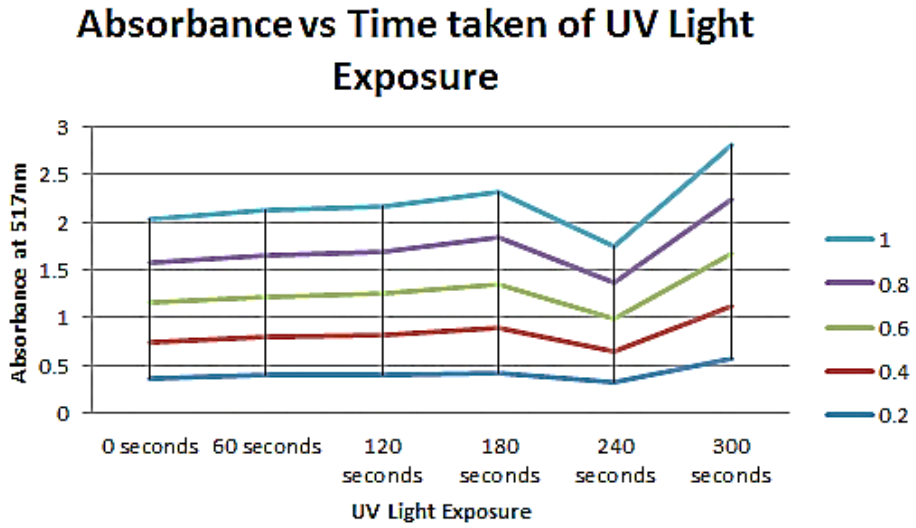

Figure 3.2: The effect of different UVC radiation on Antioxidant (DPPH).

The antioxidant activity has shown that the samples are exposed under the UV light for 180 seconds have less amount percentage of inhibition. The lower the percentage of inhibition the better cause free radicals are lesser. Free radicals can cause damaged to the cell. The higher of free radical, the more chance for cancer to happen in the body. The samples that are exposure for 240 seconds show the highest percentage of inhibition of DPPH. If the Medjool dates are exposed to long under UVC radiation free radicals tend occur a lot in the dates. It's dangerous for human to consume the food that has a lot of free radicals. At 300 seconds the percentages of inhibition have drop cause the reaction of the free radical and the antioxidants in Medjool dates fruit. This is because antioxidants can help to protect cells from damaged caused by free radicals.

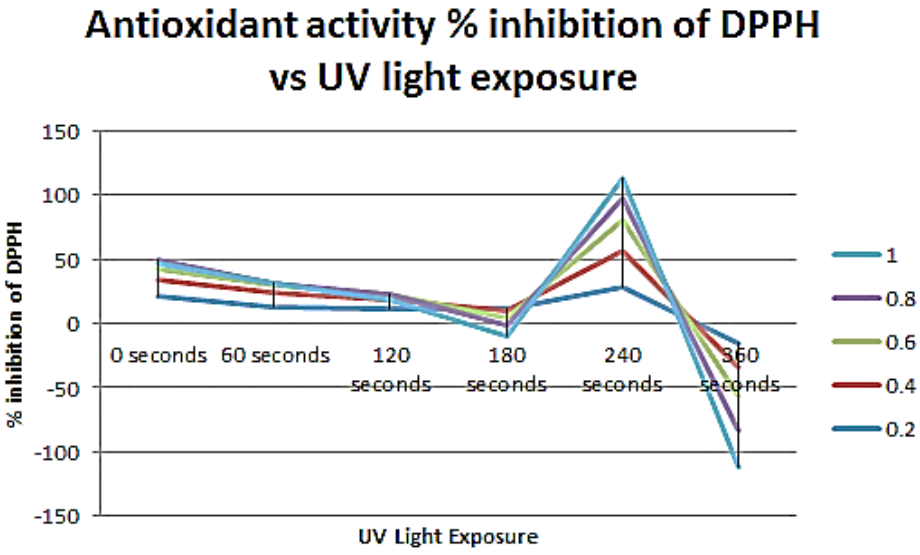

Figure 3.3: The percentage inhibition of DPPH activities of Medjool dates fruit.

5. Conclusions

It can be concluded that Medjool date fruit is a good source of natural polyphenolic compounds and have higher antioxidant potential. UVC radiation had a significant effect on the total phenolic content and antioxidant of Medjool dates fruit. From this analyze that 60 seconds to 180 seconds of exposure to UVC radiation promoted the accumulation of total phenolic content and antioxidant activity. The longer the treatment time, the higher the amount of total phenolic content and antioxidant.

At 180 seconds, the UV radiation exposure was more effective than other time limit to increase the antioxidant and total phenolic content in Medjool date fruits. UV radiation can activate the bioactive compounds in the dates fruit that can cause increases in total phenolic content and antioxidant. Further studies need to be conducted on the effect of food at certain point the graph of antioxidant and total phenolic drop and increase back. Three of our objective had been achieved which can conclude that UVC radiation did increase the amount of total phenolic content and antioxidant in Medjool dates fruits.

Acknowledgments
This research was supported by the Politeknik Tun Syed Nasir Syed Ismail.

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The Relationships Between the Levels of Basic Nutrients and the Variation of Charge Characteristic of Soil Minerals under Oil Palm Plantation

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ABSTRACT
Insufficient of nutrient availability to the plant is one of the important factor causing the loss in crop health and product yield. Nutrient availability to crops significant influenced by the total exchangeable cation in soil. The cations consumed by crops in the largest amounts are calcium (Ca^{2+}), magnesium (Mg^{2+}) and potassium (K^{+}). However, limited studies highlight the relationship between the nutrient availability with the exchangeable cation in soil specifically the soil in oil palm plantation. Cation exchange capacity (CEC) is the typical indicator to represent the ability of soils to retain the nutrients through the soil texture, organic matter content and the availability of total exchangeable cation in soil. Hence, this study aimed to study the relationships between the major exchangeable bases including Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} and the cation exchange capacity (CEC) using five different soil sample from different oil palm plantations in Malacca. The physio-chemical properties of these soil samples were analysed and their respectively correlation with CEC were also reported. From the analysed result, strong correlation between the exchangeable cation and CEC have been represented by a linear regression with Y=0.58+0.28X based on the selected soil samples.

Keywords: Nutrient availability, cation exchange capacity, exchangeable base, soil mineral, oil palm plantation

1. Introduction
The source of nutrient in soils consists of soil minerals, organic matter, adsorbed nutrients and dissolved ions. Soil base saturation is an important aspect of soil nutrient availability in terms of basic nutrient supply to all the living plants. Oil palm plants was found to be more sustainable with respect to maintain sufficient available nutrients in soil. This highly depends on the variation of the related cation exchange capacity in soil for supplying basic nutrients to oil palm growth. The major function of these cations is to balance the negative charges on the surface of soil minerals, so as to maintain a neutral charge balance [1].

Generally, oil palm plantations are grown in suitable soil that is characterized by soil minerals and organic matter inputs [2]. The clay minerals of soil have difference physical and chemical properties depending on the parent material from which they originated. The potential of soil to store chemicals is dependent on its textures and its organic matter content. Soil minerals also play as important role in soil fertility since those mineral surfaces serves as the potential sites for nutrient storage. However, the different types of soil minerals have different amounts of negative charge which hold and retain different nutrients levels.

In many oil palm plantation, the basic nutrients (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) are not applied regularly. The question to be answered is whether the available nutrient in existing soil still sustainable for optimal production of oil palm, even under limited supply of basic nutrient [3]. Therefore, the purpose of this study the relationships between the major exchangeable bases including Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} and the cation exchange capacity (CEC) using five different soil sample from different oil palm plantations in Malacca

2. Materials and methods

2.1 Soil Sampling

Soil samples were collected from five different of soil series which have variation of the cultivation area of oil palm in the state of Malacca. The soil series consists of Melaka series, Gajah Mati series, Terap series, Padang Besar series and Sedu series. The samples were collected by depths which are 0-10 cm, 10-20 cm and 20-30 cm. The soils were collected by soil auger and then air-dried before grinding. After grinding the soil was sieved with 2 mm sieve. The chemical and physical properties of soils was analyzed such as pH value, organic matter content and soil texture of soil samples are given in Table 1.

Table 1. Chemical and physical properties of soils

Series	Depth (cm)	pH	OM (%)	Soil Texture			Exchangeable bases (meq/100g)				CEC (meq/100g)
				Sand (%)	Clay (%)	Silt (%)	Na	Ca	Mg	K	
Melaka	0-10	4.48	0.63	70.04	20.96	9.00	0.27	1.11	0.89	1.38	8.13
	10-20	4.47	0.32	71.20	21.30	7.50	0.37	1.07	0.74	1.25	7.11
	20-30	4.45	0.30	66.68	20.32	13.00	0.38	0.86	0.66	0.96	6.66
Gajah Mati	0-10	4.48	0.86	70.04	19.60	10.00	0.33	1.10	0.83	1.81	10.67
	10-20	4.18	0.61	69.12	17.60	13.28	0.27	1.07	0.48	1.7	8.95
	20-30	4.12	0.32	65.40	16.60	18.00	0.31	0.42	0.26	2.34	8.45
Padang Besar	0-10	4.84	0.89	55.04	24.96	20.00	0.33	0.83	0.55	0.63	6.65
	10-20	4.78	0.60	61.40	22.60	17.00	0.38	0.75	0.24	0.52	6.05
	20-30	4.73	0.58	55.76	23.88	20.36	0.33	0.50	0.16	0.41	5.07
Terap	0-10	4.90	1.24	52.40	28.60	19.00	0.18	0.63	0.29	0.45	9.17
	10-20	4.85	1.01	48.04	29.96	22.00	0.22	0.57	0.27	0.67	9.89
	20-30	4.70	0.96	50.24	26.96	22.80	0.32	0.67	0.26	0.65	9.89
Sedu	0-10	4.12	1.46	35.68	17.32	47.00	0.45	2.12	0.66	1.18	9.36
	10-20	4.01	1.45	36.18	15.82	48.00	0.37	1.65	0.5	1.79	10.07
	20-30	3.74	1.20	38.40	22.15	39.45	0.63	1.54	0.75	1.54	13.41

2.1 Determination of cation exchange capacity

The cation exchange capacity (CEC) of soil was determined through extracting process by ammonium acetate ($NH_4C_2H_3O_2$) solution which used for exchangeable bases. The ammonium (NH_4^{+}) will substitute Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} in clay minerals. 100ml of ammonium acetate neutralized at pH 7 is pipette into a conical flask. 25g of soil was weighted and add into conical flask the solution of ammonium acetate and soil was shaken for 30 minutes using orbital shaker. After that, the soil that have been shaken was filter until it finishes by using the funnel, beaker and filter paper. 25 ml of the solution was taken for titration process by ammonium hydroxide solution which is to determine the exchangeable acidity when it neutralize the solution back to pH 7 and the balance of solution that filtered were keeping for continuing of determining exchangeable acidity by using Inductively Couple Plasma (ICP) measurement. ICP is use to find out the exchangeable bases (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) that contain in the soil solution was extracting by ammonium acetate. There are the basic nutrients that require by the oil palm tree for their growth development.

CEC = Total Exchangeable Bases + Total Exchangeable Acidity
Total exchangeable bases = $Ca^{2+} + Mg^{2+} + Na^{+} + K^{+}$

Example calculation of Exchangeable bases (**X**) from ICP data:

X meq/100 g = [EB mg/L / (atomic weight/charge of cation)] × (1/1000) × (100 ml/25 g) × 100 g soil

Total exchangeable acidity = volume of hydroxide (ml) × (0.2/25 ml) x (100 ml/25 g) × 100 g soil

2.2 Data analysis
The data analysis for this was present in regression and correlation which is to identify the relationship between two variables in a linear fashion. This relationship is between cation exchange capacity and basic nutrient levels (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) in different type of soil series and in variation of depth soil series.

3. Results

3.1 The relationship between basic nutrient level and cation exchange capacity
From the regression analysis was indicates that the relationship between total exchangeable bases (basic nutrients) and CEC is significant difference with the value of coefficient is positive, 0.279. Therefore, it indicates if the increase of total exchangeable base by one unit, it would increase the CEC by 0.279 units. From the linear regression analysis in Figure 1 show the equation which is Y=0.58+0.28X for the relationship of total exchangeable bases and CEC in different soil series. 0.58 represents the constant value when y is equal to zero. When the intercept in total exchangeable bases axis occur, the gradient value of linear graph is 0.28. Therefore, this equation was show the linear relationship between total exchangeable bases and cation exchange capacity that indicates low value of R^2 which is only 28.6 percent. From the Figure 1 also indicates this study between the relationship of total exchangeable bases and cation exchange capacity was split into two linear graph that indicates the other influences which contribute in variation of charge characteristics as reflected in CEC such as present of clay in soil texture from Table 1 that related in soil chemical and physical properties and soil mineralogy. Figure 2 was show the both of linear regression in relationship between total exchangeable bases and CEC. Therefore, it indicates that R^2 of both linear regression is high which are 84.6 percent and 99 percent between the relationship of total exchangeable bases and cation exchange capacity.

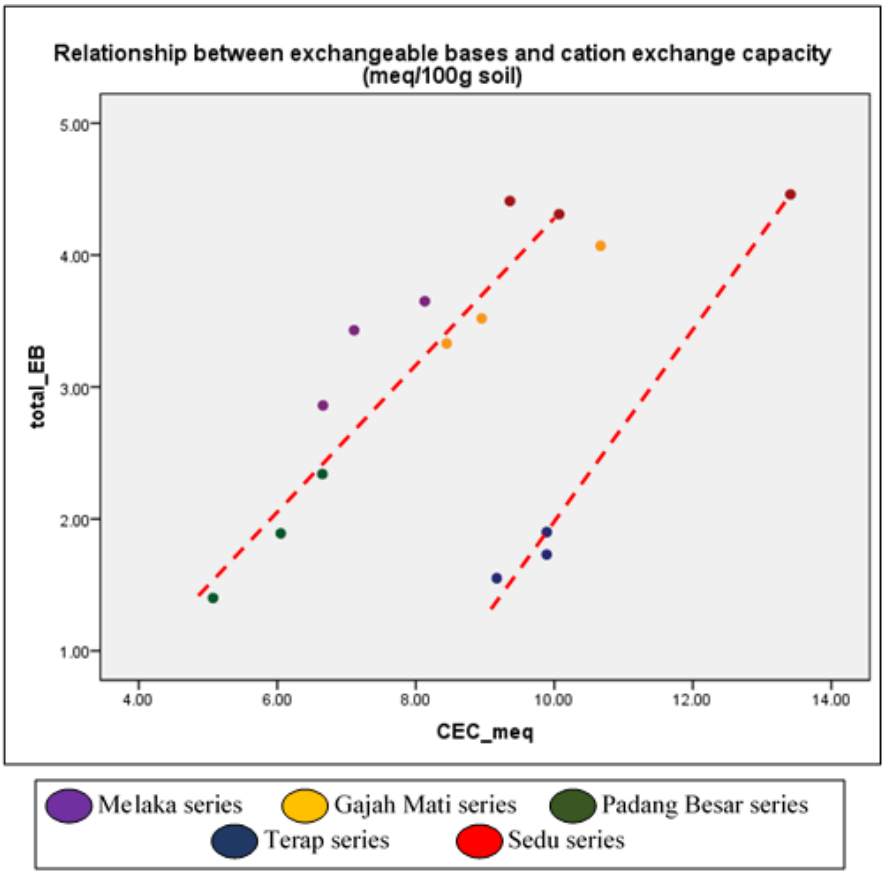

Figure 1: Relationship between total exchangeable bases and CEC

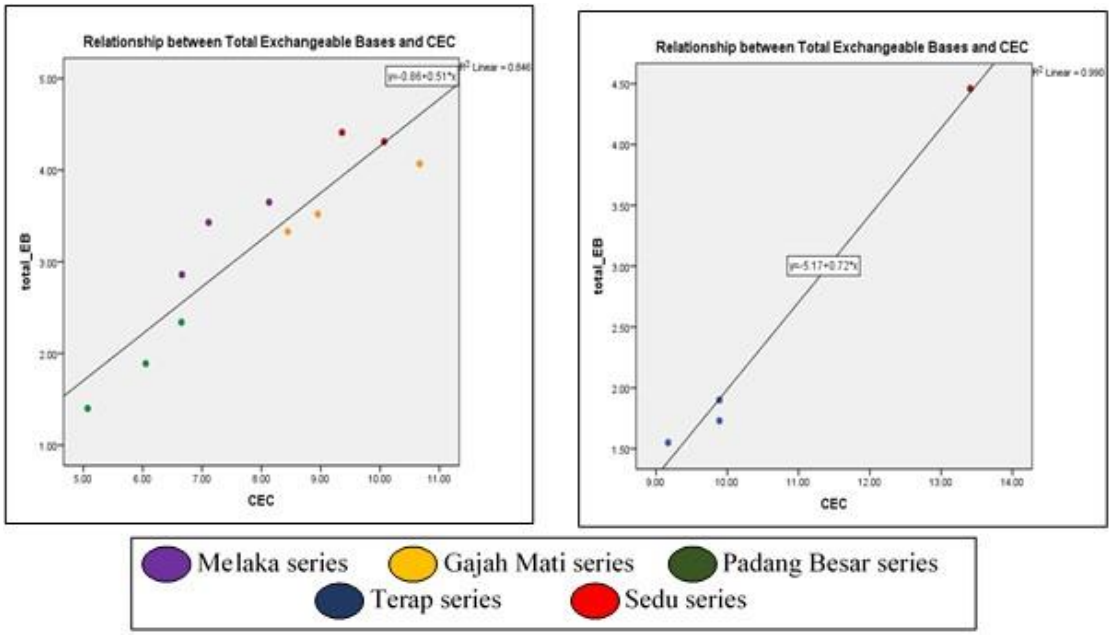

Figure 2: Linear regression between total exchangeable bases and CEC

4. Discussion

We are able to understand the relationship between basic nutrient levels and charge characteristics variations of soils under oil palm plantation. Based on the correlation analysis in the study, it was found that have significant relationship between soil pH, organic matter content, total exchangeable bases (basic nutrients) and cation exchange capacity. Therefore, this study was indicating that regression analysis of cation exchange capacity and organic matter content in different soil series is significantly difference. There have directly proportional between cation exchange capacity to organic matter content [4].

From the research objectives of study, it shows in regression analysis there have positive relationship between total exchangeable bases which is basic nutrient levels and cation exchange capacity of soils. Thus, the CEC of soils are representing the total amount of exchangeable cations (Ca^{2+}, Mg^{2+}, Na^{+} and K^{+}) that the soil can adsorb. The relative amount of each of the cations adsorb the surface of clay minerals is closely associated with important soil properties. The higher cation exchange capacity, the higher optimum level of nutrients, the more exchange sites that have to be satisfied [5].

5. Conclusions

As a conclusion, the relationship of total exchangeable bases which is basic nutrient level that require by oil palm with cation exchange capacity of soil can be represented using a linear regression models with Y=0.58+0.28X. The analyzed result also indicate that the soil organic matter content is most important factor to affect the soil of cation exchange capacity. The influence of fertilizer regime is highly recommended for further investigation as of it shown significant effect through supplied adequate nutrients to the soil.

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Simultaneous Deinking and Bioethanol Production of Office Paper Waste to Enhance Environmental Sustainability

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ABSTRACT
Approximately 65% of office paper waste could be recycled back as office paper while the other 35% which are not fit for recycling are disposed as office paper sludge. The decomposition of paper sludge releases chemicals and heavy metals that are toxic and hazardous to the environment. Office paper waste can be utilized as a carbon source, which is an important element for fermentation of glucose in bioethanol. The aim of this study was to investigate for the enzymatic deinking of the office paper waste to enhance decomposition of chemicals and heavy metals bioethanol production. The first phase of this research involved submerged cultivation of 1% (w/v) office paper sludge with a mixture of *Bjerkandera fumosa* DSMZ 4710 and *Trichoderma reesei* for deinking and cellulose degradation of office paper waste into simple sugar, respectively supplemented with 1% of yeast extract. White root fungus of *B. fumosa* DSMZ 4710 showed percentage of deinking of more than 99.25% and degradation of cellulose to glucose by *T. reesei* approximately 28.13 mM/ml from 1 % (w/v) of office paper waste. Furthermore, ethanol production using the hydrolysate by *Saccharomyces cerevisiae* was about 18.15 to 20.00 g/L with the productivity of 0.28 (g ethanol/L/h). The improved yields achieved through the pre-treatment and subsequent ethanol production suggested that the waste office paper could be a potential feedstock for the production of bioethanol.

Keywords: Deinking, office paper waste, bioethanol, white root fungus, *Trichoderma reese*

1. Introduction
Total solid waste including office paper wastes, e.g., news office papers and magazines are generated each year in Johor at an estimated 4-5 million tons in 2007 [1]. Studies has shown that the growth rate of solid waste generation will be increased 4.49% every year or even more due to increase of human population in the rural area especially for Johor Bahru. Normally, 65% of the office paper waste could be recycled back as office paper while the other 35% which are not fit for recycling gets disposed as office paper sludge [2]. Global capacity for bioethanol production from waste office paper/cardboard has been estimated at 83 billion litres per annum, replacing 5.4% fossil fuels with a greenhouse gases (GHG) emission savings of between 29% and 86%. Furthermore, the conversion of waste office paper into high quality biofertilizer also offers good business opportunities with great market potential. It is well known that the organic waste shall help maintain soil fertility and good yields via effects on physical, chemical and microbiologically [3]-[5]. The decomposition of paper sludge release chemicals and heavy metals which are toxic and hazardous to the environment. The office paper waste can be utilized as feedstock normally as carbon source that is an important element for fermentation of glucose in bioethanol because office paper primarily consists of cellulose, which is made of chains of β-glucose via glycosidic bonds [6]. The aim of this study was to investigate for the enzymatic

deinking of the recycle office paper to enhance the decomposition of chemicals and heavy metals for bioethanol production. *Trichoderma sp.* exhibits good cellulase production when office paper sludge is used as the carbon source for the process. This species should produce a complex mixture of cellulases, mainly comprised of β-glucosidases and endocellulase/carboxymethyl cellulase including other polysaccharide hydrolysing enzymes, such as xylanases, amylases and β-1,3-glucanases that contributes as plant growth promoters and biological control against plant root diseases. The enzymatic deinking of the office paper waste provides the additional benefits not limit to cellulose production but usable for agricultural practices since chemical and physical pre-treatments implicated high risk of environmental problems.

2. Materials and methods

2.1 Raw material

Office paper waste is collected from the administration office of Institute of Bioproduct Development. The office paper is then shredded using an office paper shredder before finely ground using a stainless-steel blender. The appropriate amount of water is added to make as office paper sludge.

2.2 Microorganisms

Bjerkandera fumosa DSMZ 4710, Cerrina unicolor WICC F38, *Phanerochaete chrysosporium* DSMZ 6909, *Phlebia radiate* DSMZ 2111and *Trichoderma sp.* were used in this study. The strain was activated in malt extract agar composed (g/L) of malt extract; 20, glucose; 20, peptone; 1, agar; 20. pH medium was adjusted to 5.5 before sterilization. The condition of cultivation was at 28 °C for five days. The grown colonies were preserved in 50% glycerol solution (v/v). The harvested cell suspension was pipetted in 2 ml cryovial (Nalgen Nunc. Int Rochester, N Y, USA) and the aliquots were stored at -80 °C ultra-deep freezer to minimize the productivity loss by subsequent cultivations of the cells.

2.3 Inoculum preparation

One of the vials from working cell banks of each strain was used to inoculate the malt extract agar for 28 °C for five days. Then, the mycelium plug was placed on the top of malt extract agar to develop as inoculum plate.

2.4 Screening of fungal strains with deinking ability

The mycelium plug of the fungus and *Trichoderma sp*. was placed on the top of malt extract agar which had added with 0.4, 0.6, 0.8, 1.0 and 1.2 % (v/v) of ink.

2.5 Fermentation media and cultivations

Saccharification process of office paper sludge from office paper waste as cellulosic biomass by *Trichoderma sp.* The cellulase production medium was comprised of different office paper concentrations (1.0, 1.5, 2.0, 2.5, 3.0 w/v %) of PS as a carbon source with different types of organic and inorganic nitrogen source were added to the hydrolysate and this mixture were used as the fermentation medium. The culture broth was centrifuged at 9,447 g and the supernatant was stored in a 4 °C refrigerator.

3. Results

3.1 Screening of fungal strains with deinking ability

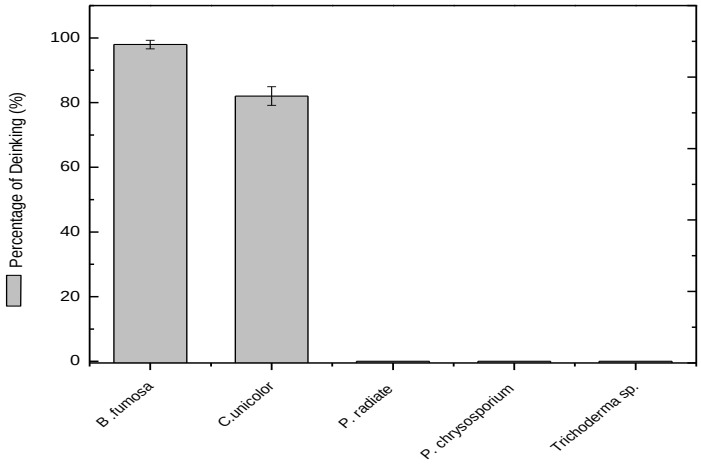

Figure 1: The percentage of deinking by different types of fungus

3.2 Screening of extracellular ligninolytic enzyme synthesis through submerged cultivation for cellulose degradation of office paper waste

Table 1. Extracellular ligninolytic enzyme synthesis

Fungal Strain	Enzymes Activity (U ml^{-1})			
	Lignin peroxidase	**Manganese peroxidase**	**Laccase**	**Cellulase**
B. fumosa DSMZ 4710	nd	24.71±0.67	74.80±4.12	0.504±0.25
Trichoderma sp.	nd	nd	nd	6.02±0.01

**nd – not detected*

3.3 Growth of Trichoderma sp. and production of glucose from office paper waste in different types of nitrogen sources

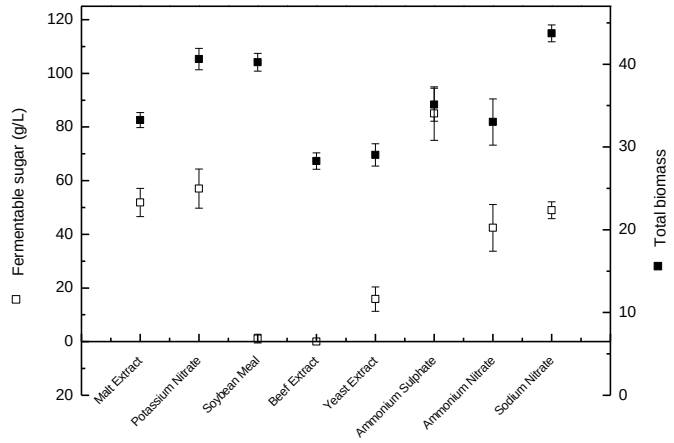

Figure 2 : Total fermentable sugar and cell growth by *Trichoderma sp*. in office paper waste

3.4 Conversion of cultivation broth of office paper waste to ethanol by Saccharomyces cerevisiae

Table 2. Initial glucose determination after cultivation of Trichoderma sp. in deinked office paper waste

No. of exp.	Absorbance value (OD) at 540 nm				Concentration of glucose(mM/ml)
	1	2	3	Average	
1	0.99	1.11	1.14	1.08	15.842
2	1.98	1.69	1.87	1.85	26.64
3	2.89	2.87	3.07	2.94	41.93

Table 3. Glucose and ethanol concentration after cultivation of *S. Cerevisae* in office paper waste (deinked paper waste sludge)

No. of exp.	Absorbance value (OD) at 540 nm				Glucose (mM/ml)	Ethanol (g/L)
	1	2	3	Average		
1	0.49	0.48	0.44	0.47	7.28	13
2	1.23	1.20	1.35	1.26	18.36	18.9
3	1.47	1.55	1.56	1.53	22.15	20

4. Discussion

The deinking capability by *B.fumosa* DSMZ 4710 was the highest with 99.25 % removal of color along with secretion of 75.5 U ml^{-1} and 24.5 U ml^{-1} of laccase and manganese peroxidase, respectively in the water to office paper ratio about 1.25 (w/v %) after five days of cultivation. Followed by *Cerrena* sp. WICC F38 about 84.07% and no deinking activity were detected by *Trichoderma sp* and others strains. Enzymatic deinking of office paper waste by *B.fumosa* was achieved due to synthesis of extracellular ligninolytic enzymes particularly manganese peroxidase & laccase. Production of glucose was the highest in *Thricoderma sp* cultivation with ammonium sulfate as the nitrogen source.

5. Conclusions

Office paper sludge had been chosen as fermentation medium for the cultivation of *Trichoderma sp* which aimed to optimize its cell biomass and cellulase production. Through the optimization process, 1% of office paper sludge with ammonium sulphate as the nitrogen source gave the highest cellulose activity. The comparison study between ammonium sulphate and inorganic nitrogen source of sodium nitrate demonstrated comparable cell growth and cellulase activity that may contribute to decrease the production cost. The findings suggest that this approach could also be useful for agricultural application owing to high minerals and enzymatic activities derived from the solid wastes produced in the end of the process.

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Intelligent Home 1.0 System Based on Arduino Platform

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ABSTRACT
The open-source hardware development platform Arduino has been growing in recent years. Nowadays, the Arduino platform has become one of the important parts in remotely control and monitoring of electrical devices (Home Automation) at home, office or company. Arduino platform has good specifications, cheap, easy to use and wide varieties of shields have been emerged with different purposes such as; Ethernet, GSM and Bluetooth support. This project was conducted using an Arduino micro-controller based system to remotely control and monitor electrical devices and sensors as power saving and security operations. With the help of the GSM and Bluetooth network, a mobile device can be used to control devices/sensors and receive alerts if there is a robbery and burglary. This system provides reduced power consumption and intrusion/alarm detections around the protected home. The system consists of two sides; the Arduino Micro-controller (AM-side) and the Mobile Phone (MP-side). The MP-side acts as a recipient to get responses from the AM-side as well as a controller for sending commands. While AM-side is responsible for reading/producing data signal/control signals from/to the devices/sensors. The Arduino Uno R3 is used in this system as a micro-controller. The SIM900 GPRS/GSM module was used to communicate between the micro-controller unit and the mobile phone unit. The system could be installed at any mobile phone supporting the SMS service. The system consists of some sensors, which are used as motion detector. The system has high potential to be implemented in residential house and agricultural facilities such as green house and indoor farming facility.

Keywords: Arduino, micro-controller, SIM900-GPRS/GSM

1. Introduction

Home automation involves the monitoring and control of activities such as lighting, air conditioning, electrical appliances, security cameras, door locks, and alarms. Home automation has various advantages, such as comfort, increased security, and energy efficiency [1]. This project also consists of designing the software part, the hardware part, interaction between software and hardware and output access via mobile Bluetooth controlling. By using the concept of Arduino Uno R3, we programmed the micro-controller to interact with the software and hardware so that we can develop a Home Intelligent system [2]. We programmed the micro-controller so that the micro-controller can responsible for reading/producing data-signals/control-signals from or to the devices/sensors. Not only that, we will make sure that the mobile phone we used can acts as a recipient to get responses from the micro-controller as well as a controller for sending commands [3].

Lately, home-breaking cases are rising, as well as the rate of electricity wastage in our country. The home-breaking case occurred because of the lack of home security systems that facilitated a thief to break in when there was no resident in the house [3]. This is also because homeowners are less sensitive to the existence of a thief who intends to break into their homes when they

are absent from home. Some parties also often tell their absence at home on social sites which cause of the home-breaking case [6].

About the meaning of rate of electricity wastage in our country, the use of electricity exceeding the limit or rate should be. Electricity wasting has often occurred recently, it is because of many consumers in Malaysia who are not concerned about the efficiency of electricity use and ignore the habit of closing the lights while out of the room, kitchen, bed room and etc. Not just that, many consumers also often complain when electricity bills are rising due to electricity wastage [1].

Essentially, there are three main objectives for this project: 1) to make sure quick action is taken when intruder is detected, 2) to own one cheap and dependable house security system and home automation system, 3) to reduce the electricity wastage.

2. Materials and Methods

2.1 Arduino Mega 2560 R3

The Arduino Mega is a micro-controller board based on the ATmega2560. It has 54 digital input/output pins (of which 14 can be used as PWM outputs), 16 analog inputs, 4 UARTs (hardware serial ports), a 16 MHz crystal oscillator, a USB connection, a power jack, an ICSP header, and a reset button. It contains everything needed to support the micro-controller, simply connect it to a computer with a USB cable or power it with a AC to DC adapter or battery to get started.

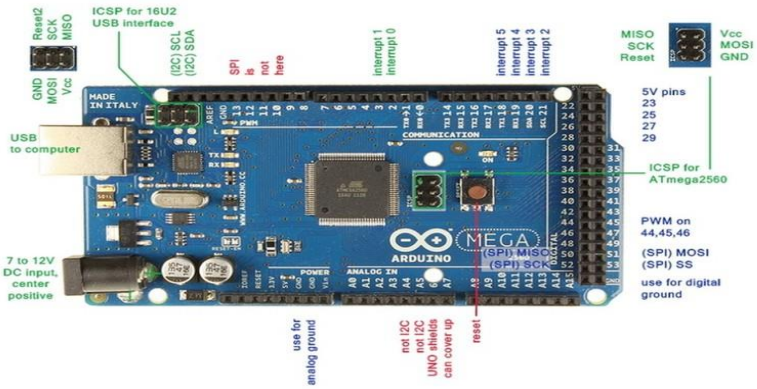

Figure 1: Arduino Mega 2560 R3

2.2 Bluetooth Module: HC-05

Serial port Bluetooth module is fully qualified Bluetooth V2.0+EDR (Enhanced Data Rate) 3Mbps Modulation with complete 2.4 GHz radio transceiver and baseband. It uses CSR Bluecore 04-External single chip Bluetooth system with CMOS technology and with AFH (Adaptive Frequency Hopping Feature). It has the footprint as small as 12.7 mm × 27 mm. Hope it will simplify your overall design/development cycle.

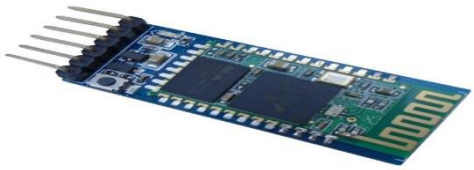

Figure 2: Bluetooth HC-05 Module

2.3 PIR Sensor

It allows you to sense motion, almost always used to detect whether a human has moved in or out of the sensors range. They are small, inexpensive, low-power, easy to use and don't wear out. For that reason, they are commonly found in appliances and gadgets used in homes or businesses. They are often referred to as PIR, "Passive Infrared","Pyroelectric", or "IR motion". PIR are basically made of pyroelectric sensor (which you can see above as round metal can with rectangular crystal in the centre), which can detect levels of infrared radiation. Everything emits some low level radiation, and the hotter something is, the more radiation is emitted. The sensor in a motion detector is actually split in two halves. The reason for that is that we are looking to detect motion (change) not average IR levels. The two halves are wire up so that they can cancel each other out. If one half sees more or less IR radiation than the other, the output will swing high or low.

Along with the pyroelectic sensor is a bunch of supporting circuitry, resistors and capacitors. It seems that most small hobbyist sensors use the BISS0001 ("Micro Power PIR Motion Detector IC"), undoubtedly a very inexpensive chip. This chip takes the output of the sensor and does some minor processing on it to emit a digital output pulse from the analog sensor.

For many basic projects or products that need to detect when a person has left or entered the area, or has approached, PIR sensors are great. They are low power and low cost, pretty rugged, have a wide lens range, and are easy to interface with. Note that PIRs won't tell you how many people are around or how close they are to the sensor, the lens is often fixed to a certain sweep and distance (although it can be hacked somewhere) and they are also sometimes set off by house pet.

Figure 3: PIR Sensor

2.4 Circuit Design with Fritzing Software

By using Fritzing software, we designed the circuit properly so that we can see how the wires were connected from components to breadboard, from breadboard to Arduino and from

component directly to Arduino. Figure below show the steps of making our circuit using Fritzing.

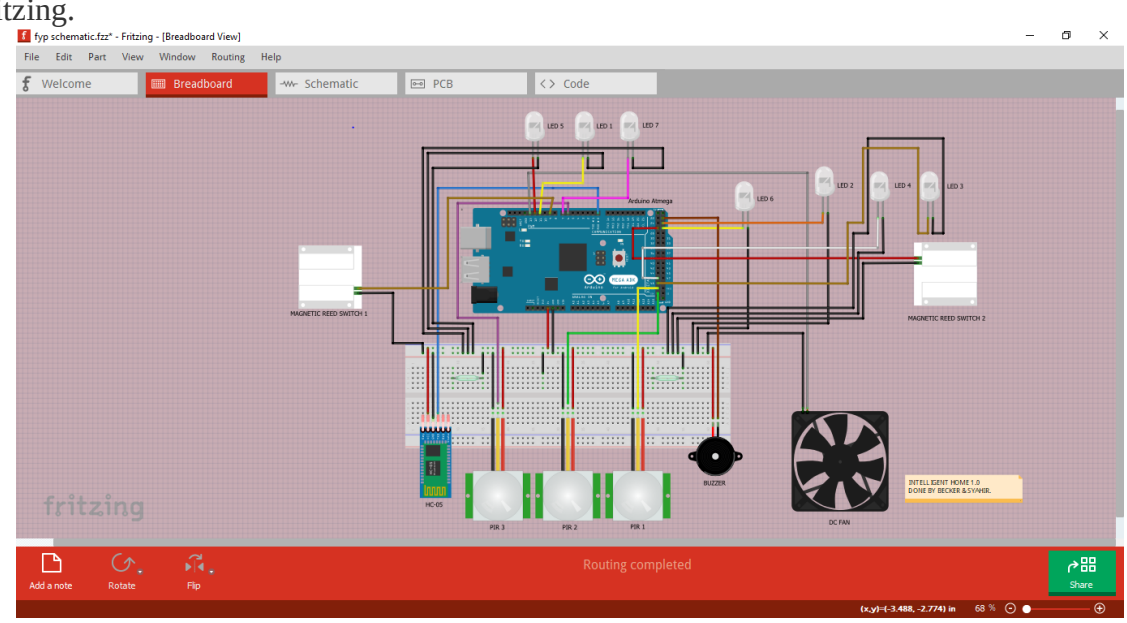

Figure 4: Final design of the circuit connection

3. Result

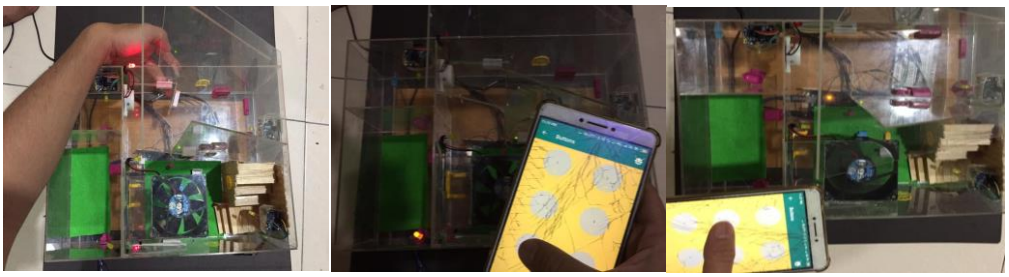

Figure 5: (a) When door is open, the buzzer will sound and the LED will light on as warning: (b) Controlling LED using Smartphone and Bluetooth connection (c) Controlling DC Fan using Smartphone and Bluetooth Conection

4. Discussion

We are able to manage and build a intelligent home by using Arduino Mega And was finally all the system is working as expected. All the cost we used to this project is almost the same as the predicted one. We also able to make the project affordable to anyone as the market price of home security and home automation is not less than a thousand ringgit Malaysia for the entire system.

We encountered lots of problems where we could not compile the source code for 2 or more sketches into one sketch in Arduino Software because it need correct code to combine 2 or more sketches into one source code. We spent more than a week to study about the solution to this problem and finally we found the way to solve it by ourselves.

The other issue we encountered was only one magnetic reed switch only will work even if we installed 2 or more magnetic reed switch at the same time. We tried to separate the output pin but we still encountered the same problem. After the research, we found out that each sensor must be named or specified correctly in the Arduino Software so that we can use as many sensor as we want in one complete circuit.

At the end of the project when we built the real prototype of house using Acrylic sheet and transfer all the wiring and connection as well as all components, we had a problem where the Bluetooth can not transfer the data from Android phone and the Arduino was not able to receive any command from Android phone. We also had problem like the PIR sensor was not working as per we seen at prototype house. After the troubleshooting of every component, we found out that our Breadboard is not working good anymore which causes low voltage flow from source to component. As soon as we troubleshooted these problems, finally our project able to work as we expect

5. Conclusion

It can be concluded that our Intelligent Home 1.0 project was a success. This project consists of Arduino, sensors, bluetooth module, LED, buzzer, and an app to control the light and fan. This project is very user-friendly where it is not going to harm the environment and human.

It's confirmed that our project has met our objectives where we:

a) Control the light and fan using smartphone
b) The light will turn on automatically when detect a human and turn off automatically when there is no detection.
c) The alarm will ring when the door is open by intruder and the red light will turn on automatically to warn the intruder.

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The Preparation of Pour Point Depressant of Waxy Crude Oil

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ABSTRACT
Waxy crude oil is usually a common problem that occurs at offshores and petroleum refinery plants especially for transporting issues. Today, the treatment of pour point reduction is by adding pour point depressant (PPD) to the waxy crude oil. The function of PPD is to enable the oil to remain fluid even at low temperature. In a precise way, PPD is a synthesis chemical which prevent coagulation. It offers effective and economical alternative way for dewaxing. The main reason of PPD is that it reduces the pour point depressant for waxy crude oil. Besides, it is a medium to lower the energy consumption as well as to ensure safety and cost effectiveness in pipeline transportation of the waxy crude oil. Pour point of waxy crude oil is found by the observation of pouring properties of waxy crude oil based on the reduction of temperature. After waxy crude oil could not be poured, the last temperature is recorded as the pour point. The excellent PPD is determined by the lowest pour point compared to other PPD results. Based on results are obtained, PPD of waste palm oil showed the excellent pour point of waxy crude oil with 28 °C at 2500 ppm compared to both of plant oils. Other that than, PPD of waste palm oil also indicated the lowest specific gravity at 0.78 compared to others.

Keywords: Pour point depressant, waxy crude oil, pour point

1. Introduction

Crude oil is transported from deep inside the ocean to the ground by using pipes. Crude oils are either in liquid form or it is in waxy form. Crude oils in liquid and less waxy form are usually easier to be sucked using pipelines compares to the waxy ones. The transportation of waxy crude oil with the production causes the pipe to be clogged and it also damages the pipe [1]. The transportation of the waxy crude oil also requires heat to change chemical composition [2]. Nowadays, many techniques are applied for waxy removal but the injection of pour point depressant in the waxy crude oil is better compared to other methods [3]. The pour point depressant (PPD) decreases the formation of the wax precipitation in waxy crude oil that still make waxy crude oil pour at low temperature. The reason of PPD is applied because the wax precipitation in waxy crude oil causes several challenges during oil extraction and pipeline transport of waxy crude oils, including wax deposition, plugging of the pipeline and damage to the oil drilling equipment. These problems cause the transportation of the waxy crude oil also requires high maintenance and takes time to fix the pipe [4].

In this research, we study to prepare the pour point depressant (PPD) from three raw materials such as palm oil, coconut oil and waste palm oil. After that, PPDs are tested by American Society for Testing and Materials (ASTM) as standard test methods of D 97 – 05 D 5853 for Pour Point of Crude Oil. Lastly, the characterization of excellent PPD is revealed by ASTM D 1298 for determining the specific gravity of PPD.

2. Materials and methods

2.1 The preparation of pour point depressant (PPD)
In this study, PPD was prepared by using materials that can be gotten easily such as palm oil, coconut oil and waste palm oil. The PPD was produced by transesterification of fatty acid from each plant oils with ethanol in ratio 0.33 to form ethyl ether [5]. After that, PPD was collected by separating it from glycerol that also produced during this reaction.

2.2 The testing of pour point depressant (PPD)
In this study, waxy crude oil sample was obtained from Petronas Penapisan Melaka (PPM) that used as a testing sample. PPD was tested by using ASTM D 97 – 05 D 5853 test method for pour point of crude oils. In this method, waxy crude oil was heated at 50 °C for 24 hours until liquid medium formed. Then, PPD was injected in the waxy crude oil sample with various concentrations (100, 500, 1000, 1500, 2000 and 2500 ppm). The excellent PPD was decided by lowest pour point of waxy crude oil.

2.3 The characterization of pour point depressant (PPD)
The excellent of PPD was decided by pour point depressant testing result. This PPD was characterized by ASTM D 1298 to determine its specific gravity that using hydrometer as the measurement apparatus. The result was used to interprete the PPD mechanism in reducing the wax precipitation in waxy crude oil.

3. Results and Discussion

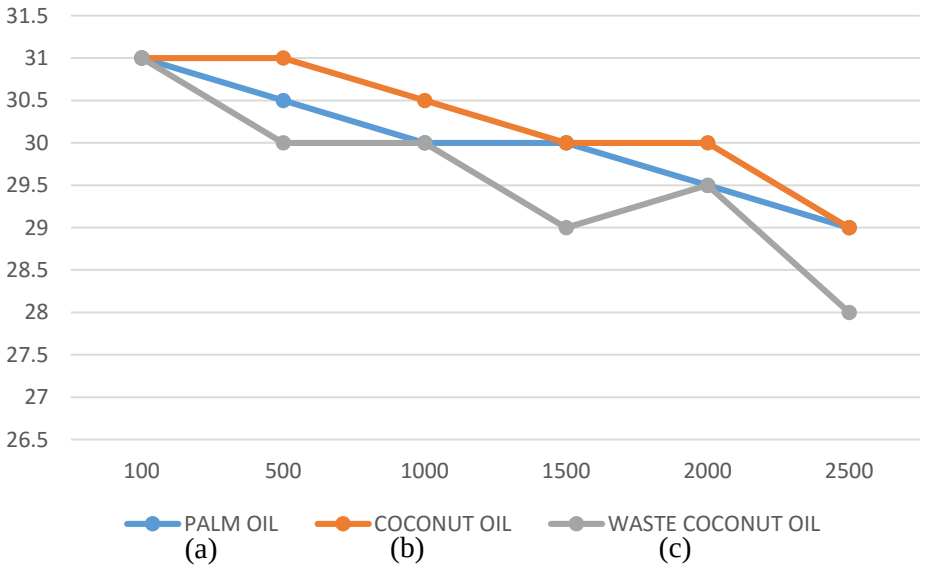

Figure 1: Pour point of waxy crude oil after treatment with (a) palm oil PPD, (b) coconut oil PPD and waste palm oil PPD.

Figure 1 shows the waxy crude oil after treated by all plant oils that indicated a same pour point at 31 °C. But it was different after the concentration was increased at 500 ppm, which waste palm oil exhibited a lower pour point of waxy crude oil at 30 °C compared to palm oil and coconut oil at 30.5 °C and 30 °C respectively. This figure also shows the trend of pour point of waxy crude oil with palm oil pour point depressant (PPD), coconut oil PPD and waste palm oil PPD were gradually decreased at 1000, 1500 and 2000 ppm. At 2500 ppm, the waxy crude oil

with waste palm oil PPD shows the lowest pour point at 28 °C was better than other plant oil PPDs at all concentrations. From this result, it revealed that the application of waste palm oil PPD in waxy crude oil was excellent compared to other PPDs [6]. Other that than, the high concentration of PPD also helped the reduction of pour point of waxy crude oil [7].

Table 1: Specific gravity of pour point depressant (PPD)

Pour Point Depressant (PPD)	Specific Gravity
Palm oil	0.83
Coconut oil	0.8
Waste palm oil	0.78

After PPD was tested with waxy crude oil, all PPDs were characterized for finding their specific gravity as the evidence that can be proven this property helps to reduce the pour point of waxy crude oil. Table 1 indicates waste palm oil PPD ha the lowest specific gravity at 0.78 but coconut oil PPD and palm oil PPD shows specific gravity at 0.8 and 0.83 respectively. Based on this analysis, a low specific gravity of PPD can contribute the reduction of pour point of waxy crude oil.

4. Conclusions

The main purpose of the implementation of this project was to decrease the formation of waxy crude oil of waxy crude oil by using ethyl ester that produced by waste palm oil, coconut oil and palm oil. The presence of pour point depressant (PPD) in waxy crude oil reduced the pour point of waxy crude oil at low temperature. The result showed PPD from waste palm oil that indicated the excellent pour point of waxy crude oil with 28 °C at 2500 ppm compared to all concentrations of other plant oils. Other that than, PPD of waste palm oil also exhibited the lowest specific gravity at 0.78 compared to others. This result revealed that the low specific gravity was important in the reduction of the pour point of waxy crude oil than others.

Acknowledgments

We wish to express our deepest gratitude and appreciation to all those who assisted us in completing this thesis and project. Above all, we thank God almighty who provide us with strength, direction and showered us with blessings throughout.

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The Effect of Solid-To-Liquid Ratio and Particle Size on the Extraction of Quercitrin from *Cosmos caudatus* (C.C)

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ABSTRACT
Cosmos caudatus (CC) or "Ulam Raja" has widely used as medical plant due to its high antioxidant properties for health benefits. Quercitrin is the major plant derived flavonoid compound in CC that possess high antioxidant activity. Hence, this study aimed to extract maximum yield of quercitrin from CC. Two most influential extraction parameters namely solid-to-liquid ratio (1/20, 1/30, 1/40 and 1/50) and particle size (355, 850, 1700 and 2500 µm) have been studied to evaluate their effect on the extraction process of CC using ultrasonic-assisted extraction (UAE). The yield of quercitrin was accomplished using HPLC equipped with diode array detector (DAD). From the analysed result, solid-to-liquid ratio at 1/20 and particle size of 850 µm were reported as the optimum extraction parameters to produce highest yield of quercitrin from CC. The quercitrin content decrease dramatically with the increment of solid-to-liquid ratio, while, particle size of 850 µm extracted the highest content. Maximum extracted quercitrin was achieved at 40.47±0.26 g/100 dw based on the optimized extraction parameters. SLR 1/20 with particle size 850 µm were considered the best processing parameters to acquire high yield of quercitrin in CC.

Keywords: Solid-to-liquid ratio, particle size, extraction, quercitrin, *Cosmos caudatus*

1. Introduction
Cosmos caudatus (CC) or locally known as "ulam raja" or "pucuk raja" is a common and popular traditional vegetable among Southeast region. CC has scientifically proven as natural source of antioxidants. Many studies had studied CC's phytochemical that give potential health benefits to human such as anticancer, anti-inflammation and antibacterial properties [1],[2]. CC has been reported to contain phenolic and flavonoid compounds and the present of these phytochemicals might contribute to pharmaceutical properties found in CC [2]. Among the phytochemicals, quercitrin was found to be the major in CC. [3],[4]. This secondary metabolites of flavonoid have been reported to serve high medicinal potential such as antioxidants agent [5].

In order to obtain high amount of quercitrin from CC, it is important to investigates the parameter involved in the extraction process. There are many factors that affect the extraction process [6],[7]. To date, limited studies has investigated the extraction of quercitrin from CC. The extraction process by ultrasonic assisted extraction (UAE) was selected because it is one of the well- known extraction technique to produce higher yield with minimum operational cost, time, and energy consumption [8],[9]. In this study, two extraction factors such as solid-

to-liquid ratio (SLR) and particle size were selected to scrutinize its effect on the extraction of quercitrin from CC using UAE. The experiment was designated using one-factor at-a time (OFAT).

2. Materials and methods

2.1 Chemical and reagent

Standard quercitrin was procured from Sigma Aldrich, (St. Louis, Missouri, United States), ethanol (reagent grade) was purchased from Across Organics (Leicestershire, UK). HPLC grade acetonitrile was obtained from Daejung Company, Ltd. (Nakdong-daero, Sasang-gu, Busan, Korea). Formic acid (HPLC) was from Sigma Aldrich, (St. Louis, Missouri, United States).

2.2 Preparation of Cosmos caudatus

The sample of CC was collected from research farm located at UTM Pagoh (Johor, Malaysia). Freshly harvested CC samples were cleaned to remove impurities. The leaves were segregated and dried by oven dryer at 40 °C and stop after four hours when the leaves were completely dried until constant weight. The dried CC was crushed and was grounded using blander. The ground materials were meshing between 550, 850, 1700 and 2500 µm (Wstyler, Mentor, OH, USA).

2.3 Extraction of quercitrin from CC by one-factor at-a time design

The extraction of quercitrin from CC was performed using UAE (WiseD, Daihan Scientific, Ltd Co, Korea). CC was extract in 80% of ethanol at temperature 45 °C in ultrasonic bath. Dried CC sample was then evaporated until dried to obtain crude extract CC. The extraction time and amplitude were fixed at 30 minutes and 40%, respectively. This experiment flow diagram as shown in Figure 1.

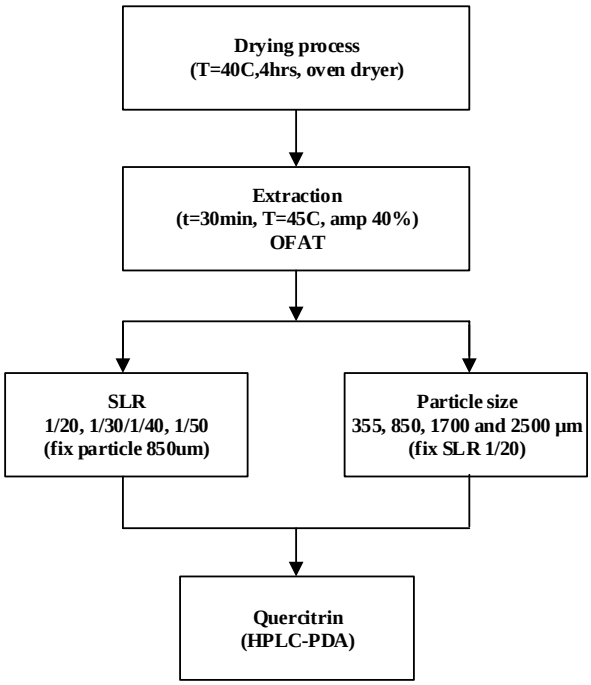

Figure 1: Process flow of the study

2.3 Quantification of quercitrin by HPLC

The quantification of quercitrin was accomplished on high performance liquid chromatography (1290 Infinity II) on Agilent Technologies (USA), in YMC- Triart C18 column (150 mm × 4.6 mm. I.D, 5 µm). The gradient system was set in mobile A (0.3% formic acid in water) and

mobile B (acetonitrile). The elution system was: 0-10 minutes, 20-50% B; 10-11 minutes, 50-100% B; 11-12 minutes, 100-20% B. Total run time was achieved in 12 minutes. CC extract was filtered with 0.45 µm nylon membrane filter before injected to the HPLC system. Detection of quercitrin was performed at 260 nm wavelength.

2.4 Statistical analysis

The mean and standard deviation was analysed using analysis tools in Microsoft Excel 2016. Data were analysed using one-way ANOVA (SPSS software 23). The graphical image was constructed using GraphPad Prism7.

3. Results and discussion

3.1 Identification of quercitrin

A representative HPLC-DAD profile of quercitrin standard and CC extract was given in Figure 2 and 3, respectively. Quercitrin peak was obtained at 6.72 minutes in a total runtime of 12 minutes. Good matched was found between UV spectra of quercitrin standard and sample.

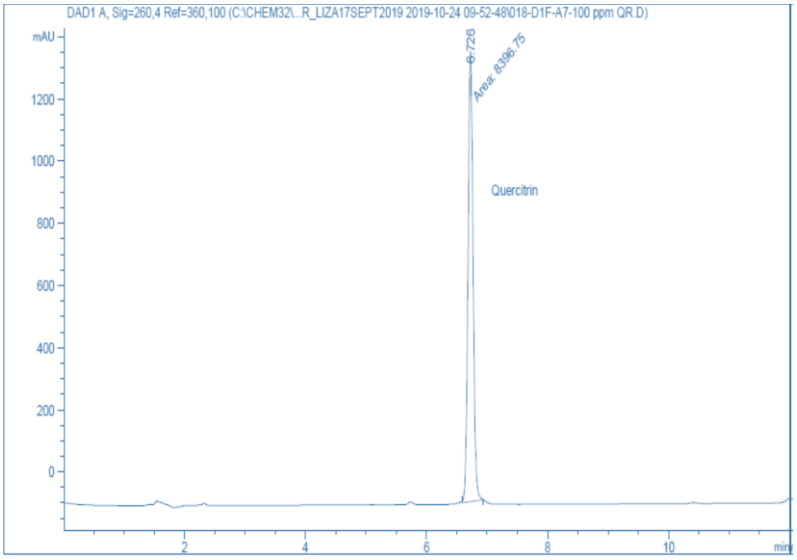

Figure 2: Chromatograms of quercitrin standard

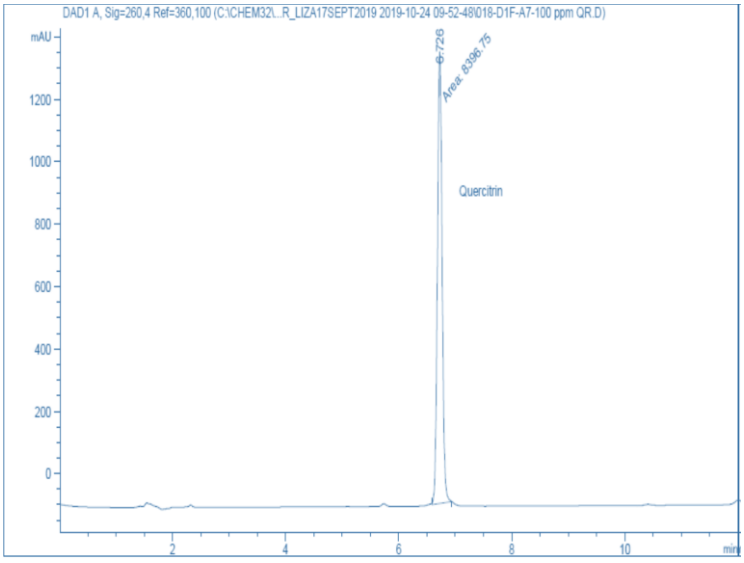

Figure 3: Chromatograms of CC extracts obtained through UAE

3.2 Effect of solid-to-liquid ratio on quercitrin yield

The effect of SLR on the extraction of quercitrin was examined at ratio 1/20 up to 1/50, respectively. The yield of queritrin showed significantly different at $p<0.05$ as shown in Figure 4. It was observed a downward trend on the yield of quercitrin at SLR 1/20 to 1/40. The highest quercitrin yield was achieved at 40.47 ± 0.26 g QR/100 dw using SLR 1/20. The result is in agreement with mass transfer principles. Following this principle, driving force is the concentration gradient between solvent and solid concentration gradient [7],[8]. Thus, the SLR 1/20 was found to be the strongest driving force to accelerate quercitrin from the plant material.

3.3 Effect of particle size on quercitrin yield

The effect of different particle size on the extraction of quercitrin were compared and presented in Figure 5. It was found that the extraction of quercitrin was affected by the particle size. The highest yield of quercitrin was obtained using particle size 850 µm (37.63±0.27 g QR/100 dw). Larger particle size of 2500 µm yielded the least quercitrin content, presented at 30.17±0.65 g QR/100 dw. The difference of the results can be explained by the smaller particle efficiently promote the mass transfer and would accelerates more phytochemical into the solution [6],[9],[10] In addition, larger surface area will create a required space for concentration gradient thus slower the diffusion rate.

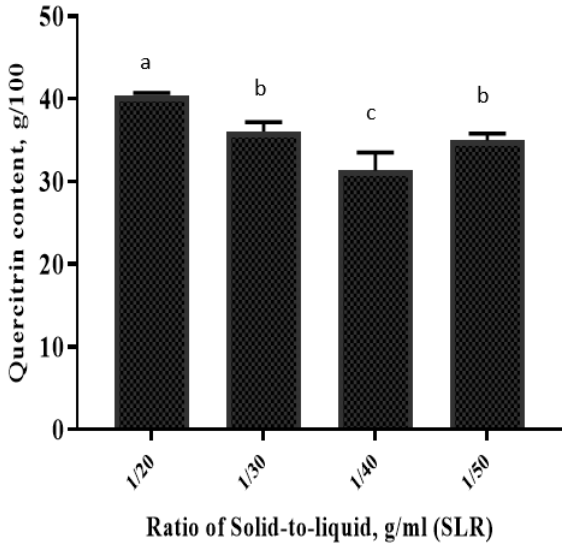

Figure 4: Quercitrin yield at different SLR. Different letters represent significant different at $p<0.05$.

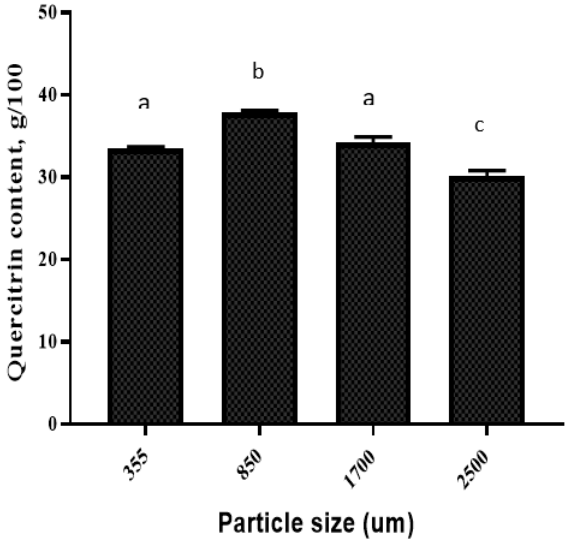

Figure 5: Quercitrin yield at different particle size. Different letters represent significant different at $p<0.05$.

4. Conclusions

The results proposed that yield of quercitrin can be maximized using appropriate solid-to-liquid ratio and particle size. In this study, medium particle size of 850 µm and solid-to-liquid ratio 1/20 was found to be the best extraction processing parameter of CC. The results successfully verified that the use of larger particle size effect the mass transfer and bio-active solubility. In addition, solid-to-liquid ratio of 1/20 created a strongest driving force to accelerates more quercitrin from the plant material. The finding from this work could be essential guide to maximized the quercitrin from CC. Future works is warranted to examine more extraction parameter in order to produce quercitrin rich extract from CC.

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Development of an Integrative Process for the Production of Microbial Biomass Protein (MBP) from Rice Straw for Animal Feeds Application

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ABSTRACT
The large production of rice straw could eventually lead to uncontrolled air pollution due to open burning activity. Although rice straw has been used as animal feed since the 1980s, it has failed to provide sufficient amount of protein for daily ruminant growth. Hence, this study explored the use of rice straw waste for animal feed that can provide the amount of protein needed. Moreover, as silica has been discovered as the main hurdle in animal feed processing, the rice straw sample was treated with alkaline hydrogen peroxide (AHP) via batch and continuous systems. In addition to reduced silica content, it also aimed to maximize the protein content of the treated rice straw. In the continuous system, a single column packed bed with up-flow system was adapted before carrying out further biological treatment with fungi for microbial biomass protein (MBP) production. In the continuous AHP pretreatment process followed by fermentation with *Neurospora sitophila* in a single column, it was found that 84% of silica was removed with 80% delignification and 8% reducing sugar production with 10% AHP solution in a 7 cm treated and compacted rice straw bed at room temperature. Despite the limited temperature control in this continuous system, the combination of treatment was found effective compared to batch system. Furthermore, the protein content in the pretreated rice straw increased by a whopping 80%. Hence, it can be concluded the selected processes for silica removal and protein enhancement of local rice straw are indeed suitable for animal feed production.

Keywords: Microbial biomass protein (MBP), rice straw, integrative process, hydrogen peroxide

1. Introduction

The global rice straw from paddy production was upwards of 730 tonne per season. The state of Kedah alone recorded around 200,000 hectares of paddy cultivated area, based on the report from Malaysian Department of Agriculture. Rice straw is considered agricultural waste after each planting season and commonly openly burned [1]. This type of burning activity deteriorates the air quality due to the emission caused which will then pose a health threat to human. This threat is attributed to the increased concentration of atmospheric particulate and polycyclic aromatic hydrocarbon (PAH) [2]. There were attempts for rice straw to be used in

silage production for animal feed application since the 1980s. However, this was proven difficult due to several reasons. Rice straw contains a low amount of crude protein, severely below the required minimum level of 15% that was determined sufficient by the Malaysian Department of Veterinary Services for healthy growth of animals as well as standard quality of milk and meat [3]. Combination with either biological, physical or chemical supplements results in higher protein content, but such method leads to higher costs for animal feeds production [4]. Rice straw also contains a high amount of complex structure such as hemicelluloses, cellulose, lignin, and silica, making the aspect of digestibility rather low for direct animal feeds application. Pretreatment of rice straw therefore deemed necessary which include methods such as alkaline treatment and steam explosion [5]. This study was aimed to observe how a combination of pretreatment with various concentrations of alkaline hydrogen peroxide (AHP), rice straw particle sizes and fermentation with *N.sitophila* in a single column will affect the microbial biomass protein (MBP) production for animal feeds application.

2. Materials and methods

The methods were divided into batch and continuous system for comparative study.

2.1 Sample preparation

Samples were obtained from FELCRA Seberang Perak and grounded. Three (3) particle sizes were collected from a mechanical siever, i) <0.5 mm, ii) 0.5-1.0 mm, and iii) >1.0 mm. Samples were subjected to fourier transform infra-red (FTIR) Spectroscopy analysis to determine the existence of silica bond before the treatment. Field emission scanning electron microscopy (FESEM) were carried out to observe the morphological aspects of the samples. Finally, the samples were dried to determine the moisture and dry matter content, before being subjected to Kjeldahl method to determine the protein content. Molybdate silica method, phenol sulphuric acid method and Klason method were used to measure silica content, total reducing sugar content and lignin content, respectively.

2.2 AHP Pretreatment Processes

2.2.1 Batch System

The process was carried out at temperatures ranged from 30 to 60 °C. Rice straw grounds were weighed to 75 g and placed into beakers containing 1500 ml varying hydrogen peroxide (H_2O_2) concentrations of 2%, 4%, 6%, 8%, and 10% in alkaline condition (constant pH of 11.6 using sodium hydroxide, NaOH). The resulting slurry was shaken with an electric shaker at approximately 200 rpm. Liquid samples were collected at 25 minute intervals and analysed to ensure the total loss occurred during the process of incubation time had been less than 10% (w/w). The resulting solids were filtered and further analysed. The experiment was replicated 3 times.

2.2.2 Continuous system

The continuous-flow sorption experiment was conducted in a stainless steel column. The column was designed with an internal diameter of 10 cm and 12 cm in length. A 0.5 mm mesh stainless steel sieve and fibre wool were placed at the bottom of the column. The drawing for the proposed system is illustrated in Figure 1. The process starts with AHP feeds at a flow rate of 10 ml/min. The process was followed with *N.sitophila* feeds at similar flow rate. Finally, air was fed to the column to support the growth requirement of *N.sitophila*. The operating temperature a recorded at 25 °C in addition to similar H_2O_2 concentrations, pH and liquid samples collection intervals to the batch system. Two different heights (7 and 12 cm) of

compacted rice straw column, made of 70 and 100 g rice straw (<0.5 mm particle size), respectively were observed.

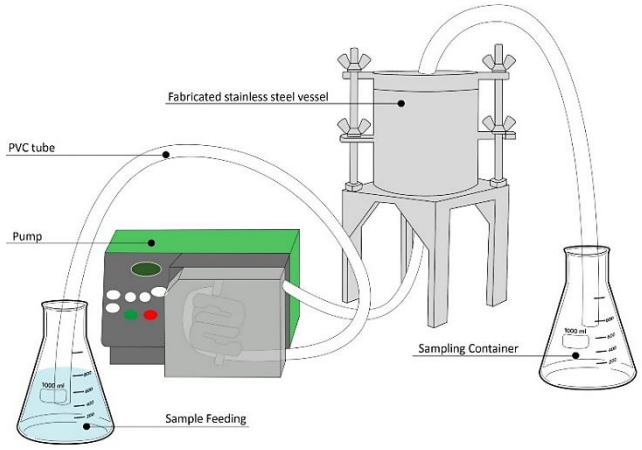

Figure 1: Proposed continuous system experimental set up

2.3 Post pre-treatment sample analysis

2.3.1 Quantitative silica analysis

A series of silica standards (0%, 20%, 40%, 60%, 80%, and 100%) was first prepared with Si(IV) solution. Serial solutions were then used to treat the standard to produce the final yellowish solution. The standard solution was measured with a UV-Vis spectrophotometer at 815 nm.

2.3.2 Determination of total reducing sugar and lignin

Phenol sulphuric acid method and Klason method were used to measure total reducing sugar and lignin content, respectively.

2.4 Preparation and biological treatment with Neurospora sitophila

Neurospora sitophila starter beads culture was enumerated on double layer pour plate with potato dextrin agar. The plate was sealed and incubated for 3 days at 37 °C. The formation of the colonies was indicated by the growth of orange and white masses on the surface of the agar. The strain was streaked and weighted on an empty plate to ensure constant weight for each treatment before it was inoculated in potato broth and incubated at 37 °C for 24 hours. This was followed by the inoculation of the pretreated rice straw samples.

2.5 Determination of fungal biomass

Fungal biomass was estimated by determining the glucosamine content of mycelial cell wall calorimetrically.

2.6 Determination of protein content

Protein content was determined by using the Kjeldahl method.

2.7 Determination of mean, standard deviation and standard error

All values were computed mathematically based on sample size and observed values.

3. Results and Discussion

3.1 Characteristics of rice straw

3.1.1 FTIR Analysis

Rice straw samples in this study exhibited a high level of silica bonding and appearance. There was a high percentage of Si-O bond stretching before the pretreatment with AHP. Some Si-O and Si-O-Si bonds were still present after the pretreatment, however the resulted FTIR spectra at 895 cm^{-1} and 1036 cm^{-1} only showed a significant removal of insoluble silica. The result also showed the elimination of aromatic ring vibration of lignin with the absence at 1500-1550 cm^{-1} as well as a drastically increased cellulase peaks at between 800 to 1300 cm^{-1}.

3.1.2 Morphological analysis

The structure was initially broad and smooth when observed at 200X magnification before the pretreatment. Some crystalline regions indicated the well bonded silica and organic material in rice straw in order to function as a coating agent in relation to lignin. There were some uneven droplet-like surface after the pretreatment, indicating the exposed silica and lignin. The structure also appeared looser with well separated fibres, resulted in more porous rice straw for further treatment.

3.1.3 Physical and chemical properties

Significant reduction of silica, lignin and total reducing sugar as well as protein and moisture content were observed when samples were compared between before and after the pretreatment with AHP, as summarized in Table 1.

Table 1: Physical and chemical properties of rice straw

Properties	Before Pretreatment	After Pretreatment
Silica	20.00±0.50%	4.00±0.10%
Lignin	12.00±0.05 %	5.70±0.05%
Total reducing sugar	0.07±0.78%	5.66±0.54%
Protein	7.00±0.07%	7.02±0.66%
Moisture content	6.00±0.98%	20.00±0.01%

3.2 Effects of AHP concentrations and particle size on silica removal

That the highest silica removal was observed at 10% AHP concentration regardless of particle size in batch system. However, there was no significant difference between 8% and 10% concentrations. Therefore, 8% AHP concentration was more economical and sufficient for silica removal. Smaller particle size allowed for greater silica removal due to greater surface area for physical and chemical interaction. Similar trends were observed in continuous system. Additionally, 7 cm column height exhibited between 7.8% to 31% greater removal potential at all AHP concentrations compared to 12 cm column height. These results were attributed to higher influent concentrations resulted in higher driving force for mass transfer, thus achieved the adsorbent saturation rapidly, which led to the decrease of exhaust time and adsorption zone length.

3.3 Effects of AHP concentrations and particle size on total reducing sugar production and delignification

Similar trends to silica removal in batch system were observed, where the smallest particle size at 8% AHP concentration produced the greatest amount of reducing sugar. Particle size might also affect sugar hydrolysis due to more accessible surface area. A maximum delignification of

80% was observed at 10% AHP. However, there was no clear trend with the particle size. The present of NaOH was more prevalent in breaking up ester bond cross-linking, degrading the lignin and increasing the porosity. Following the similar trends, 7 cm column height in continuous system also produced the maximum value of total reducing sugar (7.88%) at 8% AHP concentration. Additionally, the same column height also showed a higher lignin removal potential across all AHP concentrations. This was due to the upward flow of AHP interacting with the majority of packed bed of rice straw at the bottom layer which showed higher level of delignification than the top layer.

3.4 Effects of AHP concentrations and particle size on fungal biomass and MBP

Glucosamine content for the expression of fungal biomass peaked at day 5 at 8% AHP concentration for both systems. The highest protein production (80%) was observed in continuous system at day 5 at 8% AHP concentration, after which the protein value dropped, indicating the starvation stage of *Neurospora sitophila* due to depleted reducing sugar content at from day 4 of inoculation. Reduced particle size of <0.5 mm further improved the protein production, due to easier enzyme adhesion compared to on larger particles. The effects of the availability of reducing sugar content towards the fungal biomass and protein production is expressed in Figure 2.

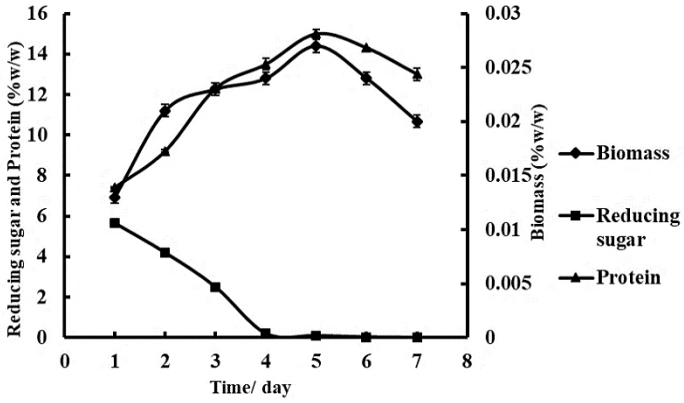

Figure 2: The effects of the availability of reducing sugar content towards the fungal biomass and protein production

4. Conclusions

The use of an integrative process suggested in this study was found to be more effective than commonly used batch process through lower operating temperature and increased protein enrichment, hence advantageous in economic sense.

Acknowledgments

The author wishes to thank FELCRA of Seberang Perak for contributing the rice straw samples needed for this study.

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Development and Sensory Attributes of Mixed Bitter Gourd (*Momordica charantia*) and Green Apple Juice

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ABSTRACT
Momordica charantia (MC) or locally known as "peria katak" is widely known for its various medicinal value. However, the bitter taste of MC maybe undesirable for some people. The purpose of this study was to conduct a sensory test to assess the palatability and acceptability of MC juice mixture among the consumers. In the experiment, the MC raw juice and green apple were obtained and mixed together with different volume ratio which were Sample 297 (25:75), Sample 981 (50:50), Sample 706 (75:25) and Sample 333 (100:0). Then the juice mixtures were evaluated by 30 panellists. Each of the panellist was given a questionnaire sheet and required to give evaluation based on smell, flavour, bitterness for each sample. Sensory analysis was performed for each sample by using the hedonic scale method. Based on the hedonic scales, the overall mean hedonic scores for Sample 706 and Sample 333 were 3.27±1.82 and 1.78±2.47, respectively, which were considered as "dislike moderately" and "dislike extremely". While for Sample 297 and Sample 981, there was no significant differences found for the overall mean hedonic scores, smell and flavour ($p>0.05$). The sensory evaluation shows that the MC-green apple juices percentage ratio of 25:75, 50:50 were considered acceptable among in terms of smell, flavour, bitterness among the panellist.

Keywords: bitter melon, sensory testing

1. Introduction

Momordica charantia (MC), a family member of Cucurbitaceae is also known as bitter gourd, bitter melon, balsam pear or peria katak. This plant grows well in the tropical and subtropical area including Asia, Africa, Caribbean, and several parts in Amazon Basin [1]. MC is an annual, slender climber herbaceous plant that has bright-green colour and lobed leaves with small flowers in yellow colour. The fruit size ranging 2-7 cm long and has a resembling feature of a cucumber; oblong and green but with large smooth warts [2]. All parts of MC plant are bitter especially when the fruit is ripening which turns from green to orange-yellow [3]. Despite its bitter taste, it is consumed traditionally for its beneficial health effect. The stem and green leaves are usually boiled in hot water and often drink as tea. The fruit is usually stir-fried or blended into juice. The MC juice is often blended with other fruits to reduce its bitter taste.

The MC contains alkaloids and polyphenolic compounds which contributed to the bitter taste of the fruit [5],[6]. There were previous reports on the effort to reduce the bitter taste of MC juice including treatment with sodium chloride, blanching or Arabic gum addition to improve the MC palatability [7]-[10]. Snee *et al.* (2011) in their study had incorporated several types of samples to MC juice for pilot palatability testing such as chili, tomato sauce, curry, chicken stir fry and hummus [11]. The finding of their studies suggested that, the sour flavour of tomato were able to mask the MC bitter taste and considered acceptable by most panellist [11]. On the

other hand, Bhardwaj and Pandey suggested that blending of two or more fruit and vegetable juices together provides convenient alternative for the utilization of these bitter fruits and vegetables [12]. In local Malaysia market, the MC juice is commonly blended together with other fruit juice, usually the one with the sour taste fruit such as green apple to reduce the bitter taste of the MC juice. Green apple contains malic acid or sometimes called as apple acid, which contributes to the sour taste of the green apple fruit [13]. Malic acid is the dominant acid in apple fruits, which makes up to 90% of the total organic acids [14]. In the current study, a sensory test on mixed MC and green apple juice at different volume ratio was carried out to evaluate the palatability of the mixed juice.

2. Materials and methods

2.1 Raw material

The fresh MC fruits were purchased from a local wholesaler based in Pagoh, Muar, Johor. The fruits were harvested 8 weeks after sowing, unripe, 15 cm average length with wart surface. A voucher specimen was obtained from Assoc. Prof. Dr. Christophe Wiart, Ethnobotanist from University of Nottingham Malaysia campus. The voucher specimen (UNMC5600/19) then had been deposited in the Herbarium, School of Pharmacy, Faculty of Science, University of Nottingham Malaysia.

2.2 Preparation of juice using a slow juicer

4 kg of MC fruits were washed thoroughly then cut into halves in elongated shape and the seeds were discarded. The fruits then were inserted into the slow juicer (Hurom, H-AA Series, Korea) to obtain juice. Then, the obtained juice was centrifuged using a refrigerated centrifuge (Kubota 7000, Japan) at 9,000 rpm, 4 °C for 15 minutes. The supernatant was collected and further centrifuged (Eppendorf 5810R, Germany) at 4 °C, 4,000 rpm for 10 minutes to remove any residual fibre

2.3 Preparation of mixed MC and green apple juice in different concentration

5 ml of mixed MC and green apple juice sample were prepared according to Table 1. Green apple was selected for this study as the sour taste of the fruit able to reduce the bitter taste of the MC juice. All mixtures were stirred to ensure that the mixtures were homogenize. Then, all mixture samples were coded randomly and not in gradual sequence to avoid bias assumption among the panellists during sensory testing activity.

Table 1: Mixed MC-green apple juice with different concentrations preparation

Sample ID	MC-green apple ratio (v/v)	MC juice volume (ml)	Green apple juice volume (ml)
297	25:75	1.25	3.75
981	50:50	2.50	2.50
706	75:25	3.75	1.35
303	100:0	5.00	0.00

2.4 Sensory testing

30 panellists were recruited for this activity. Each panellist was given with four samples of mixed MC and green apple juice (5 ml each) with different sample ID. All panellist also were given a questionnaire form and a bottle of drinking water to rinse before and between sample testing. The parameters were selected for this study were smell, flavour and bitterness. Each panellist was instructed to give scale from 1 to 9 for each sample. After sample testing by the

panellist was done, the questionnaire was collected and the statistical significance was calculated. The Hedonic scale used for this activity was as follows:

Hedonic scale method

1 = dislike extremely
2 = dislike very much
3 = dislike moderately
4 = dislike slightly
5 = neither like nor dislike
6 = like slightly
7 = like moderately
8 = like very much
9 = like extremely

2.5 Data analysis

All results were reported as means with standard deviation (SD) as indicated and performed in triplicates. The data were analysed using the Student's t-test, ANOVA followed by Dunnett's post-hoc test. A $p<0.05$ value is considered significant. All graphs were constructed using Prism 7 (Graphpad Software, Inc.).

3. Results

3.1 Panellists demographic

A total of 30 panellists were selected for this sensory testing [15]. All panellist were all healthy at the time of the experiment based on the personal communication before the activity commenced. Also, none of the panellist was pregnant. Each of the panellist was given a questionnaire form and four samples of MC-green apple juices. The questionnaire required each panellist to evaluate all samples according to the Hedonic scale (1 = dislike extremely, 9 = like extremely). The Table 2 showed the demographics of panellists (n=30). The gender distribution among panellist were male (53%) and female (47%), where 93% of the panellist were Malay, and 7% were Chinese. The average age was 27.5 ±7.7 years old.

Table 2: Demographic characteristics of participants (n=30)

Characteristic	No. of participants, n	Percentage, %*
Gender		
Female	14	47
Male	16	53
Age		
20-24	13	44
25-29	5	17
30-34	7	23
35-39	2	7
40-44	3	10
Ethnicity		
Malay	28	93
Chinese	2	7

* Percentages may not add up to 100 due to rounding.

3.2 Hedonic scores for MC-green apple juice ratio

The average hedonic scores for specific attributes and overall acceptability were shown in Table 3. While the Figure 1 showed the frequency distribution among hedonic scores for the overall liking scores for each MC-green apple juice ratio. Based on the result, Sample 297 (25:75 (v/v) ratio of MC: green apple) with the lowest volume of MC juice received the highest overall mean Hedonic scores, followed by sample 981 (50:50 ratio) (v/v), Sample 706 (75:25 ratio) (v/v) and

Sample 303 (100:0 ratio) (v/v). The overall mean Hedonic scores for Sample 706 and Sample 303 were 3.27±1.82 and 1.78±2.47 respectively, which were considered as "dislike moderately" and "dislike extremely". In addition, these two samples also received the significant lower scores for smell, flavour and bitterness, as compared to Sample 297. The participants found that Sample 981 was bitter than Sample 297 ($p<0.05$). However, no significant differences were found for the overall mean hedonic scores, smell and flavour between Sample 297 and Sample 981 ($p>0.05$).

Table 3: Hedonic scores for MC-green apple juice in different ratio

Sample ID	MC-green apple ratio	Smell	Flavour	Bitterness	Overall
297	25:75	5.20±1.99	4.70±2.26	4.30±2.12	5.00±2.32
981	50:50	4.83±1.88	4.13±1.68	3.37±2.01*	4.20±1.65
706	75:25	4.47±1.94***	2.77±1.59***	2.63±1.69***	3.28±1.82***
303	100:0	3.73±1.93***	2.03±1.30***	1.83±1.23***	1.78±2.47***

Values were given as mean ± SD (n = 30). *p<0.05, **p<0.01, ***p<0.001 were obtained from Student's t-test with Bonferonni correction comparing data with Sample 1.

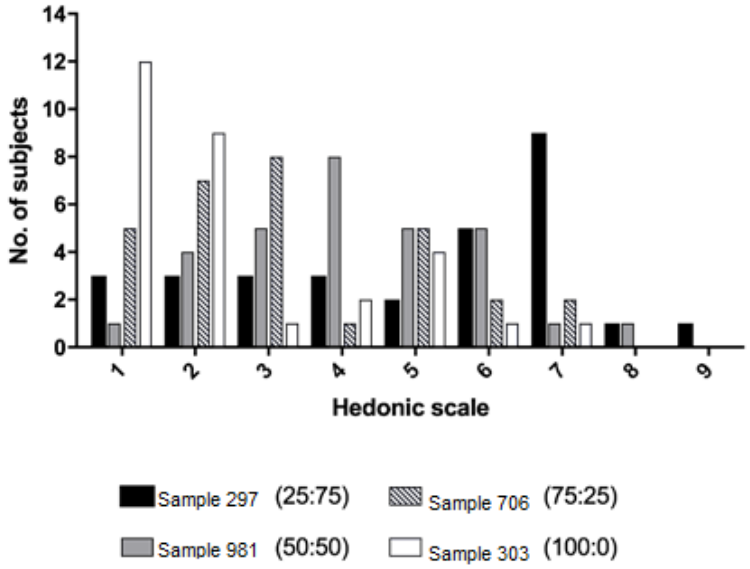

Figure 1: Hedonic scores frequency distribution for the overall liking scores for each MC-green apple juice ratio (v/v) (n=30). The MC-green apple ratio ranging from 25:75 (Sample 297), 50:50 (Sample 981), 75:25 (Sample 706) and 100:0 (Sample 303). Data represented the Hedonic score distribution: 1 = extremely dislike; 5 = neither like nor dislike; 9 = extremely like.

4. Discussion

The bitterness of the MC plant is due to the presence of non-toxic alkaloid substance, momordicine which can be found in the leaves and fruit [6],[16],[17]. Although the MC plant has many medicinal properties, its bitter taste is often unacceptable to some people thus leading to a limited number of clinical trials feasibility [11]. Bhardwaj and Pandey (2011) suggested that blending of two or more fruit and vegetable juices for the preparation of ready-to-serve beverage may provide a convenient alternative for consumption of high acidity, astringency, bitter fruits and vegetables [12]. Additionally, the blending of fruit juices may give an improved physicochemical composition and leads to new product development [18].

The bitterness of MC can be eliminated or masked through several debittering processes including treatment with sodium chloride, blanching or Arabic gum addition, which make the resulting MC more palatable [7]-[10]. These processes also enhance dietary fibre, improve nutritional value, reduce the oxidation process of food products and enhance the physical features of the product [8],[19],[20]. The debittering process also can be carried out through the microbial process, for example by biotransformation of MC fruit juice via *Lactobacillus plantarum* fermentation. In the fermentation process, the lactic acid bacterium isolated from MC fruit itself help to reduce bitterness and sugar content and produced aglycones and phenolic compounds from glycosidic momordicoside degradation. The bioprocess also improved its putative anti-diabetic α-glucosidase inhibitory potential compared to fresh unfermented juice [21].

5. Conclusions

The sensory evaluation shows that the MC-green apple juices percentage ratio of 25:75 (Sample 297), 50:50 (Sample 981) were considered acceptable among in terms of smell, flavour, bitterness among the panellist.

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Vertical Cultivation System for Sustainable Farming

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ABSTRACT
Conventional farming has been successful for high throughput crop production, but there is an increasing concern about the excessive usage of synthetic chemicals, and the impact of monoculture on food quality, soil health and the environment. In this study, we had developed a novel vertical farming technique to address the issue. In addition, a polyculture environment was created by intercropping mung bean plants and water spinach. Water spinach was found able to increase mung bean production (+25% in optimized condition). One of the key advantages of the vertical polyculture system is the significant reduction in pest problem. The cultivation system holds a promising prospect for sustainable farming.

Keywords: Vertical farming, polyculture, intercropping, cultivation system, sustainable

1. Introduction

In recent years, the importance of agriculture has been reinforced in many countries due to the concern of food security and self-sufficiency. Innovative technologies have been developed to advance the agricultural activities, including cultivation of high-quality breeds, advanced cultivation technologies, breakthroughs in disease control and prevention, as well as innovative systems such as vertical farming, soilless cultivation, and genetic improvement.

While the current farming system promise high throughput production, there is an increasing concern due to the usage of synthetic chemicals, but also the impact of monoculture on the food quality, soil health and the environment. Organic farming has emerged as a widely accepted alternative to overcome part of the problems, but the limit of fertile cropland remains an issue to feed the growing world population. On the other hand, vertical farming is an attractive option for plantation in urban area, but the power demand required to supplement the natural light may be prohibitively expensive. Therefore, in the current study, a novel vertical farming concept had been developed which can lead to a self-sustainable mini ecosystem, suitable for urban farming as well as high throughput farming.

The developed system applied carefully designed intercropping method to create a symbiotic environment for plants. Intercropping is the practice of integrating more than one crop together that have different ecological niches to promote beneficial interactions in a close proximity [1]. The different niches among the plants are likely to avoid resource competition thus it is increase production per unit area, improve resource utilization, greater profitability, enhance biodiversity and reduce the usage of external agrochemicals [2],[3]. Intercropping design viability relies on several factors including crop spatial organization, plant architecture, crop density, and plant maturity period [4],[5]. Intercrop can be arranged in several spatial design

including (i) mixed intercropping, (ii) row intercropping, (iii) row strip/relay intercropping, (iv) strip intercropping [4].

Legume intercropping is a popular cropping system in many countries and well received as it more sustainable and less risky practice. This cropping system plays important role in efficient resource utilization as it is not only increase yield, but also increase nitrogen compound through its biological nitrogen fixation process and less soil nutrient uptake compared to monocropping [6],[7]. Legume integration to cropping system also helps to improve nutrient retention, fixing nitrogen, preventing weeds, breaking disease cycle and developing soil carbon (C) [8]-[10]. Mung bean is part of legume family and shares the same genus with adzuki and cowpea. Two most recognizable type of mung bean are green gram, which mainly for human food consumption and golden gram that cultivated for green manure or cover crop [11],[12]. Mung bean also cultivated as fodder crop to satisfy forage demand especially during summer due to its drought and salinity tolerance [13]-[15].

2. Materials and methods

2.1 Optimization of symbiotic plants combinations

For the vertical farming system, the symbiotic plants combinations were optimized based on two complementary methods. Firstly, it is based on the review of list of companion plants from literature. Secondly, the combination is selected based on intercropping method, as shown in Figure 1. To create a sustainable farming system, legume plant (including mung bean, long bean plants) was included due to its nitrogen fixing activities. A number of local vegetables (including spring onion, water spinach, *sawi Hong Kong*, *sawi Jepun*, *bayam hijau*, *bayam merah*) were intercropped with legume plant to examine potential symbiotic activities. Following selection, the spring onion and water spinach were further studied for incorporation with mung bean plant cultivation.

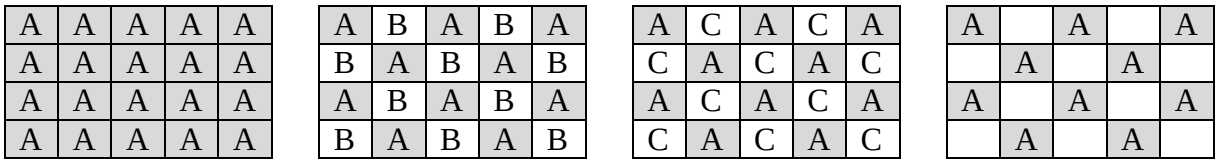

Figure 1: Strip plot design for a preliminary experiment studying effect of intercropped plant (A: mung bean plant; B: spring onion; C: water spinach or locally known as *kangkung*).

2.2 Field test of the prototype and design refinement

The developed prototypes with optimized plants combinations were field-tested in urban setting at Dataran Cemara, Institute of Bioproduct Development, Universiti Teknologi Malaysia, Johor Bahru, Johor. The growth of the plant was closely monitored, and any possible technical difficulties faced in the field had led to refinement of the prototype design.

2.3 Statistical analysis

Data obtained from the current experiments were analysed using using standard t-test and ANOVA, where p-value less than 0.05 is considered significant.

3. Results and Discussions

For intercropping experiment, intercropping with spring onion significantly reduced (-13.22%) its production ($p < 0.01$, t-test), while intercropping with water spinach resulted in 5% increase in production ($p < 0.001$, t-test). The result suggested that careful selection of companion plants

may lead help in the management of pest and disease, and at the same time, may even have positive effect on mung bean production.

The mung bean plant grew well under Malaysian condition and produced mung bean after two months of experiment. The experimental plots of T1, T2, T3, and T4 produced a total weight of mung bean of 82.98 g, 72.00 g, 87.12 g, and 83.26 g, respectively. Surprisingly, the production of plot T1 was found to be comparable with all three other plots despite the fact that the number of mung bean plants in plots T1 is two times as compared to T2, T3 and T4. The preliminary experiment condition is capable to produce more than 0.8 tonne/h of mung bean per season, although the production of this un-optimized condition is less than 1.2 tonne/h reported earlier [16]. Notably, in later stage of the project, the cultivation condition for mung bean-water spinach intercropping had been optimized, leading to production of 159.79g per plot, equivalent to 1.47 tonne/h.

By comparing the average mung bean weight per tree, we noticed that, T3 produced the highest average mung bean weight followed by T4, T2 and T1. Through our observation, the combination of mung bean and water spinach in plot T3 produced more average yield. Besides, we also noticed that although T4 contained less number of mung bean tree planted compared to T1, plot T4 was able to produce more yield due to less competition for nutrient and mineral sources.

4. Conclusions

In the current study, a symbiotic polyculture environment had been created by intercropping mung bean plants and water spinach, and applied on the developed vertical farming system. Mung bean plants not only produce mung beans; it also helps to sustain soil health due to its nitrogen fixing property. In addition, water spinach was found able to increase mung bean production (between +5% in first trial and increased up to +25% in optimized condition). One of the key advantages of the vertical farming system is the significant reduction in pest problem. The mung bean plants in the developed system also showed longer production cycle. A number of recommendations can be made for further plans for the project, including a time-control automatic water irrigation system to enable better management of the system. Additionally, the planting medium can be further optimized to suit the requirement for mung bean plant and water spinach. It is believed that the developed system holds a promising prospect and can contribute towards a sustainable farming future.

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Energy Conversion from Human Heat into Electricity Using Thermoelectric Generator

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ABSTRACT
High requirement of energy supplies is considered as worldwide concern problem due to rapid development of modern mobile terminal devices. Natural source of energy such as water, wave and solar are insufficient to allow unlimited electricity operating for all population across the world. Hence, conversion of energy directly from human body into electricity has gained increasing interest to satisfy the energy demand. In this study, heat from human body as an alternative power source is proposed to create as a new source of energy. High release of heat from human body especially during daily sport activity will be studied. A thermoelectric generator (TEG) is applied to harvest the released heat or energy from human body and transform into electric. A portable charging device incorporated with TEG was created and compared the analysed result with the theoretical and experimental data. A step-by-step study including the expansion of problem statement, development of conceptual design and the specification of device's design and final design using Computer Aided Design (CAD) is reported.

Keywords: Thermoelectric generator (TEG), human heat conversion, energy, electricity

1. Introduction
Basically renewable energies consist of solar energy, wind energy, hydro energy, tidal energy, and etc. All these energies are capable to produce electricity in different forms using different generating method. But, these energy source possess different of disadvantage during harvesting process. For example, solar energy is extensively applied in household industrial by collecting sunlight to generate electricity. However, there is no electricity to be produced if there is no sun light. Hence, there is a need to propose alternative method to best storing the available energy source and convert it to ready use electricity. To date, harvesting the generated heat from human body received growing interest among the researchers as of the human body heat is more sustainable and economically save. Thermoelectric energy generator (TEG) also serves as one of the most popular device to convert the readily human heat energy to electricity. TEG is used to convert thermal energy (heat) into electricity based on "Seebeck effect" directly [1]. TEG require smaller space and less material cost if compared to others energy generator device. Normally, TEG is applicable in jet engine parts, IC engines parts, furnace cover, refrigerator and computer device. There is limited studies investigate the converting heat energy from human body using TEG. Therefore, this research work is proposed to develop a new device to convert heat energy from human body to electricity incorporated using TEG as the energy generator.

2. Materials and methods

The fabricated TEG from the manufacture were used to fabricate the proposed prototype. The proper procedure is including:

a) Designation of the prototype device that attached to human skin for transferring the human heat to electricity using technical drawing software.
b) Fabrication of the prototype as presented in Figure 1 and Figure 2.

Figure 1: Designed Prototypes incorporated with TEG

Figure 2: Prototype incorporated TEG device on human

2.1 Design of circuit & Simulation

In this research work, a simulation design was developed using Multisim software. “Multisim” is a computer software that used to simulate the circuit diagram and analyse the variation of temperature on the increment of voltage. Besides, a circuit had developed with an input of electronic component with 800 mV voltage and alerted using LED. The electronic components were including transistor, resistor, capacitor, diode, inductor, circuit board, TEG plate and LED light as presented in Figure 3.

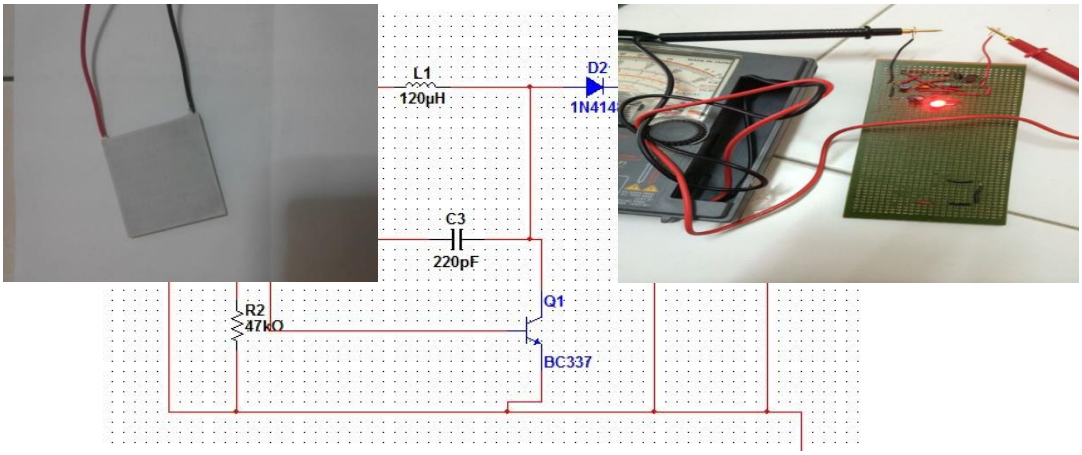

Figure 3: Circuit Diagram (800 mV Input)

2.2 Comparison the theory and experiment result

2.2.1 Theoretical result

From the theory of this thermoelectric generator, the fabricated TEG was attached to the human body and generated electrical power of 50 nW when the temperature difference between the human body and ambient air was 7 °C [2]. The typical TEG was not suitable to attach at human body directly because most typical TEGs are composed of thermocouples on a rigid substrate. On the other hand, flexible TEGs transduce the human body heat efficiently since the flexible TEGs can be tightly attached on the skin. TEG which comprises PDMS and thermoelectric materials was proposed. The proposed harvester was highly flexible through the PDMS structure and was simply fabricated by dispenser printing technology.

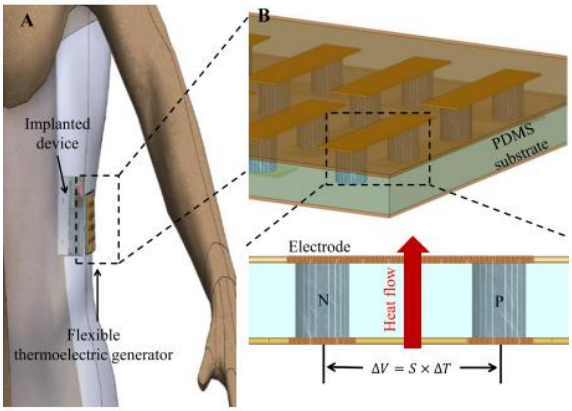

Figure 4: Schematic of TEG system

A schematic of the proposed TEG system is shown Figure 4. This TEG system was comprised of thick PDMS film with thermocouples. PDMS is a flexible polymer with a low thermal conductivity. Thus, the use of PDMS was to reduce the thermal losses during heat flows through the thermoelectric material.

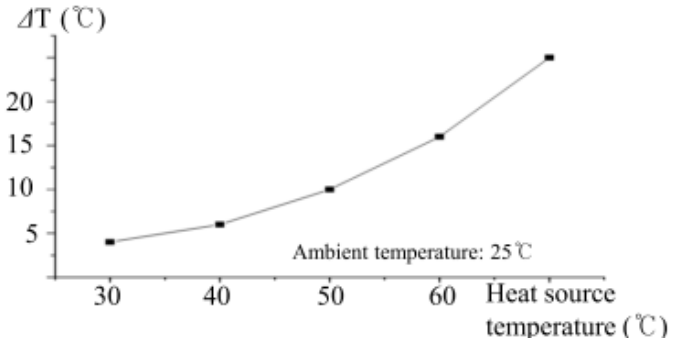

Figure 5: Variation of Temperature between TEG system and human body

Figure 5 shows the temperature difference as a function of the temperature of the heat source when the ambient temperature was 25 °C. It was possible to retain the temperature difference between top and bottom layer (ΔT) when the heat source temperature was close to the human body temperature [2].

2.2.2 Experiment result

From the experiment that been held, the TEG can generate the voltage directly before attach to the circuit as shown in Figure 6. It can produce 5 mV with the hot side need high temperature to generate the electricity. From that situation, the temperature different between hot side and cold side must in large range. Figure 3 show that the voltage goes up when the TEG directly touch with the multimeter with hot water as hot side and ceramic as cold side. The voltage gains up to 6.25 mV when the temperature different between hot side and cold side is larger.

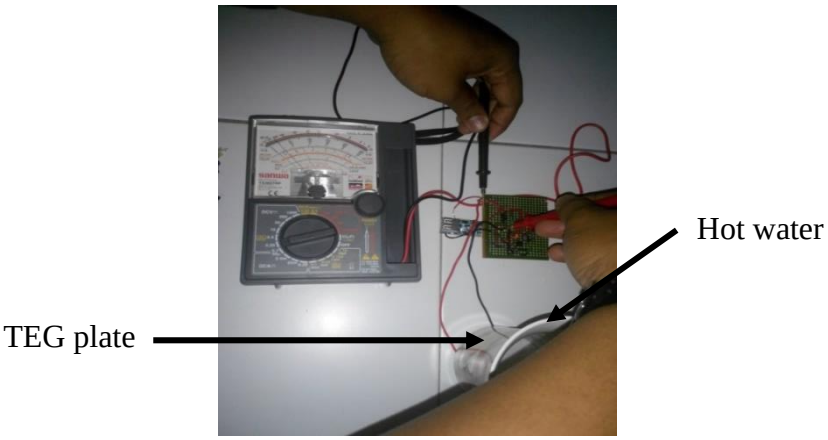

Figure 6: TEG plate direct contact with hot water

Basic formula to calculate the generated voltage from human heat is

$$\Delta V = n \times S \times \Delta T$$

Where ΔV the generated voltage (V) is, n is the number of thermocouples, S is the Seebeck coefficient of the thermoelectric materials (V K−1) and ΔT is the temperature difference between the hot and cold junctions (°C). From the theory, there use one flexible TEG and harvest for 50 nW if the ambient in 25 °C. This theory can be used to apply at the future concept which is to attach at the other sports apparel such as cloth. Human will energize more heat when in active mode; from that particular condition there more efficiency voltage will be produce.

5. Conclusions

As a conclusion, human body heat is capable to produce electricity. However, the temperature is normally too low than the optimum temperature of over 50 °C for energy generation. Although this experiment result may not completely same as the theoretical, the experiment result was closed on certain aspect such as the criteria of the element of the TEG.

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