POME: An Alternative Nutrient Source for Bio-Organic Plant Tissue Culture Media

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Abstract. One of the methods in sustainable agriculture is transforming crop wastes into beneficial products such as bio-fertilizer, which will improve the soil fertility, preserving the natural sources, and cost-effective practices. Currently, these practices also need to be applied in plant tissue culture technology to enhance an eco-friendly and sustainable resources which are abundant and locally available. To date, the new bio-organic media derived from different types of agricultural wastes, especially from fruit wastes were successfully established. The objective of this study was to determine the efficiency of agricultural waste, i.e. Palm Oil Mill Effluent (POME) through a natural fermentation process as a potential nutrient source to substitute the existing standard and synthetic plant tissue culture media (PTC) such as Murashige and Skoog’s (1962) (MS) Medium, Woody Plant Medium (WPM), N6 (Chu) Medium and Gamborg’s (B-5) Medium, available in the market. 100 mL (v/v) POME solution was added with 100 mL (v/v) molasses and then were fermented naturally for 4 weeks in dark condition at room temperature. The results showed that the nutritional value of the fermented POME solution was higher than the synthetic MS basal salt medium. The new POME PTC medium was successfully formulated as a new bio-plant tissue culture media for in vitro regeneration of Musa acuminata.

1. Introduction

Plant tissue culture industry benefits many agricultural and plantation sectors as the technology able to produce a large amount of a high quality with disease free and true-to-type offspring derived from a single explant. Plant tissue culture or micropropagation is a method used to grow the entire plants from one cell (i.e. totipotency cell), which is genetically identical to a parent plant, on a formulated nutrient medium, under in vitro conditions. The formulated plant tissue culture media such as Murashige and Skooq’s (MS) (1962) Medium, Woody Plant Medium (WPM) (1981), Gamborg’s B-5 Medium (1981), and Chu (N6) (1975) medium are generally made up of macronutrients, micronutrients, vitamins, amino acids, sugar, other undefined organic supplements, solidifying agents and plant growth regulators as stated in Table 1. In plant tissue culture technology and micropropagation process, the macronutrients and micronutrients are compulsory for plant development and the plants require an accurate combination of macronutrients and micronutrients in order to live, grow and reproduce. The dynamic factor that contributed to the effectiveness of plant...
tissue culture medium is the presence of various types of ion rather than the compound itself [1] because, too few nutrients will cause the deficiency of plant uptake and too many nutrients will cause toxicity to the plant [2].

The ultimate aim in plant tissue culture technology is for the mass propagation of elite and uniform tissue cultured plant within scarce land available. The plant tissue culture is defined as an in vitro culture method for mass production of selected plant at a competitive price [3]. Even though, the benefit of plant tissue culture technology is widely applied over the countries, the cost of commercialising still incurred high operational cost. The chemicals requirement in plant tissue culture practices was identified as the main causal of high production cost [4]. This was supported by [5] where the expensive cost in the plant tissue culture method restrict its technology as a good advantage to a few institutions. Therefore, the cost effective approach in plant micropropagation techniques is required to produce an elite clonal seedling.

Table 1. The Standard Plant Tissue Culture Medium Components.

| Medium Components | MS [6] | G5 [7] | WPM [8 & 9] | N6 [10 & 11] |
|-------------------|--------|--------|-------------|--------------|
| **Macronutrients** |        |        |             |              |
| Ca₃(PO₄)₂         | 1650.0 | 2500.0 | 2830.0      |              |
| NH₄NO₃            | 1900.0 | 400.0  | 2300.0      | 1660.0       |
| KNO₃              | 370.0  | 250.0  | 1850.0      |              |
| CaCl₂ • 2H₂O      | 440.0  | 96.0   | 1660.0      |              |
| MgSO₄ • 7H₂O      | 170.0  | 170.0  | 400.0       |              |
| KH₂PO₄            | 134.0  | 463    |             |              |
| (NH₄)₂SO₄         |        |        |             |              |
| NaH₂PO₄ • H₂O     | 150.0  |        |             |              |
| CaNO₃•4H₂O        |        |        |             | 556.0        |
| K₂SO₄             |        |        |             | 990.0        |
| **Micronutrients** |        |        |             |              |
| KI                | 0.83   | 0.75   | -           | 0.8          |
| H₃BO₃            | 6.20   | 3.0    | 6.2         | 1.6          |
| MnSO₄ • 4H₂O      | 22.30  | -      | -           | 4.4          |
| MnSO₄ • H₂O       | 10.0   | 29.43  | 3.3         |              |
| ZnSO₄ • 7H₂O      | 8.6    | 2.0    | 8.6         | 1.5          |
| Na₂MoO₄ • 2H₂O    | 0.25   | 0.25   | 0.25        |              |
| CuSO₄ • 5H₂O      | 0.025  | 0.025  |             |              |
| CoCl₂ • 6H₂O      | 0.025  | 0.025  |             |              |
| Co(NO₃)₂ • 6H₂O   | 37.3   | 37.3   | 37.3        | 37.3         |
| Na₂ EDTA          | 27.8   | 27.8   | 27.8        | 27.8         |
| Vitamins and other supplements |        |        |             |              |
| Inositol          | 100.0  | 100.0  | 100.0       | 0.0          |
| Glycine           | 2.0    | 2.0    | 2.0         | 2.0          |
| Thiamine HCl      | 0.1    | 10.0   | 1.0         | 1.0          |
| Pyridoxine HCl    | 0.5    | 0.5    | 0.5         |              |
| Nicotinic acid    | 0.5    | 0.5    | 0.5         |              |
| Ca-panthothenate   |        |        |             |              |
| Cysteine HCl      |        |        |             |              |
Riboflavin
Biotin
Folic acid

Prakash, Hoque and Brink [12] highlighted there are several options to minimize the production cost either by turning into cheaper source or improving the efficiency in method and materials without affecting the plant quality. Alternate for inexpensive resources which performed a similar function like laboratory sucrose in substitute with local commercialize sugar for in vitro micropropagation of potato (Solanum tuberosum L.) were reported [13], or the previous study reported the potential of Isabgol-husk to substitute laboratory solidifying agent for in vitro rooting of Balanites aegyptica where the Isabgol-husk decreased the production cost by ~44% and proposed that table sugar can be used in commercial scale as both of the plantlet; Balanites aegyptica and Phyllanthus emblica responded well for in vitro rooting [14]. [15] also identified the effectiveness of coconut water, papaya, tomato and banana juices in regenerating shoots of Celosia sp. Whereas, [16] found that the addition of coconut water and banana homogenate produced high number of shoot and early plantlet development of Cymbidium pendulum.

Ogero et al. [17] used vegetative fertilizer containing both macro and micronutrients were successful on in vitro regeneration of Ipomea batatas (L.) Lam to substitute MS media. In addition, [18] found that in vitro growth of Mentha sps. on low cost plant tissue culture media KFA and KFA-plus derived from fly ash was almost similar to the artificial MS media as the clonal plants successfully produced in large numbers and had high rate of plant survival during acclimatization. Furthermore, in a recent study by [19], it was found that replacing the whole standard MS media preparation where MS salt, sucrose, and gelling agent changed to much cheaper sources resolved the cost ineffective ingredients in producing in vitro banana plantlets and the waste disposal issues.

Therefore, the present study was done to determine the effectiveness of fermented palm oil mill effluent solutions to substitute the MS medium for in vitro regeneration as the fertilizing properties in palm oil mill effluent are desirable for plant growth. According to previous studies conducted by [20], where the treated POME culture medium potentially promoted the growth of H. callitrichoides compared the full strength MS medium due to its ability in subdue the plant morphological changes. Since 2002-2011, these effluent wastes increased 1.6 times and 60 million tons of POME were generated in 2012, later, the waste will be overflowing to approximately 70-110 million tons in 2020 if improperly managed [21]. Embrandiri et al. [22] pointed out POME is a free and non-toxic material which contains valuable macronutrients required for plant growth. POME also consists of high total nitrogen sources which increase the composting period efficiency by degrading the cellulose and hemicellulose components of empty fruit bunch (EFB). In addition, the organic nutrient constitution in POME and EFB are desirable by plant which it is able to reduce the usage of inorganic fertilizer as part of environment protection [23]. However, there are few reports on POME utilization as a nutrient substitution for plant micropropagation techniques. Thus, this study was conducted to substitute the synthetic plant tissue culture media, with agricultural wastes such as POME, which is locally abundant in the palm oil mill and this will also minimizing the production cost of clonal plant without compromising the quality of regenerant produced.

2. Materials and Method

2.1. Establishment of POME Solution for Fermentation Process

In this study, POME sample was obtained from the FELDA palm oil mills in Pahang, Malaysia. POME sample was collected from the main receiving tank as a main nutrient source. Molasses was purchased from small entrepreneur in Raub, Pahang, Malaysia. The POME sample and molasses were measured approximately 100 (v/v) mL and 100 (v/v) g, respectively at a ratio of 1:1. Then, the molasses were added into the POME solution. The container (24cm x 13cm x 11cm) was sealed with a mesh cloth and wrapped with an aluminum foil to begin the fermentation process. The fermentation process was
carried out for 4 weeks in dark condition (24 hours darkness) at room temperature. The initial pH value and final pH values were weekly recorded.

2.2. Preparation of Fermented Solution for Nutrient Analysis
Some text. The fermented POME solution was extracted manually by squeezing using filter to remove soils and debris from the crude solution. Then, the fermented crude solution was filtered using filter paper to remove any fine particles. Subsequently, the filtered fermented crude nutrient and mineral content was analyzed using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) instrument at the Soil Laboratory, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, Malacca, Malaysia.

2.3. Preparation of Bio-Plant Tissue Culture Medium
Some text. The fermented POME solution was taken accordingly to the composition for the establishment culture media as mention in Table 2. Each formulation was supplemented with 30.00 g/L laboratory sucrose, 2.00 g/L solidifying agent, 1.00 g/L activated charcoal, and 2.00 mg/L Benzylamipurine (BAP). The MS basal salt medium was served as standard control medium. All culture media were adjusted to pH 6.4 prior to autoclaving at temperature of 121˚C for 20 minutes. Totally, there are 6 treatments including control.

| Treatment | Composition |
|-----------|-------------|
| MS<sub>0</sub> | MSBS + Sucrose + Agar + Plant Hormone |
| PM<sub>1</sub> | 1% concentration + Sucrose + Agar + Plant Hormone |
| PM<sub>2</sub> | 2% concentration + Sucrose + Agar + Plant Hormone |
| PM<sub>3</sub> | 3% concentration + Sucrose + Agar + Plant Hormone |
| PM<sub>4</sub> | 4% concentration + Sucrose + Agar + Plant Hormone |
| PM<sub>5</sub> | 5% concentration + Sucrose + Agar + Plant Hormone |

2.4. In Vitro Regeneration of Musa acuminata
Some text. Healthy suckers of *Musa acuminata* were obtained from the banana farm at Tanjung Karang, Selangor, Malaysia. The leaves and roots were removed and washed with tap water. Then, the suckers were trimmed to the sizes of 10.0 cm length and 5.0 cm diameter before washed with liquid detergent and later, rinsed off the liquid detergent with tap water. The clean suckers were placed under running tap water for 30 minutes before being transferred to the laminar air flow chamber. The explants were surface sterilized by treated the explants with 50% (v/v) chlorox (a.i. 5.25% sodium hypochlorite) solutions added with a few drops of Tween20 for 30 minutes and then rinsed thoroughly with sterile distilled water once. The exposed explants area with chlorox were excised and dipped into 95% (v/v) alcohol for 3 minutes, followed by the solutions of 100% chlorox (v/v) (a.i. 5.25% sodium hypochlorite) with a few drops of Tween20 for 5 minutes. Lastly the explants were rinsed three times with sterile distilled water and dried on autoclaved tissue paper for 15 minutes to remove the excess moisture before the explants were trimmed to the final size of 2.0 cm length and 2.0 cm diameter. The half cut explants were cultured on the MS basal salt media and the formulated POME PTC media. All the explants were incubated for 3 months under the temperature at 25±2˚C, photoperiod condition of 16 hours with light intensity of 2000 lux per day for shoot initiation. The day for explants changed into green and explant responses on different concentrations of formulated POME PTC media were observed and recorded.

3. Results and Discussion

3.1. Fermentation of POME
The fermentation process was successfully completed after 4 weeks in the dark condition at room temperature. The weekly pH values were measured as provided in Figure 1. Visual and physical changes in the fermented solution for 4 weeks were observed. The first week fermentation, the solution released a sweet and sour odor almost like a rotten fruit. Subsequently, the solution exhibited bubble/foam on top of the liquid and disappeared at the end of the fermentation period. This finding was also supported by the conducted experiment where the fermented solution of the banana peel form bubbles at the second days, the banana peel floated, liquid settle at the bottom, and lastly the sweet and sour smell produced [24]. This indicated that the microorganism actively decomposed and transformed all the sugars, nutrient and oxygen efficiently into ionic macro and micro nutrients. There was no change in term of physical colour whereby the fermented solution is still maintained black in colour as similar with the original POME.

Figure 1 shows that the fermented solution recorded an alkaline pH value during the early phase of fermentation which is pH 7. The pH value of the fermented solution started to decrease rapidly to pH 5.61 after being fermented for one week. It is indicated that during the first week of fermentation, the microorganism using the food source provided. Subsequently, the pH value decreased in week 2 and week 3 to pH 4.23 and pH 4.19 respectively. Then, the final (week 4) pH slightly increased to pH 4.45. The fermentation process was considered completed as the pH begins to increase due to the microorganism had achieved stationary and death phase. Hence, the fermented POME solution was an acidic solution with pH 4.45 at the end of fermentation.

The reduction in pH proved that the POME solution was succeeded in the fermentation process. Nanson and Fields [25] reported that the fermentation caused a decreasing in pH value. Cokgor et al. [26] highlighted that the significant factors affecting the efficiency of sludge fermentation are pH and temperature. According to the Sundberg, Smârs, and Jöhnson [27], the final stage of composting causes the pH to decline to the below neutral value. This is because the formation of organic acid discharges a large amount of carbon dioxide (CO₂). In addition, the pH reduction is caused by the acidification process where the carbohydrates are being converted into alcohols or acids. This trend is also supported by [28] whom reported that during the glucose fermentation, the pH values decreased due to the higher production of acetic acid or butyric acid as the pH values remains constant at pH 4.68-4.85 over the time of fermentation period. Therefore, the fermented solutions of the POME may offer high amounts of nutrient content similar as the decomposition of organic materials.

![Figure 1. The pH profile of the POME solution after 4 weeks of fermentation process.](image)

### 3.2. Macro and Micro Nutrient Analysis

The fermented POME solution was analysed to determine the presence of macronutrient by using Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) instrument.
macronutrients are potassium (P), phosphorus (K), magnesium (Mg), calcium (Ca), and sulphur (S). Meanwhile, the determination of selected micronutrients is iron (Fe), copper (Cu), manganese (Mn), and sodium (Na).

Table 3 and Figure 2(a) showed that the concentration of macronutrients in fermented POME solution was relatively higher than the MS basal salt media. The total nutrient content of P and K in the fermented POME solution were found to be at 84.16 % and 55.82% respectively higher than in MS basal salt concentration. While, the total nutrient content of Mg, and Ca in fermented POME solution was also found to be at 87.97 %, and 74.99 % relatively higher than in MS basal salt concentration. All these macronutrients content were increased by an average of four times during the fermentation process.

Table 4 and Figure 2(b) showed that the concentration of micronutrient in fermented POME solution was relatively higher than the MS basal salt media. The total nutrient content of Fe, Cu, and Na were found to be at 95.23 %, 97.62 % and 89.27 %. However, the total Mn content is lower than the required concentration in MS basal salt. The Mn concentration was 21.68 % lower than the MS basal salt concentration. The lower concentrations of Mn in the fermented POME solution are probably caused by specific nutrients suppressed the Mn enhancement during the fermentation process. According to the Alam, Kamei, and Kawai [29] the significant decreased of Mn toxicity level in shoots and roots of barley (Hordeum vulgare L. cv. Minorimuig) by elevating the Fe concentration where the plant regained its constituents. Alam et al. [30] also conducted the relationship the effect of potassium (K) on absorption and translocation in Mn uptake where high K assist in preventing the plant to intake excessive Mn amount. These findings showed that iron (Fe) and potassium (K) attributed in repressing the plant toxicity especially for Mn level. Therefore, the Mn concentration below the level apparently caused by the presence of elevated K and Fe in fermented POME solution. All these micronutrients content were increased by an average of sixteen times during the fermentation process.

The conversion process occurs during fermentation synthesizes vitamins, minerals, nutrient, and other bioactive compounds better compared than the raw materials. The successful fermentation of agricultural waste, i.e. POME solution was proven by the declining of pH value and other characters present. Addition of molasses as substrate acts as a source of energy for the microorganism growth and survival during the fermentation process [31]. Kawaguchi and Minamisawa [32] highlighted the symbiotic interaction between the natural occurring microbes during the fermentation process improved some mineral intake by plants. These nutrients act as synthetic elements in cultures and it may differ for each plant species and genotype [33]. This is consistent with previous studies done by [34] reported the correlation between the fermentation process of agricultural waste and pH reduction directly enhanced the quality of fermented waste. The nutrient enhancement in fermented POME solution is vital in promoting the plant growth especially in sterile environment because the fresh POME lacking in primary macronutrients [35] nutrient composition in the fermented POME was found relatively higher compared to the MS medium. In addition, the stationary phase of fermentation process offers various metabolites for biotechnological interest [31]. The action of microorganisms during the fermentation process changed the structure, texture and added flavour and aroma of the product. In some cases, fermentation enhanced the contents of mineral, vitamins and better digestibility than the raw materials [36].

Table 3. Macro nutrient contents (mg/L) of the MS basal salt and the fermented POME solution after 4 weeks of fermentation.

| Elements     | MS basal salt | POME          |
|--------------|---------------|---------------|
| Potassium (K)| 695.88        | 1575.20       |
| Phosphorus (P)| 21.88        | 138.16        |
| Calcium (Ca) | 107.78        | 431.04        |
| Magnesium (Mg)| 19.38        | 161.20        |

Note: Slight instability of mineral content reading may happen due to the manual extraction method which may cause instability pressure during extraction. However, the subject used the pressure is the same to decrease potential error.
Table 4. Micro nutrient contents (mg/L) of the MS basal salt and the fermented POME solution after 4 weeks of fermentation.

| Elements       | MS basal salt | POME     |
|----------------|--------------|----------|
| Iron (Fe)      | 4.34         | 90.96    |
| Copper (Cu)    | 0.05         | 1.68     |
| Manganese (Mn)| 4.47         | 3.92     |
| Sodium (Na)    | 9.99         | 93.12    |

Note: Slight instability of mineral content reading may happen due to the manual extraction method which may cause instability pressure during extraction. However, the subject used the pressure is the same to decrease potential error.

Figure 2. The Comparison on nutrient content between the fermented OME solution and MS basal salt for (a) and (b)

3.3. The Effects of Fermented POME Solution on In Vitro Regeneration of Musa acuminate

Some text. Bio-organic plant tissue culture medium was formulated according to the different concentration requirements of the fermented POME solutions. The concentration levels started with 1%, 2%, 3%, 4%, to 5% respectively. The formulated bio-organic media displayed a light yellowish medium to a thick yellowish medium as the concentration level increased compared to the translucent and clear MS basal salt medium. Even though the formulated bio-organic media were yellow in color, the media still translucent which was similar to MS basal salt medium.

In addition, the pH of formulated bio-organic media was set to pH 6.40 prior to the autoclaving instead pH 5.80 as optimum pH in most standard plant tissue culture media. This is because, the pH of formulated bio-organic media tends to drop in average of 0.9±1 after autoclaving as the pH condition of formulated media became an acidic again (pH 4.30±0.5). An acidic plant tissue culture media is not preferable in plant microporpagation techniques to promote plant growth and development where optimum pH for plants to grow is pH 5.5-6.5.

After 3 months of culture initiation, the aseptic plantlets in the formulated bio-organic media ranges from 1%, 2%, 3%, 4%, and 5% showed a positive response. The days of plantlet to green was observed in different concentration levels and standard MS basal salt media as mention in Table 4. The result showed that the explants in the MS0 took 20-25 days to transform into green colour, while the explants in PM1, PM2, and PM3 took 18-25 days compared to PM1 and PM2. Hence, this indicated that the high concentration level of formulated POME media had similar days to MS basal salt for the explants to transform into green colour than the lower concentration. In addition, each explant cultured into the formulation POME PTC media and MS media were replicated into 10 and the total of responsive explants were recorded and it is shown that more than 50% of the culture positively
responded on the formulated POME PTC media. Further statistical analysis on the mean number of plantlet height and number of shoot produced will be analysed and recorded.

Table 5. Days for explants of Musa acuminata responded to morphogenesis process and exhibited green colour after being cultured into MS basal salt media and formulated POME PTC media within 3 months of culture initiation.

| Treatment | Concentration | Explant response (day) |
|-----------|---------------|-------------------------|
| MS₀       | -             | 25                      |
| PM₁       | 1%            | 45-60                   |
| PM₂       | 2%            | 30-36                   |
| PM₃       | 3%            | 18-25                   |
| PM₄       | 4%            | 18-25                   |
| PM₅       | 5%            | 18-25                   |

Figure 3. Establishment of bio-organic POME PTC media, according to its composition and supplemented with 30.0 g/L sucrose, 2.00 g/L gelling agent, and 1.0 g/L active charcoal.

Several researchers had identified the usage of agricultural wastes such as bio-organic plant tissue culture media to promote plant growth. A study conducted by [37] whom using two types of fruit waste which were banana and papaya through a fermentation process had resulted a better in vitro seed germination of pitaya than the synthetic media. In addition, the outcome of research conducted by [38] also proved that the commercial cooking agar and cocopeat has successfully reduced solidifying agent cost by 97.289% and 99.937% respectively to promote pitaya growth. The potential use of banana and papaya as nutrient replacement was continued by [34] by adding pineapple, calamansi lime, and key lime peels as new fruits waste combination to promote growth of different plant species. The formulated fruits waste solutions media showed the significant interaction of Lycium barbarum (Goji) and Aquilaria malaccensis Lamk. (Agarwood) in promoting the plant growth. Nevertheless, the application of formulating fruits waste solutions was proven to promote plant growth as both of the plant positively responsive to the formulated fruits waste media. Subsequently, [39] improved the usage of fruit waste by choosing the fruit peel waste in developing bio-organic plant tissue culture media. The fruit peel waste was selected as a main nutrient source because the fruit peel wastes have a high content of bioactive compound which can be transformed into potentially valuable products [40]. The low concentration of the formulated fruits peel media showed a greater performance in plant height than the higher concentration. Hence, Musa acuminata cv. Berangan (AAA) were successfully regenerated on the developed media of fruit peel waste. The constrained in former researches were the availability of fruit wastes. Even though it is easy to find the fruit waste due to the fruit is a daily consumption routine, but they are not available in abundant amount. Hence, the agricultural waste that produces in mass volume and easy accessible are required and POME meets all the requirements indicated. Biotransformation of POME into valuable products especially in plant tissue culture techniques was a profitable industry and more approachable sources which in agreement with [40] point out that three key elements to achieve good development in sustainable agriculture which were economic thriving, eco-friendly, and favorable to human.
4. Conclusion

The fermented POME solution was successful in enhancing the nutrient and mineral content and supported the theory of increasing nutrient quality through fermentation process. Hence, the fermented POME solution fulfills in providing important macronutrient, micronutrient and essential vitamins for the satisfactory plant growth in the aseptic condition. Furthermore, the preliminary study showed that POME PTC media were succeeding in regeneration of *Musa acuminata* in *in vitro* condition. The fermented POME solution contained nutrient needed by the tissue cultured plant as the nutrient uptake by the tissue cultured plant physiologically similar to the optimum nutrient of the standard plant tissue culture media, depending on the different needs of plant species. The collected data on plantlet height and number of shoot will be further analysed using one-way analysis of variance (ANOVA) by SPSS programme to determine its significant difference for *in vitro* regeneration of *Musa acuminata*.

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