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Effect of bio-liquid organic fertilizer on the growth of *Dipterocarpus alatus* Roxb seedlings in the pot experiment

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ABSTRACT

Aims: The objective of this research was to study the effect of bio-liquid organic fertilizer on the growth of *Dipterocarpus alatus* Roxb seedlings (30 days old) in the pot experiment.
Methodology and results: For the production of bio-liquid fertilizers, distillery slop; molasses and bio-methane waste water were fermented with plant growth promoting bacteria, which had potentials for nitrogen fixing, phosphate solubilizing and potassium solubilizing properties. It was found that treatment no. 13 (molasses + three bacterial isolates (PGPB), 30 days of fermentation) presented the best result on the growth parameters of *D. alatus* Roxb including root length (21.67 cm), shoot height (20.33 cm), root fresh weight (1.49 g), shoot fresh weight (3.61 g) and total biomass (4.13 g). Moreover, using liquid organic fertilizer produced from molasses supplemented with bacteria had higher growth-promoting effects on *D. alatus* than the effective microorganisms (EM).
Conclusion, significance and impact of study: To covert agricultural residues to the valuable product was the aim of this work. In our experiment, we found that molasses and bio-methane waste water were suitable for using as a material to produce liquid organic fertilizers which were beneficial for promoting growth of *D. alatus* seedlings.

Keywords: Bio-liquid organic fertilizer, *Dipterocarpus alatus* Roxb, seedling, plant growth promoting microorganisms

INTRODUCTION

Recently, the use of microorganisms in organic and sustainable agriculture has grown tremendously. Specially, plant growth promoting microorganisms (PGPMs) are able to promote the growth of plants. PGPMs are bacteria in genus *Bacillus, Pseudomonas, Azospirillum, Azotobacter, Burkholderia, Serratia* and *Streptomyces* (Kloepper, 1992; Jeon *et al*., 2003; Park *et al*., 2005). In a study on the effects of PGPMs on growth of *Dipterocarpus alatus* (Sangwanit and Sangtian, 1994), the plants were inoculated with chopped fruiting bodies of 3 ectomycorrhizal fungi i.e. *Russula aeruginea* Lindbl, *R. albida* Peck. and *R. sanguinea* Fr. Height and the diameter at root collar of the seedlings were measured monthly. Shoot and root dry weights were determined at 7 months old. The results found that height and diameters at root collar, shoot and root dry weights of all treatments were significantly different. The seedlings inoculated with *R. aeruginea* presented the highest values of all growth parameters mentioned above, followed by the seedlings inoculated with *R. sanguinea*, *R. albida* and non-inoculated controls, respectively. However, there was no significant difference in shoot to root dry weight ratios in all treatments. This experiment indicated that *R. aeruginea* and *R. sanguinea* were good in root colonization of *D. alatus* seedlings.

Distillery slop, molasses and bio-methane waste water were the waste from Agro-industry factory. They are excellent sources of nutrients suitable for use as a substrate for producing organic fertilizers. Therefore, to reduce these wastes by converting to high valuable agricultural products is the aim of the factories (Makkar and Cameotra, 1997; Kumar *et al*., 1997; Joshi *et al*., 2008; Joshi *et al*., 2008; Mohana *et al*., 2009; Tyagi *et al*., 2013).

PGPM could promote the growth of plant by direct or indirect mechanisms such as fix nitrogen, solubilize phosphate and potassium in soil for plant, produce phytohormone and enzyme and has the antagonistic activity. Moreover, many kinds of these microbes are cellulolytic microorganisms which were able to degrade cellulose in plant and by-products are glucose and some nutrients for plant growth and themselves Agricultural residues are the waste from the farm and Agro-industry. They are various types, such as molasses, distillery slop,

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as a source of energy and nutrients. To reduce these wastes, it can convert to valuable product such as organic fertilizer and to get the best organic fertilizer product which has the high potential on promoting the growth of plant and enhance the quality of soil, PGPMs are selected to supplement into the organic fertilizer.

Due to the good benefit of PGPMs, the aim of this study was to produce bio-liquid organic fertilizer by using agro-industry residues such as distillery slop, molasses and bio-methane waste water supplemented with plant growth promoting bacteria for enhancing the growth of *D. alatus* Roxb seedling.

MATERIALS AND METHODS

All agro-industry residues (distillery slop, molasses and bio-methane waste water) were provided by the Sugar factory at Khon Kaen province in Thailand. Plant growth promoting bacteria isolate B8 and B24 were screened from rhizosphere soil sample (Riddech *et al.*, 2017) and isolate BDS31 was isolated from distillery slop sample of ethanol factory. (Wongkoon *et al.*, 2014). Both of bacterial isolates were able to fix nitrogen, solubilize phosphate and potassium in the soil.

Bio-liquid organic fertilizer production from agricultural residues

Agro-industry waste materials including distillery slop, molasses and bio-methane waste water were supplemented with the mixture of plant growth promoting bacteria isolate B8, B24 and BDS31 with the inoculum density 10^8 CFU/mL in the ratio 1:1:1 (v/v/v). The fermented material was analyzed for pH, electrical conductivity, total N, total P and total K and enumerated total microorganism.

The six formulas of bio-liquid organic fertilizer were produced with different substrates as in the detail below:
Formula 1 distillery slop: tap water 1:50 (DS) (v/v)
Formula 2 distillery slop: tap water 1:50 (v/v)+ the mixture of 3 bacteria isolates (DS+B)
Formula 3 molasses: tap water 1:50 (M) (v/v)
Formula 4 molasses: tap water 1:50 (v/v)+ the mixture of 3 bacteria isolates (M+B)
Formula 5 bio-methane waste water: tap water 1:50 (Bi) (v/v)
Formula 6 bio-methane waste water: tap water 1:50 (v/v) + the mixture of 3 bacteria isolates (Bi+B)
The mixtures were contained in plastic cans, incubated at ambient temperature for 30 days. Bio-liquid organic fertilizers were sampled at day 0, 15 and 30 for chemical and microbiological characteristic analysis. The bio-liquid organic fertilizers were then applied for promoting the growth of *D. alatus* Roxb seedling.

The chemical parameters measurement in bio-liquid organic fertilizers

All the bio-liquid organic fertilizers at 0, 15 and 30 days of fermentation were determined the pH by pH meter (OHAUS, starter 2100and electrical conductivity (EC) by the EC meter (METTLER TOLEDO, FiveEasy FE .(1-30 Total nitrogen was determined by using Kjedahl method (Kirk, 1950). Total phosphorus was analyzed by using wet digestion spectrophotometer method at the wavelength of 420 nm. Analysis of total potassium was performed by using Flame photometer.

The microbiological parameters measurement in bio-liquid organic fertilizers

All the bio-liquid organic fertilizers at 0, 7, 15 and 30 days of fermentation were counted the number of Nitrogen fixing bacteria using nitrogen free culture medium (Ashby's medium) (Atlas, 2004) by spread plate technique, Phosphate solubilizing bacteria was enumerated on Pikovskaya medium (Surange, 1997) supplemented with tricalcium phosphate by spread plate technique, Potassium solubilizing bacteria was counted on Aleksandrov medium (Hu *et al*., 2006) supplemented with potassium aluminium silicate by spread plate technique. Total microorganisms were determined on plate count agar (PCA) by spread plate technique.

Application of bio-liquid organic fertilizers for enhancing the growth of *Dipterocarpus alatus* Roxb seedling

Nursery bags (20 × 21 cm) filled with sieved soil, were sown with freshly collected seeds of *D. alatus* Roxb. After that, after 4 months of seeds were transplanted into the green house for cultivating at the seedling pot. The experimental design was Completely Randomized Design with 15 treatments, five replications. The treatments (T) were as follows : tap water (T_1), bacteria 3 isolates (T_2), Effective Microorganisms solution (EM) (T_3), bio-liquid organic fertilizer for Bi at 15 days fermentation (T_4), bio-liquid organic fertilizer for Bi+B at 15 days fermentation (T_5), bio-liquid organic fertilizer for M at 15 days fermentation (T_6), bio-liquid organic fertilizer for M+B at 15 days fermentation (T_7), bio-liquid organic fertilizer for DS at 15 days fermentation (T_8), bio-liquid organic fertilizer for DS+B at 15 days fermentation (T_9), bio-liquid organic fertilizer for Bi at 30 days fermentation (T_{10}), bio-liquid organic fertilizer for Bi+B at 30 days fermentation (T_{11}), bio-liquid organic fertilizer for M at 30 days fermentation (T_{12}), bio-liquid organic fertilizer for M+B at 30 days fermentation (T_{13}), bio-liquid organic fertilizer for DS at 30 days fermentation (T_{14}), bio-liquid organic fertilizer for DS+B at 30 days fermentation (T_{15}). Bacteria suspension, EM solution and fertilizer (fertilizer:water ;1:100) 200 mL was added every 7 days. After that the harvesting was done at 30 days after transplanting (DAT). The effects of each treatment on the growth of the *D. alatus* Roxb were determined by indicating on these parameters: root length, shoot length, shoot fresh and dry weight, and root fresh and dry weight and biomass (total plant dry weight). The comparisons were made with the control and among the treatments.

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Statistical analysis

The data was analyzed variance ANOVA using STATISTIX 8. The significant data was analyzed with least significant differences (LSD) between treatments means at *P*<0.05 and *P*<0.01.

RESULTS

Bio-liquid organic fertilizer production from agricultural residues

Chemical properties of the original substrates were analyzed prior to being used in liquid fertilizer production and the results are presented in Table 1. The pH values of distillery slop, molasses and bio-methane waste water were extremely acidic (pH 4.20), neutral (pH 6.42) and moderately alkaline (pH 7.75) respectively. The EC values were in the range of 24-39 d*S*/m which were considered as being salty. The nutritional properties showed total potassium (K) was higher than total nitrogen (N) and total phosphorus (P). The initial amounts of microbial populations were similar.

All three substrates were used for producing the bio-liquid organic fertilizer production supplemented with plant growth promoting bacteria composing of isolates B8, B24 and BDS31 and fermented for 30 days. The chemical properties were investigated at 0, 15 and 30 days of fermentation to evaluate the nutrient contents, pH and EC in Bio-liquid organic fertilizer. It was revealed, in Table 2, that formula 4 [molasses: tap water 1:50 (v/v) + the mixture of bacteria 3 isolate (M+B)] contained the highest N, P, K content compared to all other formula at 0, 15 and 30 days of fermentation due to the higher nutrient contents of the molasses compared to those of the bio-methane and distillery slop. The changes in pH of bio-liquid organic fertilizers with fermentation duration are shown in Figure 1a. During fermentation, the pH of distillery slop and distillery slop supplemented with bacteria remained stable at the basic pH at 7.86 and 7.97 respectively. The pH of molasses and molasses supplemented with bacteria became slightly more acidic at 15 days of fermentation, then increased to close to the original pH after 30 days. The most drastic change in pH was found in bio-methane and bio-methane supplemented with bacteria in which the pH steadily increased from 3 to 6 after 30 days of fermentation. After 30 days of fermentation, the pH values of bio-fertilizers made from molasses, bio-methane waste water and distillery slop in the treatment non add bacteria were 4.85, 6.07 and 7.78, respectively. The pH values of bio-fertilizers made from molasses, bio-methane waste water and distillery slop add bacteria were 5.23, 5.79 and 7.58, respectively. The EC values in the bio-liquid organic fertilizer were about 1-4 d*S*/m (Fig 1b) which presented less salinity than in the original substrate after fermentation.

Table 1 The characteristics of the original substrates (distillery slop, molasses and bio-methane waste water).

Characteristic	Distillery slop	Molasses	Bio-methane waste water
pH	7.75 ± 0.23	6.42 ± 0.31	4.20 ± 0.15
EC (d*S*/m)	32.63 ± 0.60	39.8 ± 1.15	27.40 ± 1.25
Total microorganisms (log CFU/mL)	6.78 ± 0.65	7.25 ± 0.36	7.29 ± 0.44
Total nitrogen (N) (%)	0.30±0.01	0.92±0.01	0.29±0.03
Total phosphorus (P) (%)	0.0047±7.88	0.04±0.03	0.0207±2.83
Total potassium (K) (%)	1.54±0.02	5.25±0.02	1.62±0.02

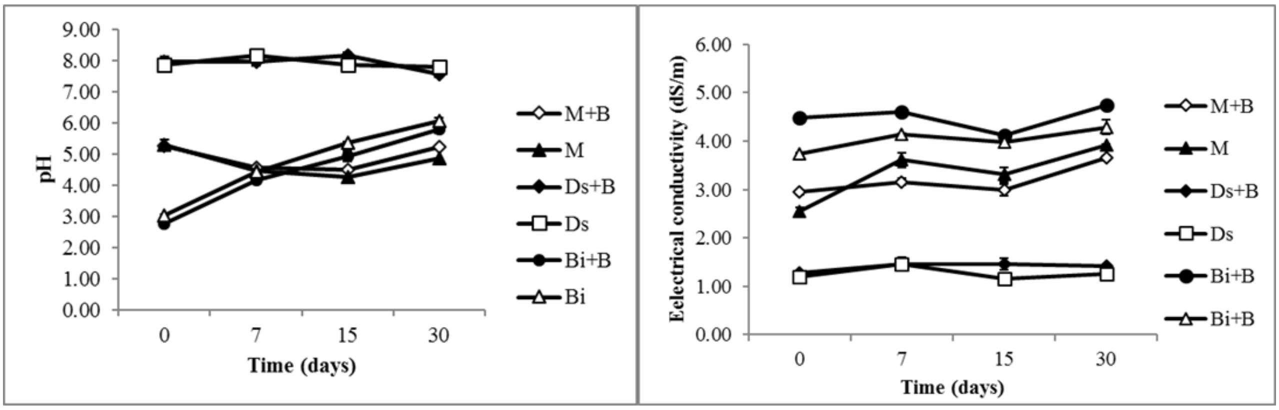

Figure 1: pH (a) and EC (b) values of bio-liquid organic fertilizers (M-Molasses, Ds-Distillery slop, Bi-Bio-methane waste water and B- the mixture of bacteria isolates) after 0, 7, 15 and 30 days of fermentation.

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Table 2: The nutrient contents in bio-liquid organic fertilizer production.

Bio-liquid organic fertilizer	0 day			15 day			30 day		
	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)	N (%)	P (%)	K (%)
1. Formula 2 distillery slop: tap water 1:50 (v/v)+ the mixture of 3 bacteria isolates (DS+B)	0.3047b	0.0051b	1.5450b	0.0171b	0.0002c	0.0257b	0.0164b	0.0002b	0.0270b
2. Formula 4 molasses: tap water 1:50 (v/v)+ the mixture of 3 bacteria isolates (M+B)	0.7697a	0.0575a	3.4035a	0.0341a	0.0010a	0.0886a	0.0364a	0.0009a	0.0930a
3. Formula 6 bio-methane waste water: tap water 1:50 (v/v) + the mixture of 3 bacteria isolates (Bi+B)	0.0151c	0.0004b	0.0244c	0.0155b	0.0004b	0.0244c	0.0164b	0.0002b	0.0270b
F-test	**	**	**	**	**	**	**	**	**
%CV	2.45	2.38	2.82	5.69	7.71	1.40	3.67	7.45	3.33

Notes: The values showed in this table were the mean.
Mean values within a column followed by different letters were significantly different according to the LSD test, $P \leq 0.01$ (∗∗).

Changes in microbial population in bio-liquid organic fertilizers during fermentation

Microbial populations in the original substrates and in the bio-liquid organic fertilizer were enumerated on plate count agar by spread plate technique. The result showed that high number of indigenous and the mixture of microorganisms were found in the bio-methane waste water substrate and in the fertilizer formula 6. Also, the data form statistical analysis showed that it was a non-significantly different in the formula 4 molasses: tap water 1:50 (v/v)+ the mixture of 3 bacteria isolates (M+B) which compared with formula 6 bio-methane waste water: tap water 1:50 (v/v) + the mixture of 3 bacteria isolates (Bi+B) (Table 3).

Table 3: Microbiological analysis of bio-liquid organic fertilizer after 0, 7, 15 and 30 days of fermentation (log CFU/mL).

Bio-liquid organic fertilizers	Total microorganism on days of fermentation (log CFU/mL)			
	0 days	7 days	15 days	30 days
Formula 1 (DS)	6.80b	6.38e	6.68b	6.40b
Formula 2 (DS+B)	8.08a	7.66bc	8.43a	6.83b
Formula 3 (M)	6.65b	7.22cd	7.38b	6.98b
Formula 4 (M+B)	7.41ab	7.79ab	8.95a	9.56a
Formula 5 (Bi)	7.11b	6.85d	7.18b	7.21b
Formula 6 (Bi+B)	8.06a	8.14a	8.41a	8.32a
F-test	*	**	**	**
%CV	8.73	4.20	6.22	8.02

Note : The values showed in this table were the mean
**Means with different letters indicate significant differences, LSD test (P<0.05)
* Means with different letters indicate significant differences, LSD test (P<0.01)
(Ds-Distillery slop, M-Molasses, Bi-bio-methane waste water and B-bacteria 3 isolates)

Effects of the application of bio-liquid organic fertilizer on the growth of *D. alatus* Roxb seedling

All formulas of 15 and 30 days old fermented bio-liquid organic fertilizers supplemented with plant growth promoting bacteria were applied to *D. alatus* Roxb seedlings and plant growth parameters 30 days after fertilizer application were evaluated. The results showed that the root length in treatment 13 (M (molasses) + B 30 day) was the best for the growth of *D. alatus* Roxb seedlings.The root length was 21.67 cm, the stem height was 20.33 cm, the fresh root weight was 1.49 g, the fresh weight was 3.61 g and the biomass was 4.13 g. (Table 4).

DISCUSSION

The molasses had high nutrient contents as compared to distillery slop and bio-methane waste water because it is a by-product of sugar cane production. According to the research of Jimenez *et al*. (2004) the composition of molasses consisted of carbohydrates, minerals and various types of nutrients. Thus, molasses is a good raw material from industry to be utilized in agricultural production. Among the six formula of bio-liquid organic fertilizers produced, we found formula 4, molasses: tap water 1:50 (v/v)+ the mixture of 3 bacteria isolates (M+B) had higher nutrient contents i.e. total nitrogen, total phosphorus and total potassium than other formulations. Molasses is an important energy source for the growth of microorganisms. Molasses was one of the components in liquid organic fertilizer production which provided a source of energy and encourages the growth of beneficial microorganisms. These microorganisms decompose substrates in the liquid organic fertilizer and become the rice source of organic and inorganic nutrients for plant (Apai and Thongdeethae, 2002).

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Table 4: Effect of the application of Bio-liquid organic fertilizer on the *Dipterocarpus alatus* Roxb seedling.

Treatments	Root Length (cm)	Shoot length (cm)	Root fresh weight (g)	Shoot fresh weight (g)	Biomass (g)
T1(control)	12.67^{f}	15.33^{ef}	0.88^{d}	3.28^{abc}	2.24^{e}
T2 (Bac)	16.33^{df}	14.17^{f}	1.15^{bcd}	2.77^{bcdef}	2.72^{cde}
T3 (EM)	20.33^{abc}	17.20^{bcde}	1.16^{abcd}	3.52^{ab}	2.89^{bcde}
T4 (Bi 15 day)	18.00^{bcd}	16.50^{cde}	1.17^{abcd}	3.27^{abc}	3.01^{bcde}
T5 (Bi+B 15 day)	18.97^{abcd}	17.90^{bcd}	1.25^{abc}	2.99^{abcde}	3.77^{ab}
T6 (M 15 day)	14.17^{ef}	15.33^{ef}	1.34^{ab}	2.78^{bcdef}	2.65^{de}
T7 (M+B 15 day)	16.33^{df}	17.00^{cde}	1.29^{ab}	3.15^{abcd}	2.94^{bcde}
T8 (Ds 15 day)	19.00^{abcd}	18.00^{bcd}	0.94^{cd}	2.45^{def}	3.11^{bcde}
T9 (Ds+B 15 day)	21.00^{ab}	18.33^{abc}	1.08^{bcd}	2.50^{cdef}	3.42^{abcd}
T10 (Bi 30 day)	19.00^{abcd}	17.33^{bcde}	0.89^{cd}	2.00^{f}	2.22^{e}
T11 (Bi+B 30 day)	20.33^{abc}	18.33^{abc}	1.17^{abcd}	2.31^{ef}	3.39^{abcd}
T12 (M 30 day)	19.00^{abcd}	17.67^{bcd}	1.31^{ab}	2.41^{def}	3.07^{bcde}
T13 (M+B 30 day)	21.67^{a}	20.33^{a}	1.49^{a}	3.61^{a}	4.13^{a}
T14 (Ds 30 day)	17.60^{cd}	16.00^{def}	1.11^{bcd}	2.59^{cdef}	3.02^{bcde}
T15 (Ds+B 30 day)	20.67^{abc}	19.33^{ab}	1.28^{ab}	3.40^{ab}	3.55^{abc}
F-test	**	**	*	**	**
%CV	10.31	8.03	17.03	16.58	17.57

Notes: The values showed in this table were the mean.
**Means with different letters indicate significant differences, LSD test (*P*<0.05)
* Means with different letters indicate significant differences, LSD test (*P*<0.01)
(M-Molasses, Ds-Distillery slop, Bi-Bio-methane waste water and B- bacteria 3 isolate)

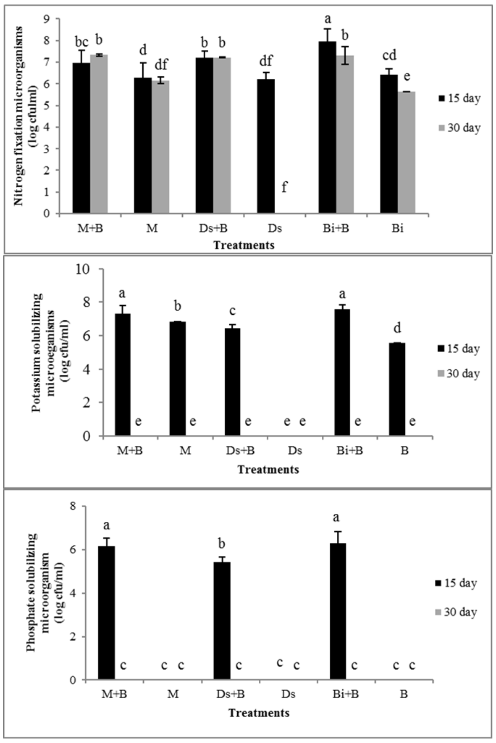

Figure 2: Nitrogen fixing (a), potassium solubilizing (b) and phosphate solubilizing (c) bacteria in bio-liquid organic fertilizer after 15 and 30 days of fermentation (logCFU/mL). The vertical bar indicated significant difference values at *P*<0.05. (M-Molasses, Ds-Distillery slop, Bi-Bio-methane waste water and B- bacteria 3 isolate).

Figure 3: Effect of the application of Bio-liquid organic fertilizers on the growth of *Dipterocarpus alatus* Roxb seedling.

Moreover, we also found high amount of microorganisms on this formula and in bio-methane waste water: tap water 1:50 (v/v) + the mixture of bacteria 3 isolate (Bi+B). These microorganisms had the main role on the degradation of substrates to produce the energy and nutrient source the growth of microorganisms. In addition, they acted as plant growth promoting microorganism through several mechanisms such as nitrogen fixing, phosphate solubilizing and potassium solubilizing. It was reported that these microorganisms were useful for plants growth and it improved the nutrient rotation in the soil (Ratneetoo, 2012).

The experiment on the effects of application of Bio-liquid organic fertilizer on the growth of *D. alatus* Roxb seedlings was performed by using pouring technique. We found that the quality of liquid organic fertilizer depended on the content of nutrients in original substrates and the supplementation with plant growth promoting bacteria was able to help the degradation process of the materials to become the beneficial compounds for plant growth.

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The results showed that Treatment 13 (M + B, 30 days) was the best bio-liquid fertilizer that promoted the growth of *D. alatus* seedlings as determined from root length and biomass. All growth parameters including root length, stem height, fresh root weight, fresh shoot weight and biomass were found significantly different among all treatments. According to previous research (Yokota, 1992; Tjandraatmadja *et al*., 1994) that used fermented molasses as an additive growing plant, it was found that plants had a higher dry matter content than non-molasses treatments. Moreover, the research of Netpae (2012) showed that liquid organic fertilizer production containing molasses, and molasses and microorganism (EM) fermented for 12 weeks promoted growth of kale better than chemical fertilizer. Sangwanit and Sangtian (1994) used non-autoclaved soil for producing the diverse ectomycorrhizal (ECM) fungi for enhancing the growth of *D. alatus* seedlings. The diverse ECM fungi were able to stimulate the growth of seedlings and could proliferate in field soil after plantation of the seedlings, which would be important for reforestation with dipterocarps. The similar results were reported Kaewgrajang *et al*. (2014) on the use of pure mycelial culture of *Astraeus odoratus* which was promising for easy preparation of the inoculum and the positive effect on seedling growth of D. *alatus.* It is well known that ECM formation enhances host-plant growth through the increased uptake of soil nutrients. Indeed, some studies have revealed positive effects of ECM fungal colonization on growth and phosphorus uptake of dipterocarp seedlings (Sangwanit *et al*., 1991; See and Alexander, 1994; Yazid *et al*., 1994; Lee *et al*., 2008).

CONCLUSION

Agricultural residues from the agro-industry are increasing every year which causes growing environmental problems. To convert these wastes to the valuable products was the aim of this study. It was found that molasses and bio-methane waste water were suitable for using as a material to produce liquid organic fertilizers which were beneficial for promoting growth of *D. alatus* seedlings.

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