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Effect of biogas effluent from pig manure and Longan (*Dimocarpus longan*) residues on growth of Marigold (*Tagetes erecta*)

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Abstract The efficiency of biogas effluent fermented with pig manure and longan residues on growth of marigold was investigated. Treatments were control (no fertilizer), and four concentrations (10%, 20%, 30% and 40%) of biogas effluent and chemical fertilizer. Plants were watered with 200 ml of each biogas effluent concentrationt every 4 days. Chemical fertilizers were applied as 15-15-15 and 8-24-24. Results showed that the growth of marigold in the control was lowest as compared to the other treatments. There was no significant difference in the plant height, stem diameter, bush diameter, fresh weight of blooming flowers and initiation of first flower between biogas effluents and chemical fertilizer. Fresh weight of whole plant and number of blooming flowers resulting from the chemical fertilizer treatment was highest compared to the control and effluent treatments. Dry weight of whole plant resulting from the chemical fertilizer treatment was not significantly different compared to 10%, 20% and 30% biogas effluent treatments. Total chlorophyll and chlorophyll per unit leaf area in the chemical fertilizer did not differ as compared to 30% and 40% biogas effluent treatments.

Keywords: biogas effluent, longan, marigold, growth

Introduction

Thailand is well-known as an agricultural country where fertilizer is necessary for farmers to increase their agricultural productivity. Thailand imports chemical fertilizers from abroad every year. In 2014, Thailand imported 5,415,020 tons of chemical fertilizers from abroad valuedat 2,030 million dollars (Department of Agriculture, 2015). Therefore, searching for chemical fertilizer replacement is important to reduce the expense of purchasing and importing chemical fertilizer.

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At present, the animal husbandry in Thailand has grown to meet the demand for meat. However, the expansion of animal husbandry has caused environmental pollution problems in farms and nearby communities, such as bad smell, flies, wastewater and diseases resulting from animal waste and other wastes from the unsuitable management of farm systems. Therefore, biogas production from animal waste was promoted for solving the pollution problems and the biogas is also a renewable energy source in farms. After biogas production is completed, the fermented waste or effluent can be used as organic fertilizer to improve the soil and increase crop yields (Furukawa and Hasegawa, 2006; Ausungnoen *et al.*, 2014; Stinner, Moller and Leithold, 2008; Hossain *et al.*, 2014). Therefore, using effluent from biogas fermentation as bio-fertilizer is markable strategy for reducing the expense and trade deficit from the import of chemical fertilizer.

Prathumyot *et al.* (2017) reported that the biogas effluent fermented with pig dung, peels and seeds of longan (*Dimocarpus longan*) contains nitrogen, phosphorus and potassium that are important nutrients for plant growth. However, the utilization of effluent after biogas production as bio-fertilizer has not been studied. Thus, in this experiment the effects of biogas effluent fermented with pig manure, peels and seeds of longan on the growth of marigold (*Tagetes erecta*), an attractive yellow flower that can tolerate and adapt to various environmental conditions, were investigated for potential use as bio-fertilizer.

Materials and methods

The experimental design was a Completely Randomized Design (CRD) with 4 replications. Six treatments were control (no fertilizer), four concentrations of biogas effluent (10%, 20%, 30% and 40%) and chemical fertilizer (15-15-15 and 8-24-24). Marigolds were planted in cell plug trays with peat moss. The plants were transferred to pots containing 2.4 kg of soil mixed with husk and burned husk at a ratio of 2:1:1, 10 days after planting (DAP). The chemical properties of soil and biogas effluent are shown in Table 1. Marigolds were watered with a total of 200 ml/pot of 10%, 20%, 30% and 40% biogas effluent solution every 4 days. The chemical fertilizer treatment consisted of 5 g of 15-15-15 formula fertilizer dissolved in 400 ml water to water pots 16, 23 and 30 days after planting (DAP) and 5 g of 8-24-24 formula fertilizer dissolved in 400 ml of water to pots 37, 44, 51, 58 and 65 DAP by following the cultivation method of the Department of Agricultural Extension (2002). Four hundred ml of water were added daily to all pots. The experiment was conducted for 69 days at the Agricultural Technology Faculty at Rambhai Barni Rajabhat University.

Table1. Chemical properties of soil and biogas effluent at the start of the experiment

Chemical properties	**Soil**	**Biogas effluent**
pH	5.44	5.57
Nitrogen concentration (ppm)	1033	4900
Phosphorus concentration (ppm)	161.68	1300
Potassium concentration (ppm)	754.48	3600

Soil pH was recorded at the beginning and the end of the experiment using a IQ-150 pH meter (IQ Scientific Instruments Inc., Carlsbad, CA). Data of plant height, stem diameter and bush diameter were collected weekly. After flowering, flower number, fresh weight and dry weight of blooming flowers was measured daily. The data of flower diameter was collected from 1st to 10th blooming flower. Total chlorophyll, chlorophyll a and chlorophyll b concentrations were measured by the method of Mackinney (1941). At the end of experiment, plants were sampled and washed in water. The fresh plants were weighted for collecting the data of fresh weight. Then plants were dried in an oven at 80 ℃ for 48 hours and measured dry weight of roots, branches and leaves. Results were analyzed by analysis of variance and means separated by Duncan’s multiple range test (DMRT).

Results

Soil pH

Soil pH at the start of experiment (15 DAP) was not statistically different among the treatments and ranged from 5.40 to 5.44. However, the results showed a statistical difference in soil pH at 63 DAP. The soil pH was decreased by the chemical fertilizer and 40% effluent treatments compared to the remaining treatments (Table 2).

Growth parameters

There was a significant difference in plant height after 28 DAP until the end of the experiment. The chemical fertilizer and all effluent treatments increased in plant height compared to the control at 69 DAP. The control treatment resulted in the lowest plant height of 36.66 cm. There was no significant difference in plant height between all biogas effluent and chemical fertilizer treatments at 69 DAP and ranged from 68.16 cm to 77.66 cm. However, the plant height resulting from the 40% effluent treatment tended to be lower than the other effluent treatments (Fig. 1).

Table 2. The effect of treatments on soil pH, plant height, stem diameter and bush diameter at the end of experiment

Treatment	Soil pH		Stem diameter (mm)	Bush diameter (cm)
	15 DAP	63 DAP		
Control (0%)	5.44±0.15^{a}	5.44±0.25^{a}	16.00±1.00^{c}	4.03±0.21^{b}
Effluent 10%	5.44±0.03^{a}	5.45±0.05^{a}	38.50±1.80^{b}	11.37±1.12^{a}
Effluent 20%	5.36±0.26^{a}	5.37±0.09^{a}	46.33±5.50^{a}	12.02±1.36^{a}
Effluent 30%	5.42±0.04^{a}	5.31±0.14^{a}	44.00±4.58^{ab}	11.99±1.35^{a}
Effluent 40%	5.40±0.02^{a}	4.60±0.33^{b}	39.00±3.46^{b}	10.86±0.86^{a}
Chemical fertilizer	5.41±0.08^{a}	3.72±.08^{c}	42.66±1.52^{ab}	12.36±1.24^{a}
F-test	ns	*	**	**
CV (%)	1.92	8.38	9.04	10.58

Means with different letters in each column are significantly different according to DMRT.
** = significant at P≤0.01. * = significant at P≤0.05. ns = not significant at P≤0.05.

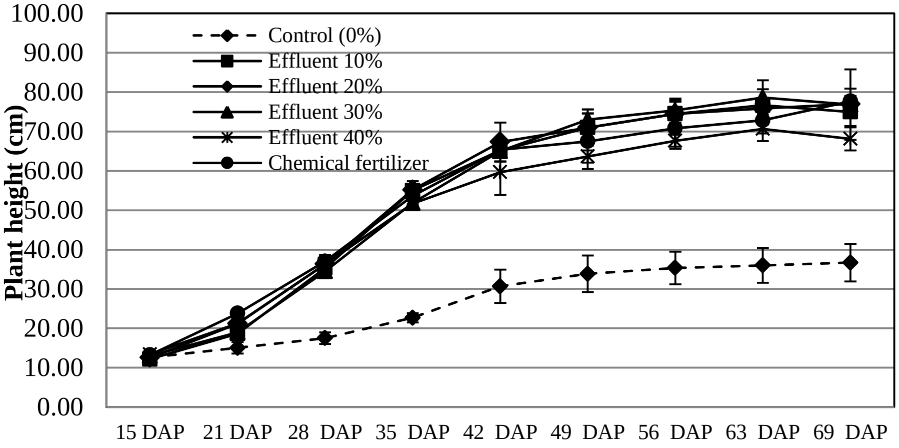

Figure 1. The effect of treatments on plant height during the experimental period

The greatest stem diameter resulted from the 20 % effluent treatment (46.33 mm) which did not significantly differ from the chemical fertilizer and 30% effluent treatments. The control treatment resulted in the lowest stem diameter of 16 cm (Table 2). Bush diameter was significantly increased by the chemical fertilizer and all effluent treatments compared to the control which resulted in the lowest bush diameter of 4.03 cm. There was no significant difference in bush diameter among all biogas effluent and chemical fertilizer treatments and ranged from 11.37 cm to 12.36 cm (Table 2).

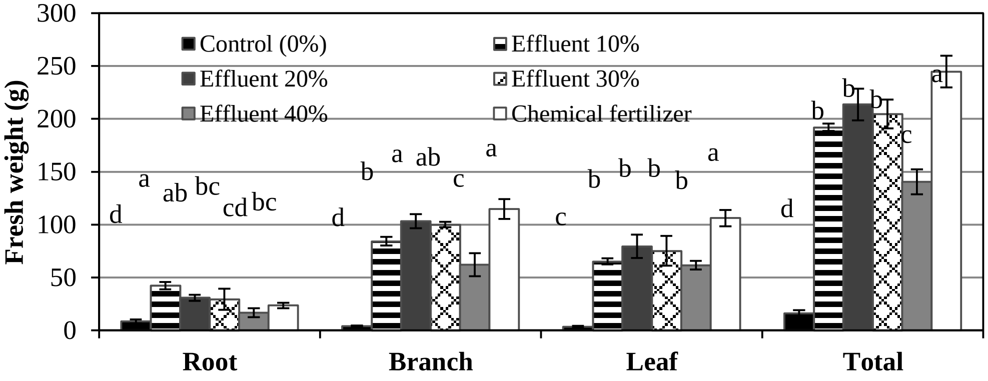

Figure 2. The effect of treatments on Fresh weight of root, branch, leaf and total of marigold plants at the end of the experiment (69 DAP). Bars with different letters in each plant part indicate significant differences in different treatments at the 0.01 probability level, according to DMRT.

Root fresh weight was significantly increased by the chemical fertilizers and all effluent treatments compared to the control. Root fresh weight resulting from the 10% effluent treatment was significantly higher than that resulting from the chemical fertilizer treatment. Effluent treatment of 20%, 30% and 40% increased root fresh weight to similar levels compared to the chemical fertilizer treatment (Fig. 2). In the case of branch fresh weight, the control treatment resulted in the lowest branch fresh weight of 3.96 g. 20% and 30% effluent treatments significantly increased branch fresh weight equivalent to the chemical fertilizer treatment and ranged from 100.12 to 114.90 g. Branch fresh weight resulting from 40% effluent treatment was lower compared to the remaining effluent treatments (Fig. 2).

Leaf fresh weight was significantly increased by the chemical fertilizer treatment compared to the other treatments. The control treatment resulted in the lowest dry weight of leaf and followed by the effluent treatments (Fig. 2). Total plant fresh weight was significantly increased by the chemical fertilizers and all effluent treatments compared to the control. Total fresh weight was greater in the chemical fertilizer treatment. Effluent treatment of 40% resulted in the lowest total fresh weight compared to the other effluent treatments. The control treatment resulted in the lowest total fresh weight of 16.16 g (Fig. 2).

Root dry weight was increased by the 10% and 30% effluent treatments compared to the chemical fertilizer treatment. Root dry weight of the control was similar compared to the chemical fertilizer and 40% effluent treatments (Fig. 3).

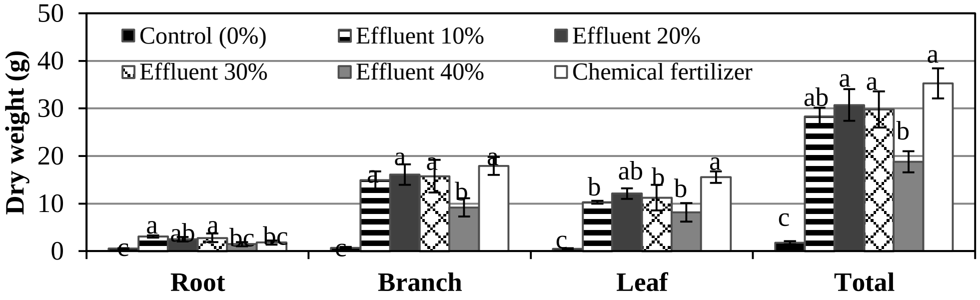

Figure 3. The effect of treatments on dry weight of root, branch, leaf and total of marigold plants at the end of the experiment (69 DAP). Bars with different letters in each plant part indicate significant differences in treatments at the 0.01 probability level, according to DMRT.

Branch dry weight was significantly increased by the chemical fertilizer treatment and the 10%, 20% and 30% effluent treatments. Effluent treatment of 40% resulted in the lowest branch dry weight compared to the other effluent treatments. The control treatment resulted in the lowest branch dry weight of 0.70 g (Fig. 3). The 20% effluent treatment increased leaf dry weight the same as the chemical fertilizer treatment. There was no difference in leaf dry weight among effluent treatments. Leaf dry weight was reduced by the control and was 0.51 g (Fig. 3). Total dry weight was increased by all effluent and chemical fertilizer treatments compared to the control. Total dry weight in the chemical fertilizer treatment did not differ as compared to 10%, 20% and 30% effluent treatments. Total dry weight in 40% effluent treatment and the control was significantly lower as compared to the chemical fertilizer treatment. The lowest total dry weight was 1.76 g in the control (Fig. 3).

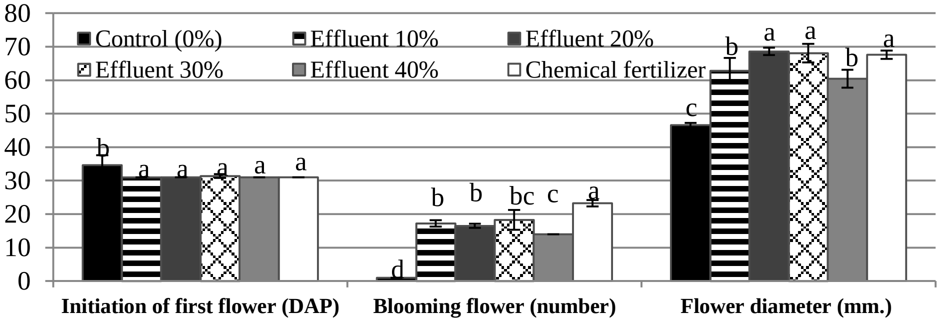

Figure 4. The effect of treatments on initiation of first flower, blooming flower number and flower diameter of marigold at the end of experiment. Bars with different letters in each plant part indicate significant difference in treatments at the 0.05 probability level, according to DMRT.

There was a significant difference in the initiation of first flower, blooming flower, and flower diameter. The chemical fertilizer and all effluent treatments significantly reduced the initiation of first flower compared to the control. The initiation of first flower in the chemical fertilizer and all effluent treatments was 31 DAP and 34.67 DAP in the control (Fig. 4). Blooming flower number was increased by the chemical fertilizer as compared to the remaining treatments. Number of blooming flowers resulting from the 40% effluent treatment tended to be lower compared to the other effluent treatments. The control treatment resulted in the lowest number of blooming flowers (Fig. 4). In the case of flower diameter, the control treatment resulted in the lowest flower diameter followed by 40% effluent and 10% effluent. Flower diameter was similar in plants treated with 20% and 30% effluent and the chemical fertilizer (Fig. 4).

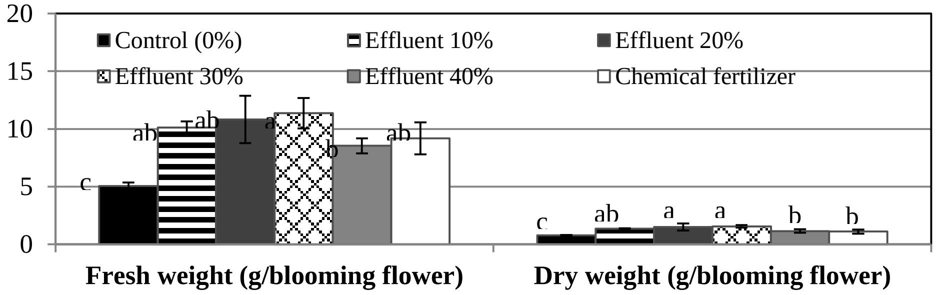

Figure 5. The effect of treatments on fresh weight and dry weight of blooming flower at the end of experiment. Bars with different letters in each plant part indicate significant difference in treatments at the 0.01 probability level, according to DMRT.

Fresh and dry weight of blooming flowers was increased by all treatments compared to the control. In case of fresh weight, the chemical fertilizer treatment did not differ significantly when compared with the effluent treatments. The lowest fresh weight was in the control at 5.06 g (Fig. 5). Dry weight of blooming flower in 20% and 30% effluent treatments was significantly higher than that in the chemical fertilizer treatment. The 10% and 40% effluent treatments resulted in dry weight similar to the chemical fertilizer treatment. The control resulted in the least dry weight of 0.77g (Fig. 5).

Plant physiological parameters

There was a significant difference in the concentrations of total chlorophyll, chlorophyll a and chlorophyll b per unit leaf area among treatments. Total chlorophyll concentration resulting from the chemical fertilizer treatment was similar to the 30% and 40% effluent treatments. The 20% and 10% effluent and control treatments showed the lowest total chlorophyll concentration as compared to the chemical fertilizer treatment (Fig. 6). Similarly, the difference in chlorophyll a concentration per unit leaf area did not differ among the chemical fertilizer, 30% and 40% effluent treatments. Chlorophyll a concentration resulting from the 20% and 10% effluent treatments and the control was less than chlorophyll a resulting from the chemical fertilizer treatment (Fig. 6). Chlorophyll b concentration in the chemical fertilizer treatment did not differ significantly when compared to the effluent treatments. The lowest concentration of chlorophyll b was in the control at 4.92 µg/cm^2 (Fig. 6).

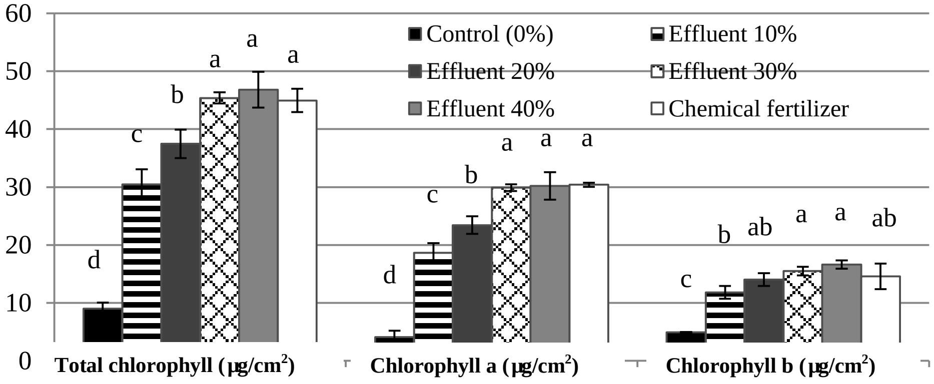

Figure 6. The effect of treatments on the concentrations of total chlorophyll, chlorophyll a and chlorophyll b per unit leaf area. Bars with different letters in each plant part indicate significant difference in treatments at the 0.01 probability level, according to DMRT.

Discussion

Nitrogenous fertilizers decrease soil pH (Liu *et al.*, 2010; Hatiet *et al.*, 2008; Darusman *et al.*, 1991). This is mainly due to the fact that most fertilizers supply N as NH_4^+ first, which upon oxidation releases H^+ ions (Magdof *et al.*, 1997). The soil chemical properties of this experiment showed that soil pH was reduced by the chemical fertilizer treatment compared to the remaining treatments. It may be due to the chemical fertilizers used in this experiment, 15-15-15 and 8-24-24, which are nitrogenous fertilizers. These results were in agreement with the findings of Chit-aree *et al.* (2017), who also reported the effect of chemical fertilizer on soil acidity.

Moreover, the results in this experiment also showed that soil pH in 40% effluent treatment decreased as compared to the other effluent treatments. This may be also due to the 40% effluent treatment consisted of the highest amount of nitrogen which affected soil acidity. However, this result was different as compared to the report of Chit-aree *et al.* (2017) which found that biogas effluent did not affect soil pH. This difference may due to high nitrogen concentration in biogas effluent of this experiment (4900 ppm) while it was 1000 ppm in the experiment of Chit-aree *et al*. (2017).

In this experiment, it was found that dry weight of roots in the control was the same as the dry weight of roots in the chemical fertilizer and 40% effluent treatments. These results agree with the research of Chit-aree *et al.* (2017) who found that the dry weight of marigold roots was decreased by soil acidity which was 3.72 and 4.60 in the chemical fertilizer and 40% effluent treatments, respectively. The dry weight of branch in 10%, 20% and 30% effluent treatments did not decrease as compared to the chemical fertilizer treatment. On the other hand, growth of leaf was reduced by all effluent treatments when compared to the chemical fertilizer treatment. The reduction of growth by the biogas effluent treatment was most severe in the leaf as compared to root and branch. Finally, total fresh and dry weights as influenced by all effluent treatments tended to be lower than total fresh and dry weight resulting from the chemical fertilizer treatment.

Plant nutrients are necessary for plant growth and survival. Nitrogen, phosphorus and potassium are essential nutrients for plant growth (Taiz and Zeiger, 2006). In this experiment, total amount of nitrogen, phosphorus and potassium which plants received from the chemical fertilizer treatments was 4.25, 8.25 and 8.25 g, respectively, while plants treated with 30% biogas effluent received 3.82 g of nitrogen, 1.01 g of phosphorus and 2.81 g of potassium. The total amount of nitrogen, phosphorus and potassium applied to plants gradually decreased with the concentration of biogas effluent. The treatment containing the lowest nutrient concentration was the control treatment. Thus, the lower nitrogen, phosphorus and potassium concentrations in 10%, 20% and 30% effluent treatments as compared to the chemical fertilizer treatment may be the cause of growth reduction resulting from these effluent treatments.

On the other hand, total amount of nitrogen, phosphorus and potassium which plants received from 40% biogas effluent treatment was 5.10, 1.35 and 3.74g, respectively. It showed that total amount of nitrogen, phosphorus and potassium was higher than that of 10%, 20% and 30% effluent treatments. However, total fresh and dry weights in 40% effluent treatment were lower as compared to 10%, 20% and 30% effluent treatments. A report by Chaikachang *et al*. (2017) showed that the high concentration of effluent was also high in electric conductivity of effluent. When this high EC effluent was used to water the soil, soil EC also increased and resulted in low plant growth. The EC of 10%, 20%, 30% and 40% effluent in this experiment was 3.49, 4.40, 2.50 and 7.11 ds/m, respectively. The EC of 40% effluent was highest as compared to the other concentration. This may be the reason for growth reduction by the 40% biogas effluent treatment.

In this experiment, it was found that low plant nutrients in biogas effluent treatments effected to the number of blooming flowers while it did not affect initiation of first flower, fresh and dry weights of blooming flowers. These results were to agree with the findings of Chit-aree *et al*. (2017), who also reported the effect of low nutrient concentration in biogas effluent on blooming flower number.

Nitrogen is an important component of chlorophyll which is the major pigment for photosynthesis (Taiz and Zeiger, 2006). Results showed that total chlorophyll and concentrations were decreased by the control, 10% and 20% effluent as compared to the remaining treatments. This may be due to the low nitrogen concentration resulting from the control, 10% and 20% effluent treatments as explained above. This low chlorophyll concentration resulting from the control, 10% and 20% effluent treatments may also influenced total fresh and dry weights of marigold.

The growth parameters of marigold in this experiment were enhanced to a greater degree compared to the results of Chit-aree *et al.* (2017). For example, plant height, stem diameter, bush diameter, total chlorophyll, chlorophyll a and b concentrations and branch dry weight in 30% effluent treatment of this experiment was equivalent to those resulting from the chemical fertilizer treatment while they were lower those of the chemical fertilizer treatment in the report of Chit-aree *et al*. (2017). It may because of the biogas effluent in this experiment contained higher concentration of nitrogen as compared to the report of Chit-aree *et al*. (2017), 24% biogas effluent had only 1.70 g of nitrogen as bio-fertilizer. Moreover, the number of blooming flowers was still low in this and Chit-aree *et al*. (2017) experiments. Such results may be due to the low concentration of phosphorus and potassium in the effluent of both experiments. Therefore, identifying materials that can produce biogas and that contain high concentration of phosphorus and potassium should be further investigated.

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