Chiang Mai J. Sci. 2018; 45(3) : 1257-1273
http://epg.science.cmu.ac.th/ejournal/
Contributed Paper

# The Effects Bio-fertilizer and Liquid Organic Fertilizer on the Growth of Vegetables in the Pot Experiment

**Nguyen Thi Huynh Nhu [a], Ng Lee Chuen [b] and Nuntavun Riddech* [c,d]**

[a] Graduate School of International Program of Biological Science, Faculty of Science, Khon Kaen University, Thailand.

[b] School of Food Science and Technology, Universiti Malaysia Terengganu, Malaysia.

[c] Salt-tolerant Rice Research Group, Khon Kaen University, Khon Kaen, 40002, Thailand.

[d] Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen, 40002, Thailand.

* Author for correspondence; e-mail: nunrid@kku.ac.th

Received: 15 January 2017
Accepted: 7 September 2017

## ABSTRACT

Bio-fertilizer has been used as an alternative of the chemical fertilizer, *Klebsiella oxytoca* in organic fertilizer can convert nutrients in the soil to the available form for plant growth. This study was aimed to investigate the effects of *Klebsiella oxytoca* in the forms of bio-fertilizer and liquid organic fertilizer on the growth of *Ipomoea aquatica*, *Brassica integrifolia* and *Brassica rapa chinensis*. These trials were carried out in four replications of the randomized complete block design (RCBD) in the greenhouse. Results showed that by applying organic fertilizer in *I. aquatica,* plant growth parameters, soil nutrients and microbial activities were approximately in chemical fertilizer treatment excepted for the nutrient content in plant. The nutrients in *B. integrifolia* plant that grown in liquid organic fertilizer was lower than soil that apply chemical. On the other hand, these values in *B. rapa chinensis* which applied with liquid fertilizer was equal (applying fermentation fruit waste) and significant higher (applying molasses) than in the chemical fertilizer application. The result from this study indicated that *B. rapa chinensis* responsed better *B. integrifolia* when applied liquid organic fertilizer. The microbial activities of microorganisms were in inverse ratio to the amount of chemical applying. The highest number nitrogen fixing bacteria, phosphate and potassium solubilization bacteria, and *Klebsiella oxytoca* were found in the soil supplemented with liquid fertilizer. The treatment of 50% of chemical fertilizer in combination with liquid fertilizer could increase the *I. aquatica* yield and improved soil nutrient.

**Keywords:** bio-fertilizer, liquid organic fertilizer, *Klebsiella oxytoca, Ipomoea aquatica, Brassica integrifolia*, *Brassica rapa chinensis*

## 1. INTRODUCTION

In recent years, increase of food demand due to global overpopulation has been a burden for agriculture, which is causing the overuse of chemical pesticides and fertilizers [1]. Consequently, the ground water, air and soil have been polluted with industrial substances such as potassium (K), phosphorus (P) and nitrogen (N) which cause serious

threat to human health and environment [2]. Over-use of ammonium sulfate causes the decline of soil fertility due to the lowering of soil pH. In addition, overuse of inorganic nitrogen and phosphate releases the harmful compound into the soil, water and atmosphere, a typical example is phosphorus and nitrate eutrophication that cause death of animal and the dense growth of plant by lack of oxygen. In general, plants absorb only 10-40% of total fertilizer applied. In other words, 60% to 90% of fertilizer is lost. To improve this problem, microbial inoculation is necessary to manage integrated nutrient for green environment and sustainable agricultural production [3].

In the innovative point of view, the alternatives to agro-chemicals are biological products based on organic fertilizer [4]. Bio-fertilizer is defined as a biological product that contains living organisms to support for plant growth and improve the natural fertility of soil. There are two forms of bio-fertilizers, the solid and liquid form. The good carriers for the solid form should be low cost, nontoxic, easy to sterilize, available in sufficient amount, good absorption and maintenance of moisture, free of lump-forming and easy to process, suitable for seed germination and good at buffering capacity of pH [5-6]. Liquid bio-fertilizer is considered as the potential strategy to increase the shelf-life of bio-fertilizer [7]. Compared with the solid form, liquid form allows the manufacturer to increase the amount of nutrients and inducers. The processing cost of the solid form is significantly lower than that of the liquid form. However, bio-fertilizers in the solid form are less thermo-tolerant compared with liquid form, which can tolerate the high temperature of 55 °C [8]. Therefore, improve the quality of bio-fertilizer can be success by the application of formulation in liquid bio-fertilizer.

Beneficial microorganisms in bio-fertilizer can improve the nutrient in soil by their potential of nitrogen fixation, phosphorus metabolisms and provide plant hormones such asindole-3-acetic acid (IAA), gibberellic acid (GA), abscisic acid (ABA), 1-minocyclopropane-1-carboxylate (ACC) deaminase, salicylic acid and siderophore. Based on the characteristics and function as the plant growth promoting rhizobacteria (PGPRs), *Klebsiella* sp. are classified as diazotrophs or nitrogen-fixers [9], and has been predominantly used for the production of liquid organic fertilizer and bio-fertilizer. *Striga hermonthica* seeds germination was stimulated by the mixture of *Klebsiella oxytoca* ($5 \times 10^7$ cfu $ml^{-1}$) in both laboratory and green-house [10].

In addition, *Ipomoea aquatica* is a high value vegetable and consider as local medical for human, food for pigs and animals in farm and has been apply widely to purify eutrophic water. The people in the Southeast Asia called water spinach or water cabbage, other common name is swam spinach, kang kong or water convolvulus. *Brassica integrifolia* and *Brassica rapa chinensis* provide leaves, stems, edible root, buds, seed and flowers. They provide vitamin A, carotenes, vitamin K, flavonoid anti-oxidants and mineral [11]. The main product of *B. integrifolia* is leave, and *B. rapa chinensis* is petiole.

Bio-fertilizer has been applied not only to enrich macro- and micro- nutrient of soil but also to support plant growth and to increase soil organic matter by releasing bio-degradation and antibiotics [12]. Bio-fertilizer can be applied to soil or seed inoculants, for multiple crop production and nutrient cycling [13]. The additional benefits of the use of bio-fertilizer are no side effects to ecosystem even prolonged application of organic substances [14].

Unfortunately, many beneficial PGPRs are still not actively used, because agriculturists and ecologists have only limited knowledge about PGPRs. The main reason is the insufficient awareness of bio-fertilizer protocol application in the field and technology transfer to the farmers. Recent development of biotechnology, microbial science, genomics and plant pathogen interaction will be believed to optimize bio-protocols [15]. The success of sustainable agriculture will be affected by the success of bio-fertilizers strategies research, following by the inventions of PGPRs properties and their mechanism in enhancing plant growth and soil fertilities.

To reduce the usage of chemical fertilizers, new strategies are need to be developed for suitable and green agriculture. The main aim of this study was to determine the effect of bio-fertilizer on the growth of *I. aquatica*, *B. integrifolia* and *B. rapa chinensis*, the effect of liquid organic fertilizer on *I. aquatica* plant and the microbial activities on these soil treatments that cultivated three vegetables by fluorescein diacetate method in the greenhouse.

## 2. MATERIALS AND METHODS

This research was conducted from August to December 2015 in the greenhouse of the Faculty of Agriculture, Khon Kaen University, Thailand. The ambient temperature range was 28-36 °C and the moisture range was 67-79%. The photo period was 11-12.6 hours.

### 2.1 Materials

The *Klebsiella oxytoca* strains LC136922 has high potential on plant growth promoting properties and it has good characteristics in nitrogen-fixer, phosphate solubilization, potassium solubilization, ACC deaminase activities, ammonium production, IAA and gibberellic acid production [16]. It provided from the Microbial Fertilizer Laboratory group, Department of Microbiology, Faculty of Science, Khon Kaen University, Thailand, and was used to produce bio-fertilizer and liquid organic fertilizer.

To make bio-fertilizer, rice straw was ground in the small piece (5 mm) and inoculated with the broth culture of *Klebsiella oxytoca* with the initiate cell density at $10^8$ CFU/ml and incubated at 30 °C for 2 months [17]. Two kinds of liquid organic fertilizers were produced by inoculating *Klebsiella oxytoca* (the initiate cell density was $10^8$ CFU/ml) into sterile molasses(diluted 100 times with sterile distill water) or fermented fruit waste substrate (1 month old of sterile extracted liquid organic fertilizer form) then incubated for 15 days [17].

Three vegetables used in this study were *Ipomoea aquatica, Brassica integrifolia,* and *Brassica rapa chinensis*. Their seeds were purchased from Chia Tai Company Limited, Thailand, and were stored in the packet at 18 °C and 50% relative humidityto retain 98% germination and purity quality.

### 2.2 Methods

#### 2.2.1 Soil, seed and plant preparation

Soil was ground, dried under the sun, mixed well with rice husk ash at the ratio of 4:1, and 2.5 kg of soil was put into the pot (14 cm × 18 cm, high: 15.5 cm). Total N, P, K, organic matter, the pH and electrical conductivity of the soil samples were analyzed before and after cultivation.

The experiments were conducted by using plastic pots placed in the green house as a randomized complete block design with four replications. Fifteen seeds were placed in each pot, and only 10 strongest seedlings were chosen for further cultivation. The plants were watered every day in the early morning. In the strong sunny day, plants were watered twice in the early morning and late afternoon.

Fresh and dry weight, shoot height; root length, number of leaf, and nutrient content (N, P, K) of the vegetables were measured after harvest at the designated days. The data of plant and soil were both taken on the day 30 for *I. aquatica* and on day 40 for *B. integrifolia* and *B. rapa chinensis*. Chlorophyll concentration was extracted by acetone and followed by the equation [18]

Chl a (mg g-1) = [(12.7 × A663) - (2.6 × A645)] × ml acetone/mg leaf tissue

Chl b (mg g-1) = [(22.9 × A645) - (4.68 × A663)] × ml acetone/mg leaf tissue

Total Chl = Chl a + Chl b

The number of microbes and microbial activities in soil was also enumerated using two methods before cultivation and at the day of harvest.

The first method was the colony counting method using selective medium to grow nitrogen-fixing [19], potassium solubilizing [20] and phosphate solubilizing [21] bacteria. Total number of the *Klebsiella oxytoca* isolate was determined by enumerating the colonies on plate count agar by spread plate technique and re-checked this strain by Gram staining compare to original strain [22]. In the second method, total microbial activity in the soil was determined by using flourescein diacetate (FDA) hydrolysis [23 -24]

For this method, 60 mM potassium phosphate buffer pH 7.6, 2:1 chloroform/methanol, 1000 µg/ml fluorescein diacetate stock solution, 2000 µg/ml fluorescein sodium salt stock solution. 20 µg/ml FDA standard solution were prepared to make the standards, dilute a standard stock solution (1 µl of 2 mg/ml FDA in acetone) of fluorescein sodium salt was perform. Stock solution was added to a 100 ml volumetric flask and the contents made up to the mark with 60 mM potassium phosphate buffer pH 7.6. The standards 1 to 5 µg/ml were prepared from the standard solution by dilution with 60 mM potassium phosphate buffer pH 7.6. The protocol was shown in Figure 1.

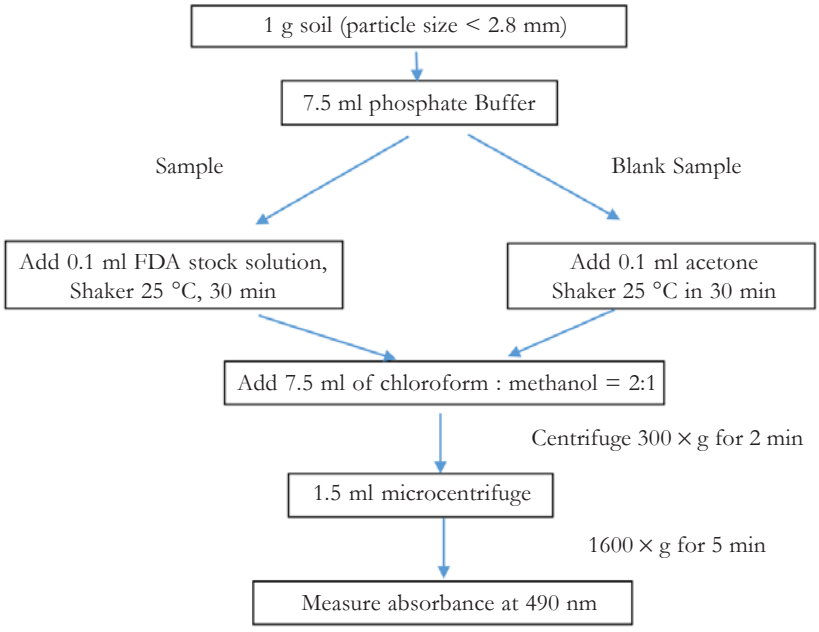


**Figure 1**. FDA method protocol.

**2.2.2 Evaluation of the effects of various fertilizers on the growth of three vegetables**

The vegetable seeds were immersed in 1% (v/v) sodium hypochlorite solution for 10 minutes for sterilization of their surface, and then rinsed three times with sterile distilled water. The seeds were soaked in *Klebsiella oxytoca* supernatant (the solution of bacteria at log phase after centrifuged) for 60 min and used for treatments, excepted control treatments. The seeds soaked in sterilized distilled water were used for positive control which treated with chemical fertilizer (NPK) and negative control treatments. There were six treatments; 1) negative control without fertilizer (Cont), 2) positive control treated with chemical fertilizer (NPK, 15-15-15), 3) treated with bio-fertilizer of rice straw inoculated with *K. oxytoca* for 2 months (B), 4) treated with liquid organic fertilizer which was made from fruit waste (fermentation solution after 1 month) inoculated with *K. oxytoca* for 2 weeks (L), 5) treated with liquid organic fertilizer which was made from molasses (diluted 100 times by sterilized water) inoculated with *K. oxytoca* for 2 weeks (M), and 6) treated with the combination of bio-fertilizer and fruit waste liquid organic fertilizer (C-L).

The amount of fertilizers given to the pots was as follows: Water was applied to the control and bio-fertilizer treatments pots every day. NPK was given at the dose of 0.2 g/ pot (according to the recommendation of the maker) on the first day of cultivation. For the two kinds of liquid organic fertilizers 10 ml was sprinkled to the plant every 5 days in the evening (around 18:30 -19:00).

**2.2.3 Determination the effect of liquid organic fertilizer and chemical fertilizer ratio on the growth of *I. aquatica***

The *I. aquatica* seeds were sterilized. The seeds that were soaked 60 minutes in *Klebsiella oxytoca* strain H1-702 supernatant was used for treatments, others which soaked in sterilized distilled water were used for NPK and control treatments. There were six treatments including: NPK which applied chemical fertilizer (15-15-15) 0.2g/pot; Cont which applied only applied water, 75% NPK + L which applied chemical fertilizer 1.5 gram/pot and liquid fertilizer (10ml/pot/5days); 50% NPK + L: applied chemical fertilizer 1 gram/pot and liquid fertilizer (10ml/pot/5days); 25 % NPK + L which applied chemical fertilizer 0.5 gram/pot and liquid fertilizer (10 ml/pot/5days); and L which applied liquid fertilizer (10 ml/pot/5days)

The data of plant and soil were taken out at day 30 of cultivating.

**2.2.4 Statistical analysis**

The experiments were designed as the randomized complete block with four replications in the greenhouse. The IBM SPSS Statistics 19 software was used for data analysis with least significant difference test (LSD) atthe probability level 0.05 and analysis of variance (ANOVA).

## 3. RESULTS AND DISCUSSIONS

### 3.1 Effects of Various Fertilizers on the Growth of Three Vegetables

The nutrient content in soil and rice husk ash before mixing was very low. The content of organic matter in soil was also very low (0.7%) (Table 1).

**Table 1**. The nutrient contents in original soil and rice husk ash.

| | N (mg/kg) | P (mg/kg) | K (mg/kg) | OM% |
|---|---|---|---|---|
| soil | 168.12 | 609 | 1998 | 0.703 |
| rice husk ash | 0.009 | 0.47 | 0.91 | |

**3.3.1 The effect of various fertilizer on the growth of *I. aquatica***

The shoot lengths of *I. aquatica* treated with chemical (NPK group) or liquid organic fertilizers (L and M groups) were significantly longer than the other three groups (Cont: control, B: biofertilizer-treated and BL: mixed fertilizer-treated) (Table 2). Compared with the control (23.3 cm), the root length of the fertilizer-treated groups was about 25 cm or longer, but no significant difference was seen among all fertilizer treatments. Likewise, the leaf number, leaf width and leaf length of the NPK and L-treated groups were significantly higher than those of Cont, B and BL treated groups, although no difference was seen in between NPK and L groups. The fresh weight of the NPK and L treated groups were significantly higher than the other treatment groups. The dry weight was highest in NPK treated group (Table 2). In this study, the *I. aquatica* root was established in the first 15 days, the average root length of *I. aquatica* slowly increased from day 15 to day 30 of cultivating; therefore, the interaction between plant root and microorganisms in the first stage of growing was very important. However, bacteria need time to adjust and activate themselves in the soil, thus the plant treatment which apply chemical fertilizer was growth better in the first stage. Thereafter, the plant grew nearly twice in the next 15 days when the nutrient contents plays a major role. Application of biological fertilizer, bio-humus products on the growth of organic *I. aquatica* increased 45.83% of yield by comparing to the natural control [25].

**Table 2**. Growth parameters of *I. aquatica* plants 30 days after cultivation.

| Treatments | shoot length (cm) | root length (cm) | leaf number | leaf width (cm) | leaf length (cm) | Fresh weight (g) | Dry weight (g) | Cla (mg/g) | Clb (mg/g) | Total Cl (mg/g) |
|---|---|---|---|---|---|---|---|---|---|---|
| NPK | 23.62a | 25.64ab | 7.35a | 1.27a | 10.67a | 26.78a | 2.66a | 0.74a | 0.24 | 0.98a |
| Cont | 20.08bc | 23.25b | 6.61bc | 1.10bc | 9.25bc | 20.37bc | 1.74bc | 0.63ab | 0.21 | 0.84ab |
| Carr | 18.41c | 25.74ab | 6.08c | 1.06bc | 8.54cd | 20.70bc | 2.10b | 0.55ab | 0.25 | 0.80ab |
| L | 23.26ab | 25.71ab | 7.18a | 1.20ab | 9.93ab | 24.17ab | 2.06b | 0.63ab | 0.22 | 0.85ab |
| M | 21.44abc | 27.17a | 6.87ab | 1.17ab | 9.55b | 22.18bc | 1.98bc | 0.56ab | 0.19 | 0.75ab |
| CL | 19.07c | 24.78ab | 6.45bc | 0.99c | 8.30d | 18.37c | 1.50c | 0.54b | 0.19 | 0.73b |
| T-test | ** | * | ** | ** | ** | ** | ** | ** | ns | * |
| %CV | 2.76 | 2.13 | 0.54 | 0.12 | 0.93 | 3.58 | 0.46 | 0.1 | 0.07 | 0.15 |

Note. The evalues with the same letter in each column were ns: non-significant; *: significant at P<0.05; **: significant at P<0.01. NPK: chemical fertilizer, C: control, Carr: applying carrier, L applying liquid fertilizer, M: applying molasses as liquid fertilizer, CL: combination of carrier and liquid fertilizer.

The chlorophyll-a content of *I. aquatica* was highest in NPK treated group (0.74 mg/g) compared with the lowest content of 0.63 mg/g in L-treated and control groups. There was no significant difference in the chlorophyll b and total chlorophyll content in all treatments except for the BL treated group, which had the lowest total chlorophyll content (Table 2).

NPK-treated group has the lower number of NFB, PSB and *Klebsiella oxytoca*, whereas the B treated group showed the highest number those bacteria (Figure 2). The interesting thing to note in this study was that the number of bacteria in the bio-fertilizer was highest among all treatment; however, the plant growth on bio-fertilizer was limiting growth and their yield lower than the control.

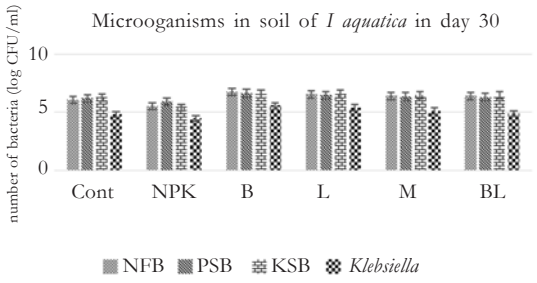


**Figure 2**. The number of microorganisms in soil of *I. aquatica* 30 days after cultivation. NPK: chemical fertilizer, Cont: control, B: bio-fertilizer, L: fruit waste liquid fertilizer, M: molasses liquid fertilizer, BL: combination of bio- and fruit waste liquid fertilizer. NFB: nitrogen fixing bacteria, PSB: phosphates solubilizing bacteria, KSB: potassium solubilizing bacteria.

The results of soil analysis showed that the total nitrogen nutrient was significantly higher in BL followed by B treatment. There was a little variation in the total phosphorus. The highest value was seen in NPK treated group and the lowest in B treated group (Table 3). The potassium content was not significantly different among various fertilizer treatments, although they were significantly higher than that of the control group. The OM content was highest in BL treated group. Soil pH was slightly alkaline regardless of the treatments. Similarly, soil EC was around 8-9 dS/m in all treatments, except for that of M-treated group (Table 3). During microbial degradation of rice straw in soil, organic acid or di-hydro sulfur ($H_2S$) might be produced and cause toxic effects on the plant root [26]. Bio-fertilizer provided a high content of nutrient and number of useful microorganisms, nevertheless the carrier must be qualified in the best way before applying in the greenhouse. The biomass of *I. aquatica* was enhanced in the artificial soil that mixed subsoil with 4% of horticultural compost (wet weight/wet weight) and 2% of sewage sludge (dry weight/ wet weight) [27].

Figure 3 showed the nutrients in *I. aquatica* plant 30 days after cultivation. Compared to nitrogen and phosphorus, potassium content was very high in regardless of the type of treatments, even in control group (Figure 3). The nutrient values of the NPK-treated plants were significantly higher than those of the other two groups.

**Table 3**. Soil analysis 30 days after cultivation of *I. aquatica*.

| Treatments | Total N mg/kg | TotalP mg/kg | Total K mg/kg | OM % | pH (soil) | EC (dS/m; soil) |
|---|---|---|---|---|---|---|
| C | 459.51c | 9398.00c | 2289.67b | 0.99c | 7.39b | 8.45ab |
| Carr | 598.26b | 8877.33d | 3608.00ab | 1.48b | 7.74ab | 8.12ab |
| NPK | 412.34c | 10902.67a | 3278.33ab | 1.04c | 7.63ab | 9.13ab |
| L | 429.59c | 9629.67c | 3453.00ab | 0.84d | 7.83a | 1.06a |
| M | 386.75c | 9340.00cd | 3239.67ab | 0.85d | 7.77ab | 7.89b |
| CL | 700.07a | 10289.33b | 3724.67a | 1.64a | 7.62ab | 8.92ab |
| T-test | * | ** | * | ** | ** | ** |
| %CV | 34 | 22.9 | 44.7 | 0.04 | 0.22 | 1.35 |

Note: The means values in each column: *: significant at P<0.05; **: significant at P<0.01. NPK: chemical fertilizer, C: control, Carr: applying carrier, L applying liquid fertilizer, M: applying molasses as liquid fertilizer, CL: combination of carrier and liquid fertilizer.

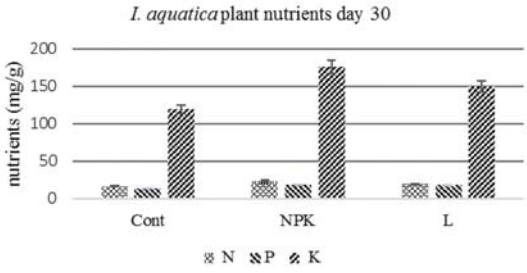


**Figure 3**. The nutrients in *I.aquatica* after 30 days of cultivation. NPK: applying chemical fertilizer, Cont: control, L: applying fruit waste liquid fertilizer; N: total nitrogen, P: total phosphorus, K: total potassium.

By applying fruit liquid organic fertilizer, the growth parameters of *I. aquatica* plants were approximate plants that grew in chemical fertilizer, excepting nutrient contents. This result was similar to another research [28], liquid fertilizer inoculum with PGPR1 *Klebsiella planticola* presented the highest yield of strawberry under greenhouse condition, the application of PGPR1 to soil also gave the highest number of total microorganisms. In this study, the number of *Klebsiella oxytoca* and total microorganisms in soil that was treated with liquid organic fertilizer (both fermented fruit waste and molasses) was higher than other treatments. The microorganisms were also growth in the control but chemical inhibited growth in the soil that apply. In another research, the farmers should apply 15 liters liquid organic fertilizer at per hectare in the early tasseling for increasing the maize grain yield [29].

### 3.3.2 The effects of various fertilizers on the growth of *B. integrifolia*

In day 40 of cultivating *B. integrifolia*, the fresh weight and dry weight in NPK group -, liquid and molasses treatments - was significantly higher than the second group - control, B and BL treatments (Figure 11). There were no significantly difference in the content of chlorophyll b and total chlorophyll in all six treatments at the harvesting day, however, the chlorophyll a content in control was significantly lower than the other 5 treatments. B resulted in the lowest number of leaf, leaf width and leaf length followed by BL treatment (Table 4). Shoot length was lowest in B treatment (12.36 cm) and root length was significant lower in B and control treatment (19.07 and 23.73 cm,

respectively) while other treatments were not significantly different in these values (Table 4). The plant of *Brassica* spp. was harvested on day 40 of cultivation. *Brassica* spp. were also growth root in the first stage, the average root of *B. integrifolia* (14.52 cm) was higher *B. rapa chinensis* (11.86 cm). In all observation data of tree vegetables, the yields of plants that were treated with fruit waste liquid fertilizer were comparable with those of the plants treated with chemical fertilizer. No significant difference in the pH and EC values of soil used to grow *B. integrifolia* after 20 days. The pH was lowest in molasses soil (7.56) and EC was lowest in BL soil (80.73) while no significant difference among the other treatments at day 40 was observed (Table 4). However, the chlorophyll content was similar in all treatments of *Brassica* in this experiment. To *Brassica*s species, bio-fertilizer (carrier) was rather toxic for their growth because their yield was lower than control, this might be due to toxic compound produced by insufficient degradation.

**Table 4**. Growth parameters of *B. integrifolia* plants 40 days after cultivation.

| Treatments | Shoot length (cm) | Root length (cm) | number of leaf | leaf width (cm) | leaf length (cm) | Fresh weight (g) | Fry weight (g) | Cla (mg/g) | Clb (mg/g) | Total Cl (mg/g) | pH (soil) | EC (dS/m; soil) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPK | 15.92a | 22.42ab | 4.40a | 3.81a | 6.07a | 26.06a | 3.00a | 0.38ab | 0.26 | 0.65 | 7.76a | 13.28ab |
| C | 15.14a | 22.40ab | 4.18a | 3.55ab | 5.69ab | 16.30b | 1.64cd | 0.25b | 0.30b | 0.55 | 7.67ab | 11.93ab |
| Carr | 12.36b | 19.07b | 3.47b | 2.62b | 4.13c | 14.58b | 1.38d | 0.43a | 0.21 | 0.63 | 7.71ab | 13.03ab |
| L | 15.66a | 23.75a | 4.27a | 3.70ab | 5.86ab | 28.16a | 2.76ab | 0.39ab | 0.23 | 0.61 | 7.69ab | 14.92a |
| M | 15.65a | 21.16ab | 4.11a | 3.46ab | 5.58b | 24.30a | 2.25bc | 0.45a | 0.19 | 0.65 | 7.56b | 10.92ab |
| CL | 12.38b | 21.64ab | 3.71b | 2.62b | 4.35c | 17.47b | 1.57d | 0.36ab | 0.22 | 0.58 | 7.58ab | 8.07b |
| T-test | ** | * | ** | ** | ** | ** | ** | * | ns | ns | * | ** |
| %CV | 1.72 | 2.43 | 0.39 | 0.53 | 0.81 | 6.09 | 0.73 | 0.11 | 0.07 | 0.11 | 0.12 | 3.09 |

Note. The values in each column:ns: non-significant; *: significant at P<0.05; **: significant at P<0.01; NPK: chemical fertilizer, C: control, Carr: applying carrier, L applying liquid fertilizer, M: applying molasses as liquid fertilizer, CL: combination of carrier and liquid fertilizer.

The numbers of NFB, PSB, KSB and *Klebsiella oxytoca* on day 40 of the culture were the highest in the soil treated with fruit waste liquid fertilizer, followed by the soil treated with B and BL fertilizers. The lowest values were seen in NPK treated group (Figure 4). Comparing between two kind of liquid organic fertilizers, fruit waste liquid organic fertilizer gave better production than did the molasses one. This difference might be due to higher nutrient content in fruit waste fertilizer. Nitrogen liquid organic fertilizer could enhance the nitrogen uptake of corn [30].

There was no significant difference in the nitrogen and phosphorus content in between NPK and fruit waste liquid fertilizer treated groups. The potassium content was highest in the plants of NPK group treated with chemical fertilizer (Figure 5). In both *Brassica* plant the potassium content was very high comparing to nitrogen and phosphorus, back to the nutrient content in raw soil the amount of potassium was very rich and the number of potassium was also higher the other in soil. *Klebsiella oxytoca*

is known to have a potential to solubilize potassium in laboratory experiment [31] and in other research, *Klebsiella* sp. SBP-8 protected the plants against adverse effects of salt and temperatures tress [32].

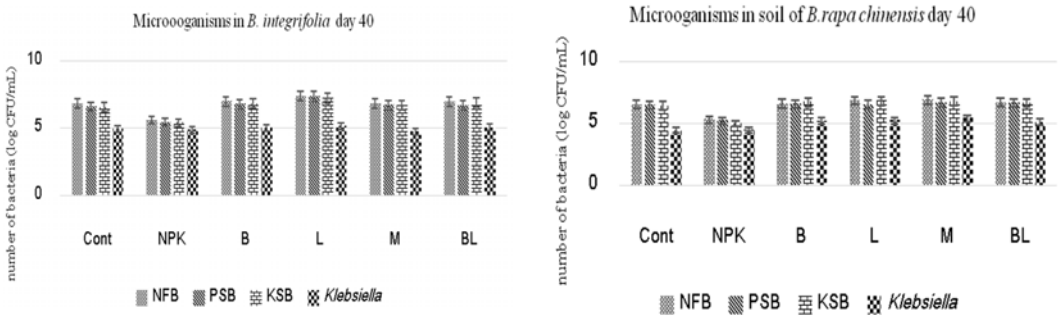


**Figure 4**. Number of bacteria in the soil of *B. integrifolia* and *B. rapa chinensis* 40 days after cultivation. NPK: chemical fertilizer, Cont: control, B: bio-fertilizer, L: fruit waste liquid fertilizer, M: molasses liquid fertilizer, BL: combination of bio- and liquid fertilizer.

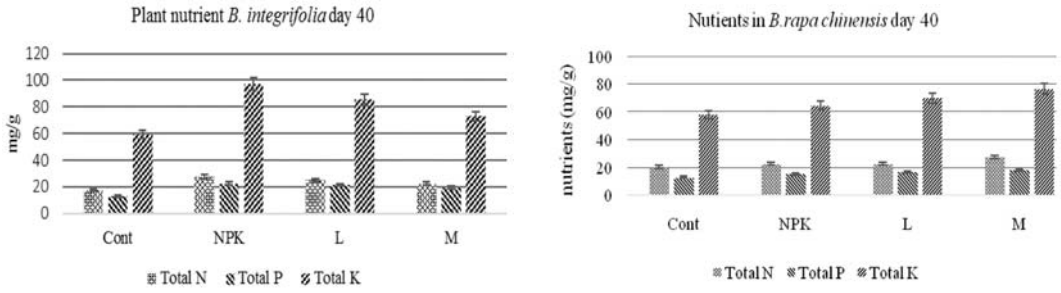


**Figure 5**. The nutrient content of *B. integrifolia* and *B. rapa chinensis* 40 days after cultivation. NPK: chemical fertilizer, Cont: control, L: fruit waste liquid fertilizer, M: molasses liquid fertilizer; Total N: nitrogen, Total P: phosphorus, Total K: potassium.

### 3.3.3 The effect of various fertilizers on the growth of *B. rapa chinensis*

The shoot length of *B. rapa chinensis* was high in the groups treated with NPK, fruit-liquid and molasses treatments, compared with the other group (Figure 11). The root length ranged from 21.84 cm in B treatment to 26.31 cm in NPK treatment. The leaf number, leaf width and leaf length in the plants that applied NPK, fruit-liquid and molasses fertilizer was higher bio-fertilizer (B) treatments (Table 5). The fresh weight number was no significantly different among treatments, excepted B (13.73 g); in addition, the dry weight was similar trend.

**Table 5**. Growth parameter of *B. rapa chinensis* plants on day 40 of cultivation.

| Treatments | shoot length (cm) | root length (cm) | number of leaf | leaf width (cm) | leaf length (cm) | Fresh weight (g) | Fry weight (g) | Cla (mg/g) | Clb (mg/g) | Total Cl (mg/g) | pH (soil) | EC (dS/m; soil) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPK | 13.08a | 26.31a | 5.64a | 2.73ab | 4.87ab | 19.39a | 1.86 | 0.69ab | 0.24 | 0.94ab | 7.61a | 10.61 |
| Cont | 11.14b | 24.11ab | 5.32ab | 2.35bc | 4.29b | 16.57ab | 1.56 | 0.51b | 0.18 | 0.69b | 7.44a | 10.15 |
| Carr | 9.25c | 21.84b | 4.89b | 1.85d | 3.51c | 13.73b | 1.46 | 0.60ab | 0.20 | 0.80ab | 7.51a | 11.89 |
| Liquid | 13.34a | 25.91ab | 5.79a | 2.78ab | 5.14a | 20.68a | 1.92 | 0.76a | 0.24 | 1.00a | 7.2b | 8.75 |
| Mollasse | 13.18a | 24.12ab | 5.71a | 2.92a | 5.15a | 21.67a | 2.06 | 0.51b | 0.23 | 0.74ab | 7.49a | 8.96 |
| CL | 11.19b | 22.34ab | 5.60a | 2.28cd | 4.33b | 19.47a | 1.69 | 0.23c | 0.16 | 0.40c | 7.46a | 12.21 |
| T-test | ** | * | ** | ** | ** | * | ns | ** | ns | ** | ** | ns |
| %CV | 1.70 | 2.84 | 0.48 | 0.46 | 0.70 | 4.17 | 0.42 | 0.19 | 0.06 | 0.23 | 0.16 | 3.22 |

Note. The values in each column:ns: non-significant; *: significant at P<0.05; **: significant at P<0.01; NPK: chemical fertilizer, C: control, Carr: applying carrier, L applying liquid fertilizer, M: applying molasses as liquid fertilizer, CL: combination of carrier and liquid fertilizer.

There was no significant difference in the chlorophyll b content, therefore, the range of total chlorophyll was similar the chlorophyll a content which the highest value belonged to liquid fertilizer treatments (1.0 mg/g total chlorophyll and 0.76 mg/g chlorophyll a) (Table 5). The soil that cultivated with NPK chemical, control, bio-fertilizer and molasses had similar pH and the soil in BL treatment was the lowest pH at day 20 of *B. rapa chinensis* cultivation. The soil analysis results show that pH was lowest in liquid fertilizer treatments, there was no significant difference in the EC value among all treatments on day 40 (Table 5). During the growth of three vegetables the pH and EC did not change much in all soil treatment. When bacteria grow in the soil, they might adjust pH to the neutral to make soil suitable for plant growth, the standard compost was pH 5.5 - 8.5 [33].

Chemical fertilizer inhibited the growth of microorganisms (Figure 4), the number of bacteria was proximally 6 log CFU/mL and *Klebsiella oxytoca* number was proximally 5 log CFU/mL. The highest nutrients content in *B. integrifolia* was the plant that applied chemical fertilizer. The nutrients value in *B. rapa chinensis* was highest in the plant that apply molasses liquid fertilizer while those data in the plant that applied liquid organic fertilizer (applying fermentation fruit waste) and the chemical fertilizer application were equal. As a result, *B. rapa chinensis* responded better than *B. integrifolia* when apply liquid organic fertilizer. Such success was positive signal for applying liquid organic fertilizer for *Brassica* spp. PGPRs in bio-fertilizer supplying sufficient both minor and major nutrients and plant growth regulating substances [34] which could enhance the growth and yield of vegetable. In other research, the combination of liquid organic fertilizer and bio-pesticide increased the calcium content, vitamin C and glucose content in apple fruit [35].

On the day of harvest, the potassium content was highest among three analyzed nutrients in *B. rapa chinensis*. The plant that apply molasses which contained *Klebsiella oxytoca* got the highest amount of nitrogen, phosphorus and potassium by 27.55, 18.08 and 76.45 mg/g, respectively, followed by liquid applying treatment which was

significantly higher than NPK treatment in total P and K value (6.58 and 69.94 mg/g, respectively) (Figure 5). By addition encapsulated *K. oxytoca* Rs-5 in the salt stress condition, the results were positive effect comparing to free living cell [36].

### 3.3.4 The effect of combined chemical and organic fertilizers on the growth of *I. aquatica*

In this experiment, the amount of chemical fertilizer was reduced by application of fruit-liquid fertilizer. On the day of harvest, the shoot length and root length of all treated groups were significantly higher than those of the control group, although no statistical differences were seen among the treatments (Figure 11). The number of leaf, leaf width and leaf length of NPK, L, 50%L and 75% L treated groups were higher than those of control and 25% L treated groups. The fresh weight was highest in 50%L treated group, and dry weight was highest in NPK treated group, but no significant difference was seen in between NPK and 50%L treated groups (Table 6). The chlorophyll a and b contents at day 30 were higher in 50%L and 70%L treated group compared with those of control group (Table 6).

**Table 6**. Growth parameters of *I. aquatica* plants after 30 days cultivation in the chemical : liquid ratio test experiment.

| Treatments | shoot length (cm) | root length (cm) | number of leaf | leaf width (cm) | leaf length (cm) | Fresh weight (g) | Dry weight (g) | Cla (mg/g) | Clb (mg/g) | Total Cl (mg/g) | pH (soil) | EC (dS/m) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| NPK | 18.85a | 26.40ns | 7.90a | 1.22a | 10.19a | 24.97a | 2.71a | 0.70ab | 0.24ab | 0.94bc | 7.85 | 2.12 |
| Cont | 14.98b | 24.49ns | 6.73c | 0.91b | 8.44c | 16.46c | 1.65c | 0.58b | 0.20b | 0.78c | 7.77 | 8.23 |
| L | 19.22a | 25.11ns | 7.78ab | 1.14a | 9.78a | 23.08ab | 2.19b | 0.59b | 0.26ab | 0.85bc | 7.66 | 8.24 |
| 25%L | 17.61ab | 24.36ns | 7.43b | 1.00b | 9.13b | 19.66bc | 1.84bc | 0.72ab | 0.24ab | 0.96abc | 7.82 | 7.29 |
| 50%L | 19.48a | 26.55ns | 7.66ab | 1.13a | 9.74a | 21.76ab | 2.24ab | 0.82ab | 0.26ab | 1.08ab | 7.48 | 6.05 |
| 75%L | 19.71a | 26.19ns | 7.93a | 1.19a | 10.14a | 21.42ab | 1.93bc | 0.90a | 0.30a | 1.20a | 7.53 | 7.05 |
| F-Test | ** | ns | ** | ** | ** | ** | ** | ** | ** | ** | ns | ns |
| %CV | 2.35 | 1.93 | 0.47 | 0.13 | 0.70 | 3.59 | 0.45 | 0.19 | 0.06 | 0.23 | 0.17 | 1.42 |

Note. The values in each column:ns: non-significant; **: significant at P<0.01; NPK: chemical fertilizer, C: control, L applying liquid fertilizer, 25%L: liquid fertilizer +25% chemical, 50%L: liquid fertilizer + 50% chemical, 75%L: 75% chemical + liquid fertilizer.

In terms of physicochemical nature of soil, pH was low in 50% L and 75% L treated groups and no significant difference in other treatments (Table 6). EC in soil in all treatments were in the range of 2.12 to 8.2 dS/m. On day 30 of cultivation, pH in soil of *I. aquatica* was not significantly different from each other in all treatments. In all treatments that applied chemical fertilizer, liquid organic fertilizer and the combination fertilizer, the plant growth parameters were not significantly different; however, the number of microorganisms was in inverse ratio to the amount of chemical applying. Increasing chemical inputs not only affect plants but also decrease microbial communities through changes of soil pH [37].

At day 30, the number of microorganisms was lowest in the soil treated with chemical

fertilizer. Liquid treatment got the highest number of *Klebsiella oxytoca*, NFB, PSB and KSB (Figure 6).

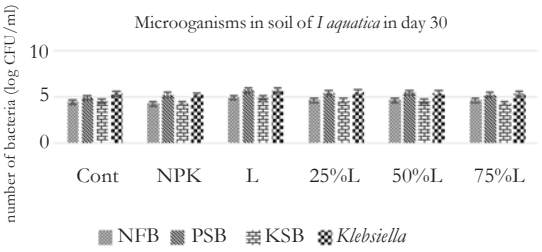


**Figure 6**. Number of bacteria in soil sample of *I. aquatica* treated with various proportion of fruit waste liquid organic fertilizer and chemical fertilizer and cultivated for 30 days. NPK: chemical fertilizer, Cont: control, L: fruit waste liquid organic fertilizer, 25%L: liquid fertilizer +25% chemical, 50%L: liquid fertilizer + 50% chemical, 75%L: 75% chemical + liquid fertilizer.

The nutrient content was highest in NPK treatment with 24.21 mg/l nitrogen, 23 g phosphorus and 155.85 mg/g potassium, followed by L and the lowest nutrient content belonged to control treatment (Figure 7).

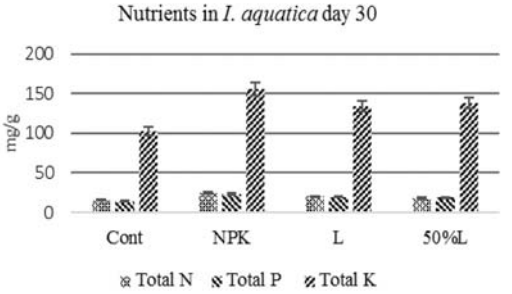


**Figure 7**. The nutrient content of *I. aquatica* 30 days after cultivation with chemical fertilizer, fruit waste liquid organic fertilizer, and combination of both (NPK: chemical fertilizer, C: control, L: liquid fertilizer, 50%L: 50% liquid fertilizer + 50% chemical).

### 3.2 Microbial Activity Determined by FDA Method in the Soil Treated with Various Fertilizers After Growing Three Vegetables

#### 3.2.1 Microbial activity in the soil after growing *I. aquatica*, *B. integrifolia* and *B. rapa chinensis* treated with various fertilizers

Microbial activity in soil samples was determined using the FDA method on the day of harvest. Regardless of the species of plants grown, the significantly higher microbial activitythan control was seen in bio-fertilizer (B) treated soil followed by the soil treated with thefruit waste liquid fertilizer (L) and combination of the bio-fertilizer with fruit waste liquid fertilizer (BL). Thus, application ofbio-fertilizer andliquid organic fertilizer can support the growth of microoganisms in soil that growing *I. aquatica* (Figure 9).

While microbial activity was higher in *B. integrifolia* than in *B. rapa chinensis* in NPK, Cont, L and M treatments, opposite resultswere observed in B and BL treatments. Bacteria in soil could not survive well under the presence of chemical fertilizer or in the control treatments (Figure 9). Fruit waste and molasses fertilizers could enhance the microbial activity in the soil for the cultivation of *B. integrifolia*.

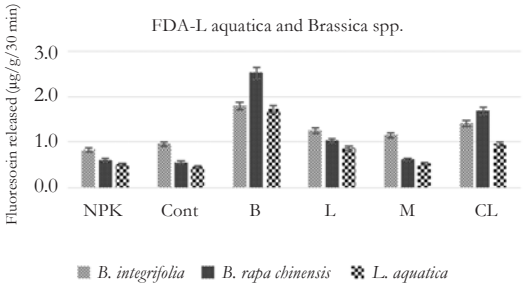


**Figure 9**. FDA in the soil after growing *I. aquatica* with supplementation of various fertilizers. NPK: chemical fertilizer, C: control, B: bio-fertilizer, L: fruit waste liquid fertilizer, M: molasses liquid fertilizer, CL: combination of carrier and fruit waste liquid fertilizer.

Using FDA method, the total microbial activity of the treatments was determined. The use of100 % fruit waste liquid fertilizer gave the highest microbial activities. Thus, fruit waste liquid fertilizer could be applied to enrich soil and recover the soil ecosystem. Since combination of 50% each of chemical and fruit waste liquid fertilizer could increase the yield of *I. aquatica* in association with the increase of soil nutrient (Table 6). Similar to the present results, the combination of multifunctional bio-fertilizer (mixture of *P. rubiacearum*, *B. erythropolis*, *B. pumilus* and *B. subtillis*) [38] and 50% of chemical fertilizer gave the highest plant growth, dry matter and crop yield of maize. The combination of chemical fertilizer and *rhizobacteria* gave the highest dry matter, plant growth and crop yields of maize [39].

### 3.2.2 Microbial activity in the soil grown *I. aquatica* under treatment with the combination of liquid organicfertilizer and chemical fertilizer

The supplementation of liquid organic fertilizer could reduce the amount of chemical fertilizer in soil and increase theuseful microoganisms in soil in a dose dependent manner (Figure 10). In a new study, hazardous agricultural chemicals may be decreased by beneficial PGRBs which considered as agro-ecosystems [40]. In addition, *K. oxytoca* JM26 was found as bacteria inhibition and had potential in bioenhancer of biofertilizer producing [41].

FDA method is a rapid and useful tool to determine total microbial activities in soil samples. The combination of liquid organic fertilizer with chemical fertilizer can increase the yield of *I. aquatica,* reduced chemical and supporting microorganism survival.

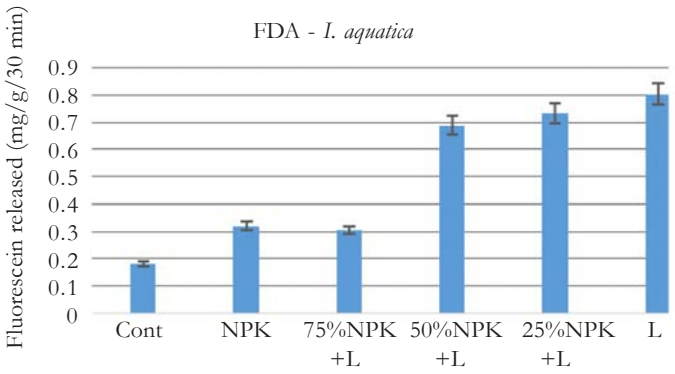


**Figure 10**. FDA in soil after cultivation of *I. aquatica* with supplementation of liquid organicfertilizer to chemical fertilizer; NPK: chemical fertilizer, C: control, L applying liquid fertilizer, 25%NPK+L: 25% chemical and liquid fertilizer , 50%NPK + L: 50% chemical and liquid fertilizer, 75% NPK+L: 75% chemical and liquid fertilizer.

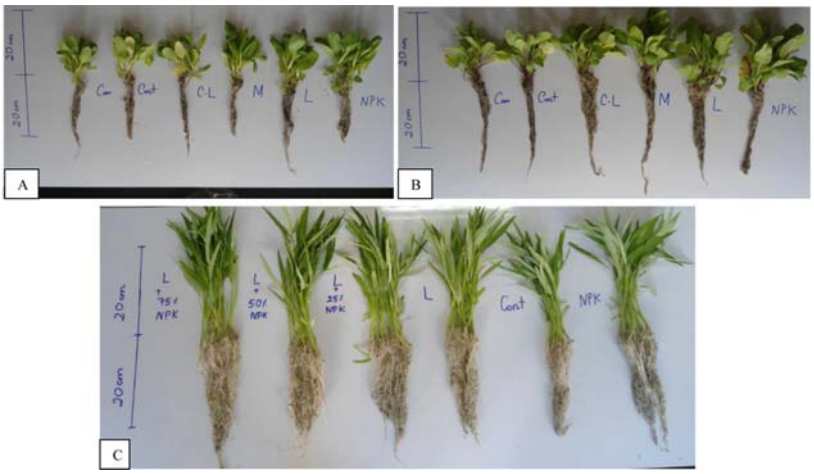


**Figure 11**. Growth of plants at the day of harvesting
**A**. *Brassica rapa chinensis* at 40 days of cultivating
**B**. *Brassica integrifolia* at 40 days of cultivating
**C**. *Ipomoea aquatica* at 30 days of cultivating

## 4. CONCLUSION AND SUGGESTION

In the greenhouse experiment, the liquid organic fertilizer prepared from fruit wastes substrate and *Klebsiella oxytoca* could enhance growth of three vegetables, *I. aquatica*, *B. integrifolia* and *B. rapa chinensis*. Fruit waste liquid organic fertilizer improved the soil nutrient, biological properties and the number of bacteria in the soil. Molasses liquid organic fertilizer also gave positive results for plant growth. In contrast, the application of bio-fertilizer in the form of rice straw carrier caused rather toxic effect on plant growth; this might be due to toxic compound produced by insufficient degradation [42]. Chemical fertilizer had a side effect for the microbial activity of the soil.

Further study is required to produce high quality liquid organic fertilizer of commercial level based on their good quality and long shelf life. Incomplete degradation of carrier may produce organic toxic substances to cause suppression of plant growth. More research on the application of *K. oxytoca* in liquid organic fertilizer and bio-fertilization should be carry out under the field condition.

## ACKNOWLEDGEMENTS

This research was supported by the Faculty of Science (Scholarship of Academic Year 2015), Department of Microbiology and Salt-tolerant Rice Research Group, Khon Kaen University, Thailand. We would like to thank Prof. Dr. Yukifumi Nawa, Invited Professor, Faculty of Medicine, Khon Kaen University, for his critical reading of the manuscript.

## REFERENCES

[1] Santos V.B, Araújo A.S.F., Leite L.F.C., Nunes L.A.P.L. and Melo W.J., *Geoderma*, 2012; **170**: 227-231. DOI 10.1016/j.geoderma.2011.11.007.

[2] Bhardwaj D., Ansari M.W., Sahoo R.K. and Tuteja N., *Microb. Cell Factories*, 2014; **13**: 66. DOI 10.1186/1475-2859-13-66.

[3] Adesemoye A.O. and Kloepper J.W., *Appl. Microbiol. Biotecnol.*, 2009; **85**: 1-12. DOI 10.1007/s00253-009-2196-0.

[4] Raja N., *Biofertilizers Biopesticides.*, 2013; **4(1)**: 1-2. DOI 10.4172/2155-6202.1000e112.

[5] Somasegaran P. and Hoben H.J., *Handbook for Rhizobia: Methods in Legume Rhizobium Technology*, Springer, Berlin Heidelberg New York, USA., 1994: 217-218.

[6] Bashan Y., *Biotechnol. Adv.*, 1998; **16(4)**: 729-770. DOI 10.1016/S0734-9750(98) 00003-2.

[7], [8] Brar S.K., Sarma S.J. and Chaabouni E., *Biofertilizers Biopesticides*, 2012; **3(5)**: 3-5. DOI 10.4172/2155-6202.1000e109.

[9] Mahl M.C., Wilson P.W., Fife M.A. and Ewing W.H., *J. Bacteriol.*, 1965; **89(6)**: 1482-1487.

[10] Babalola O.O., Berner D.K. and Amusa N.A., *Afr. J. Agric. Res.*, 2007; **2(1)**: 27-30.

[11] Pokluda R., *Hortsci.*, 2007; **34(3)**: 123-128. DOI 10.17221/1891-HORTSCI.

[12] Sinha R.K., Valani D., Chauhan K. and Agarwal S., *J. Agric. Biotechnol. Sustan. Development*, 2010; **2(7)**: 113-128. DOI 10.1023/A:1007796609378.

[13] Singh J.S., Pandey V.C. and Singh D.P., *Agric. Ecosyst. Environ.*, 2011; **140**: 339-353. DOI 10.1016/j.agee.2011.01.017.

[14] Megali L., Glauser G. and Rasmann S., *Agronomy for Sustainable Development*, 2014; **34 (3)**: 649-656. DOI 10.1007/s13593-013-0187-0.

[15] Bhardwaj D., Ansari M.W., Sahoo R.K. and Tuteja N., *Microb. Cell. Factories*, 2014; **13**: 1-10. DOI 10.1186/1475-2859-13-66.

[16] Nguyen T.H.N. and Riddech N., *The Philippine Agricultural Scientist*, 2016; **99(4)**: 287-293.

[17] Nguyen T.H.N., *Effect of Bio-fertilizer and Liquid Organic Fertilizer on Vegetable Production on the Pot Experiment*, MS Thesis, Khon Kaen University, Thailand, 2016.

[18] Arnon D.I., *Plant Physiol.*, 1949; **24**: 1-15.

[19] Dobereiner J. and Day J.M., *Associative Symbioses in Tropical Grasses: Characterization of Microorganismsand Dinitrogen-fixing Sites*; in: Newton W.E. and Nyman C.J., eds. *Proceedings of the 1st International Symposium on Nitrogen Fixation, Vol. 2. Pullman, WA: Washington State University Press.*, 1976; 518-538.

[20] Sugumaran P. and Janarthanam B., *World J. Agric. Sci.*, 2007; **3(3)**: 350-355.

[21] Nautiyal C., *FEMS Microbiol. Lett.*, 1999; **170(1)**: 265-270. DOI 10.1111/j.1574-6968.1999.tb13383.x.

[22] Jacobs M.B. and Gerstein M.J., *Hand-Book of Microbiology*, New York, 1960.

[23] Adam G. and Duncan H., *Soil Biol. Biochem.*, 2001; **33**: 943-951. DOI 10.1016/S0038-0717(00)00244-3.

[24] Schnurer J. and Rosswall T., *Appl. Environ. Microbiol.*, 1982; **4**: 1256-1261.

[25] Sibounnavong P., Sysouphanthong P., Xay Ly., Phoutasay P., Promrin K., Pongnak W. and Soytong K., *J. Agric. Technol.*, 2006; **2(2)**: 177-189.

[26] Koschorreck M., *FEMS Microbiol. Ecol.*, 2008; **64(3)**: 329-342. DOI 10.1111/j.1574-6941.2008.00482.x.

[27] Stabnikova O., WANG J.Y., BO Ding H. and Joo-Hwatay., *Bioresour. Technol.*, 2005; **96**: 747-751. DOI 10.1016/j.biortech.2004.06.022.

[28] Pasakovíc M., Karaklajíc-Stajíc Z., Milenkovíc S. and Mitrovíc O., *Scientia Horticulturae*, 2013; **150**: 238-243. DOI 10.1016/j.scienta.2012.11.016.

[29] Enujeke E. and Ojeifo I., *Asia Econom. Social Soc.*, 2013; **3(4)**: 186-192.

[30] Kasim S., Ahmed O.H. and Majid N.M.A., *Afr. J. Biotechnol.*, 2011; **10(12)**: 2274-2281. DOI 10.5897/AJB10.1402.

[31] Nguyen T.H.N. and Riddech N., *The Philippine Agric. Sci.*, 2016; **99(4)**: 287-293.

[32] Singh R.P., Jha P. and Jha P.N., *J. Plant Physiol.*, 2015; **184**: 57-67. DOI 10.1016/j.jplph.2015.07.002.

[33] Kham-Iam S., Kanalerk C., Soonthornpat S., Boonkerd and Santadwoot C., *Hand-Book of Compost Thai Agricultural Standard Tas.*, Thailand, 2005.

[34] Zaidi A., Ahmad E., Khan M.S., Saif S. and Rizvi A., *Scientia Horticulturae*, 2015; **193**: 231-239. DOI 10.1016/j.scienta.2015.07.020.

[35] Leksono A.S. and Yanuwiadi B., *Int. J. Agron. Agric. Res.*, 2014; **5(5)**: 53-58.

[36] Wu Z., Peng Y., Guo L. and Li C., *Eur. J. Soil Biol.*, 2014; **60**: 81-87. DOI 10.1016/j.ejsobi.2013.11.008.

[37] Geisseler D. and Scow K.M., *Soil Biol. Biochem.*, 2014; **75**: 54-63. DOI 10.1016/j.soilbio.2014.03.023.

[38] Young C.C., Lai W.A., Shen F.T., Hung M.H., Hung W.S. and Arun A.B., *Proceedings of the 6th ESAFS International Conference: Soil Management Technology on Low Productivity and Degraded Soils,* Taipei, Taiwan, 2003; 25-27.

[39] Umesha S., Srikantaiah M., Prasanna S., Sreeramuiu K.R., Divya M. and Lakshmipathi R.N., *Curr. Agric. Res. J.*, 2004; **2(1)**: 55-62. DOI 10.12944/CARJ.2.1.08.

[40] Ahemad M. and Kibret M., *J. King Saud Univ. Sci.*, 2014; **26**: 1-20. DOI 10.1016/j.jksus.2013.05.001.

[41] Martínez-Rodríguez J.D.C., Mora-Amutio M.D.L, Plascencia-Correa L.A., Audelo-Regalado E., Guardado F.R., Hernández-Sánchez E., Peña-Ramírez Y.J., Escalante A., Beltrán-García M.J. and Ogura T., *Braz. J. Microbiol.*, 2014; **45**: 1333-1339. DOI 10.1590/S1517-83822014000400025.

[42] Son T.T.N., Man L.H., Diep C.N., Thu T.T.A. and Nam N.N., *Omonrice*, 2008; **16**: 57-70.