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Contributed Paper

Effect of Effective Microorganisms (EM) with Liquid Organic Fertilizer on the Growth of Pak Choy (*Brassica rapa* var. *chinensis*) Seedlings

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ABSTRACT

The effect of 'effective microorganisms' with liquid organic fertilizer on the growth and quality of Pak Choy seedlings was investigated. Several different methods of treating the seeds/seedling were employed in this study, including a control with no fertilizer; spraying liquid organic fertilizer (LOF); pouring LOF; soaking seeds in LOF; spraying the mixture of effective microorganisms (EM) and LOF; pouring EM+LOF; and soaked seeds in EM+LOF. Among the treatments, spraying LOF resulted in the greatest growth, with the root length of Pak choy being 1.1 times greater than that of the control after 15 and 30 days. After each harvest time, spraying LOF also had the highest root and shoot dry weight compared to other treatments, with1.27-fold and 1.11-fold increases compared to the control treatment, respectively. Meanwhile, soaking seeds in LOF increased vitamin C content by almost 2 times from the first to the second harvest. Spraying LOF+EM benefitted the Pak choy through the reduction of leaf senescence as the chlorophyll content of the vegetable remained almost the same after two harvests, compared to the obvious loss of chlorophyll content with other treatments.

Keywords: liquid organic fertilizer, effective microorganism, pak choy seedlings

1. INTRODUCTION

Microorganisms have long been known for their positive effects on agricultural practices and environmental protection [1]. Several studies have demonstrated that using beneficial microorganisms as a form of fertilizer offers a solution to the environmental pollution caused by excessive use of chemical fertilizers and inappropriate handling of human and animal waste, thus promoting a more sustainable form of agriculture [1]. In 1991, Professor Higa, University of the Ryukyus, Okinawa, Japan, developed the concept of "effective microorganisms" (EM), which is usually applied to the land as a microbial inoculant consisting of mixed cultures of beneficial microorganisms [2]. Previous studies have shown that EM inoculant can enhance soil fertility as it reduces soil pH and EC [3-5]. Inoculation of many different kinds of vegetables with EM not only improves growth, but also quality and yield [6,7]. El-Shafei *et al.* (2008) found that using EM compost can increase rice grain weight and hardness [3]. Olle and Williams (2015) showed that soaking seeds in EM can decrease the nitrate content in vegetable transplants [7]. There are numerous other reports on the influence of EM treatment on plants qualities - such as their nutrient content including N, P, Ca, and K, in addition to their chlorophyll content [8-10]. However, few reports have determined the effect of EM supplements on the vitamin C content in vegetables. Herein, the quality of vegetables grown with EM is investigated through their chlorophyll content and the effect of such treatments on their vitamin C content is also studied.

Liquid organic fertilizers (LOF) are obtained from fresh organic materials in nature. These materials can be derived from animal dung or plant residues and are then processed into a liquid form which can be easily absorbed by plants. Many scientists have reported the positive effects of liquid organic fertilizers on the growth and yield of several crops such as chillies [11] and maize [12]. Using organic materials can also help improve soil physical properties and soil fertility [13,14]. The microorganisms in organic fertilizers are said to be the main factor in affecting the soil microflora and thus beneficially effecting soil and ultimately crop quality. Many reports have shown the effective use of rice straw, fish waste [11], rice hull [3] and animal manure [4] as substrates for producing liquid organic fertilizers. In this study, banana stem was selected as the feedstock for the liquid organic fertilizer due to its widespread abundance as an agricultural waste in tropical countries.

EM is known to quicken the breaking down process of organic materials and can have a positive effect on soil quality and crop productivity [15]. In liquid organic fertilizers, the organic matter or the carbon source can be a suitable environment for EM to work efficiently. The present study evaluates the effectiveness of this combination (LOF and EM) on the growth of Pak Choy (*Brassica rapa* var. *chinensis*) seedlings, a popular leafy vegetable in Southeast Asia. The study also compares the effect of using liquid organic fertilizers alone and a mixture of this with EM in growing Pak Choy. Previous studies have shown the positive effects of different methods of fertilizer application on plants such as soaked seeds [7] and spraying on leaves [11,14]. However, limited research has been conducted on the effect of pouring the fertilizer directly to the soil surrounding the plants. Therefore, this study evaluated three fertilizer application methods, including soaking seeds, spraying leaves and pouring over the plants.

2. MATERIALS AND METHODS

This study was conducted in the Microbiology Laboratory, Faculty of Science and the Green house, Faculty of Agriculture, Khon Kaen University during two months from July to August, 2017. Pak Choy seeds and commercial EM (Chawngarm brand, Thailand) was purchased from the Faculty of Agriculture, Khon Kaen University.

2.1 Production of Liquid Organic Fertilizer (LOF)

Banana stem was collected from the campus of Khon Kaen University.

Approximately 10 kg of banana stem was cut into small pieces (size:1-2 cm). Liquid organic fertilizer was prepared by mixing 3 kg of banana stem, 1 kg of molasses and 1 litre of filtrated water (3:1:1), with three replications. Each replication of the mixture was contained in a plastic bucket, covered with a lid and kept at room temperature for 15 days.

2.2 pH and EC Measurements

For pH and EC measurement, OHAUS Starter 2100 pH meter and METTLER TOLEDO EC meter were used respectively. The pH and EC of molasses and banana stem were measured in a 1:5 substrate-water suspension. pH and EC of liquid organic fertilizer were determined during and after the fermentation process by using its extract. EM contains photosynthetic bacteria, lactic acid bacteria and yeast, obtained from living microorganisms in diluted molasses. pH and EC of EM were measured in a 1:50 EM-water solution. After the fermentation process of LOF, pH and EC of the mixture of LOF and EM (1:1 ratio) were also determined. The mixture of LOF and EM was also diluted at 1:5 ratio with water prior to measurement.

2.3 Microbiology Test

Plate count agar (PCA), Nitrogen free agar (NFA), Pikovskya agar and Alexsandrov agar media were used to test the presence of total bacteria, nitrogen fixing bacteria, phosphate solubilizing bacteria and potassium solubilizing bacteria of LOF and EM. LOF and EM solutions were diluted with sterile distilled water until a 10^{-6} dilution was reached. 100 μL of each solution (10^{-4}, 10^{-5} and 10^{-6} dilutions) was pipetted into each petri dish containing PCA for counting the total numbers of bacteria present. Diluted LOF and EM solutions were pipetted into each petri dish containing NFA, Pikovskya agar and Alexsandrov agar. After 2-5 days, the petri dishes were taken out of the incubator, and the colonies counted.

2.4 Seed Germination Test

Seed germination was tested on the two types of fertilizers used in this study - LOF only and the mixture of LOF and EM. Germination was performed according to the procedure of Rani *et al.* [16]. The seven treatments were designated as 0% (control), 1% dilution, 10% dilution and non-dilute LOF, 1% dilution, 10% dilution and non-dilute LOF+EM mixture. Each treatment was replicated five times. Pak Choy seeds were soaked in alcohol 95° for 5 minutes and washed with sterile water three times to remove the alcohol. Then the seeds were treated with fertilizers for an hour. Ten seeds of each treatment were planted in each petri dish on paper moistened by 5 ml of the fertilizer solution. The germination rate of each treatment and the length of root and shoot of each seedling were recorded after 7 days of incubation in the dark. Germination index was calculated using the following formulae of Zucconi *et al.* [17]:

$$\frac{\text{\%of germination in treatment} \times \text{root length in treatment} \times 100}{\text{\%of germination in control treatment} \times \text{root length in control treatment}}$$

2.5 Application of Fertilizers on the Growth of Vegetable Seedlings

The fertilizer dilution which yielded best seed germination was selected for the application of fertilizers on the growth of vegetable seedlings. 1% dilution of LOF and of LOF+EM mixture was chosen.

Pak Choy seeds were cultivated in trays before being transplanted into pots. Seeds were soaked in either distilled water (for the control treatment and spraying and pouring fertilizer treatments) or in the 1% fertilizer solutions (for soaked seeds in fertilizer treatments) for an hour. After that, 3-5 seeds were sown into each well of the tray containing a mixture of soil and rice husk ash (1:0.25 ratio). The trays were put under sunlight and watered every day. When the seedlings had developed two pairs of leaves, they were transferred into 500 ml pots filled with 1.5 kg soil:rice husk ash mixture (4:1 ratio) and grown for 30 days. Three seedlings were sown into each pot.

The experimental design was completely randomized with seven treatments; each treatment was replicated seven times. All the treatments were harvested on day 15 and day 30 for measurements, thus requiring 98 pots in total. The seven treatments in the experiment were:

Treatment 1: soil without fertilizer (control).

Treatment 2: seed inoculation with 10% LOF+EM mixture.

Treatment 3: spraying plants with 10% LOF+EM mixture

Treatment 4: pouring 10%LOF+EM mixture to the soil.

Treatment 5: seed inoculation with 10% LOF.

Treatment 6: spraying plants with 10% LOF.

Treatment 7: pouring 10% LOF to the soil.

For treatments 3, 4, 6 and 7, the fertilizers were sprayed on the leaves or poured into the root zones of the seedlings at day 0 (to all pots) and day 14 (only to pots that were harvested on day 30). Approximately 10 mL were poured and 7-8 mL of fertilizers was sprayed on these pots. For the control and seed inoculation treatments, 10 mL of water was poured into each pot. Watering was carried out on a daily basis.

2.6 Plant Measurements

Plants were harvested at day 15 and 30. Four pots of each treatment were selected for the measurement of root and shoot, these included dry weight, fresh weight, length, and leaf number.

The three remaining pots of each treatment were taken for the measurement of vitamin C and chlorophyll content. Estimation of vitamin C was carried out following the procedure proposed by Rahman *et al.* [18].

To determine chlorophyll content of Pak choy seedlings, 0.1 g of the leaves of each treatment was collected. Then, 5 mL of 80% acetone was added to the test tubes containing leaves. These test tubes were incubated in the dark for 48 hours at room temperature. The resulting extract was used to measure absorbency using a spectrophotometer, against an 80% acetone blank at 645 nm and 663 nm. Total chlorophyll content was determined following this formula (according to Yadegari et al. [19]): Total Chlorophyll = $(20.2A645 + 8.02A663) \times (V/(1000 \times w))$ (mg/g fresh weight)

In which, A663 was absorbance at 663 nm and A645 was absorbance at 645 nm.

2.7 Soil Measurements

Fluorescein diacetate (FDA) hydrolysis was carried out to measure the total microbial activity in soil samples by using the method proposed by Adam *et al.* [20]. pH and EC of soil were determined in 1:5 soil-water suspensions and its extract.

2.8 Data Analysis

Data analyses were carried out using the linear model procedure of Statistix version 8 to test the pH and EC of fertilizers, pH, EC and FDA of the soil, the effects of LOF and LOF+EM on the growth (root length, shoot length, leaf number, root and shoot fresh weight, root and shoot dry weight) and quality (Vitamin C and chlorophyll content) of Pak choy seedlings. All analysis was based on a randomized design. F-tests were used to determine the significance of differences between means of treatments. Statistical significance was considered at $P \leq 0.05$.

3. RESULTS

3.1 pH and EC

pH and EC of EM, liquid organic fertilizer and the mixture of liquid organic fertilizer and EM are shown in Table 1. There were significant differences in both pH and EC in all types of fertilizer. The lowest value of pH and EC was found in LOF, where a pH of 3.467 and EC value of 4.463 mS/cm were observed. The highest values of these two parameters belonged to EM. pH and EC of the mixture were in between these values of each component.

pH and EC values of soil of 0 days (not treated with any kinds of fertilizers), 15 days and 30 days are illustrated in Table 2. There were significant differences in pH values in both sampling times. The pH values of all the soil treatments, with the exception of pouring LOF, showed a slight decrease over 30 days. The largest decline of 0.43 in pH value was found in the treatment of pouring LOF+EM. The lowest pH value at day 15 was found in the soil sprayed with LOF+EM (6.88), while the lowest pH value of day 30^{th} was recorded in the treatment of soaking seeds in LOF. There was no significant difference in EC among the treatments over the testing period. EC value also decreased after 30 days. Soil used to plant seeds soaked with LOF experienced the biggest decline in EC value, from 0.2017 mS/cm to 0.079 mS/cm.

3.2 Microbiology Test Result

The counts of nitrogen fixing, phosphate and potassium solubilizing and total microbes are shown in Table 3. Microorganisms were not recorded in every microbiology test for each fertilizer. With LOF, only total microbes and nitrogen fixing microbes were recorded with the numbers of 52.7×10^6 cfu/mL and 39.3×10^4 cfu/ml, respectively. Meanwhile, when the EM was initially tested for the presence of microbes in these media and no colonies were observed. Therefore, the EM was tested again with nutrient broth and then only total microbes could be recorded with 86×10^6 cfu/mL.

3.3 Seed Germination

Table 4 shows the result of the seed germination test of Pak Choy treated by

Table 1. pH and EC of EM, LOF and the mixture of LOF and EM.

	pH	EC (mS/cm)
EM	$4.037^a \pm 0.015$	$72.167^a \pm 0.513$
LOF	$3.467^c \pm 0.038$	$4.463^c \pm 0.051$
LOF + EM	$3.697^b \pm 0.012$	$31.533^b \pm 0.404$
F-test	**	**
%CV	0.66	1.05

**: values marked with the same letter do not differ statistically at $p \leq 0.01$, separately for each of means.

Table 2. pH and EC values of soil at 0, 15 and 30 days.

Time	Treatment	pH	EC (mS/cm)
0 days	Soil	6.47 ± 0.23	0.447 ± 0.076
15 days	Control	7.09^{a} ± 0.07	0.0834 ± 0.023
	LOF, soaked seeds	6.89^{cd} ± 0.08	0.2017 ± 0.143
	LOF, spraying	6.79^{d} ± 0.13	0.2289 ± 0.044
	LOF, pouring	6.91^{bcd} ± 0.09	0.1935 ± 0.044
	LOF+EM, soaked seeds	6.99^{abc} ± 0.06	0.1241 ± 0.019
	LOF+EM, spraying	6.88^{cd} ± 0.02	0.122 ± 0.002
	LOF+EM, pouring	7.04^{ab} ± 0.02	0.127 ± 0.015
	F-test	**	ns
	%CV	1.1	39.07
30 days	Control	6.89^{a} ± 0.14	0.083 ± 0.001
	LOF, soaked seeds	6.6^{b} ± 0.13	0.079 ± 0.018
	LOF, spraying	6.91^{a} ± 0.04	0.140 ± 0.098
	LOF, pouring	6.71^{ab} ± 0.13	0.117 ± 0.033
	LOF+EM, soaked seeds	6.88^{a} ± 0.21	0.122 ± 0.064
	LOF+EM, spraying	6.76^{ab} ± 0.06	0.092 ± 0.018
	LOF+EM, pouring	6.61^{b} ± 0.12	0.104 ± 0.013
	F-test	*	ns
	%CV	1.91	44.93

*: Values marked with the same letter do not differ statistically at $p \leq 0.05$, separately for each of means

**: Values marked with the same letter do not differ statistically at $p \leq 0.01$, separately for each of means

ns: Means are not significantly different

Table 3. Bacterial counts of LOF and EM in different media.

CFU/mL				
Fertilizer	Plate count agar	Nitrogen free agar	Pikovskya agar	Alexsandrov agar
LOF	52.7×10^{6}	39.3×10^{4}	-	-
EM (+NB)	86×10^{6}	-	-	-

"-" means no colonies were recorded.

different dilutions of fertilizer. The treatment of (LOF+EM) 1% showed the greatest germination rate; while the seeds treated with LOF 100%, (LOF+EM) 10% and (LOF+EM) 100% did not germinate during the 7-day testing period. Mean root length and mean shoot length of the control treatment (not shown in the table) were 44.45 mm and 51.59 mm, respectively. The mean root length and the Germination index of LOF 1% were the highest among the three treatments, which were 28.49 cm and 64.1%, respectively.

Table 4. Seed germination test results with different dilution of LOF and the mixture of LOF and EM.

Treatment	Shoot length mean (mm)	Root length mean (mm)	Germination rate (%)	GI (%)
LOF 1%	65.6 ± 4.55	28.49 ± 2.01	98	98
LOF 10%	35.05 ± 4.73	17.24 ± 2.43	44	44
LOF 100%	-	-	-	-
(LOF+EM) 1%	60.3 ± 5.97	17.82 ± 1.1	100	100
(LOF+EM) 10%	-	-	-	-
(LOF+EM) 100%	-	-	-	-

"-" means no seeds germinated.

3.4. Growth Parameters

3.4.1 Root length and shoot length

Root length and shoot length increased in all the treatments over two sampling dates (Table 5). There were significant differences in root length in the first harvest time and shoot length in both harvest times, but there was no significant difference in root length in the second harvest time. The treatment of spraying LOF onto the plants yielded the longest root length over both harvest times, which were 31.1 cm and 32.6 cm for 15 and 30 days respectively. During the 15-day period, the plants treated by soaking their seeds into LOF showed the highest shoot growth (18.22 cm) compared to all other treatments. However, the biggest improvement in shoot length was recorded in the treatment of spraying LOF+EM on the vegetables, from 15.41 cm in the first harvest to 22.55 cm in the second harvest, which made the shoot length of this treatment the highest in the 30-day period.

3.4.2 Leaf number

Four out of seven treatments (LOF, soaked seeds; LOF, spraying; LOF, pouring and LOF+EM, pouring) showed an increase in the number of leaves per plant after two harvests (Table 5). There were significant differences among the treatments during the 15-day period but limited variation after the 30-day period. Inoculation of seeds in the mixture of LOF+EM produced the highest number of leaves (4.7) in the 15-day period while plants sprayed with LOF+EM recorded the highest number of leaves (4.23) in the 30-day period. The highest increase of 0.63 leaves was found in plants treated by pouring with LOF+EM over the testing period.

3.4.3 Root and shoot fresh weight

Fresh weight of root and shoot of the vegetables is shown in Table 6. A large improvement was observed in shoot and root fresh weight over the 30-day period. There were significant differences in root fresh weight in the first harvest time and shoot fresh weight in both harvest times, but there was no significant difference in root fresh weight in the second harvest time. Root fresh weight was highest in the seeds soaked in LOF treatment (4.63 g for first harvest and 6.51 g for second harvest) for both harvests. Maximum shoot fresh weight in both harvests was recorded for those plants treated by soaking their seeds in LOF+EM

Table 5. Root length, shoot length and leaf number of Pak Choy over the experimental period.

Time	Treatment	Root length (cm)	Shoot length (cm)	Leaf number/plant
15 days	Control	$28.10^{ab} \pm 0.36$	$17.03^{bc} \pm 0.39$	4.30^{a}
	LOF, soaked seeds	$27.30^{ab} \pm 3.04$	$18.22^{a} \pm 0.56$	3.70^{b}
	LOF, spraying	$31.10^{a} \pm 5.15$	$16.46^{cd} \pm 0.5$	$3.10^{c} \pm 0.17$
	LOF, pouring	$22.07^{c} \pm 1.42$	$17.97^{ab} \pm 0.63$	$3.67^{b} \pm 0.35$
	LOF+EM, soaked seeds	$25.60^{bc} \pm 3.15$	$17.8^{ab} \pm 0.4$	4.70^{a}
	LOF+EM, spraying	$26.70^{abc} \pm 2.15$	$15.41^{d} \pm 0.8$	$4.57^{a} \pm 0.23$
	LOF+EM, pouring	$25.60^{bc} \pm 1.65$	$15.65^{d} \pm 0.85$	$3.37^{bc} \pm 0.58$
	F-test	*	**	**
	%CV	10.54	3.63	7.10
30 days	Control	28.90 ± 2.93	$19.93^{b} \pm 0.87$	3.10 ± 0.17
	LOF, soaked seeds	30.50 ± 3.03	$19.91^{b} \pm 0.63$	4.00 ± 0.3
	LOF, spraying	32.6 ± 2.5	$21.23^{ab} \pm 1.04$	3.70
	LOF, pouring	26.23 ± 3.92	$19.79^{b} \pm 1.24$	3.77 ± 1.08
	LOF+EM, soaked seeds	28.30 ± 1.04	$19.83^{b} \pm 0.65$	3.77 ± 0.5
	LOF+EM, spraying	28.33 ± 3.38	$22.55^{a} \pm 0.55$	4.23 ± 0.68
	LOF+EM, pouring	26.90 ± 5.08	$20.21^{b} \pm 1.28$	4.00 ± 1
	F-test	ns	*	ns
	%CV	11.56	4.57	17.25

*: Values marked with the same letter do not differ statistically at $p \leq 0.05$, separately for each of means.

**: Values marked with the same letter do not differ statistically at $p \leq 0.01$, separately for each of means.

ns: Means are not significantly different.

(12.19 g and 18.43 g, first and second harvest, respectively). However, the largest rise of over 100% in shoot fresh weight was recorded in plants sprayed with LOF.

3.4.4 Root and shoot dry weight

Table 6 demonstrates the growth in root and shoot dry weight that occurred over 30 days. There was no significant difference in root and shoot dry weight in the first 15-days for any treatment, but significant differences in root and shoot dry weight were observed over a 30-day period. Plants sprayed with LOF showed the highest root dry weight over two harvests, 0.27 g for the first harvest and 0.62 g for the second harvest. For the first harvest, both spraying LOF and soaking seeds in LOF+EM had the highest shoot dry mass, which was 0.74 g. However, over the 30-day period, spraying LOF made a dramatic growth of 1.22 g and recorded the highest shoot dry weight in the second harvest.

3.4 FDA Activity

FDA activities in soil of the different treatments are demonstrated in Figure 1.

Table 6. Root and shoot fresh weight and root and shoot dry weight of Pak Choy over the experimental period.

Time	Treatment	Root fresh weight (g)	Shoot fresh weight (g)	Root dry weight (g)	Shoot dry weight (g)
15 days	Control	$3.04^{bc} \pm 0.98$	$9.47^{b} \pm 1.16$	0.23 ± 0.09	0.71 ± 0.04
	LOF, soaked seeds	$4.63^{a} \pm 0.95$	$12.09^{a} \pm 0.31$	0.24 ± 0.05	0.71 ± 0.08
	LOF, spraying	$4.18^{ab} \pm 0.16$	$8.12^{b} \pm 1.6$	0.27 ± 0.02	0.74 ± 0.16
	LOF, pouring	$2.66^{c} \pm 0.09$	$8.22^{b} \pm 0.38$	0.18 ± 0.02	0.60 ± 0.02
	LOF+EM, soaked seeds	$3.01^{bc} \pm 1.38$	$12.19^{a} \pm 2.57$	0.22 ± 0.07	0.74 ± 0.18
	LOF+EM, spraying	$2.38^{c} \pm 0.43$	$8.58^{b} \pm 0.07$	0.15 ± 0.03	0.61 ± 0.05
	LOF+EM, pouring	$2.45^{c} \pm 0.85$	$9.07^{b} \pm 1.61$	0.25 ± 0.04	0.50 ± 0.11
	F-test	*	**	ns	ns
	%CV	25.64	14.27	22.73	16.43
30 days	Control	6.32 ± 1.35	$16.08^{ab} \pm 1.82$	$0.49^{b} \pm 0.06$	$1.76^{ab} \pm 0.42$
	LOF, soaked seeds	6.51 ± 0.69	$15.74^{abc} \pm 2.33$	$0.59^{ab} \pm 0.06$	$1.67^{ab} \pm 0.2$
	LOF, spraying	6.19 ± 2.1	$16.36^{ab} \pm 2.99$	$0.62^{a} \pm 0.14$	$1.96^{a} \pm 0.47$
	LOF, pouring	5.27 ± 0.87	$12.77^{bcd} \pm 1.14$	$0.61^{ab} \pm 0.04$	$1.33^{bc} \pm 0.08$
	LOF+EM, soaked seeds	5.59 ± 0.5	$18.43^{a} \pm 2.27$	$0.50^{ab} \pm 0.03$	$1.78^{ab} \pm 0.16$
	LOF+EM, spraying	2.70 ± 1.42	$11.11^{d} \pm 3.93$	$0.20^{c} \pm 0.05$	$1.02^{c} \pm 0.47$
	LOF+EM, pouring	5.71 ± 1.77	$11.83^{cd} \pm 0.49$	$0.49^{ab} \pm 0.07$	$1.04^{c} \pm 0.27$
	F-test	ns	*	**	*
	%CV	24.81	16.31	14.49	21.96

*: Values marked with the same letter do not differ statistically at $p \leq 0.05$, separately for each of means.
**: Values marked with the same letter do not differ statistically at $p \leq 0.01$, separately for each of means.
ns: Means are not significantly different.

The figure shows an increase in FDA hydrolysis in all the treatments. The amount of fluorescein hydrolysed in the treatment of LOF, soaked seeds increased almost five times from the 15th to the 30th day. This treatment had the highest FDA activity over the 30-day period. Despite having the highest FDA activity in the 15-day measurements, the control treatment demonstrated 0.29 μg/g increase in FDA activity, which was the smallest, throughout the 30 day testing period.

3.5 Vitamin C Content

Figure 2 illustrates the effect of applying different treatment on vitamin C production in vegetables. Vitamin C content of the vegetables was found to increase in all treatments, except the control treatment after 30 days. The highest vitamin C content of the first harvest (61.611 mg/100g fresh weight) was found in the treatment of pouring the mixture of LOF and EM to the plants. The lowest vitamin C content after 15 days of growing Pak Choy was 28.876 mg, in the treatment of spraying

LOF+EM to the plants while the lowest amount of Vitamin C in the second harvest was found in the control treatment (38.277 mg/100g fresh weight). The biggest increase in vitamin C content after two harvest times was recorded in plants whose seeds were soaked in LOF, which was about 42.3 mg. This made the seeds soaked in LOF the treatment with the highest vitamin C content over the 30-day period.

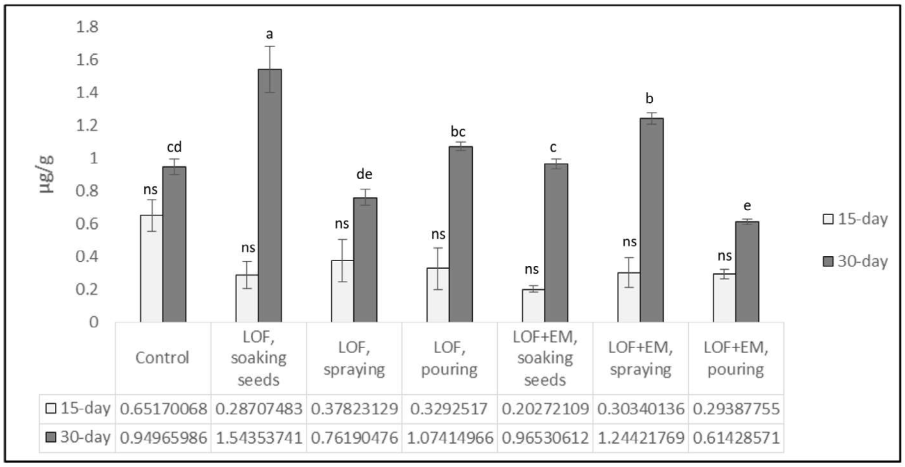

Figure 1. FDA activity in the soils after growing Pak Choy plants for 15 and 30 days. ns: non-significant. Means followed by the various letters of 30-day harvest are significant (P < 0.01), according to F-test. The same letter was showed non- significant different.

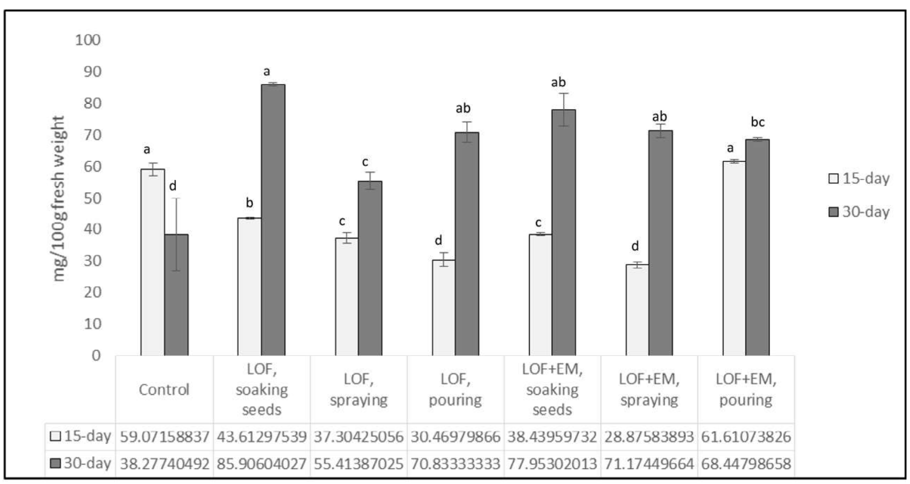

Figure 2. Vitamin C content in the leaves of Pak Choy after 15 and 30 days of growth. Means followed by the various letters of each sampling time are significantly different (P < 0.01), according to F-test.

3.6 Chlorophyll Content

Chlorophyll content was found to be higher when the vegetables were harvested after 15 days of growth rather than after 30 days (Figure 3). The highest relative chlorophyll content (1.498 mg/g fresh weight) of the first harvest was recorded for seeds soaked in LOF, while the smallest amount of this pigment was found in vegetables grown by pouring the mixture of LOF and EM. In the 30-day harvest, the treatment of pouring LOF on Pak Choy was found to have the highest amount of chlorophyll (1.148 mg/g fresh weight) and the control plants showed the lowest content. The smallest decrease in chlorophyll content was seen in the treatment of spraying LOF+EM to the vegetables, with only 0.025 mg/g fresh weight.

4. Discussion

4.1 Soil Characteristics

The pH and EC value of all treatments decreased after 30 days. The pH value of the treatments using LOF and the mixture of LOF+EM as fertilizers was slightly lower than that of the control treatment. This could be because of the high acidity of LOF and EM, shown in Table 1. El-Shafei *et al.* (2008) and Yaduvanshi (2003) demonstrated comparable results when treating soil with EM compost and organic manure, respectively [3], [13]. No significant differences were found in soil salinity among treatments. However, the soil salinity of all treatments was reduced after 30 days. The reduction in these values is an expression of better soil fertility [3, 5].

FDA activity in the soils rose after two harvest times for all treatments, indicating that total microbial activity in the soil had increased. Higher microorganism levels can help in enhancing soil fertility and plant quality [19]. However, there are significant differences in the increase of FDA activity among the treatments. The soil on which plants treated with LOF or LOF+EM had greater improvement in microbial activity as compared to the control treatment. This result concurs the findings of Packialakshmi and Yasotha [20].

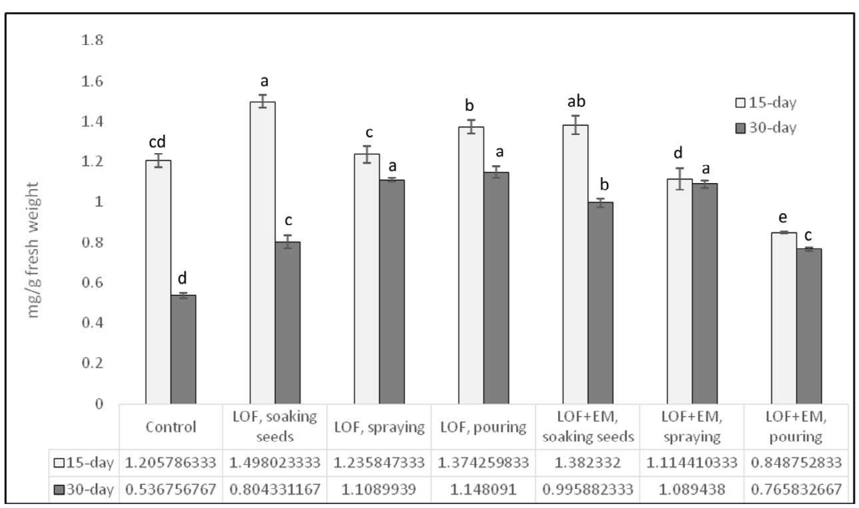

Figure 3. Chlorophyll content in the leaves of Pak Choy after growing for 15 and 30 days. Means followed by the various letters of each sampling time are significantly different (P < 0.01), according to F-test.

4.2 Seed Germination

The seeds treated with high concentrations of fertilizers (LOF 100%, (LOF+EM) 10% and (LOF+EM) 100%) could not germinate during the seed germination test. This may be due to the high electrical conductivity values of LOF and EM. However, the seeds germinated well in the treatment of (LOF+EM) with the highest germination rate (100%) and shoot length of seeds treated with LOF 1% and (LOF+EM) 1% were higher than that of the control treatment (51.59 mm). This means LOF and LOF+EM are potential fertilizers to improve plant growth and quality.

4.3. Plant growth and quality

Root length of all treatments improved after 30 days of cultivation. Although in the first 15 days the root systems of plants treated with fertilizers (except for the spraying LOF treatment) were shorter than that of the control treatment, a greater improvement was observed after 30 days. The treatment of pouring LOF on Pak Choy showed the biggest increase of root length after the 30-day period. This could be attributed to the direct application of the fertilizer to the root zone of the vegetable. Seeds soaked in LOF produced the longest shoot among all treatments in the first sampling dates while spraying LOF+EM to the plants led to a remarkable increase in shoot length after two harvests. However, these treatments did not yield a noticeable increase in root length. This could be because of the allocation of nutrients and resources of the plant to shoots rather than roots [6]. The control treatment had a high number of leaves after the first 15 days. However, after 30 days, the number of leaves per plant in the control treatment reduced dramatically, perhaps due to the fact that Pak Choy is a fast growing vegetable so the senescence of leaves occurs quickly. On the other hand, using LOF in three ways – seed inoculation, spraying and pouring – could increase the number of leaves. Increases in leaf number can help enhance photosynthesis rate and therefore plant growth.

Seeds soaked in either LOF or LOF+EM resulted in high shoot fresh weight (Table 6) for the first harvest while spraying LOF and seeds soaked in LOF+EM demonstrated the best results for the second harvest. These results are in agreement with those of Kleiber [10]. Noticeably, after 30 days of growth, the treatment of spraying LOF had the most significant increase in shoot and root dry weight. Moreover, spraying LOF also produced the highest figures in root length for both harvests. This is in line with a report by Ji *et al.* who found that using liquid organic fertilizer could dramatically improve root growth [21]. The reason why spraying LOF is the most effective fertilizer could be because foliar application of fertilizer facilitates quick absorption and limits the loss of nutrients through leaf tissues [22, 23].

After 15 days of growth, the root and shoot dry weight of Pak choy were not significantly different among treatments due to the short amount of time for the seedlings to grow. This is in agreement with the experiment with Pigweed by Muthaura *et al.*, where the first 14 days of growth also experienced only slight differences between the treatments and it was only after 42 days were the data of the treatments demonstrated significant differences from each other [6].

Almost all growth parameters of Pak choy were smaller when treated with LOF+EM treatments than the other treatments. This could be due to a lack of beneficial microorganisms in EM, as observed in the microbiology test.

Furthermore, the salinity (EC value) of EM was very high (Table 1). These factors could affect the development of vegetables as it dramatically reduced plant growth [24].

Vitamin C content increased with sampling dates with the exception of the control treatment. This result is in line with that obtained by *Daiss et al.* [25]. Seeds soaked in LOF could significantly improve the quality of plants, or in this case, the plants' vitamin C content. In the first sampling at 15 days, chlorophyll content is higher in the seeds soaked in LOF and also the mixture of LOF+EM, as compared to other treatments. This result is similar to that of Muthaura *et al.* [6]. However, in the second harvest, all of the treatments showed a decrease in chlorophyll content. This due to yellowing of the Pak Choy leaves at the harvest time of 30 day.

5. Conclusion

This study proved the beneficial effects of using liquid organic fertilizer and a mixture of this fertilizer with EM. Among the seven treatments, spraying LOF is the best for plants in terms of roots and shoots dried weights as it significantly increased both during two sampling times. Applying LOF+EM was not beneficial for the growth of Pak choy as all three ways of applying it (soaking, spraying and pouring) did not demonstrate greater root and shoot weight (fresh and dry) compared to the control treatment at both sampling times. On the other hand, the best treatment for increasing the vitamin C content of Pak choy is soaking seeds in LOF. Meanwhile, spraying the mixture of LOF + EM is the best treatment to reduce the senescence of leaves. In general, applying liquid organic fertilizer and EM can be attributed to the better quality content (vitamin C and chlorophyll) of Pak choy. However, the effectiveness of the commercial EM could not be proven in this research.

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References

[1] Higa T. and Parr J.F., *Beneficial and Effective Microorganisms for a Sustainable Agriculture and Environment*, Vol. 1, Atami, Japan: International Nature Farming Research Center, 1994.

[2] Higa T., *Proceedings of the First International Conference on Kyusei Nature Farming*, US Department of Agriculture, Washington, DC, USA, 1991; 8-14.

[3] El-Shafei A., Yehia M. and El-Naqib F., *Misr. J. Agric. Eng.*, 2008; **25(3)**: 1067-1093.

[4] Zydlik P. and Zydlik Z., *Nauka Przyroda Technologie*, 2008; **2(1)**: 4.

[5] Fan L., Zhou X., Li Y., Ji L., Wu G., Li B. and Zou L., *Appl. Sci.*, 106; **6(6)**: 168. DOI 10.3390/app6060168.

[6] Muthaura C., Musyimi D.M., Ogur J.A. and Okello S.V., *J. Agric. Biol. Sci.*, 2010; **5(1)**: 17-22.

[7] Olle M. and Williams I., *J. Adv. Agric. Technol.*, 2015; **2(1)**. DOI 10.12720/joaat.2.1.25-28.

[8] Frlszczak B., Kleiber T. and Klama J., *Afr. J. Agric. Res.*, 2012; **7(43)**: 5756-5765. DOI 10.5897/ajar12.145.

[9] Daly M.J. and Stewart D.P.C., *J. Sust. Agric.*, 1999; **14(2-3)**: 15-25. DOI 10.1300/J064v14n02_04.

[10] Kleiber T., Starzyk J., Gorski R., Sobieralski K., Siwulski M., Rempulska A. and Sobiak A., *Acta Scientiarum Polonorum-Hortorum Cultus*, 2014; **13(1)**: 79-90.

[11] Deore G.B., Limaye A.S., Shinde B.M. and Laware S.L., *Asian J. Exp. Biol. Sci.*, 2010; **(Spl)**: 15-19.

[12] Enujeke E.C., Ojeifo I.M. and Nnaji G.U., *Asian J. Agric. Rural Dev.*, 2013; **3(4)**: 186.

[13] Yaduvanshi N.P.S., *J. Agric. Sci.*, 2003; **140(2)**: 161-168.

[14] Soumare M., Tack F.M.G. and Verloo M.G., *Bioresour. Technol.*, 2003; **86(1)**: 15-20. DOI 10.1017/S0021859603002934.

[15] Higa T. and Wididana G.N., *Proceedings of the 1st International Conference on Kyusei Nature Farming*, US Department of Agriculture, Washington, DC, USA, 1991; 153-162.

[16] Rani M.U., Arundhathi and Reddy G., *Afr. J. Biotechnol.*, 2012; **11(32)**: 8085-8091. DOI: 10.5897/AJB10.2166

[17] Zucconi F., Pera A., Forte M. and Bertoldi M., *Biocycle*, 1981; **22**: 54-57.

[18] Rahman M.M, Khan M.M.R and Hosain M.M., *Bangladesh J. Sci. Ind. Res.*, 2007; **42(4)**: 417-424. DOI 10.3329/bjsir.v42i4.749

[19] Yadegari L.Z., Haidari R. and Carapetian J., *J. Biol. Sci.*, 2007; **7(8)**: 1436-1441. DOI 10.3923/jbs.2007.1436.1441.

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

[21] Ji R., Dong G., Shi W. and Min J., *Sustainability*, 2017; **9(5)**: 841. DOI 10.3390/su905084.

[22] Hassan H.S.A., Sarrwy S.M.A. and Mostafa E.A.M., *Agric. Biol. J. North Am.*, 2010; **1(4)**: 638-643.

[23] Fageria N.K., Filho M.B., Moreira A. and Guimaraes C.M., *J. Plant Nutr.*, 2009; **32(6)**: 1044-1064. DOI 10.1080/01904160902872826

[24] Ashkan A. and Jalal M., *Int. J. Agric. Crop Sci.*, 2013; **5(22)**: 2669-2676.

[25] Daiss N., Lobo M.G., Socorro A.R., Bruckner U., Heller J. and Gonzalez M., *Eur. Food Res. Technol.*, 2008; **226(3)**: 345-353. DOI 10.1007/s00217-006-0543-2.