J. Agrobiotech. **Vol. 9** (1S), 2018, p. 154–165.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)

The effect of *Bacillus* sp. (B43) on the growth of Pak choy and Red tilapia in aquaponics system

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Keywords:
Oreochromis sp
Brassica rapa
aquaponics
water quality

ABSTRACT

The objective of this study is to determine the effect of *Bacillus* sp. (B43) which were cultured into aquaponics system to enhance the red tilapia (*Oreochromis sp.*) and Pak Choy (*Brassica rapa*) growth performance. Six aquaponics sets have been setup, three (3) for control (without the addition of *Bacillus* sp. (B43) culture) and another three (3) for bacteria treatment. The plant seedlings were transplanted into the aquaponics set a week after the fish were released into the tank. The experiment started by culturing the *Bacillus* sp. (B43) into the tank at $4x10^5$/ml. Parameters such as fish length, fish body weight, plant height, number of leaves, ammonia content, nitrate content, pH, Dissolved Oxygen (DO), were recorded weekly. The dry weight of the plants was recorded at the end of the experiment. All the data has been analysed by using IBM SPSS Statistic version 25. Independent Sample T-test was chosen to compare the mean of every data collected by weeks. For plant height, the result showed that there are no significant differences between the control and bacteria treatment. However plant height mean for control at week fourth was 5.678 ± 1.366 g lower than bacteria treatment 5.700 ± 1.211 g. Analysis for a number of leaf and dry weight of Pak Choy also show no significant difference between bacteria treatment and control. However, the dry weight mean for control was 0.904 ± 0.355 g and for bacteria treatment is 0.947 ± 0.573 g. The fish weight and fish length showed there is no significant difference between the bacteria treatment and control. As a conclusion, the addition of *Bacillus* sp. (B43) at $32x10^6$/80ml into at 190 liter aquaponics system of cultivating red tilapia (30 tails) and Pak Choy (6) does not show any significant difference in fish and plant growth as well as the water quality for ammonia and nitrate content.

Keywords: *Oreochromis* sp.*, Brassica rapa,* aquaponics, water quality,

ABSTRAK

Objektif kajian ini adalah untuk menentukan kesan *Bacillus* sp*,* (B43) yang dikultur di dalam sistem akuaponik dalam peningkatan pertumbuhan tilapia merah (*Oreochromis* sp.) dan Pak Choy (*Brassica rapa*). Enam set akuaponik telah disediakan, tiga untuk kawalan (tidak ditambah kultur *Bacillus* sp. (B43)) dan tiga lagi untuk rawatan bakteria. Biji benih dipindahkan ke dalam set akuaponik selepas seminggu ikan diletakkan di dalam tangki. Kajian dimulakan dengan $4x10^5$/ml *Bacillus* sp.dikultur di dalam tangki Parameter seperti panjang ikan, berat ikan, tinggi tumbuhan, bilangan daun, kandungan ammonia, kandungan nitrat, pH, oksigen terlarut direkodkan setiap minggu. Berat kering tumbuhan direkodkan dihujung kajian. Kesemua data telah di analisis menggunakan IBM SPSS Statistik versi 25. "Independent sample T-test" dipilih untuk membandingkan purata setiap data yang telah direkodkan setiap minggu. Untuk ketinggian pokok, keputusan kajian menunjukkan tiada perbezaan ketara di antara kawalan dan rawatan bakteria. Walaubagaimanapun, min untuk kawalan di minggu ke-empat adalah 5.678 ± 1.366 g lebih rendah berbanding rawatan bakteria 5.700 ± 1.211 g. Analisis bagi bilangan daun dan berat kering untuk Pak Choy juga menunjukkan tiada perbezaan ketara diantara kawalan dan rawatan bakteria. Walaubagaimanapun, min bagi berat kering untuk kawalan adalah 0.904 ± 0.355 g dan untuk rawatan bakteria adalah 0.947 ± 0.573 g. Berat ikan dan panjang ikan menunjukkan tiada perbezaan ketara diantara rawatan bakteria dan kawalan. Kesimpulannya, penambahan $32x10^6$/80ml kultur *Bacillus* sp. (B43) ke dalam 190 liter sistem akuaponik yang diternak tilapia merah (30 ekor) dan Pak Choy (6 pokok) tidak menunjukkan apa-apa perbezaan pada pertumbuhan ikan dan pertumbuhan pokok serta kandungan ammonia dan nitrat di dalam air.
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Kata kunci: *Oreochromis* sp.*, Brassica rapa,* akuaponik*,* kualiti air*,*

INTRODUCTION

As global population growth are forecast to increase, an enormous amount of pressure will be put on our natural resources and food supplies (Battersby, 2012; MacDevette *et al*., 2009). Currently high-input, resource-intensive farming systems have caused massive deforestation, water scarcities, soil depletion and high levels of greenhouse gas emissions. Rapid development of country gives an effect to agriculture because land of agriculture was destroyed in order to make new buildings and residential area. It is urgent that the agricultural sector produces more, with less by following principles of efficient resource use. To this end, proper understanding and deployment of new comprehensive strategies that can accommodate the needs and well-being of present and future populations are required (UN, 2014; FAO, 2016; 2017). One strategy to achieve this is by urban agriculture where agriculture production can be cultivated, process and distributed from villages, town and cities (Dumlao, 2012). In an urban agriculture, it provides an alternative to finite resources such as land and water as in agriculture (Connolly and Trebic, 2010). One way of practicing urban agriculture is known as aquaponics.

Aquaponic can be explained as a combination of hydroponic system and aquaculture (Goldstein, 2015). The term aquaponic system were first introduced by Watten and Busch (1984) in their publication entitled Tropical Production of tilapia (*Sarotherodon aurea*) and tomatoes (*Lycopersicon esculentum*) in a small-scale recirculating water system. Since then, there were a growing number interest of researcher working on the development and diversification in this field. Aquaponic also refers to as growing of plant and cultivation of fish together in a closed system with re-circulating water system which use natural bacteria to convert waste secreted from the fish into the non-toxic nutrient that can be adsorbed by the plant (Bernstein, 2011). Aquaponic can be separated into three components such as: 1) Bacteria : In an aquaponic system, fish will secrete ammonia waste into the water as a product of its metabolism. However, the release of NH_3 steadily would cause the NH_3 content to increase. High concentrations of ammonia at 0.5-1ppm are lethal to fish (Robert, 1997); 2) Aquatic Animals: Using aquaponics system, freshwater organism can be raised such as freshwater fish, prawn and crayfish (Diver, 2006); 3) Plants : As in hydroponics, usually green leafy vegeatables such as lettuce, spinach and chinese cabbage are used. This is because green leafy vegeatables requires low nutrient requirements.

In aquaponics environment, nitrification process occurs naturally as toxic ammonia (NH_3 or NH_4^+) from the fish waste are converted to nitrite (NO_2^-) and nitrate(NO_3^-) by *Nitrosomonas* sp. and *Nitrobacter* sp bacteria (CropKing, 2005 ; Nelson, 2008). *Nitrosomonas* bacteria are chemoautotrophic, which means that it uses energy gained from oxidation of ammonia to nitrites in its metabolism to fix carbon dioxide into an organic compound (http://microbewiki.kenyon.edu/index.php/Nitrosomonas). *Nitrobacter* falls under a gram-negative and chemoautotropic bacteria (www.bioconslab.com/nitribactfacts.html).where it converts nitrite (NO^-_2) to nitrates (NO^-_3) in a nitrogen cycle. *Nitrobacter* metabolizes nitrite to nitrate and uses the energy during the conversion as a part of its needs (www.bioconslab.com/nitribactfacts.html).

Red Tilapia or *Oreochromis* sp. is one of the freshwater fish which is commonly cultivated in the aquaponic system due to its texture which the fish has the ability to grow in a short period of time (James, 2014). With increasing waste water from the cultivated fish, it will increase the ammonia level in fish tank resulting in nitrification increase contributing to highly crop production by nutrient sufficiency. (John & Craig, 2004). Pak Choy is a light green leaf cabbage under the Cruciferae family and contains high nutrients such as Vitamin A, C, protein and calcium (Myers, 1998). Normally, in Malaysia Pak Choy is a main or additional ingredient in dishes such as soups and other, while in Japan or Korea, Pak Choy is used as kimchi or other dishes. Pak Choy can be made to prepare dishes such as Korean barbecued flank steakon, hot and sour slaw salad, mandarin beef buns, and miso soup egg drops (Jo et al., 2012).

MATERIALS AND METHODS

Materials

Aquaponic System

For this study six aquaponic system were set up where it consisted of two (2) treatment with three (3) replicates/treatment. A control and bacteria treatment cultured with *Bacillus sp.* (B43) were set up with each aquaponic system consisted of a 250L fish tank, aeration tube, wooden table, multitray, polybag, pipe system, plant seed, 30 of red tilapia, water pump and plant media. Cocopeat were selected as a media in the system due to its capacity in trapping water and water retention. Cocopeat or coir fiber is the most effective wetting agent where it is reported that this material are able to trap water nutrient and also can retain water 8-9 times its weight and slowly release the water to the plants through its feeding root (Horsfall, 2014).

Plant Materials

Pak Choy was used as the experimental plant to be planted in the aquaponic system. There were six (6) replicate of Pak Choy for each of the aquaponic system. Peatmoss were used as the germination media for Pak Choy seeds. The germination process take around seven days, where the seedling were transfered into the aquaponic media, after the sprout of its third leaf. The transfer would initiate the experiment.

The Red Tilapia

The red tilapia or *Oreochromis sp.* were collected at the Setiu Red tilapia breeder. The Red Tilapia fingerlings were bought at RM 0.40 per tail and have the size between 4 cm – 6 cm.

Experimental design

The experiment was conducted to determine the suitability and effect of *Bacillus sp.* (B43) for aquaponic system. The *Bacillus sp.* (B43) was cultured into three tanks for bacteria treatment whereas for control the tanks were filled only with water without any addition of fertilizer or bacteria. The aquaponic systems were setup first before the experiments were carried out. All the tanks were filled with water and the water pumps were initiated in order to increase its Dissolve Oxygen (DO). The fishes were then transferred into each tank contained 30 red tilapia as the nutrient source for our plant. For fish growth, parameters such as the Body Weight (g) and Total Length (cm) were recorded. Ten (10) of the fishes were randomly selected and recorded weekly.

The study was conducted for five consecutive weeks whereas the seedling process of Pak Choy starts at the second week. Parameters for Pak Choy's growth such as Plant Height (cm), Leaf Width (cm) were collected on weekly basis while its Dry Weight (g) were collected at the end of experiment. In aquaculture, water quality plays an important role where it determined the mobility of the fish in the system. If the ammonia level is too high, then it will lead to fish dead. So the content of ammonia content (ppm), temperature (°C), pH, nitrate (NO_3-) content and Dissolved Oxygen (DO) were observed weekly to make sure that the condition for the fish to grow were optimum. The HORIBA LAQUA 100 Series Handheld water quality instruments were used to obtain DO, pH and temperature of every tank. API Ammonia NH_3/NH_4^+ test kit were used to determine the ammonia content in the water while LAQUAtwin HORIBA compact NO_3^- were used to obtain the Nitrate content.

DATA ANALYSIS

In the study, the data was analyzed by using general linear model and chart model in SPSS followed by the comparison of mean for all parameter by using the Independent sample T-test. Levene's test was selected to assess the equality of variances for a calculated variable of two or more groups.

RESULTS AND DISCUSSION

Fish performance

The Red Tilapia, *Oreochromis* sp. were successfully cultured for 35 day, from 28th January 2018 to 3rd March 2018. They were fed with commercial pellet based on their average body weight for all tank. The growth performance of *Oreochromis sp* fingerlings were observed based on their body weight and body length (Table 1 and Table 2). Figure 1 and Figure 2 shows the graph on fish body weight and body length of both treatments.

Table 1 Mean of fish body weight in control and bacteria treatment

Treatment	Week 1	Week 2	Week 3	Week 4
Control	5.63g ± 2.57g	6.54g ± 2.65g	8.75g ± 3.42g	9.77g ± 3.96g
Bacteria Treatment	5.44g ± 2.072g	6.31g ± 2.24g	7.90g ± 3.03g	10.76g ± 3.97g

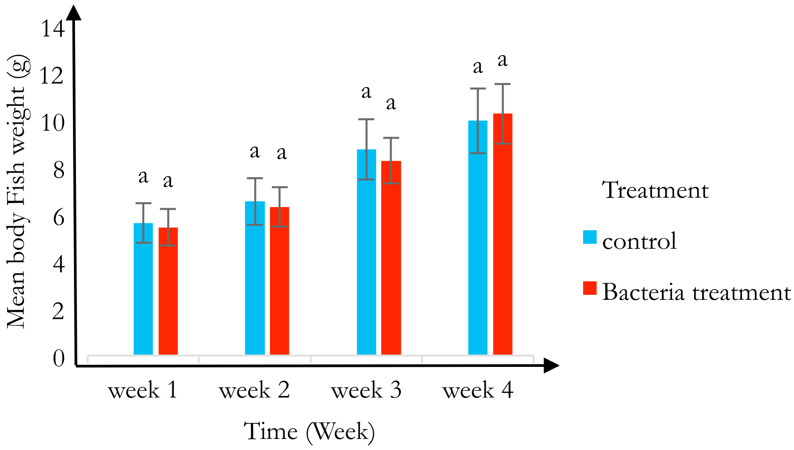

Figure 1 Comparison of means of fish body weight by week by using SPSS at 95% confidence. Error bar indicates standard error of means.

The body weight of red tilapia between control and bacteria treatment at week 1 showed the *p* value was at 0.33 (p>0.05). The fish body weight was not significantly different by comparing the mean of body weight between control and bacteria treatment. At week 4 the mean of the fish body weight for bacteria treatment were observed at 10.76 ± 3.97g higher than control (9.77g ± 3.96 g). However the analysis showed the p value for week 4 is at 0.86 (p>0.05). The fish body weight for week 4th showed no significant difference between the control and bacteria treatment. However the graph showed fish body weight increased consistently from the first week to the 4th week.

Table 2 Mean of fish body length in control and bacteria treatment

Treatment	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	6.59cm ± 0.96	6.99cm ± 1.08	7.69cm ± 1.07	7.98cm ± 1.07
Bacteria Treatment	6.49cm ± 0.75	6.94cm ± 0.85	7.62cm ± 0.81	8.29cm ± 1.12

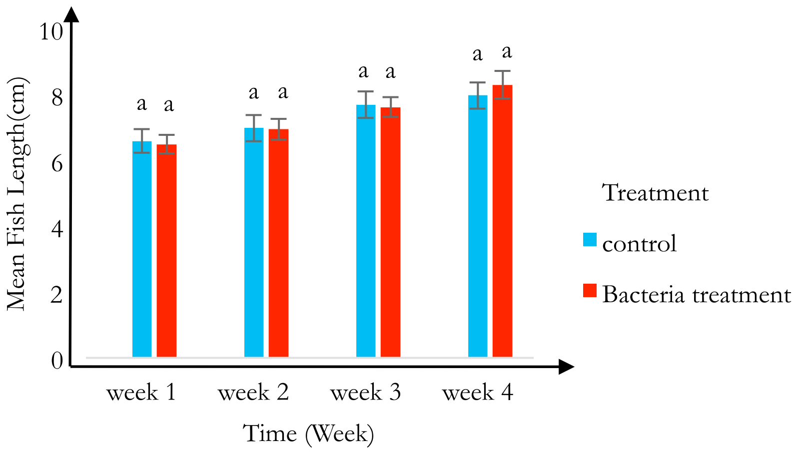

Figure 2 Comparison of means of fish length by week by using SPSS at 95% confidence. Error bar indicates standard error of means.

Figure 2 showed the fish length for control and bacteria treatment increasing consistently from the first week till the fourth week. The first week showed the *p* value was at 0.20 and the second week was at 0.23 followed by third and fourth week at 0.28 and 0.87 (p>0.05) perspectively. All the Levene`s test were not significantly different. Consulting our t-value, df and two-tail significance, again no significant differences were appear at p>0.05. There is no significant difference in the fish length supplemented with *Bacillus sp.* (B43) and the control. The graph also show the same trend as the graph at Figure1 where at week four, the mean of fish length in bacteria treatment was higher (8.29 ± 1.12 cm) than control (7.98 ± 1.07cm).

Plant performance

The Pak choy (*Brassica rapa*) has been successful cultivated in aquaponic system for 28 days after a week it germinated in a peatmoss. The plant height and number of Pak choy leaves has been recorded from the day it was transferred into aquaponic system at 6th February 2018 till 4th March 2018. After that, the plants were harvested and its dry weight was calculated. Table 3 and Table 4 below showed the mean ± Standard Deviation on the plant height and number of leaves. The Figure 3 and Figure 4 showed the graphs of plant height and number of leaves. The graph of Dry Weight of Pak Choy (*Brassica rapa*) supplemented with *Bacillus sp.* (B43) and control was shown in Figure 5.

Table 3 Mean of Plant Height of Pak Choy in control and bacteria treatment

Treatment	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	2.73 cm ± 0.70	4.32 cm ± 0.71	4.87 cm± 0.89	5.67 cm ± 1.36
Bacteria Treatment	2.40 cm ± 0.65	4.47 cm ± 0.55	5.03 cm ± 0.66	5.70 cm± 1.21

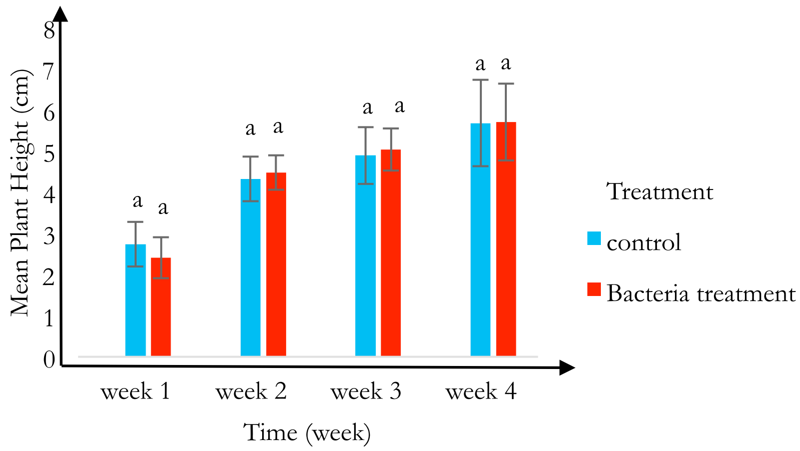

Figure 3 Comparison of Plant Height (cm) by week using SPSS at 95% confidence. Error bar indicates standard error of means.

Plant height in first week for both bacteria treatment and control were observed at 2.40 cm ± 0.65 and 2.73 cm ± 0.70 respectively where its *p* value was 0.81 (p>0.05). The length of Pak Choy was not significantly different between the bacteria treatment and control. However at fourth week the mean for the bacteria treatment was observed at 5.70 cm ± 1.21 higher than control at 5.67 cm ± 1.36, but the Levene`s test has a probability greater than 0.05 which can be assumed the population are relatively equal.

Table 4: Mean number of leaves of Pak Choy in control and bacteria treatment

Treatment	**Week 1**	**Week 2**	**Week 3**	**Week 4**
Control	3.56 ± 0.52	4.67 ± 0.50	5.56 ± 0.88	6.44 ± 0.72
Bacteria Treatment	3.44 ± 0.73	4.89 ± 0.93	5.56 ± 0.88	6.67 ± 0.86

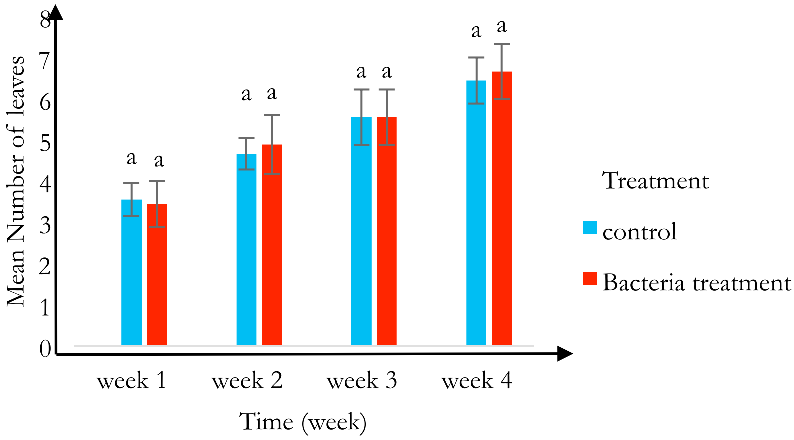

Figure 4 Number of leaves means compared weekly using SPSS at 95% confidence. Error bar indicates standard error of means.

Figure of number of leaves of Pak Choy showed that the number of leaves increasing consistently from the first week to the fourth week. At third week the number of leaves mean of the plant was same and their p value was 1.00 (p>0.05). The number of leaves does not showed any significant difference between the bacteria treatment and control. At the fourth week number of leaves mean in bacteria treatment was 6.67 ± 0.86 higher than control 6.44 ± 0.73, however there were no significant difference because the p value for week 4 was 0.40 (p>0.05).

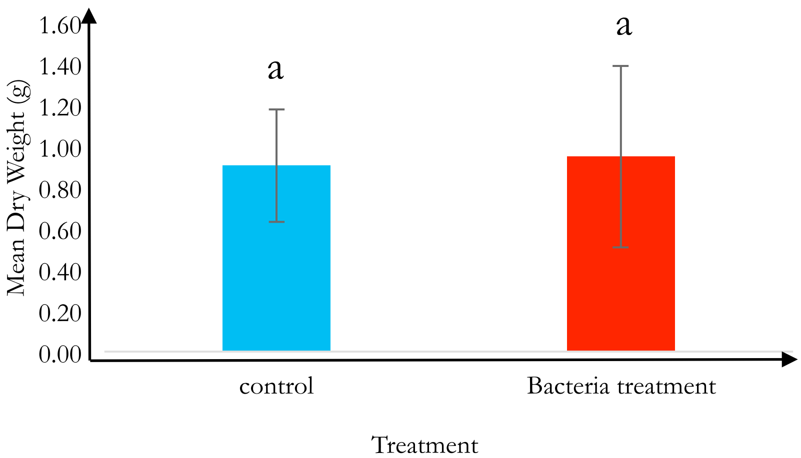

Figure 5 Comparison of means of dry weight of Pak choy by week by using SPSS at 95% confidence. Error bar indicates standard error of means.

Figure 5 showed the dry weight of Pak Choy growth in aquaponic system. The mean value showed the bacteria treatment was higher than control. Based on the output data from SPSS it given that Lavene`s test has probability greater than 0.05, it can be assumed that the population variances are relatively equal. Consulting the t-value, df and two-tail significance, again there is no significance difference are apparent ($p>0.05$). Thus, there is no significant difference in bacteria treatment and control for the comparison of its Dry weight(t (16) = -0.19, $p>0.05$).

Water Quality

In aquaponis system, water quality plays an important factor that must be given a top priority. This is because water is one of the agent that help distribute the nutrient from fish waste to the plant. Parameters on water quality which are ammonia content, temperature (°C), pH, nitrate (NO_3-) content and Dissolved Oxygen (DO) were recorded for four (4) consecutive weeks. The reading on nitrate content (NO^-_3) for bacteria treatment increased constantly from first week till fourth week. While ammonia content reduces from week one to week fourth. At the week 2 the ammonia level for control is higher than bacteria treatment. The nitrate and ammonia graph showed a correlation where the increasing of ammonia content will be followed by reducing of nitrate content. From Fig. 6 and Fig. 7, it showed that the aquaponics system treated with *Bacillus sp.* (B43) showed a low ammonia content and a high nitrate content (NO_3^-) compared to control. This results is in paralel with Frinchu and Dumitrache (2016) where in aquaponic operational system of 30 days it is capable of developing Nitrosomonas and Nitrobacter population. This results with the decrease of ammonia (NH_3) followed by increase of nitrate (NO^-_3) in both treatment and control. However, tanks added with *Bacillus sp* (B43) showed a decrease of NH_3 lower than control. This is because *Bacillus sp* (B43) is a Nitrosomonas bacteria which converts ammonia (NH_3) to nitrite (NO_2^-).The reading for DO showed the same trend where the bacteria treatment has a higher mean than control but at the week 3 there is some error where mean for control is higher than bacteria treatment.This is due that on week 2nd-3rd it was rainy season. However, for both of the treatments, it showed an overall decreasing trend in DO which is correlated with the increasing fish weight (Fig. 1). A decrease trend of DO indicates a high stocking density of fish which volume of the fish tank inadequately match the number of fish and fish growth (Sace and Fitzsimmons, 2013). According to Rakocy et al.,(2006), aeration and frequent or continous water exchange of tank water can be adopted in order to renew dissolve oxygen supplies as well to increase the beneficial bacterial growth. . Researcher John and Craig (2004) reported that different regeneration time of bacteria growth does not contribute any difference to ammonia conversion of nitrogen fixing bacteria. A vital factor is a suitable condition and environment such as temperature and DO in the water which will affect the growth of bacteria in the water. To obtain a better result for further study of these experiment, it is suggested that an optimum condition for the bacteria of *Bacillus sp.* (B43) need to be studied before it is added into the aquaponics system.

For pH and temperature the value between the control and bacteria treatment does not showed any difference from the first week until the end of experiment.From the experiment, the pH of the aquaculture was maintained in the range of 6.5-7.5. The pH in this range are onsidered as desirable for fish, plant and bacterial growth. This is because fish and bacteria prefer pH ranging from 7.0-9.0 for optimum growth while plant grows well within the range of 5.8-6.8 (Tyson et al., 2007 ; Sace and Fitzsimmons, 2013). From Figure 10 the minimum temperature was monitored at 28 °C and the maximum temperature was 33 °C. Temperature ranging from 20^{o}C-30^{o}C provides an optimum temperature for both *Nitrosomomonas* sp. and *Nitrobacter* sp. growth (http://microbewiki.kenyon.edu/index.php/Nitrosomonas). However, Grundman et al. (2000) stated *Nitrobacter* sp. grows optimum at temperature of 38^{0}C. The very low or very high temperature can affect the plant and the fish growth because at a very low temperature at 15 °C, it may kill or make the nitrifying bacteria become dormant. While at temperature as high as 49^{o}C would cause *Nitrobacter* sp. death (www.bioconslab.com/nitribactfacts.html). This will cause the ammonium to build the toxic level in the fish tank and plant (Mattson and Leatherwood, 2009). Increase in temperature and pH would also result in the increase of NH_3 content (Emmerson et al., 1975).

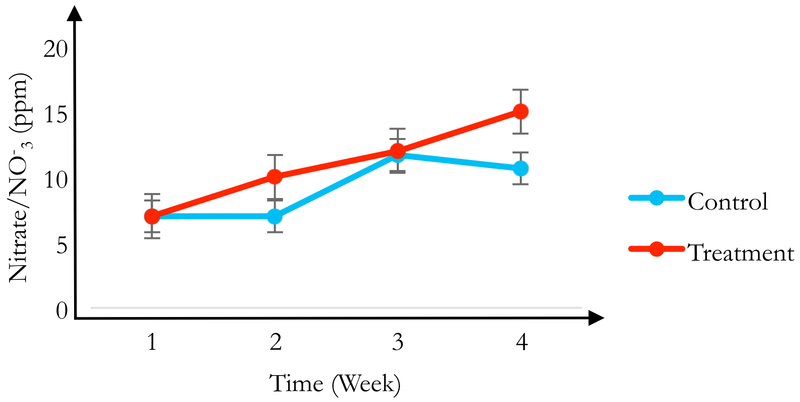

Figure 6 Comparison of means of Nitrate by week by using SPSS at 95% confidence. Error bar indicates standard error of means.

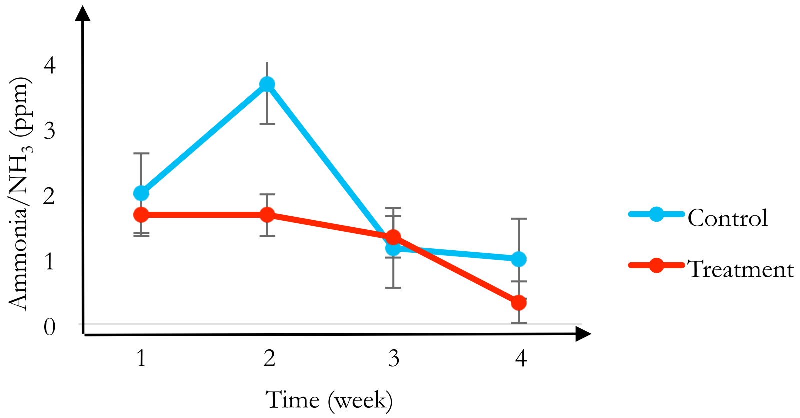

Figure 7 Comparison of means of Ammonia by week by using SPSS at 95% confidence. Error bar indicates standard error of means.

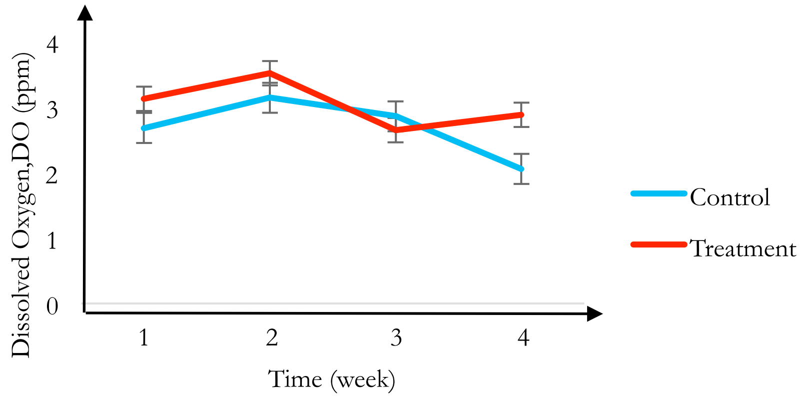

Figure 8 Comparison of means of Dissolved Oxygen by week by using SPSS at 95% confidence. Error bar indicates standard error of means.

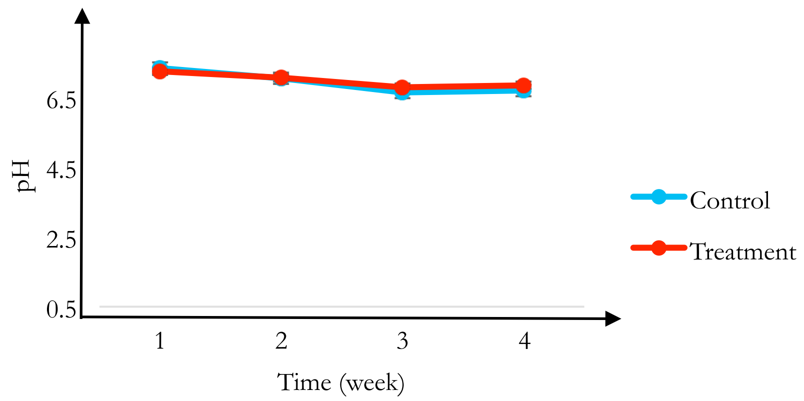

Figure 9 Comparison of pH in control and treatment tank by weeks using SPSS at 95% confidence. Error bar indicates standard error of means.

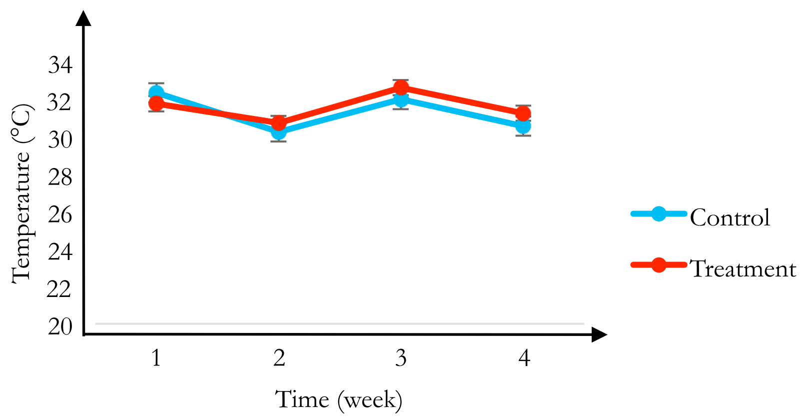

Figure 10: Graff of means of Temperature by week using SPSS at 95% confidence. Error bar indicates standard error of means.

The growth of the red tilapia depend on the feeding rate where in this experiment the feeding rate was set at 7% of the fish body weight per day and the fish is feed three times a day (El-Sheriff, M. S., and El-Feky, A. M. I, 2009). From Fig.7, it was observed that NH_3 content were monitored above 1ppm from week 1st to week 3rd. In a balance and and functioning aquaponic system, concentration of ammonia should be maintained at 0-1ppm [http://www.fao.org/3/a-i402le/i4021e05.pdf].The plant growth in aquaponics system was not very healthy because the leaves of the Pak choy were yellowish green compared to normal growth grow at the soil there were big different between the colour. In comparison, between the bacteria treatment and control there was no major difference of the leaf colour. Park (2011) stated that leafy vegetable that don't get enough nutrient will show the nutrient deficiencies symptom like stunted growth and yellowish green leaf on the old leaf it can cause by the lack of nitrogen in soil or their bed. However, since that the water quality in the aquaponics showed a high level of NH_3 and NO^-_3, the symptom of lack nutrient deficiencies might also indicates that the plants were lacked of other nutrients and minerals. As reported by many researchers, aquaponic which rely only on fish waste as its nutrient supplement for plants showed a low levels of phosphorus, potassium, iron, manganese and sulphur (Adler et al., 1996; Seawright et al., 1998 ; Graber and Junge, 2009).

CONCLUSION

Addition of *Bacillus sp.* (B43) at $32x10^6$/80ml into a 190 L aquaponics system of cultivating 30 tails of red tilapia and six Pak Choy showed that the growth was higher when supplemented with the *Bacillus sp.* (B43). However there are no significant difference in fish and plant growth as well as the water quality for ammonia and nitrate content for both treatments. For further research, this study can be repeated by using other type of bacteria that has the characteristic in converting the ammonia (NH_3 or NH_4^+) from the fish waste to nitrite (NO_2^-) and nitrate (NO_3^-). It is also recommended that the a further study can be set up by using a consortium of nitrogen fixing bacteria as well as using the same bacteria but with different inoculum density.

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