

Effects of Fish Waste Extract on the Growth, Yield and Quality of *Cucumis sativus* L.

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ABSTRACT

This study was conducted to evaluate the potential effects of fish waste extract on the growth, yield and quality of cucumber which is a popular cucurbitaceae fruit vegetable. Different treatments were arranged according with CRD layouts with five replicates for each treatment. All the six (6) treatments viz; control, NPK, 10 ml, 20 ml, 40 ml and 50 ml of fish waste extract were applied to the potting media. The results showed that the application of 20 ml of fish waste extract increased the vine length, number of leaf, chlorophyll content (SPAD), stomatal conductance and leaf TSS content of cucumber compared to other treatments and control. Treatment with 50 mL of fish waste extract produced the highest leaf area. The chlorophyll concentration, carotene content, chlorophyll fluorescence and photosynthetic yield also positively affected with fish waste extracts. It was also found that number of flower, number of fruit, and weight of individual fruit of cucumber also significantly increased by fish waste extracts. It can be concluded that 20 ml fish waste extract was the best treatment for improving the growth, yield and quality of cucumber. Foliar application of fish waste effluent on Cucurbitaceae vegetables is merit to further study.

Keywords: Fish waste, growth, development, cucumber, growing media

ABSTRAK

Kajian ini dijalankan untuk menilai kesan potensi ekstrak sisa ikan terhadap pertumbuhan, hasil dan kualiti timun, iaitu sejenis, sayur buah cucurbitaceae yang popular. Rawatan yang berbeza diatur mengikut susun atur CRD dengan lima replikasi untuk setiap rawatan. Kesemua enam (6) rawatan ini; kawalan, NPK, 10 ml, 20 ml, 40 ml dan 50 ml ekstrak sisa ikan diaplikasi pada tanaman. Keputusan menunjukkan bahawa penggunaan 20 mL ekstrak sisa ikan meningkatkan panjang urat, jumlah daun, kandungan klorofil (SPAD), konduktansi stomatal dan kandungan daun TSS daripada timun berbanding dengan rawatan dan kawalan lain. Rawatan dengan 50 ml ekstrak sisa ikan menghasilkan luas daun yang paling tinggi. Kepekatan klorofil, kandungan karotena, pendarfluor klorofil dan hasil fotosintesis juga positif dipengaruhi oleh ekstrak sisa ikan. Ia juga mendapati bahawa jumlah bunga, bilangan buah, dan berat buah individu timun juga meningkat dengan ketara oleh ekstrak sisa ikan. Dapat disimpulkan bahawa ekstrak sisa ikan 20 mL adalah rawatan terbaik untuk meningkatkan pertumbuhan, hasil dan kualiti timun. Penggunaan foliar efluen sisa ikan pada sayur-sayuran Cucurbitaceae adalah merit untuk melanjutkan pelajaran.

Kata kunci: Sisa ikan, pertumbuhan, pembangunan, timun, media berkembang

INTRODUCTION

Cucumber (*Cucumis sativus* L.) is an important vegetable and a well-known member from the family, Cucurbitaceae (Lower and Edwards, 1986). It is thought to be one of the oldest vegetables cultivated by man with historical records dating back 5, 000 years (Wehner and Guner, 2004). In Asia, cucumber is the fourth most important vegetables after tomato, cabbage and onion (Tatlioglu, 1993). The crop is a very good source of vitamins A, C, K, B₆, potassium, pantothenic acid, magnesium, phosphorus, copper and manganese (Vimala et al., 1999). The ascorbic acid and caffeic acid contained in cucumber help to reduce skin irritation and swollen (Okonmah, 2011). Cucumber is also a high nutrient demanding crop and show poorly in nutrient deficient soils leading to low yields, bitter and misshapen fruits (Grubben and Denton, 2004). In order to sustain high yield of cucumber, there is a need to augment the nutrient status of the soil to meet the crop's need, thereby maintaining soil fertility.

Organic agricultural production seeks to provide top quality food while respecting the environment and maintaining soil fertility through optimal use of resources. Organic agriculture promotes the recycling of nutrients to minimize the quantity of nutrients imported to the farm. Application of organic waste in soil is a suitable method for the maintenance of soil organic matter, improve soil fertility and supply nutrients needed by plants (Davarinjad et al., 2004). Organic fertilizers are organic materials that are more environmentally friendly compare to chemical fertilizer. Large quantities of agricultural wastes such as animal wastes, agriculture and industrial effluents, municipal waste water, and bio solids that are enrich with plant nutrients. It is include about

15 million tons of municipal wastes, 20 million tons of crop residues and 275.5 million of animal manure (El-Shemy, 1997). The fishing sector produces large amounts of waste in fish markets and processing industries (López-Mosquera *et al.*, 2011). Fish remains have also been traditionally used as fertilizer, given their wealth of nutritive elements (principally N and P) and their rapid decomposition. Nearly 75% of the total weight of the fish was generated as solid waste in the form of gut, head, skin, bones, fins and frames after processing. The fish wastes rich in nitrogen, potassium, phosphorus and trace minerals (Ghaly *et al.*, 2013) can serve as raw material for the production of many nutritive and nonnutritive products.

MATERIALS AND METHODS

An experiment was carried out at farm of Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut Campus, 22200 Besut, Terengganu Darul Iman to see the effects of fish waste extract on the growth, yield and quality of cucumber plant under polybag condition.

Planting of seeds and transplanting of seedlings

Cucumber seed was collected from hypermarket and was used for germination. The seed was planted in the germination tray that fills the holes with peat moss as germination medium. All the germination trays were kept in normal environmental condition for germination. The peat moss was damped with water before sowing the cucumber seed and each hole was sown with one seed only. The seed was germinated in three days and around two weeks the seedling was grown in germination tray. After three (3) weeks of germination, all the seedlings were transplanted into the polybags contained coco peat. The planting media was sprayed with water to make it easy to pull out from the tray. A small hole was dug at the center of the polybag and each polybags contain one seedling only. The experiment was carried out at the research plot of Faculty of Bioresources and Food Industry, Universiti Sultan Zainal Abidin, Besut campus, 22200 Besut, Terengganu. The temperature was about 21-30 °C, with maximum PAR of 500-1000 $\mu\text{Em}^{-2}\text{s}^{-1}$ and relative humidity of 70% to 90%. The weeding was done manually by using hand tools once a month to keep the polybags free of weed.

Preparation of fish waste extract

Fish waste (head, scale, intestine, gall bladder, etc.) was obtained from local fish market in Besut, Terengganu. Fish waste extract was prepared and then blended around 30 minutes by using blender. Approximately 1 kilogram of fish waste was mixed with 1 litre of water for the preparation of fish waste effluent. Filtered fish waste extracts of 10 ml, 20 ml, 40 ml and 50 ml were applied on top of coco peat.

Measurement of plant morph physiological parameters

Vine length was measured starting from the base of the stem to the tip of the highest leaf by using measuring tape and ruler in unit cm as per method used by Grant and Todd (2001). The measurement for the vine length was taken every week for six weeks. The number of leaves per plant was counted by manually observation. For the number of leaf was measured until the flowering stage. The leaf area was measured by using ruler in formula Length x Width (L x W). For the measurement of leaf area was used in unit cm^2 . The number of flower per plant was counted by manually observation. The measurements were recorded.

Measurement of plant physiological parameters

The chlorophyll content in the leaves was measured by using SPAD-502 portable chlorophyll meter (Minolta Japan). Before using this meter, it was calibrated about 15 minutes to take accurate readings. The measurements were taken by meter which was simply clamped over leafy tissue. After that, meter showed an indexed chlorophyll content reading in less than 2 seconds. The reading has scale from -9.9 to 199.9 and then the readings were recorded. The chlorophyll fluorescence was measured by using Junior-Pam chlorophyll fluorometer. WinControl-3 software was installed before take the readings. USB cable was used to connect the Junior-Pam to a computer. The leaf clip and magnetic leaf clip was clamp over the leaf. The readings were observed on the WinControl-3 software and the result was recorded. It was represented in F_0 , F_m and F_v and photosynthetic yield (F_v/F_m). Leaf chlorophyll content and chlorophyll fluorescence were measured according to the methods described in Khandaker *et al.* (2017).

Stomatal conductance of leaf was measured by using portable Porometer (Leaf Porometer, Model SC-1, USA) as per method described in Khandaker *et al.* (2018). Before measurements are taken, it was calibrated so

that readings can be taken accurately. The leaf chamber was kept at an ambient temperature for 10 to 15 minutes to maintain sunlight adaptation. After that, a leaf chamber was attached to one leaf and the readings were showed on the screen of porometer. The detailed procedure was carried out Carotenoid content was measured by using spectrophotometer. The leaves samples were collected for each treatment. Then, the samples were cleaned and air dried. The veins of the leaf were removed and the leaf was cut into small pieces. The samples were weighed about one g by using electronic balance for each treatment. The weighed samples were crushed by using mortar and pestle and homogenized in 10 mL of 80% acetone for each 0.25 g samples. The homogenate was filtered and then poured in 3 mL cuvette and its absorbance was measured in wavelengths of 663 nm, 645 nm and 480 nm wavelengths for measurement of chlorophyll *a*, chlorophyll *b* and carotene content, respectively. These readings were taken by spectrophotometer devices. The concentrations of the chlorophyll and carotene content were calculated by using the formula of Arnon (1949).

The formulas to calculate the pigments concentration are shown below:

Chlorophyll *a* (mg/L) = $12.7 \times A_{663} - 2.69 \times A_{645}$

Chlorophyll *b* (mg/L) = $22.9 \times A_{645} - 4.68 \times A_{663}$

Carotene content (μg/g) = $[A_{480} + (0.114 \times A_{663}) - (0.638 \times A_{645})] / 112.5$

Total soluble solids (TSS) content

The TSS content of leaf was evaluated by using Atago 8469 hand refractometer (Atago Co. LTD., Tokyo, Japan) as per method described in Khandaker et al. (2013) and was expressed as percentage Brix (% Brix). The leaves samples were collected for each treatment. Then, the samples were cleaned and air dried. The veins of the leaf were removed and the leaf was cut into small pieces. The samples were weighed about 1 g by using electronic balance for each treatment. The weighed samples were crushed by using mortar and pestle and 1 ml of distilled water was added to produce leaf juice. Two drops of leaf juice were placed to the refractometer sensor. The readings showed in percentage and the data was recorded.

Fruits growth and weight of individual fruit

The number of fruits was counted manually and the results were recorded as per method described in Dalorima et al. (2018). The weight of individual fruit was measured by using weighing balance. The readings were observed and recorded.

Experimental design

The experiment was carried out with Completely Randomized Design (CRD) layouts that consist of six (6) treatments (Control, positive control (NPK), 10 ml, 20 ml, 40 ml and 50 ml of fish waste extract. The experiment consists of five replicates for each treatments and a single plant was taken as an experimental unit. The treatment was applied seven days interval until the flowering stage.

Statistical analysis

The data obtained from the experiment was analyzed by using SPSS Statistics version 25 software. A one-way repeated analysis of variance (ANOVA) was applied to evaluate significant differences in the studied parameters in the different treatments. Means comparisons were performed with Tukey's test, significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

In this study, several physiological and growth parameters were recorded for evaluating the effect of fish waste extract on the growth, yield and quality of cucumber under potted condition. The result was described into one aspect, which is treatment effect based on repeated measure analysis of variance (ANOVA) and graph trend.

Plant morph-physiological properties of cucumber

Based on Figure 1A, the result for vine length, 20 mL of fish waste extract was showed the highest reading with 247.57 ± 8.31 cm followed by 40 mL of fish waste extract with 238.87 ± 9.53 cm. While the lowest reading of

vine length was comes from control treatment with 108.57 ± 18.52 cm. It showed that 20 mL of fish waste extract was the best treatment of vine length. The means differences of vine length was showed a significant different at $p < 0.05$ level (Figure 1A). Our results are supported by the findings of Oladimeji *et al* (2018), who reported that application fish waste increase vine length of pumpkin.

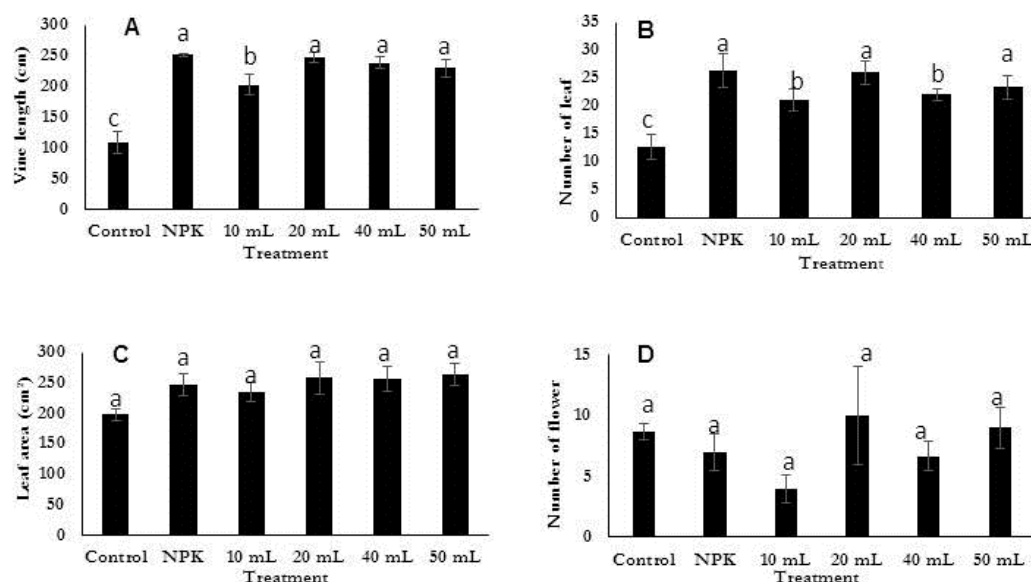


Figure 1 The effects of fish waste extract on vine length (A), number of leaf (B), leaf area (C) and number of flower (D) of cucumber plant. Bars indicate \pm SE.

The fish waste content important plant nutrient or plant growth regulators which enhance the growth of the vine. It has been also reported that application of plant growth regulators increased the stem or vine length of plant (Moneruzzaman *et al.*, 2010a). Shoot or vine length increase probably due to the effect on early cell division and cell expansion (Saifuddin *et al.*, 2009a).

Based on Figure 1B, 20 mL of fish waste extract showed the highest number of leaf with 26.00 ± 2.08 followed by 50 and 40 mL of fish waste extract with a value of 23.33 ± 2.03 and 22.00 ± 1.15 , respectively. While the lowest number of leaf was showed in control treatment with 12.67 ± 2.33 . The means differences number of leaf was showed significant different at $p < 0.05$ level (Figure 1B). Our results are supported by the findings of Oladimeji *et al.* (2018), who reported that application fish waste increase the number of pumpkin leaf. The applied fish waste increased the activity of microorganism present in the soil, increased the root growth and plant growth regulators activity. Thus, number of cucumber leaf is increased. Khandaker *et al.* (2013) reported that application of growth regulators increased the vegetative growth of flowering and fruiting plants.

Based on Figure 1C, there was not statistically different at $p > 0.05$ level in case of leaf area. The higher value of leaf area was showed in 50 mL of fish waste extract with 263.94 ± 17.97 followed by 20 and 40 mL of fish waste extract with a leaf area 258.47 ± 26.88 and 256.57 ± 19.92 , respectively. The smaller leaf was observed in control treatment with 198.42 ± 9.89 . In our study, we did not notice the significant effect of fish waste extract on leaf area. On the other hand, Oladimeji *et al* (2018) reported positive significant effect of fish effluent on leaf area of pumpkin plants. The fish waste extract elevated the levels of plant nutrient and plant growth regulators which increased the leaf area cucumber. Another study reported that application of gibberellin and phloemic stress significantly increased the leaf area of flowering plant (Saifuddin *et al.*, 2009b). Even though exogenous application sugar to the leaf increase the quality of plant products (Moneruzzaman *et al.*, 2010b) Based on Figure 1D, there was not statistically different at $p > 0.05$ level in case number of flower. The highest number of flower (10.00 ± 4.00) was recorded in 20 mL of fish waste extract followed by 50 mL with a value of 9.00 ± 1.73 .

The treatment of 10 mL of fish waste extract was showed the lowest number of flower with 4.00 ± 1.15 followed by 40 mL of fish waste extract with 6.67 ± 1.20 . We found that higher concentration of fish waste increase the flower number of cucumber but at lower concentration did not produce any effect. It has been reported earlier that fish waste extract contains the growth regulators and these plant growth regulators may be play a significant role to develop the flower bud. Moneruzzaman et al. (2013) also reported that localized application of gibberellin increased the number of flower of wax apple plants.

Plant physiological properties of cucumber

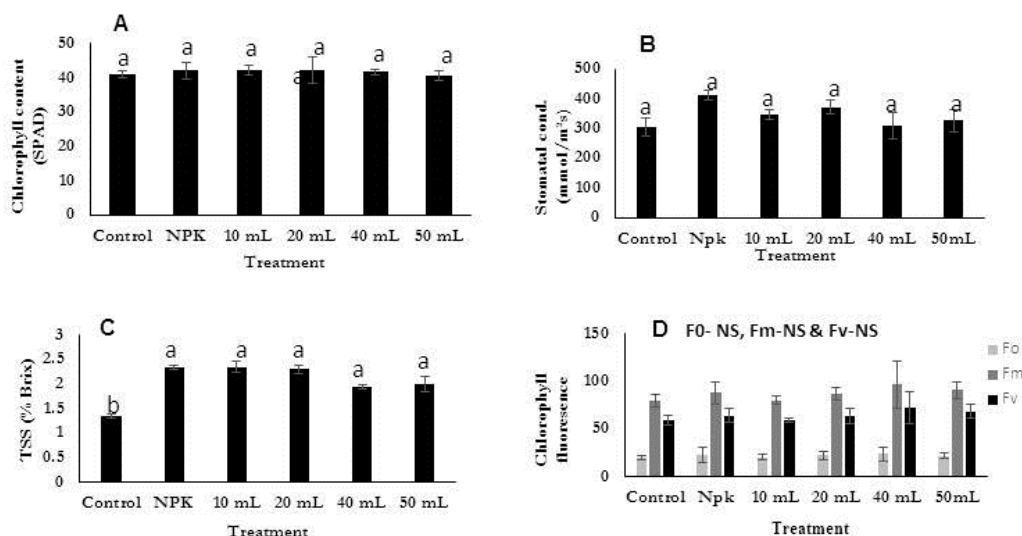


Figure 2 The effects of fish waste extract on chlorophyll content (SPAD) (A), stomatal conductance (B), TSS content (C) and chlorophyll fluorescence (D) of cucumber plants. Bars indicate \pm SE. NS-Non Significant.

Figure 2A represents the mean of chlorophyll content (SPAD) of cucumber leaf. The results showed the highest reading of chlorophyll content was showed in 20 mL of fish waste extract with 42.20 ± 3.84 followed by 10 and 40 mL of fish waste extract with 42.17 ± 1.49 and 41.73 ± 0.85 , respectively. The lowest reading of chlorophyll content was showed in 50 mL of fish waste extract with 40.57 ± 1.52 . The means differences of chlorophyll content (SPAD) was showed there was not significantly different at $p > 0.05$ level. Our results are supported by the findings of Khandaker et al (2017), who reported that application of fish effluent the chlorophyll content of Red Chilli Kulai. Chlorophyll component is made up from nitrogen and promoting the vegetative growth and green coloration of plant foliage.

As showed in Figure 2B, the highest reading of stomatal conductance was showed in 20 mL of fish waste extract with 371.95 ± 26.45 followed by 10 and 50 mL of fish waste extract with a value of 345.55 ± 19.25 and 325.40 ± 46.90 , respectively. While the lowest reading comes from control treatment with 304.80 ± 37.70 . The means differences of stomatal conductance was not significantly different at $p > 0.05$ level. Similar findings was also reported by Khandaker et al. (2017), stated that application of fish effluent can increase the stomatal conductivity on Red Chilli Kulai. Carbon dioxide (CO_2) is important for the production of carbohydrates during photosynthesis process.

The TSS content of cucumber leaf was significantly affected at $p < 0.05$ level by different fish waste extract (Figure 2C). The highest value of TSS content was observed in 10 mL of fish waste extract with 2.35 ± 0.15 followed by 20 mL of fish waste extract with 2.30 ± 0.10 and 50 mL of fish waste extract with 2.00 ± 0.20 , respectively. The lowest value was observed in control treatment with 1.35 ± 0.05 . Our results showed in agreement with the findings of Shaheen et al. (2012), who reported that application organic fertilizer increased the TSS content of Seedless Grapevines.

The chlorophyll fluorescence has become one of the most powerful and widely used techniques available to plant physiologist and ecophysiologist (Al-Saif et al., 2011). Figure 2D represents the chlorophyll

fluorescence of leaf of cucumber at different treatments. The best treatment in chlorophyll fluorescence was showed in 40 mL of fish waste extract for F_0 , F_m and F_v . The results showed that the highest value for lower fluorescence (F_0) in 40 mL of fish waste extract with 24.33 ± 7.36 followed by 20 mL of fish waste extract with 23.00 ± 4.51 . The lowest value recorded control treatment with a value of 20.33 ± 2.84 . Lower fluorescence was not significantly affected with the treatments at $p > 0.05$ level. In case of higher fluorescence (F_m), the results showed that the highest reading in 40 mL of fish waste extract with 97.00 ± 24.52 followed by 50 mL of fish waste with 91.33 ± 8.74 . The lowest reading was showed in control treatment with 79.33 ± 6.49 . But the effect of fish waste extract was not significantly different at $p > 0.05$ level. The result for relative variable fluorescence (F_v) has the highest reading in 40 mL of fish waste extract with 72.67 ± 17.17 followed by 50 mL of fish waste extract with 69.00 ± 7.57 . The lowest reading comes from control treatment with 59.00 ± 5.03 . The results also not significant among the treatment and control different at $p > 0.05$ level. Our results related to chlorophyll fluorescence are in an agreement with the findings of Dalorima *et al.* (2018), who reported that application of organic matter and biostimulant increased the chlorophyll fluorescence and photosynthetic yield of watermelon in field condition.

Physiological and reproductive characteristics of cucumber

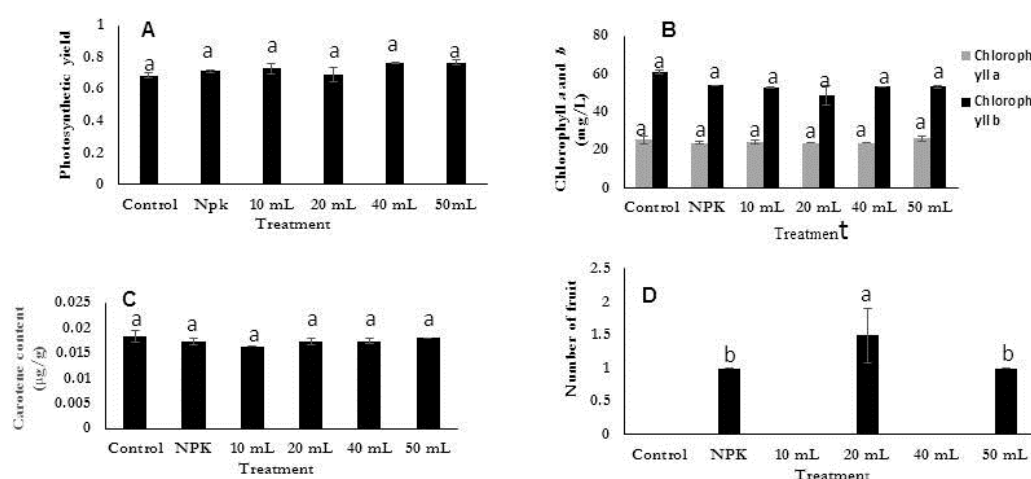


Figure 3 The effects of fish waste extract on photosynthetic yield (A), chlorophyll a and b (B), carotenoid content (C) and number of fruit (D) of cucumber plants. Bars indicate \pm SE.

Based on Figure 3A, 40 mL and 50 mL of fish waste extract was showed the highest reading with 0.77 ± 0.01 and 0.77 ± 0.03 . While the lowest was showed in control treatment with 0.69 ± 0.02 . The means differences were not significantly different at $p > 0.05$ level. However, Dalorima *et al.* (2018) reported significant positive effects of organic matters on the photosynthetic yield of watermelon plants.

It has been stated that leaf chlorophyll contents, are a good indicator of photosynthesis activity and plant productivity, are of special significance to precision agriculture (Shafiee *et al.*, 2013). Based on Figure 3B, for chlorophyll *a*, 50 mL of fish waste extract was showed the highest value with 26.04 ± 1.56 mg/L followed by control treatment with 25.42 ± 2.72 mg/L. The lowest value comes from 20 mL and 40 mL of fish waste extract which have the same value with 23.81 ± 0.08 mg/L. There was not significantly different at $p > 0.05$ level. While for chlorophyll *b*, the highest value was showed in control treatment with 61.40 ± 1.19 mg/L followed by 50 mL of fish waste extract with 53.50 ± 0.79 mg/L. The lowest value was showed in 20 mL of fish waste extract with 48.88 ± 6.29 mg/L. There was not significantly different at $p > 0.05$ level (Figure 3B). Conversely, Khandaker *et al.* (2017) reported that application of fermented fish extract increased the chlorophyll content of chilli plants under potted condition.

Based on Figure 3C, the means differences of carotene content was showed not significantly different at $p > 0.05$ level. The highest value was showed in control treatment with 0.0184 ± 0.0013 µg/g. The lowest value

Based on Figure 3D, there was three treatments comes from 10 mL of fish waste extract with 0.0164 ± 0.0000 $\mu\text{g/g}$. On the other hand, Cruz et al. (2012) reported that soil enrichment with organic matter increased the carotene content in lettuce leaves. was showed the reading which were NPK, 20 mL and 50 mL of fish waste extract. Other treatment was not showed any reading due to no fruit presence in cucumber plant. The highest reading number of fruit was showed in 20 mL of fish waste extract with 1.50 ± 0.50 . The means differences number of fruit was significantly different at $p < 0.05$ level (Figure 12). On the other hand, Khandaker et al. (2017) also reported positive significant effect of fish effluent on number of fruit of Red Chilli Kulai.

Weight of individual fruit (g)

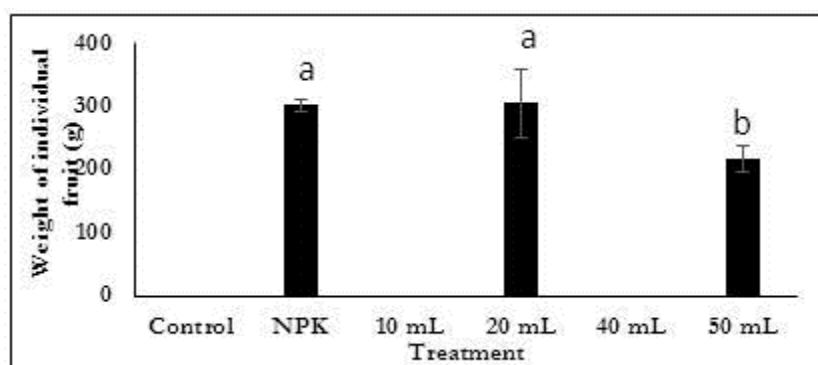


Figure 4 The effects weight of individual fruit of cucumber as affected by different treatments. Bars indicate \pm SE.

Based on Figure 4, there was significantly different at $p < 0.05$ level. The highest fruit weight was recorded in 20 mL of fish waste extract with 305.57 ± 67.67 g. treatments. Positive control (NPK) and 50 mL fish waste extract also produced fruit but fruit weight was lower than 20 mL treatment. Besides, control treatment did not produce any fruits. Out results showed that plant physiological activities, flowering and fruit formation of cucumber significantly affected by fish waste extract application. The finding of this study supported by the results of Moneruzzaman et al (2012), who reported that fruit size is affected by the cultural practice including use of fertilizers and plant growth regulators. The results of this study supported by the findings of Mahmoud et al. (2009), who reported that the application of organic waste improve the plant physiological activities of cucumber plants.

CONCLUSION

From the above results, we concluded that tested concentrations of fish waste extract, particularly 20 mL fish waste extract can increase the vine length, number of leaf, chlorophyll content (SPAD), stomatal conductance, number of flower, number of fruit, and weight of individual fruit of cucumber. Leaf chlorophyll content, carotenoids, TSS ontent and chlorophyll fluorescence of cucumber plant also improved with fish waste extract. It can be concluded that 20 mL fish waste extract are promosing for enhancing the growth, development and quality of cucumber under potted condition.

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