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Eating Behavior of Imago *Aulacophora similis* Oliver on Cucumber (*Cucumis sativus* L.) with Treatment Plant Growth Promoting Rhizobacteria (PGPR)

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KEYWORDS

Aulacophora similis Oliver; Cucumber Plant; *Plant Growth Promoting Rhizobacteria.*

Abstract The cucumber harvests failure occurred mainly due to the destruction of crops by the leaf-eating beetle *A. similis* Oliver. Many ways are taken especially pesticides that are synthesized widely because it is considered the fastest and most potent to overcome pest disorders. Based on, one of the control techniques that can be used is PGPR, which is a group of bacteria that can be utilized to strengthen plants against pest attacks and plant diseases, so research needs to be done by using this technique to change the feeding behavior of the *A. similis* Oliver beetle on cucumber plants. The research was conducted at the Green House Department of Pest and Plant Diseases University of Brawijaya Malang in September 2018 to March 2019. It was conducted using Randomized Block Design (RBD) 5 treatment, i.e., PGPR, urea fertilizer and control (without treatment). Based on the results of the study shows that: Application of *PGPR* on cucumber plant can reduce damage on either leaves or flower due to the eating behavior of imago *A. similis* Oliver and Application of *PGPR* can increase the cucumber plant growth during the eating behavior of imago *A. similis* Oliver.

Introduction

Cucumber is a vegetable that many people consume because it is a source of nutrients, vitamins and minerals needed by the body. The needs and demand for cucumber commodities will increase in line with the increasing population and consumption of cucumbers for fresh consumption, and the cosmetics and medicines industry (Oktaviana, 2016). Therefore, farmers must work efficiently in managing cucumber farming to achieve higher production and more significant profit.

In 2013 to 2014, the growth percentage of cucumber production decreased by 2.78% (Ministry of Agriculture, 2015). Pest organisms can also cause decreased production of cucumbers. The rate of crop loss varies from 30% to 100%, depending on the variety of crops and the growing season (Dhillon *et al.*, 2005). The most significant damage reaches 25% and occurs in a population of 15 tails per plant. The highest percentage of leaf damage occurs at the plants age, running 7 to 13 days after planting (hst), when the damage reaches 17%. At the age of 25 hst, the damage decreased to 4% and increased again at the age of 45 and 65 hst, an increase of 5% and 7%. The percentage of economic loss caused by *A. similis* Oliver reached 21.76% in the number of stricken crops, which reached 54.47% (Tarno, 2003).

Many ways are taken to deal with disruptive pests using resistant varieties, holding crop golirans, simultaneous planting and pesticide use. The use of pesticides in particular that are synthesized is widespread because it is considered the fastest and most potent to overcome pest disorders. However, its use turns out to cause losses such as pest

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resistance, pest resurgence, the killing of natural enemies, and environmental pollution problems and is very dangerous to humans (Kardinan, 2001). One of the control techniques used is PGPR, a group of bacteria that can be used as biofertilizers to help plants in nutrient supply and strengthen against pest attacks as well as plant diseases (Soesanto, 2008). The excess use of PGPR can increase plant productivity with nutrient mobilization, growth hormone production, nitrogen fixation or pest and disease resistance mechanisms (Wei *et al*., 1996); (Thakuria *et al*., 2004). Studies of PGPR administration of larvae's feeding behavior and imago pests *A. similis* Oliver, both single and combination have not been widely reported. Therefore, research is needed on the application of PGPR to suppress the attack of larvae and imago *A. similis* Oliver on cucumber plants.

Materials and Methods

The research activity was conducted at the Green House Faculty of Plant Disease Pests University of Brawijaya, Malang City, East Java Province. The research began in January 2019 to March 2019. This study used Randomized Block Design and was conducted by making 5 treatments and repeated 5 times so that there was a total of 25 plant samples.

Preparation of The Aulacophora Similis Oliver Beetle

The preparation of *larvae A. similis* Oliver is carried out by rearing. Cucumber seeds of ballet varieties for the propagation of *A. similis* Oliver are seeded first on small plastic. Imago *A. Similis* Oliver was found on cucumber farming acreage Badang Jombang farmers land and Ploso Batu Malang Reef. After the cucumber plant aged 13 hst was transferred into a polybag size 5 kg, as much as 3 plants yang already filled the soil and compost (1:1), then put in a cage whose top is covered in kassa cloth. Then Imago *A. similis* Oliver was infested, nurtured and reproduced by releasing several pairs of beetles (males and females) per cage.

PGPR Immersion Preparation on Cucumber Seeds

Cucumber seeds are washed with clean water 3 times. Once washed clean, the cucumber seeds are soaked in small buckets with a PGPR solution according to the treatment with a concentration of 15 ml/liter of water and soaked for 15 minutes. After 15 minutes, the seeds are taken and then planted on a small plastic. After the seedlings are 7 hst or have grown leaves of 3-4 strands, the seeds are ready to be transferred on the planting medium. For the treatment of of imago *A. similis* Oliver, the planting medium is polybag sized 5 kg. After moving PGPR planting, sprayed on cucumber plants evenly using sprayer bottles according to treatment with a concentration of 15 ml/liter of water. PGPR used is obtained from the isolates Department of Pest and Plant Diseases Faculty of Agriculture University of Brawijaya Malang with a density of 10^8 CFU (Colony Forming Unit). Warning of the use of PGPR, i.e., (1) Application is carried out in the afternoon, (2) Do not mix chemical pesticides and (3) Do not use water PDAM (Regional Company Drinking Water).

Preparation of Urea Tablet Fertilizer

Before the cucumber seed was planted on the planting media according to the treatment, the mixture of soil and manure is given urea fertilizer tablets by putting the fertilizer around the plant as far as ± 10 cm from the stem with a dose of 4.5 grams/plant. For the application of urea tablet fertilizer is different from how the application of NPK fertilizer is cast first, the application of urea tablet fertilizer is directly inserted into the planting hole shortly before the plant is planted (pop up).

The Preparation of Cucumber at the Green House

Cucumber seeds were sown on small plastics containing a mixture of soil and manure

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in a ratio of 1:1. After the seed are 7 days after planting or when the roots have two leaves, they can be transplanted to the planting medium according to the treatment 14 days after planting cucumber plants are ready to use as a medium for application according to the treatment. 25 cucumber plants for the samples. 25 samples of cucumber plants were using for testing the feeding behavior of imago.

PGPR Test on Feeding Behavior of Imago A. similis Oliver on Cucumber

The PGPR application tests Function is to determine larvae feeding behavior, which causes damage to plant roots. Meanwhile, imago causes damage to leaves and flowers, killing young plants if the population is abundant. The imago test is doing by the infestation of *A. similis* Oliver.

Imago Eating Behavior That Damages Plant Leaves and Flowers

Observations are made by calculating the area of leaf damage (attack intensity) and observing the behavior of insects perching while eating by seeing whether the insects are perched on the leaf surface or under the leaf surface. Attack Intensity calculation uses the damage scale value. The value of the damage scale present in Table 1.

Table 1. Damage Scale

Scale	% Damage	Category
0	0	Not Attacked
1	≤25	Very Light Intensity
2	>25 - 50	Light Intensity
3	>50-75	Medium Intensity
4	>75	Weight Intensity

The formula for calculating the intensity of pest attacks according to (Rahayu *et al.,* 2006) is as follows:

$$P = \frac{\sum (n \times v)}{Z \times N} \times 100\ \%$$

Note:

P = Intensity of attack

N = The numbers of damaged leaves per category

v = The scale value of each attack category

N = The numbers of leaves observed

Plant Length

Measure the length of the plant by using a ruler. Do these observations once a week after planting.

Number of Leaves

Count the number of leaves by recording the number of leaves after the application of each plant. Do these Observations once a week after planting.

The number of Flowers and Number of Fruits

Do these observations once a week after planting.

Fruit Weight

I was weighing the weight of the fruit (without leaves). Do it after harvest.

Data Analysis

Analyze by using a randomized block design method. After obtaining data from the study, the next step is testing with an analysis of variance or ANOVA (F test) at a significance level of 5% to determine each treatments effect. If there is an impact on each treatment, it will display the Honestly Significant Difference (HSD) with a significance level of 5% to see the difference between treatments (Gomez and Gomez, 2005).

Result and Discussion

Testing of the PGPR application on the eating behavior of *A. similis* Oliver was carried out by immersing and infesting the imago, namely by soaking cucumber seeds using PGPR and observations were made every 24 hours after the cucumber plants reached the age of 14 days afterward. The feeding percentage of *A.*

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similis Oliver by using variance showed that the application of PGPR on cucumber plants could reduce damage to leaves and flowers and had a different effect on *A. similis* Oliver eating behavior imago. The results of the BNJ α 5% test against the eating variable of *A. similis* Oliver are presented in the Table 2.

Table 2. Average Attack of Intensity *A. similis* Oliver on Cucumber Leaves (*Cucumis sativus* L.)

Treatment	Imago Eating Average (%)					
	5 dap	10 dap	15 dap	20 dap	25 dap	30 dap
	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)
Pseudomonas	3.65±2.29 a	4.76±1.52 a	28.20±15.66 a	30.40±16.13 a	32.60±16.86 a	34.40±17.85 a
Bacillus subtilis	3.20±1.25 a	4.85±1.99 a	31.00±16.99 a	35.00±14.56 a	39.00±13.62 a	42.00±13.25 a
Pf + Bs	4.05±1.25 ab	5.05±1.24 a	33.20±9.60 a	37.20±8.70 a	41.40±8.38 a	44.80±10.01 a
Urea Fertilizer	6.82±1.52 bc	7.95±1.05 b	68.60±14.60 b	71.20±14.11 b	74.40±14.15 b	78.20±14.13 b
Control	6.23±0.60 c	7.38±0.50 b	60.40±9.56 b	63.60±8.99 b	72.60±17.37 b	75.20±16.08 b
HSD α 5%	2.22	2.20	22.53	20.72	20.53	21.43

Note:

The number followed by the same letter in the same column shows no significant difference based on the further test of Honestly Significant Difference (HSD) with an error rate of 5%.

dap = Days After Planting
X̄ = Average
SD = Standard Deviation
Pf = *Pseudomonas fluorescens*
Bs = *Bacillus subtilis*
Data 5 and 10 dap have been transformed with Arc sin √x + 0.5

The study results showed that cucumber plants aged 25 dap showed an increase in the percentage of eating imago. It is because, in Control - Not given any material, the plants wilted and died because *A. similis* Oliver larvae were found eating plant roots. The results showed that the cucumber plants defense against eating behavior induced by PGPR could reduce the eating of *A. similis* Oliver imago. Under the research of Zehnder *et al*. (1997), PGPR can reduce the consumption of *Diabrotica Undacimpunctata Howardi* Barber on cucumber plants, because of the plant defense system induced by PGPR. So, this will also show that PGPR application plants can accelerate the death of *A. similis* Oliver.

Published information about PGPR administration (reviewed by Kloepper, 1993) shows that most PGPR strains do not have a single mechanism about beneficial effects on plants, the most fundamental tool for biological control by PGPR involves the production of bacterial metabolites have detrimental effects on plant pathogens namely siderophores, HeN, antibiotics, lytic enzymes and phytoalexins.

Previous studies on *Diabrotica Undacimpunctata Howardi* Barber and *A. vittatum* "a strong positive correlation between the basin content in seedlings and *Diabrotica* beetle attack" (Ferguson *et al*., 1983). Based on (Kogan, 1977), (Andersen and Metcalf, 1986) proposed a model for Diabroticites and cucurbit

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in which the scent of flowers acts in a "host seeking phase" and gives rise to an "orientation to the host plant from a distance" and cucurbits act in a "host acceptance stage with stimulates feeding and restrains movement". Imagine that the plant physiological changes are associated with ISR due to the application of PGPR namely a shift in the metabolic pathway to produce other plant defense compounds by releasing the scent of beetle attractants or cucurbitacin, resulting in lower beetle numbers and the spread of cucurbit wilt in PGPR applied plants. To confirm this hypothesis, additional studies should be done to compare known metabolic pathways namely key enzymes, substrates and end product concentrations associated with induced and non-induced plant allelochemical production in plants.

The Effect of PGPR on Cucumber Plants Growth During the Eating Behavior Test Imago A. similis Oliver.

Testing the PGPR application on the eating behavior of *A. similis* Oliver imago can affect cucumber plants growth. Observations were made every 7 days after immersing the PGPR and planting. Observation growth consists of 5 parameters namely plant length, number of leaves, number of flowers, number of fruits and weight of fruits.

The Effect of PGPR on the Length of Cucumber Plants (Cucumis sativus L.)

The test results show that PGPR can provide and mobilize or facilitate the absorption of various nutrients in the soil synthesize and change the concentration of various growth stimulating phytohormones. The results of the analysis of variance showed that the effect of PGPR application on the length of cucumber plants during the eating behavior test of *A. similis* Oliver increased and had a different impact on the length of the cucumber plant. The average size of cucumber plants is presented in the Table 3.

Table 3. Average Length of Cucumber (*Cucumis sativus* L.) during the Eating Behavior Test of Imago *A. similis* Oliver

Treatment	Average Plant Length (cm)				
	14 dap	21 dap	28 dap	35 dap	42 dap
	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)
Pseudomonas	58.20±2.39 a	95.60±8.47 a	135.20±21.22 a	166.40±28.75 a	176.20±10.84 a
Bacillus subtilis	51.60±5.82 ab	88.40±9.32 b	121.00±15.97 ab	148.20±19.19 ab	158.00±6.52 ab
Pf + Bs	48.70±9.04 bc	81.80±15.01 b	116.30±14.54 bc	143.10±19.39 bc	150.00±8.37 ab
Urea Fertilizer	42.30±6.91 cd	77.40±11.28 bc	100.00±3.54 bc	114.00±1.41 bc	122.60±15.76 ab
Control	35.10±4.93 d	57.60±11.35 c	93.60±14.22 c	120.60±17.91 c	105.60±13.87 b
HSD α 5%	7.36	12.09	21.60	28.74	55.65

Remarks:
The number followed by the same letter in the same column shows no significant difference based on the further test of Honestly Significant Difference (HSD) with an error rate of 5%.
dap = Days After Planting
X̄ = Average
SD = Standard Deviation
Pf = *Pseudomonas fluorescens*
Bs = *Bacillus subtilis*

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The results showed that the PGPR treatment was able to increase the cucumber plants length during the eating behavior test of *A. similis* Oliver. Imago *A. similis* Oliver eats plant leaves, this causes a decrease in the productivity of Cucurbitaceae plants, so that the ability to eat *A. similis* also affects the level of plant damage caused by *A. similis* Oliver (Tuismiwati, 1995). Meanwhile, PGPR can stimulate plant growth because the rhizobacteria group can synthesize phytohormones is including IAA or auxin. The increase in IAA accumulation resulting from PGPR inoculation can enable cell extension and enlargement resulting in increased root hair growth. The increase in root hair volume increases in the absorption of nutrients in the soil (Barreto *et al*., 2011).

The Effect of PGPR on the Number of Leaves of Cucumber Plants (Cucumis sativus L.)

Testing the PGPR application on the number of cucumber leaves during the eating behavior test of *A. similis* Oliver was carried out by counting the number of cucumber leaves in each treatment. The test results show that PGPR helps obtain mineral resources (nitrogen, phosphorus and other essential minerals). The results of the analysis of variance showed that the effect of the PGPR application on the number of cucumber leaves during the eating behavior test of *A. similis* Oliver increased. However, at the end of the observation there was a decrease due to the fall of old withered leaves. The average number of cucumber leaves is presented in Table 4.

The results showed that PGPR treatment could increase cucumber leaves number during the eating behavior test for *A. similis* Oliver. According to (Cook *et al*., 2002), it is consistent with several researchers that the application of PGPR to chili seeds and chili seeds when transplanted to the field in general can increase plant height, leaf number and crop yield.

Table 4. Average Number of Cucumber Leaves During the Eating Behavior Test of Imago *A. similis* Oliver

Treatment	Average Number of Leaves					
	14 dap	21 dap	28 dap	35 dap	42 dap	49 dap
	(X̄ ± SD	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)
Pseudomonas	4.40±0.55 a	9.00±0.71 a	14.60±2.41 a	16.00±2.24 a	18.60±1.82 a	20.60±2.41 a
Bacillus subtilis	4.40±0.55 ab	8.60±0.55 a	13.00±1.58 a	15.20±1.92 b	16.60±2.70 ab	16.40±3.78 ab
Pf + Bs	4.20±0.84 ab	8.40±0.55 b	12.40±1.52 b	14.20±1.79 bc	15.40±2.79 bc	14.80±3.27 bc
Urea Fertilizer	3.40±0.45 b	7.00±0.71 b	10.40±2.30 ab	12.60±1.95 c	12,40±3.21 bc	12.20±2.59 c
Control	3.80±0.55 b	6.40±0.55 b	9.40±1.95 c	10.40±0.89 c	9.40±6.02 c	8.60±5.41 d
HSD α 5%	0.84	0.89	1.78	2.15	4.56	3.87

Remarks:
The number followed by the same letter in the same column shows no significant difference based on the further test of Honestly Significant Difference (HSD) with an error rate of 5%.
dap = Days After Planting
X̄ = Average
SD = Standard Deviation
Pf = *Pseudomonas fluorescens*
Bs = *Bacillus subtilis*

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Table 5. Average Number of Flowers of Cucumber Plants (*Cucumis sativus* L.) During the Eating Behavior Test of Imago *A. similis* Oliver

Treatment	Average Number of Flowers		
	35 dap	42 dap	49 dap
	(X̄ ± SD)	(X̄ ± SD)	(X̄ ± SD)
Pseudomonas fluorescens	5.60±3.36 a	9.60±3.13 a	12.20±2.28 a
Bacillus subtilis	3.60±2.51 a	8.60±2.70 ab	9.80±1.79 ab
Pseudomonas + Bacillus subtilis	3.40±2.19 ab	7.00±2.24 bc	9.20±1.92 bc
Urea Fertilizer	0.60±0.89 ab	2.00±3.08 bc	3.60±2.70 bc
Control	1.80±1.64 b	4.00±3.67 c	6.60±3.97 c
HSD α 5%	3.40	4.64	3.42

Remarks:
The number followed by the same letter in the same column shows no significant difference based on the further test of Honestly Significant Difference (HSD) with an error rate of 5%.
dap = Days After Planting
X̄ = Average
SD = Standard Deviation

The Effect of PGPR on the Number of Cucumber Plant Flowers (Cucumis sativus L.)

Testing the PGPR application on the number of cucumber flowers during the eating behavior test of *A. similis* Oliver was carried out by counting the number of flowers on the cucumber plant for each treatment. Observations were made when the cucumber plants reached the age of 35 dap. The test results show that PGPR has an essential role in increasing plant growth. The results of the analysis of variance showed that the effect of the PGPR application on the number of cucumber flowers at the time of the eating behavior test of *A. similis* Oliver increased so that it had a different effect on the number of cucumber flowers. The average number of cucumber flower is presented in Table 5.

Based on the results of the observation of the number of cucumber leaves at the end of the observation at the age of 56 dap, it showed that the *Pseudomonas fluorescens* treatment had a number of flowers of 14.20 which was the highest result when compared to other treatments. Meanwhile, the lowest interest rate, namely 4.40, is found in Urea Fertilizer (Table 6). The observations indicated that PGPR treatment had an important role in increasing the number of cucumber flowers. This is in accordance with the explanation according to Raka *et al*. (2012) stated that PGPR is a consortium of bacteria that actively colonizes plant roots which play an important role in increasing plant growth, crop yields and soil fertility.

PGPR effect on the Number of Cucumber Fruit (Cucumis sativus L.) During Imago Aulacophora similis Oliver Eating Behavior Test

The variance analysis on the variable number of cucumber fruit during the eating behavior test of *A. similis* Oliver with PGPR treatment showed no significant effect on plant age 42 and 56 days after planting (dap). The description of the average number of cucumber leaves presented in Table 6.

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Table 6. Average Number of Cucumber Plant Fruits (*Cucumis sativus* L.) During the Eating Behavior Test of Imago *A. similis* Oliver

Treatment	Average Number of Fruits	
		49 dap
	(X̄ ± SD)	(X̄ ± SD)
Pseudomonas fluorescens	0.20±0.45 a	2.40±0.55 a
Bacillus subtilis	1.40±1.52 a	3.20±2.28 ab
Pseudomonas + Bacillus subtilis	1.20±1.10 a	2.20±1.30 ab
Urea Fertilizer	0.40±0.55 a	1.00±0.71 ab
Control	0.40±0.89 a	0.60±1.34 b
HSD α 5%		

Remarks:
The number followed by the same letter in the same column shows no significant difference based on the further test of Honestly Significant Difference (HSD) with an error rate of 5%.
dap = Days After Planting
X̄ = Average
SD = Standard Deviation

The test results show that the effect of the PGPR application on the number of cucumber fruit fluctuated and gives a different effect at the age of 49 days after planting (dad). The result of the highest average number of fruits was found in the treatment of providing PGPR with an active ingredient of Bacillus subtilis 3.20. Meanwhile, the lowest treatment result of 0.60 was found in the control treatment - not given any materials (Table 7). The results of this study are in line with the results of research presented by Murphy *et al*. (2000) who showed that treating tomato plants with PGPR resulted in faster growth.

PGPR Effect on the Weight of Cucumber (Cucumis sativus L.) Fruit During the Eating Behavior Test of Imago A. similis Oliver

The results of the analysis of variance on cucumber fruit weight variables showed that there was no significant effect. The description of the average fruit weights presented in Figure 1.

Based on the observation, result of cucumber fruit weight showed that the PGPR application with the active ingredient *Pseudomonas fluorescens* had the highest fruit weight of 99 grams compared to other treatments. Meanwhile, the lowest fruit weight results were found in Control - Not given any ingredients, namely 52 gr (Figure 1). This is because the PGPR application can increase plant growth, one of which is fruit weight. The results of this study are commensurate with A'yun *et al*. (2013) stated that the PGPR treatment resulted in a cayenne pepper fruit weight of 2.73 grams per plant, while the weight of cayenne pepper without PGPR treatment was 1,13 grams per plant. This shows that cayenne pepper plants with PGPR treatment have an effect on fruit weight in cayenne pepper plants.

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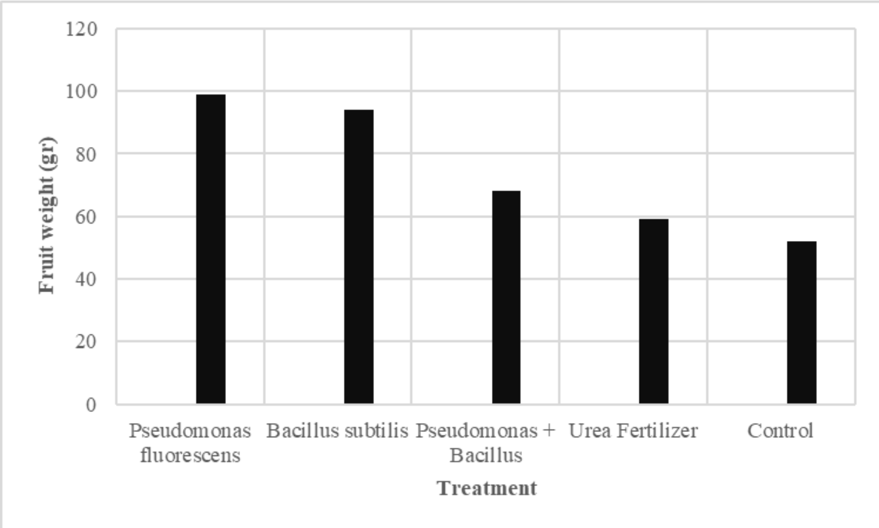

Figure 1. Average Fruit Weight (gr) of Cucumber (*Cucumis sativus* L.)

Conclusion and Suggestion

Based on observations result and discussion on this research, can be concluded that the variance analysis that application of *PGPR* on cucumber plant can reduce damage on either leaves or flower due to the eating behavior of imago *A. similis* Oliver and application of *PGPR* can increase the cucumber plant growth during the eating behavior of imago *A. similis* Oliver.

Based on the observations that have been made, the suggestion that can be given is that it is necessary to do further research on the effect of urea on the feeding behavior of *A. similis* Oliver beetle larvae by carrying out the Cucurbitasin test using HPLC (*High Performance Liquid Chromatography*).

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