Effect of Yeast Carrier Media with *Azotobacter* Addition as Biofertilizer Against Growth and Productivity of Mustard (*Brassica juncea* L.)

Effect of Yeast Carrier Media with *Azotobacter* Addition as Biofertilizer Against Growth and Productivity of Mustard (*Brassica juncea* L.)

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Abstract. The effectiveness of biofertilizer is influenced by the type of microbial constituent and the carrier media. This study is aimed to determine the effect of carrier media on microbial viability, mustard growth and productivity, and the type of carrier media with the best response to mustard. This study used three kinds of carrier media, consisting of molasses, rice water, and sucrose. Microbes used were *Candida* G3.2 and W3.8, with the addition of *Azotobacter* A10.The results showed that all biofertilizer carrier media improved microbial viability, with the highest TPC which was obtained by rice water with the value of $1.19x10^{14}$ cfu/ml, sucrose with the value of $1.18x10^{14}$ cfu/ml and molasses with the value of $9.65x10^{13}$ cfu/ml at 7 days of incubation times. Biofertilizer carrier media also gave the positive effect that was indicated by the increase of mustard growth and productivity. Molasses is the best carrier media that provides a high response in mustard growth and productivity, with the value of plant height which is 24.250 cm, the number of the leaf is 5.250, the leaf area is 2959.8 mm^2 and the dry weight is 3.90 gram.
Keywords: biofertilizer, carrier media, mustard, yeast

1. Introduction

Crop production enhancement depends largely on the type of fertilizer used to supplement the essential nutrients for the plant. Chemical fertilizers in recent decades have a great influence on farmers. However, chemical fertilizers also cause some ecological problems and damage the environment. The impact of chemical fertilizers on agricultural land is not only in terms of soil quality, but also on the survival of soil organism [1-3]. Plant mustard is one type of vegetables that are generally consumed by the Indonesian people every day. Green mustard has the potential as a producer of essential mineral elements needed by human life [4]. Along with the increase in population and the need for nutrients, the demand for green mustard is increasing [5]. Green mustard is prospective for cultivation [6]. Production of high quality of green mustard can be obtained with good cultivation techniques through proper fertilization. Fertilization with biofertilizer expected can reduce the negative impacts caused by chemical fertilizers [6]. The results of the previous research have found that yeast *Candida* G3.2 has the ability to dissolve phosphate that is shown with the ability of enzyme activity equal to 135.8 U / mL. *Candida* G3.2 has cellulase activity of 267 U / ml and optimum protease activity of *Candida* G3.2 which is 321.5 U / mL. Meanwhile, *Candida* W3.8 has the

ability of lipase enzyme activity with the value of 2357.3 U / ml [7]. On the other side, [8] *Azotobacter* A10 produces the highest nitrate concentration of 6.05% and produces IAA hormone of 9.45 ppm [9]. *Azotobacter* A10 also optimally produces siderofor compounds characterized by the formation of orange-colored siderophores around the colony [10]. The siderophor compound will bind Fe strongly then transport into cells with a specific transporter compound as the constituent component of the nitrogenase enzyme [10].

Media consisting of complex nutrients can stimulate higher growth of yeast and bacteria. Carbon is a very important source in the media. The main sources of carbon can come from molasses, rice water and sucrose. Molasses become the most preferred substrate by yeast for its growth. Molasses is a waste from the sugar industry and the rice water is an unused organic waste making it more economical to use C source from this media [11]. Based on this research, the biofertilizer formulation process consisted of organic material mixture as a carrier medium with different C source and inoculant yeast of *Candida* G3.2 and W3.8 with the addition of *Azotobacter* A10 to increase plant growth and productivity. Vegetable plants used in responding to biofertilizer formulas are green mustard. The success of the treatment is measured through the growth and productivity of mustard plants.

2. Materials and methods

2.1 Microbial isolates

Isolates used in this research were *Candida* W3.8, *Candida* G3.2 [7] and *Azotobacter* A10 [8] collection of Microbiology and Biotechnology Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Institut Teknologi Sepuluh Nopember Surabaya (ITS).

2.2 Preparation of microbial suspension

Measurements of yeast and bacteria growth were done by adding each culture in 0.9 (w/v) of physiological solution, consisting of NaCl and aquades. Absorbance was measured using a UV-Vis spectrophotometer with OD 0.5 for yeast and OD 0.2 for bacteria at λ 600 nm. The value of OD is based on the approximate number of yeast and bacterial cell densities (about 10^7 CFU/ml).

2.3 Growth of yeast and bacteria in various production media

Production medium used in this study consists of 3 medium types with different C source that are molasses, rice water and sucrose 20%. About 15 mL of microbial suspension from the previous step then inoculated in 85 mL of basal medium. Suspension consist of *Candida* G3.2 (5 ml), *Candida* W3.8 (5 ml), and *Azotobacter* A10 (5 mL). Each basal medium was added with 50 mL of molasses (50 ml molasses diluted in 100 ml distilled water, precipitated for 24 hours and about 50 ml of the supernatant was taken); 100 mL of rice water and 20 g of sucrose [11]. Czapek Dox Broth (g/L) was used as basal medium consisting of sodium nitrate (3 g); Dipotassium phosphate (1 g); Magnesium sulphate (0.5 g); Potassium chloride (0.5 g); Ferrous sulphate ($FeSO_4$), (0.01 g); Aquadest (H_2O) (1000 mL) [12]. Each production medium was homogenized on a rotary shaker for 7 days [13]. The measurement of growth was done by Total Plate Count Method to obtain maximum growth day.

2.4 Biofertilizer production

The first step of making biofertilizer was inoculating *Candida* G3.2, *Candida* W3.8 and *Azotobacter* A10 in basal media with different C sources. The measurement of each yeast and bacteria was conducted by adding culture in physiological solution 0.9 (w/v) and then absorbance value was measured at spectrophotometric with OD 0.5 at λ 600 nm [14]. About 15 ml of suspension then was inoculated in 85 ml of production medium with different C source. The medium was homogenized on the rotary shaker during certain incubation time according to the previous step. Biofertilizer treatment against mustard was done by four replications with concentration of 0 ml/L (without treatment), molasses, rice water, sucrose and positive control (using NPK fertilizer and compost).

2.5 Planting and maintenance of mustard
The mustard seeds were soaked with water for 24 hours, then seeds that were submerged in water were selected. Seeds were allowed to grow in seedling medium at least until two leaves were formed or 14 days after planting. In detail, seedlings media were inserted into the container with a thickness of 3-4 cm. Seeds were sprinkled over medium. Furthermore, the seeds were covered with a soil of about 0.5 cm. Watering is done daily to avoid dryness [15]. Biofertilizer is composted by watering 250 ml into polybags and left up to 14 days. After 14 days, the plants are transferred into polybags containing biofertilizer. Plants are grown for 5 weeks. Measurements of growth include plant height, number of leaves, leaf area, root length, and biomass (dry weight).

2.6 Plant height measurement
Plant height is measured from ground level to the highest leaf [16]. Plant height measurement was done once a week for 5 weeks.

2.7 Number of leaves calculation
The number of leaves calculation was done on leaves that had developed perfectly. Counting the number of leaves were done once a week for 5 weeks.

2.8 Measuring the leaf area
The leaf area is calculated by millimeter paper method by putting the leaves on millimeter paper and the pattern of the leaves followed. The leaf area is estimated based on the number of squares contained in the leaf pattern. The measurement of leaf area is done once a week for 5 weeks.

2.9 Root length measurement
The measurement of root length with destruction method was done by removing the whole plant and measuring the root length from root base to root by using ruler. Root length measurements were made at harvest time.

2.10 Biomass calculation
Biomass is obtained from the dry weight of the plant. Before weighing, the plant is cleaned with water and dried. The dry weight of the plant was carried out by drying at 60°C for 4 days, then was weighed using analytical balance [16]. Biomass measurements were made during harvest time.

2.11 Total soil microbe analysis
The total analysis of yeast and bacteria in the soil is carried out at the beginning of the biofertilizer watering, in the middle and the end of the harvest period. Total bacterial and yeast analysis use Total Plate Count (TPC) method.

2.12 Research design
Planting of mustard was done on polybags. Each polybag contains 1 mustard plant, 6 treatments and 4 repetitions.Research design are shown in the following figure 1.

CM.1	CO.4	A.2	C.3	CM.4
M.4	CM.3	C.2	S.3	M.1
S.1	CO.1	M.2	CM.2	A.4
C.1	M.3	CO.2	S.4	CO.3
A.1	C.4	S.2	A.3	

Figure 1. Research Design. CM.1: Chemical fertilizer repetition 1; CM.2: Chemical fertilizer repetition 2; CM.3: Chemical fertilizer repetition 3; CM.4: Chemical fertilizer repetition 4; CO.1: Compost fertilizer repetition 1; CO.2; Compost fertilizer repetition 2; CO.3: Compost fertilizer repetition 3; CO.4: Compost fertilizer repetition 4; C.1: Without biofertilizer repetition 1; C.2: Without biofertilizer repetition 2; C.3: Without biofertilizer repetition 3; C.4: Without biofertilizer repetition 4; M1: Molasses repetition 1; M2: Molasses repetition 2; M3: Molasses repetition 3; M4: Molasses repetition 4; A1: Rice water repetition 1; A2: Rice water repetition 2; A3: Rice water repetition 3; A4: Rice water repetition 4; S1: Sucrose repetition 1; S2: Sucrose repetition 2; S3: Sucrose repetition 3; S4: Sucrose repetition 4.

3. Results

3.1 Yeast and bacteria growth in various production media

The calculation of microorganisms in this study was done by Total Plate Count (TPC). TPC which is aimed to determine viability or living cells on carrier medium based on the number of colonies [17]. The microbial growth results are shown in figure 2.

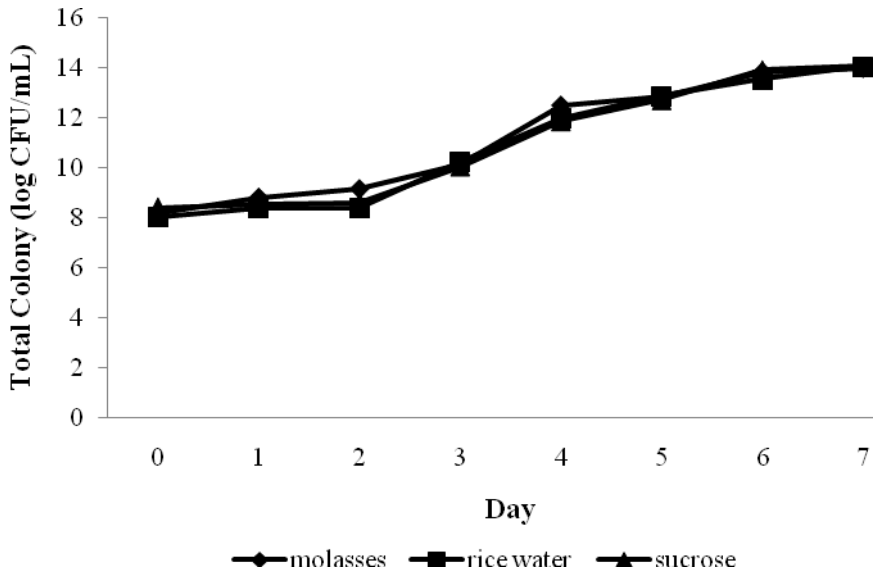

Figure 2. Result of yeast and bacteria growth in various production media.

The calculation result of Total Plate Count for 7 days on each carrier media showed that the growth increased gradually until day 7. The highest TPC value was in biofertilizer carrier media of rice water with the value of $1.19x10^{14}$cfu / mL on day 7, then were continued by sucrose with the value of $1.18x10^{14}$cfu / mL and molasses with the value of $9.65x10^{13}$cfu / ml.

3.2 Plant Growth

Measurement of plant height, number of leaves, leaf area, and root length were done to determine the growth of mustard. The results of the measurement plant height of mustard plants are shown in figure 3.

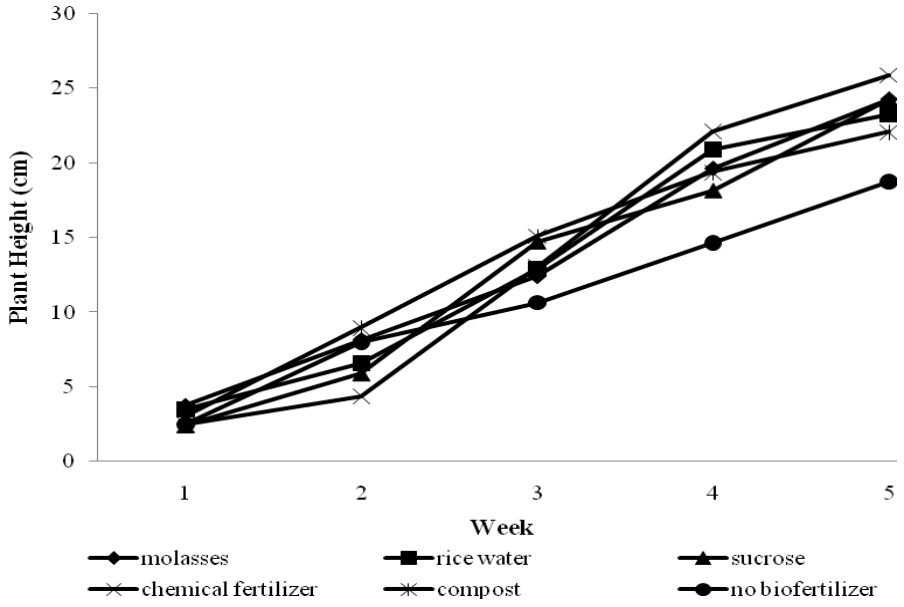

Figure 3. Result of plant height.

The results of plant height measurements based on Figure 3 show that the highest yield is mustard with chemical fertilizer treatment of 25.85 cm. Treatment of biofertilizer with medium of molasses and sucrose carrier which is equal to 24.25 cm, rice water shows the result of 23.25 cm. The lowest yield is a mustard without biofertilizer with the value of 18.75 cm. One-way ANOVA results show that different carrier media gives effect to the height of the mustard, so that the Tukey test was done at 95% ($\alpha = 0,05$) using Minitab software. Tukey test results are shown in table 1.

Table 1. Plant height.

	Plant height (cm)
Chemical fertilizer	25.850^{a}
Mollasses	24.250^{ab}
Sucrose	24.250^{ab}
Rice water	23.250^{ab}
Compost	22.075^{ab}
No biofertilizer	18.750^{b}

The results of Tukey test of mustard in table 1 show that the treatment of mustard using biofertilizer with molasses medium, rice water, and sucrose did not differ significantly compared to chemical fertilizers and compost, but did not give a significant response when compared with treatment without biofertilizer. The highest yield of plant height at week 5 of biofertilizer with three carrier medium is molasses and sucrose and the lowest is rice water. This is in accordance with the total soil microbial analysis, at week 5 the highest TPC result was in the molasses treatment which was $1.17x10^{12}$ cfu/ml while sucrose of $4.9x10^{11}$ cfu/ml and the rice water was slightly lower than sucrose and molasses which is $3.19x10^{11}$ cfu/ml so that the decomposition of organic matter in soil is not greater than molasses and sucrose. The result of the number of leaf was shown in figure 4.

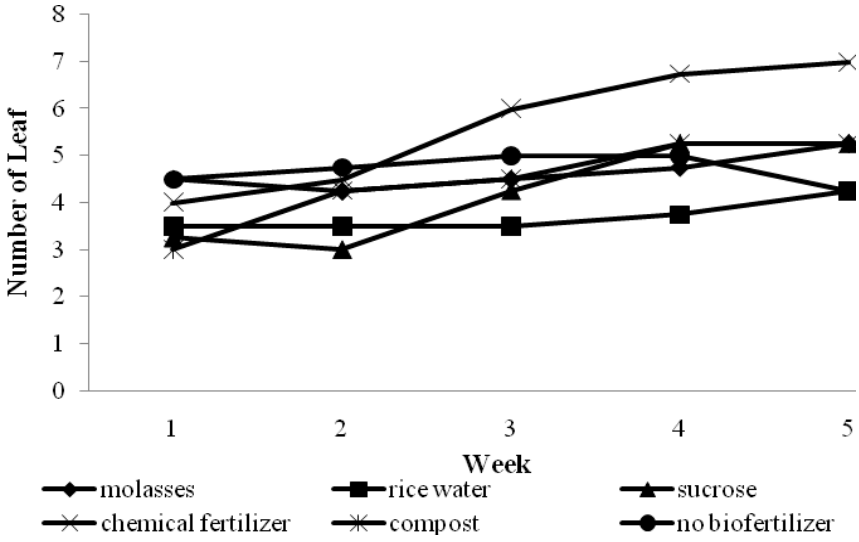

Figure 4. Result of number of leaf.

Based on figure 4 the average number of leaves of mustard plants with biofertilizer treatment with molasses and sucrose carrier media is 5.25 pieces at week 5. The number of leaves on both media does not exceed the chemical fertilizer with the average number of leaves by 7 strands at week 5. The average number of leaves of mustard plants with biofertilizer treatment with rice water carrier media equal to the treatment without biofertilizer which was 4.25 pieces at week 5. One-way ANOVA results show different carrier media influence the number of leaves of mustard plants, then was followed by Tukey test. Tukey test results are shown in table 2.

Table 2. Number of leaf.

	Number of leaf
Chemical fertilizer	7.000^{a}
Compost	5.250^{ab}
Molasses	5.250^{ab}
Sucrose	5.250^{ab}
Rice water	4.250^{b}
No biofertilizer	4.250^{b}

Based on Tukey test results in table 2 shows that the number of leaf on biofertilizer treatment with molasses carrier media and sucrose is not significant compared to chemical fertilizers. But it is not as

significant when compared with no biofertilizer. The results on biofertilizer treatment in rice water differed significantly from chemical fertilizers and did not differ significantly when compared to without biofertilizer. The result of leaf area was shown in figure 5.

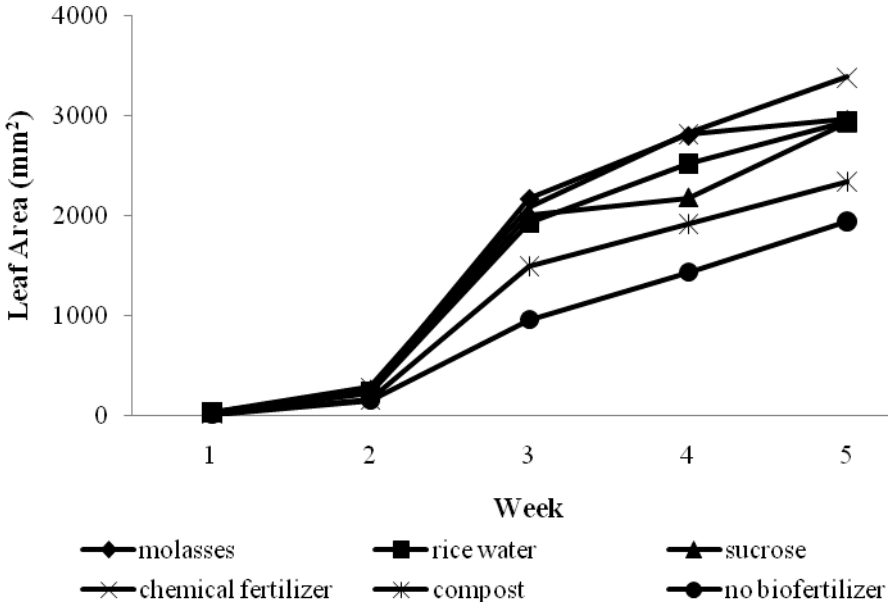

Figure 5. Result of leaf area.

The result of leaf area measurement in figure 5 shows that the leaf area in the treatment of biofertilizer with the highest molasses medium is 2959.766 mm^2, rice water is 2943 mm^2 and sucrose is 292.,899 mm^2 at week 5. The results of leaf area from biofertilizer with the three carrier media showed higher yield compared to compost and without biofertilizer, but no greater than chemical fertilizers.The result of one-way ANOVA analysis shows that different carrier media affect the leaf area of the mustard, so was continued with Tukey test. Tukey test results are shown in table 3.

Table 3. Leaf area.

	Leaf area (mm^2)
Chemical fertilizer	3384.1^{a}
Molasses	2959.8^{ab}
Rice water	2943.0^{ab}
Sucrose	2927.9^{ab}
Compost	2337.1^{ab}
No biofertilizer	1938.4^{b}

Based on Tukey test results in table 3 shows that leaf area on biofertilizer treatment with molasses carrier media, rice water, and sucrose is not significant compared to chemical fertilizers. However, it is not as significant when compared with no biofertilizer. The result of lenght of root was shown in figure 6.

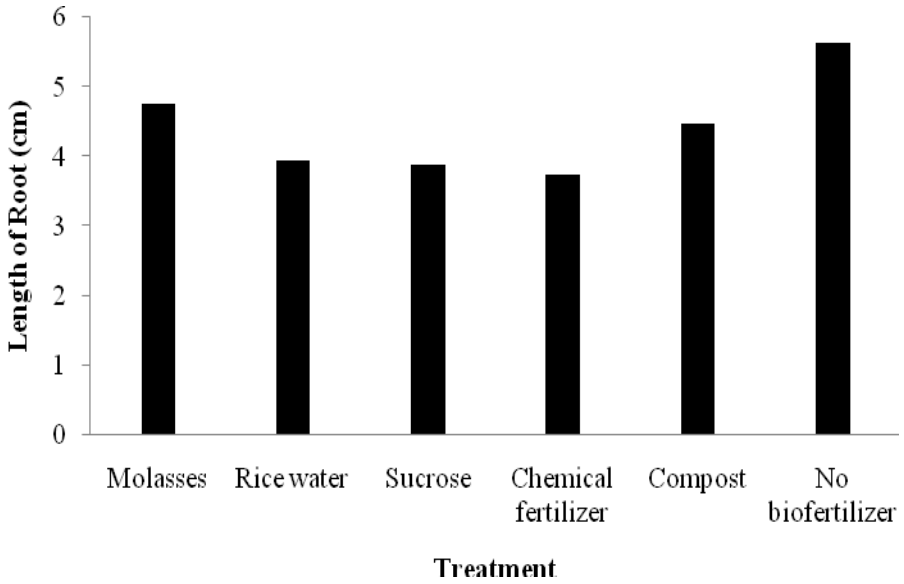

Figure 6. Result of root length.

The highest measurement result of root lenght at week 5 is without biofertilizer treatment that is 5.625 cm. The treatment of root lenght of molasses carrier media is higher than compost and chemical fertilizers but still lower than without biofertilizer. The rice water is higher than chemical fertilizers and sucrose but still lower than molasses, compost and without biofertilizer. One way ANOVA results show that different carrier media have no effect on root length of mustard plants.

3.3 Productivity of mustard

Biomass calculation was done once at harvest time (5 weeks). The calculation of biomass includes measurement of dry weight of mustard. The calculation results are shown in figure 7.

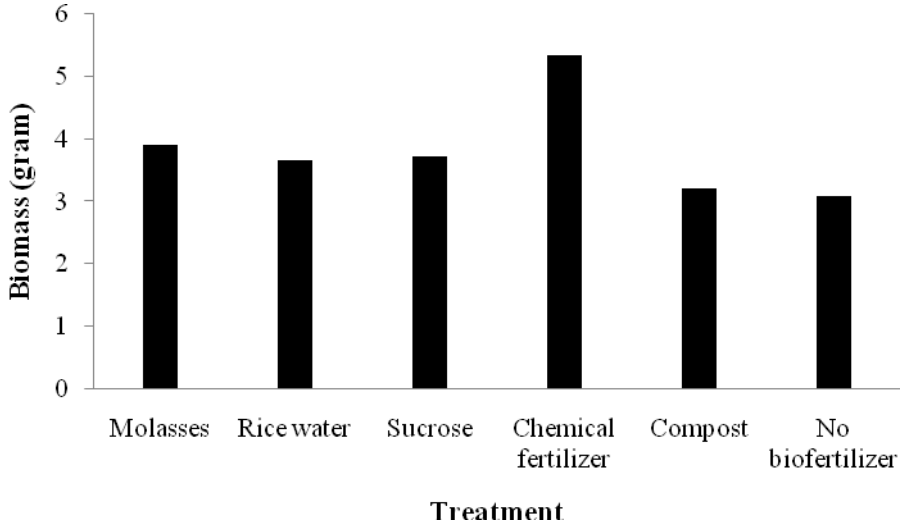

Figure 7. Result of biomass.

Biomass was measured from dried mustard plants in the oven for 4 days at a temperature of 60°C. Based on Figure 7 the results show the mustard on biofertilizer treatment with molasses medium, rice water and sucrose greater than compost and without biofertilizer. But not bigger than chemical fertilizers. One-way ANOVA results indicate that different carrier media influence the growth and productivity of mustard, then was continued with Tukey test. The results are shown in table 4.

Table 4. Biomass.

	Biomass (gram)
Chemical fertilizer	5.3414[a]
Molasses	3.9003[ab]
Sucrose	3.7209[ab]
Rice water	3.6615[ab]
Compost	3.2016[ab]
No biofertilizer	3.0923[b]

The results shown in table 4 show that in the biofertilizer treatment the three media showed unsignificant results on chemical fertilizers, but also unsignificant to compost and without biofertilizer.

3.4 Analysis of total soil microbes

Total soil microbial analysis was conducted at week 1, week 3, and week 5. The analysis was performed on all treatments. The total microbial soil analysis is shown in figure 8. The result of total soil microbial analysis in figure 8. shows that the highest total microbial increase was in biofertilizer with molasses carrier media. Rice water and sucrose also showed increase until week 5 but not higher than molasses.

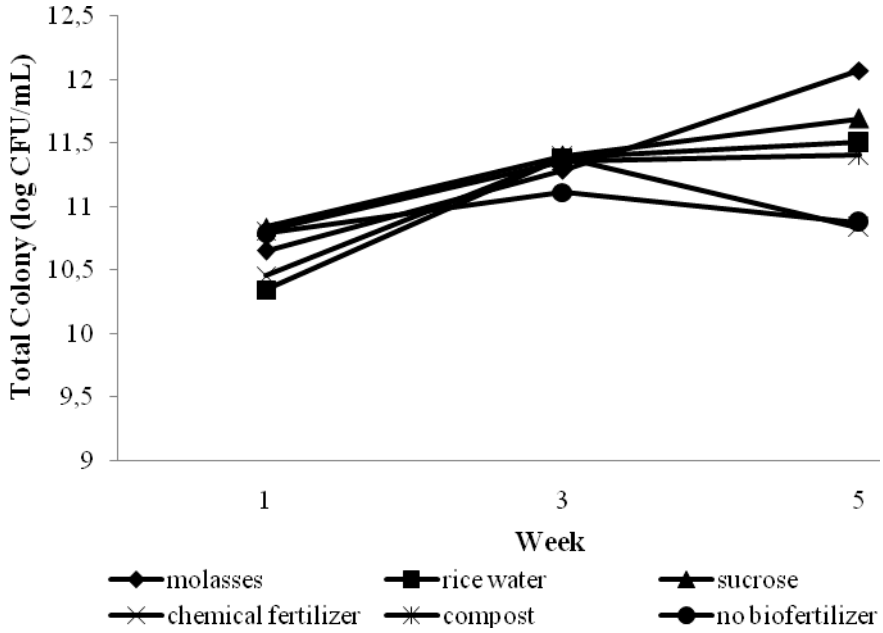

Figure 8. Result of total soil microbes.

4. Discussion

Based on Figure 2, the highest TPC value was in biofertilizer carrier media of rice water. Rice water contains carbohydrates, proteins and B vitamins (especially vitamin B1) needed in cell metabolism of microorganisms. Vitamins play a role in the process of hormone formation and function as a coenzyme. Thiamin contained in rice washing water helps the microorganisms in the release of energy and helps the regulation of metabolites. Lysine plays a role in oxidation β of long chain fatty acid [18].

The treatment of mustard using biofertilizer with molasses medium, rice water, and sucrose did not differ significantly compared to chemical fertilizers and compost, but did not give a significant response when compared with treatment without biofertilizer (table 1). This is influenced by the presence of external factors and internal factors that affect plant growth. External factors may support the work of bacterial inoculants in biofertilizer formulations, so treatment with biofertilizers has a greater effect than controls. External factors are categorized in ecosystems including climate (light, temperature, and wind), soil (texture, organic matter, altitude, water source and pH), and biological (insects, microorganisms, viruses, other disease-causing organisms). Internal factors such as genes, phytohormon activity, and photosynthetic rate [19]. Biofertilizer with molasses carrier media, rice water and sucrose showed less than maximum yield on plant height because yield was not significant compared without biofertilizer and compost. However, when compared with chemical fertilizer, the results also showed no significant difference. So that biofertilizer can give a positive effect on the increase of plant height. Carrier media serves to grow, pack and extend the storage time of biological agents [20]. The biofertilizer carrier media used in this study were molasses, rice water and sucrose as a substitute for carbon source as a nutrient provider. The availability of nutrients is an important factor for microbial growth. Sufficient nutrients will be followed by good microbial growth. The availability of less nutrients will be a barrier to microbial cell growth. One of the most important nutrients for microorganisms is carbon. Nearly 50% of the dry weight of the cell consists of carbon, therefore carbon is the most important macronutrient needed [21]. The increased growth of mustard plants is caused by the increase of microorganisms in the soil. If the number of microorganism get more and more, then decomposition of organic material will be greater. Plant height growth is caused by increased nitrogen [22,23] and phosphate [23]. Nitrogen is an element that is needed by plants for growth and also by soil microorganisms. Most of the nitrogen in the soil is in the form of complex organic molecules. This organic form will be converted to ammonium and nitrate by microorganisms, as a result of the mineralization process [24]. Nitrogen acts as a constituent of proteins, chlorophyll, amino acids and many other organic compounds, while phosphates are composers of nucleoprotein phospholipids, phosphoric sugars and energy storage [25]. The amount of nitrogen that can be used depends on the speed of the mineralization. The process of mineralization which depends on the environmental factors that affect the activity of the microorganism itself are the amount of carbon, temperature, oxygen and others [25]. Phosphorus is an important component of compounds for energy transfer (ATP and other nucleoproteins), for genetic information systems (DNA and RNA), for cell membranes (phospholipids), and phosphoproteins [19]. Plant height is also affected by the IAA hormone [26]. This study of *Candida* G3.2 plays a role in phosphate solubilitation and *Azotobacter* A10 plays a role in hormone synthesis of IAA and nitrogen synthesis.

The growth of leaves number (figure 4) is influenced by N minerals [27]. Nitrogen is an important element in the formation of chlorophyll, protoplasm, protein, and nucleic acids. This element has an important role in the growth and development of living tissue [25,28]. The study of microorganisms that play a role in nitrogen synthesis is *Azotobacter* A10.Nitrogen availability also has a significant effect on leaf area (figure 5). When enough nitrogen was supplied, the plant leaves will grow large and expand the available surface for photosynthesis.

Based on Figure 6, root length is affected by nitrogen fixation and phosphate solubilitation [23]. The role of phosphate, among others, for cell growth, the formation of fine roots and root hair, strengthen the plant [29]. Roots that grow on sufficient nitrogen are large and short, whereas root on soil with less nitrogen will be elongated and small [25].

Biofertilizer increases the biological activity on the soil and the absorption of mineral components which causes an increase in biomass production (figure 7). The dry weight of the plant is affected by the increase of nitrogen, phosphate and vegetative growth of the plant [30]. In this study, *Azotobacter* A10 has nitrogen synthesis role. Meanwhile, *Candida* G3.2 plays a role in phosphate solubilitation.The process of mineralization caused by phosphate solubilitation microorganisms have been regulated by many enzymatic processes, also acting as the activator of several enzymes, which leads to metabolism process and the formation of new cells that can promote vegetative growth. In addition, phosphorus plays a major role in protein synthesis and the formation of protoplasm leading to an increase in cell size [31].

Molasses is a type of carrier medium that provides the best response to the growth and productivity of mustard in terms of leaf area and dry weight when compared to rice water and sucrose. Molasses is a by-product of the sugar industry containing nitrogen compounds, trace elements, 34% sucrose and a total carbon content of about 37%. Molasses are also rich in biotin, pantentonic acid, phosphorus and sulfur. Sulfur has a role in protein synthesis and part of the amino acid cysteine, biotin and thiamin. Sulfur helps stabilize protein structure, helps oil synthesis and chlorophyll formation, and reduces the occurrence of disease attacking on the plant body. Phosphorus, a constituent of amino acids, coenzymes NAD, NADP and ATP, active in cell division, stimulates seed growth and flowering. Molasses also contain magnesium and calcium. Magnesium, an essential constituent of chlorophyll, serves as a cofactor in most enzymes that activate the phosphorylation process, as a bridge between the pyrophosphate structure of ATP and ADP and enzyme molecules and can stabilize the particles in the configuration for protein synthesis. Calcium, a constituent of cell walls, plays a role in maintaining cell integrity and membrane permeability [18].

Biofertilizer with molasses carrier media, rice water and sucrose showed the highest total soil microbial increase compared to without biofertilizer and chemical fertilizer (figure 8). This is thought due to an association between indigenous bacteria and bacteria added to the biofertilizer formulation. The increase in the population indicates a positive relationship or interaction. Positive interactions allow the bacterial population to live in a habitat, where individually they can not live alone. Positive interactions performed by nitrogen fixing bacteria such as exudates produced by plant roots are sufficient for bacteria to grow. While the plants will get nutrients in the form of N nutrients provided by bacteria through the process of N fixation. In addition, there is a positive interaction with decomposer bacteria in the formulation. N fixing bacteria require C element for nutrition growth in carrying out the fixation process while decomposer bacteria do not get disadvantaged [21].

Overall, the results of growth and productivity of mustard in this study are still not maximal because the results show that they are not significant when compared without biofertilizer or compost. However, when compared with chemical fertilizers, they showed good results, because the data obtained from the continued test of tukey are unsignificant. Compost fertilizer is obtained from the results of natural materials such as straw, leaves, organic waste processing plant, etc. Compost fertilizers have elemental properties, but the available macro element content (which can be absorbed by plants) is low [24]. Biofertilizer contains microbial inoculants that help the absorption of plant nutrients by converting essential nutrient elements in an available form to plants through biological processes. Biofertilizer can not produce a direct impact on the harvest. Biofertilizer, which is sustainable, plays a role in improving physical properties, soil chemistry and enhancing the nutrients essential to plant growth [32-35]. Overall, chemical fertilizers showed the best results seen from the highest values of plant height parameters, leaf number, leaf area, and biomass, since chemical fertilizers contained NPK in a large available form. It shows that nutrient availability for plant growth and productivity is very influential. Although chemical fertilizers dissolve easily so that they are absorbed faster by plants and their effects can be directly seen in the plants, but the continuous use of chemical fertilizers can leave residues which can damage the environment [1,34,36]. The long-term use of chemical fertilizers has resulted in poor soil quality, reduced production, and increased pests and diseases and environmental pollution [36]. The results of total microbial analysis showed that total

soil microbes in the treatment of chemical fertilizers and without biofertilizer were decreased. While the treatment of biofertilizer by the three carrier media was increased.

5. Conclusion

The results showed that all biofertilizer carrier media improved microbial viability, with the highest TPC which was obtained from rice water with the value of $1.19x10^{14}$ cfu/ml, then was continued by sucrose with the value of $1.18x10^{14}$ cfu/ml and molasses with the value of $9.65x10^{13}$ cfu/ml at day 7 of incubation times. Biofertilizer carrier media also gave possitive effect that was indicated by the increase of mustard growth and productivity. Molasses is the best carrier media that provides high response in mustard growth and productivity, with the value of plant height which is 24.250 cm, number of leaf is 5.250, leaf area is 2959.8 mm^2 and dry weight is 3.90 gram when compared to the rice water and sucrose media.

Acknowledgments

This work was conducted as part of "Superior and Commercial Biofertilizer of Marine Yeast and Bacteria". This research was supported by grants from Pemula Research 2017 awarded by ITS local fund 2017.

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