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The effectiveness of biofertilizer on edamame productivity

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Abstract. This study aimed at determining the effectiveness of bio-fertilizer with different concentrations on productivity soybean "Edamame" (*Glycin max*). The productivity consisted of the number of pods, empty pods, pod content, Edamame weight each plant. Bio-fertilizer in this study consists of microbial consortia (*Lactobacillus, Pseudomonas, Bacillus, Saccharomyces, Rhizobium, Azotobacter, Azospirillum,* and*Cellulomonas*). The treatment consists of 3 bio-fertilizer concentrations (25%, 50%, and 75%), as well as the negative control and positive control (100% chemical fertilizer equivalent 5g/plant). The results showed that treatment B2 (bio-fertilizer 50%) gave better results on productivity compared with other treatments.

1. Introduction

Bio-fertilizer is a fertilizer containing microbes, include *Bacillus, Pseudomonas, Rhizobium, Azotobacter, Azosprillum, Mycorrhiza,* and *Trichoderma*. The existence of these microbes can be either single or combined several types of microbes, is called microbial consortium. Microbes are used as biological fertilizer able to spur plant growth, holds the nitrogen, dissolve phosphate and inhibits the growth of plant diseases[1][2]. Bio-fertilizer is one of the numerous components that s are critical to improving the system for the supply of nutrients in agriculture. Several types of soil microbes are often used as a bio-fertilizer among other bacteria as fixation N non-bacterial symbioses, symbiotic N bacterial, mold, and bacteria mycorrhiza solvent phosphate. The soil Microbe when utilized together and right in the system of organic farming can give positive impact to the availability of nutrients needed by crops, disease and pest control, can increase the growth and the productivity of the crop [3]. Edamame (*Glycine max* (l.)) as a potential plant because that has an average production of 3.5 tons ha-1 higher than regular soy crop production has an average production of 1.7 – 3.2 ton ha-1 [1]. besides, edamame also has an extensive export market opportunity.

The request export from the country of Japan hills to 100,000 tons per year and the US amounted to 7,000 tons per year. Meanwhile, Indonesia can meet the 3% of Japan's market needs, while 97% filled by China and Taiwan [4]. Edamame can be consumed as a vegetable when young pods are still green. Edamame had a complete protein with quality equivalent to the protein content in milk, eggs or meat. Edamame is a complete protein, dietary fiber, and micronutrients, particularly folate, manganese, phosphorus and vitamin k. The balance of fatty acids in 100 grams of edamame is 361 mg omega- fatty acids 3-1794 mg of omega-6 fatty acids. Besides, the edamame also contains anti-cholesterol, so it is perfect for consumption. Chemical fertilizers are types of fertilizer is frequently used in planting soy edamame. The use of chemicals in the form of chemical fertilizers in excess doses, currently pose a serious enough problem. Using chemical fertilizers is not only harmful to agricultural but also endangers human health. The ecosystem of farmland being damaged, natural predators are gone, and the balance of nutrient elements in soil being disturbed [5]. A dose of chemical fertilizers used on edamame was too high, so, to overcome used biofertilizer with the intent to reduce high dose in the chemical fertilizer. Research on using biofertilizer to increase edamame productivity was still a bit of a do. Edamame soybean farmers mostly use chemical fertilizers with a very high dose that is 600 kg per hectare. It is feared may damage soil fertility, so the other alternative biofertilizer required to minimize the use of chemical fertilizers. Based on the background, need to be developed microbial consortium biofertilizer technology to increase crop productivity edamame (*Glycin max* (l.) Merrill).

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2. Main Body

2.1. Biofertilizer

Biofertilizer is a product of biological active consists of microbes that can increase the efficiency of fertilization, soil health, and fertility. Microbes are harnessed to increase the fertility of the soil is known as biofertilizer (microbial fertilizer = microbial fertiliz- ers). A wide range of benefits that can be obtained from the use of microbes, are: (1) provide a source of nutrient for plants; (2) pro- tect the roots of disorders of pests and diseases; (3) in order to develop rooting perfect stimulate system and extend the root; (4). The meristem tissue in mitosis gunning point of growing shoots, buds, and the stolen; (5) as an antidote to the poison of some heavy metals; (6) as growing regulatory metabolites; and (7) as bio-activator destroy organic materials. Besides, the microbes contained in biological fertilizer/biofertilizer useful as a fastening system N2 boosters and growing plants, solvents, phosphates and destroy materials organic [5].

2.2. Microbial nitrogen fastening system

Nitrogen (N) is the most crucial element for plants and plays a role in the vegetative growth of plants. Nitrogen in the soil, among others derived from organic materials, the results of binding of N from the air by microbes, fertilizers, and rain. Soil nitrogen con- tained in low generally, so should always be added in the form of manure or other sources at the beginning of each planting. In addi- tion to merely low level, N in the soil have a dynamic nature (easily changed from one form into other forms such as NH_4 NO_3 be- ing, NO, N_2O and N_2) and easily vaporized and leached lost along the water drainage [3]. Different types of bacteria in biological N_2 fixation, among others consist of *Rhizobia, Sianobakter* (blue- green algae), photoautotrophic bacteria on the surface of stagnant water and, as well as refer to heterotrophic bacteria in the soil and root zone. The bacteria can bind nitrogen from the air, either in symbiosis (root-nodulating bacteria) or nonsymbiotic (free-living nitrogen-fixing rhizobacteria). Utilization of bacterial fixation of N_2, both being applied through the soil or sprayed on the plant, capable of improving the efficiency of fertilization. Use N_2 fixation Bacterial potentially reducing the need for fertilizers, improv- ing production and N synthetic the income of farming with cheaper inputs. *Azotobacter* is N_2 bacteria fixation that able to produce glycemic promoting, *cytokinin, indole-acetic aci*d, and so it can spur the growth of the root. The population of *Azotobacter* in soils is affected by fertilization and plant type [6].

2.3. Microbe solvent phosphate

Phosphorus (P) in the soil consists of P-P and inorganic-organic that comes from organic materials and minerals containing P (apatite). Elements of P in availability land for plants because P is bind by clay, organic material, as well as oxides of Fe and Al in the soil pH, is low soil pH with a Dour 4-5.5) and by the Ca and Mg on soil that has high pH soil (neutral and alkaline with a pH of (7-8). Soil minerals that administered generally have neutral pH between 5.5 to 6.5 so that the availability of P is not a problem. Due to the massive amounts of P fertilization and over the years continually, it caused hoarding (accumulation) P in the soil [3].

One alternative to increasing the efficiency of fertilizer phosphate in overcoming low phosphate or phosphate saturation available in the soil is by utilizing microbes that can dissolve phosphate solvent phosphate unavailable become available for the plant. As expressed above, the release of phosphate of iron phosphate on the waterlogged lands can occur through the process of reduction of iron, to increase the availability of the phosphate for a plant. The increasing P availability in the soil also explains why rice requires a relatively low P fertilizer. Various types of phosphate solvent microbes, such as Pseudomonas, Micrococcus, Bacillus, Flavobacterium, Penicillium, Fusarium, and Sclerotium, Aspergillus potentially high in dissolving phosphate bound phosphate becomes available in the soil)[7].

2.4. Microbial provider growth

Some bacterial species Rizosver (around rooting) that able to improve plant growth is often called a Plant Growth Promoting Rhizobacteria (PGPR) or plant growth Boosters Rhizobacter(RPPT). RPPT consists of the genus *Rhizobium, Azospirillum, Azotobacter, Bacillus, Arthrobacter, Bacterium,* *Mycobacterium and Pseudomona*s [7]. Bacteria boosters grow producing directly to the phytohormone able to induce growth. Increasing of growing plant can occur when a rhizobacterium produces metabolites which act as a phytohormone that enhances plant growth directly. The resulting metabolites other than in the form of a phytohormone, as well as antibiotics, siderophore, cyanide, and so on. phytohormone or growth hormone produced can be produced Auxin, ethylene, cytokinin, and abscisic acid. Bacteria grow boosters indirectly also inhibit pathogens through the synthesis of antibiotics, as a biological control. Some kind of endophytic mutualistic with its host plant symbiotes in increasing resilience against insect pests through the production of a toxin, anti- microbial compounds on the side such as fungi, Pestalotiopsis microspore, Taxus and walkchyana that producing taxol (anti- cancer substances) [6].

2.5. Microbes remodel organic matter

Definition of microorganism remodel organic material or bio- decomposer is a microorganism lignin fiber, parser, and organic compounds that contain nitrogen and carbon from the organic matter (organic remnants of plants or animals that have been dying). remodel microbial organic substances composed of *Trichoderma reesei, t. harzianum, t. koningii, Phanerochaeta crysosporium, Cellulomonas, Pseudomonas, Thermospora, Aspergillus niger, A. terreus, Penicillium, and Streptomyces*. The function of remodeling organic materials generally has better ability than bacteria in decomposing the remnants of plants (cellulose, hemicellulose, and lignin). General microbes that able to degrade cellulose was also able to degrade hemicellulose. The Group of fungi showed the most apparent bio-decomposition activity, which can soon make soil organic matter decompose into simpler organic compounds, which doubles as primary ion exchangers save and release nutrients around the plant [8].

3. Edamame Soybean

Soy edamame is a species of plants which are included in the category of vegetables (green vegetable soybean) in his native country, namely Japan, edamame or Gojira as vegetables and snacks. Edamame soybeans contain high nutritional value, per 100 g seeds contain 582 kcal, protein 11.4 g, carbohydrate 7.4 g, fat 6.6 g, vitamin A or beta-carotene, 100 mg, B1 0.27 mg, B20, 14 mg, B3 1 mg of vitamin C, and 27mg, as well as minerals such as phosphorous 140 mg , Calcium 70 mg 1.7 mg, iron, and potassium 140 mg). Edamame soybeans are also rich in isoflavones which is an organic compound that is an antioxidant and helps prevent cancer. Edamame (*Glycine max* (l.) Merr.) plants that need to be developed because the potential has an average production of 3.5 tons ha-1 higher than regular soy crop production has an average production of 1.7 – 3.2 ton ha-1. Besides, edamame also has extensive export market opportunities [4].

4. Plant Productivity

Crop productivity is the ability of a plant to produce product/results seen from measurements of dry mass. In the modern production cultivation plant, the production of a plant aims to increase and maximize growth rate through genetic manipulation and the environment, to obtain maximum yields. In other words, the production of a crop could be interpreted as the result of a plant that is obtained after the growth process is completed [7].

5. Methodology

This research was conducted at two places, at the Islamic University of Jember, Laboratory and paddy fields in the village of Dukuhmencek, district Sukorambi, Jember. This research was carried out in April 2017 – August 2017. Ingredients used in this research include seed soy edamame (*Glycin max* (l.) Merrill), manure obtained from the agricultural, chemical fertilizers as positive controls (Urea, ZA, KCL), medium Nutrient Agar (NA), and Potato Dextrose Agar (PDA) 70% alcohol, methylene blue, NaCl, glucose, molasses. Some microbes are used as a biofertilizer consists of N Bacteria affixation (*Rhizobium spp., Azotobacter and Azospirillum sp*.), solvent phosphate bacteria (*Pseudomonas sp., Lactobacillus*.) and microbial decomposers (*Saccharomyces sp., Cellulomonas sp*.).

5.1 Producing of Biofertilizer
Make a solvent of 2% molasses (140 mL molasses in 6860 mL water), heating it to the boil, and leave it to cool. Then enter the respective breed bacteria and yeasts. The total number of breeds that are incorporated into the molasses that are 10% (700 mL of microbial consortium in 6300 molasses 2%). Counting of biological fertilizer in TPC media molasses is done with the smallest dilution to dilution series. In the dilution series, taking as much as 1 mL to pour plate on Nutrient Agar medium (for TPC bacteria) and Potato Dextrose Agar (for TPC, Yeast). Next, incubate at temperature 37^{O}C for 24-hours and do the calculation TPC.

5.2. Measurement of crop productivity.
Harvest soy edamame was done when most of the leaves are already yellowed, the fruit began to change color to dark green, or a solid look already contains pods. Soy edamame aged that will be harvested which is around the age of 75-100 days. Collection data of plant productivity include the number of pods, empty pods, pod content, edamame weight each plant.

5.3. Methods and analysis
This research is experimental using a complete randomized design, with 4 treatment (0, 25, 50, and 75%). Each treatment was repeated 3 times and every repetition consists of 5 plants. The data will be analyzed using ANOVA with the degree of significance of 5% and continued with Duncan's test to compare between the treatments. ANOVA test is done before, do a test of its homogeneity and normality test.

6. Results and Discussion

6.1. Result
Planting the seed of soy edamame on May 29, 2017, three days before planting, water on the land so that the land will be sprinkled with soy edamame seeds moist. 4 days after planting the plastic covering of the dike wall opened so as not to interfere with an edamame soybean germination. Plant soy edamame flowered almost simultaneously from all the treatment, that is, at the moment the plants aged 30 days after planting. Data capture productivity include the number of pods per plant, the number of empty pods, number of pods contents, fresh soy edamame and weight per plant. Harvesting is done on the date of 5-10 August 2017, precisely when the plant 67-72 day-old edamame after planting. Harvesting is done in the span of 2 days, this is due to the maturity of the soy edamame each plant is not the same. Data were analyzed with ANOVA statistics measurements with SPSS version 16. Based on the results of the analysis of the obtained data is as follows:

Table 1. The Result of analysis plant productivity with an application of consortia microbe biofertilizer

Treatment	Number of pods	Empty pods	Pods content	Edamame weight
B1	23,11±3,48	0,80±0,87^{a}	23,53±0,81^{b}	54,00±13,0^{b}
B2	25,20±3,17	0,60±0,20^{a}	24,60±3,03^{b}	55,13±2,41^{b}
B3	**27,67±4,39**	**0,53±0,23^{a}**	**6,40±5,37^{b}**	**57,87±5,31^{b}**
K+	21,87±1,01	0,80±0,53^{a}	21,87±1,81^{ab}	44,00±11,13^{ab}
K-	21,20±0,72	2,87±0,50^{b}	7,80±1,06^{a}	32,00±6,92^{a}

Information:
The numbers followed by the same letters in the same column are not significantly different, while the numbers followed by different letters in the same column are significantly different.

Productivity results were analyzed using SPSS version 16. Based on the results of the analysis it was found that all biofertilizer data on the productivity of edamame soybean plants were normally distributed and homogeneous ($\alpha > 0.05$), so that they were continued using ANOVA analysis. Based on the results of Anova's analysis, it was found that the significance of the number of pods was ($\alpha = 0.116$), so that biofertilizer administration had no significant effect on the number of edamame soybean pods because it had a significance value of $\alpha > 0.05$, so the data was not followed by Duncan's test. As for the other productivity data, the following results are obtained: the number of empty pods has a significance value of ($\alpha = 0.001$), number of filled pods ($\alpha = 0.04$), and edamame soybean weight ($\alpha = 0.024$), so that it can be said Biofertilizer fertilizer significantly affected the number of empty pods (the smallest value), the number of filled pods, and the weight of edamame soybeans per plant, because it had a significance value of $\alpha < 0.05$. The data is known to have a significant effect, so it is continued with the Duncan test. Duncan Test Results can be observed in Table 2. From the table shows that of all the biofertilizer concentration variants, the concentration of B3 (75% concentration biofertilizer) is significantly different from other concentration variations, so it can be said that the best biofertilizer for edamame soybean productivity that is at a concentration of 75%. Based on the results of the above measurement data obtained images as below:

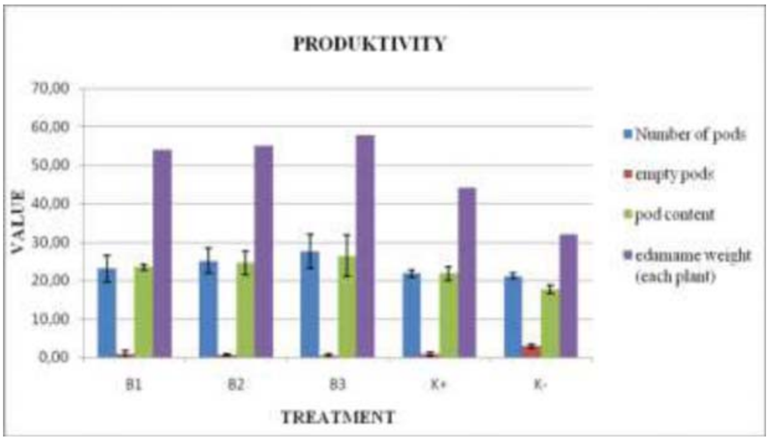

Figure 1. Edamame productivity with granting a microbial consortia

6.2. Discussion

This research microbial consortium biofertilizer, using 8 kinds of microorganisms, they were: *Rhizobium spp., Azotobacter sp., Azospirillum sp., Bacillus sp., Pseudomonas sp., Cellulomonas sp., Lactobacillus sp.* and *Saccharomyces sp.* Until now, Some bacteria are reported to have a beneficial influence for plants so that it can be classified into a group of PGPR (Plant Growth Promoting Rhizobacteria), the Group of the genus *Azoarcus sp., Azospirillum spp., Azotobacter sp.,Arthrobacter sp., Bacillus sp., Clostridium sp., Enterobacter sp., Pseudomonas sp., Gluconoacetobacter sp., and Serratia sp.*, [9]. Microbes that used in this study belongs to PGPR, whereas a provider of nutrient for plants, it can also as a producer of hormones that can spur the growth of the plant. *Azotobacter* in addition to binding the N from the air, it is also able to produce Indol Acetic Acid (IAA) in an amount directly proportional to its density. Besides, *Azotobacter* can also generate *cytokinin, gibberellin*, and *abscisic acid*[10].

One of the microbes that make up this biofertilizer fertilizer is N fixing bacteria (*Rhizobium sp., Azotobacter sp.* and *Azospirillum sp.*), Where it is known that Nitrogen (N) is the most important element for plants and plays a role in vegetative growth of plants. Nitrogen in the soil includes organic matter, the binding of N from the air by microbes, fertilizers, and rainwater. Nitrogen contained in the soil is generally low, so it must always be added in the form of fertilizer or other sources at the beginning of each crop. In addition to its low levels, N in the soil has a dynamic nature (easily changes from one form to another such as NH_4 to NO_3, NO, N_2O and N_2) and easily vaporizes and is washed away with drainage water [3].

Biofertilizer microbial consortia that used in the study included in N fixation, bacteria that can bind nitrogen from the air, either in symbiosis (root-nodulating bacteria) or non-symbiotic (free-living nitrogen-fixing rhizobacteria), so it can supply the nutrients needed by plants. In addition, the biofertilizer in this study also used phosphate (P) solvent bacteria (*Pseudomonas sp., Lactobacillus*). P element in soil availability for plants is low because P is bound by clay, organic matter, and Fe and Al

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oxides on soils with low pH (acid soils with pH 4 - 5.5) and by Ca and Mg on soil the pH is high (neutral and alkaline soil with pH 7-8). One alternative to overcome the low available phosphate or saturation of phosphate in the soil is by utilizing phosphate solvent microbes that can dissolve phosphate not available to be available to plants. So that in the presence of *Pseudomonas sp., Lactobacillus* can dissolve the phosphate bound to a free phosphate element so that it can be absorbed directly by plants. And there are microbes are having (*Saccharomyces sp., Cellulomonas sp*) that can break down lignin, fibers, and organic compounds that contain nitrogen and carbon from the organic matter (organic remnants of plant or animal tissue that had died). Generally, the microbes that can break down soil organic matter into simpler organic compounds, which doubles as primary ion exchangers save and release nutrients around the plant [11]. So the Microbe can provide nutrient elements required by plants.

Microbial consortia biofertilizer can provide soil organic matter which is very beneficial in restoring the fertility of the physics, chemistry, and biology of the soil, as useful as a binder of soil particles through soil aggregation process. Aggregation of soil formation can produce micro-pore spaces, so the aeration in the soil become better and create the optimum state for the absorption of nutrient elements that are useful to the plant. Influence of organic materials against chemical soil fertility among others against the capacity of exchange of cations and anions, increasing the activity of microbial soil through decomposition and mineralized inorganic materials. Besides, the organic matter can absorb and retain water, which in turn affect the accumulation of food substances and results of metabolism that is saved in the stem, leaf, fruit, and seeds [12].

Nutrient elements that can increase crop productivity soy edamame had been produced by Microbes that found in microbial consortium biofertilizer, thus granting biofertilizer 75% concentration of microbial consortium (B3) shows the best results productivity compared with other concentrations. This is because of the more concentrated solution of biofertilizer, increasingly many microorganisms contained in the biofertilizer. So the Microbe can provide nutrient elements in abundance, and plant soy edamame can directly absorb the nutrient elements to improve its productivity.

Figure 2. Edamame soybean plant with apply of biofertilizer (B3)

Based on the results of the above research it is known that the administration of microbial consortium biofertilizer can increase plant productivity in terms of the amount of edamame per plant, number of filled pods, number of empty pods, and edamame weight, and the best treatment was 75% (B3) biofertilizer concentration treatment.

7. Conclusion

Granting of biofertilizer effect on productivity in soy edamame, 75% concentration of biofertilizer (B3) is the most well compared with other concentrations.

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