

THE POTENTIAL OF THE CONSORTIUM (*Azotobacter* spp. and Phosphate Solubilizing Bacteria) IN INCREASING PLANT N UPTAKE, PLANT NITROGEN CONTENT, *Azotobacter* spp. POPULATION AND LETTUCE (*Lactuca sativa* L) CROP YEALD

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ABSTRACT

Indonesia is a tropical agricultural country that has an Andisol soil order which is potential to be used for the cultivation of lettuce (*Lactuca sativa* L.). To increase lettuce yields, farmers generally use excessive synthetic NPK fertilizers. This has resulted in serious pollution and land degradation. As an effort to restore soil fertility conditions that are environmentally friendly is through the application of biological fertilizers. Biofertilizer inoculation that potentially used is a form of consortium, which consists of the N-fixing group, namely *Azotobacter* spp and phosphate solubilizing bacteria (*Pseudomonas* sp.). The characteristics of the consortium inoculant that need to be investigated are its viability and effectiveness when applied in the field to lettuce cultivation. The experimental data analysis was carried out based on the linear model of the Randomized Block Design (RBD), consisting of seven treatments, namely: control; NPK 100% recommended dosage; consortium 10 l / h + 100% NPK; consortium 10 l / h + 75% NPK; ; consortium 10 l / h + 50% NPK; consortium 10 l / h + 25% NPK. The results showed that consortium inoculation of 10 l / h + 50% NPK could increase the population of *Azotobacter* spp. and the yield of fresh lettuce increased by 49.24%. Meanwhile, in the application of the consortium inoculation of 10 l / h + 25% NPK, tend increasing N absorption value. The consortium inoculant can reduce the NPK requirement by 50% followed by a higher fresh weight yield of lettuce compared to use 100% NPK, by using conventional method.

Keywords: Andisol, *Azotobacter* sp., Consortium inoculant, *Lactuca sativa* L.

1. INTRODUCTION

Increasing agricultural production leads the higher using fertilizers to ensure the need for plant nutrients. Fertilizer is a material that is supplied to plants to support plant growth and increase plant production[1]. The number of nutrients contained in fertilizers is the main factor for assessing the quality and nutrient content in fertilizers.

Indonesia is a tropical country that has the Andisol soil order which is potential to be used for the cultivation of plant commodities. The definition of Andisol based on the classification system in [2] is black or dark brown soil, crumb structure, high organic matter content, and slippery

(smeary). The subsoil is brown to yellowish brown, medium texture, porous, weak boiling, and clay accumulation is often found in the lower layer. This soil order are only found in non-coherent volcanic materials, at an altitude of 750 to 3,000 m above sea level (m asl), are found in wet tropical climates with rainfall between 2,500-7,000 mm years, spread across the islands of Sumatra, Java, Bali, Lombok, Flores, North Maluku and North Sulawesi with a total spread of 5,395 hectares, and especially Andisol has good agricultural potential, one of which is managed for the cultivation of lettuce (*Lactuca sativa* L.). The Andisol Order dominates the area of vegetable plantations in Lembang - Bandung, West Java and Lettuce cultivation has been widely practiced in that area.

Lettuce (*Lactuca sativa* L.) is an annual leaf vegetable grows optimally in the highlands, optimal growth in land that contains a lot of humus, sand or mud with a soil pH of 5-6.5 [2]. Excessive use of synthetic fertilizers, especially nitrogen, have been widely used, and leads the negative impact, i.e. spur the overhaul of soil organic matter and result in a decrease in the C-organic content. Current conditions indicate that the carbon content of both dry and wetlands has decreased drastically and is included in the low-very low (<1.5%) category. Recent studies show that 90% of the 70 million ha of agricultural land has been significantly degraded, and has even been categorized as sick and fatigue soils [3]. One of the efforts to restore soil fertility which is environmentally friendly is through the application of biological fertilizers. The application of biological fertilizers can increase plant growth because biological fertilizers can increase nutrient availability and produce growth stimulating hormone (PGPR). Plant Growth-Promoting Rhizobacteria (PGPR) are compounds produced by a number of nutrient-providing bacteria that live in the root rhizosphere [4]. Bacteria in the rhizosphere play an important role in increasing the availability of nutrients and maintaining the cycle of macro nutrients such as N and P. Inoculation of biological fertilizers that can potentially be used is a mixed inoculant or in the form of a consortium consisting of nitrogen fixing bacteria and phosphate solubilizing bacteria (PSB). The inoculant consortium application is expected to increase the production of lettuce in Andisol.

The potential microbial consortium as a biofertilizer inoculant is the non-symbiotic group of N-fixing bacteria. The genus which could potentially be used in bio-fertilizer formulations is *Azotobacter* [4]. *Azotobacter* spp. are a species of rhizobacteria as an N-fixing biological agent that converts dinitrogen into ammonium [5]. *Azotobacter* is a genus of free-living diazotrophic bacteria, mainly found in neutral to alkaline/ alkaline soils, aquatic environments, and in various types of plants. generally they growth and developed by colonizing plant roots and being in Rhizosphere soil, non-rhizosphere soil, leaf surface, and water, including gram-negative bacteria in the form of stems or cocci and can grow at an optimum temperature of 20-30°C [6]. These bacteria are heterotrophs that use organic compounds as an energy source and thrive in aerobic conditions [7]. There are four important species of *Azotobacter* including *A.chroococcum*, *A.agilis*, *A.paspali* and *A.vinelandii*. Genetically, *Azotobacter* spp. have a higher amount of DNA than other types of bacteria and has a genome size that is unique to other prokaryotes [8]. This genus also included in Plant growth promoting rhizobacteria, because it is able to produce secondary metabolites known as phytohormones such as auxin, indole acetic acid, gibberellin and other growth regulating compounds that are beneficial for plant growth [9].

Another important microbial group that can play a role in supporting the activities of *Azotobacter* spp. followed by the increase in plant growth were the phosphate solubilizing bacteria (SPB) group. The phosphate-solubilizing bacteria (PSB) are plant growth promoting rhizobacteria (PGPR) that can solubilize insoluble P and release phosphate (PO_4^-) into the soil solution where they were absorbed by plant roots [10]. The various isolates of indigenous PSB are present in the soil and associated with the rhizosphere in various types of plants [11]. The ability to phosphate solubilizing and different enzyme activities are in varying capacity for each species [12]. Screening and capacity bio-assaying of PSB isolates are needed to obtain the superior traits in solubilizing the fixed P, mineralize the organic P and produce plant growth promoting substances, such as indole acetic acid (IAA), gibberellin and other organic acids ([13].

The characteristics of the two microbial groups as described above illustrate that the application of the two groups of microbes in the form of a consortium inoculant is an appropriate strategy to be applied to increase productivity and quality of Lettuce plants in the Andisol soil order. However, the viability of the inoculant, the effectiveness of microbes in the consortium's condition still needs to be investigated more deeply to obtain its potential and effectiveness as a superior biofertilizer. This research was focused on investigation of the effectiveness of consortium inoculation (*Azotobacter* spp. and phosphate solubilizing bacteria) combined with NPK dosage variations on *Azotobacter* spp. density in rhizosphere, N uptake, N content and lettuce fresh biomass.

2. MATERIALS AND METHODS

2.1. The experimental design

The research was carried out on the land of the Center for Agricultural Training located in Kayuambon Village, Lembang District, Bandung Regency, West Java with an altitude of 2,084 meters above the surface. Consortium inoculants are obtained and produced in the Laboratory of Soil Biology, with a microbial composition consisting of *Azotobacter chroococcum*, *Azotobacter vinelandii*, and Phosphate Solubilizing Bacteria (*Pseudomonas* sp.). Each bacterial density used was 10^8 CFU mL^{-1} in liquid culture media. The experimental design used was a randomized block design (RBD) which consisted of seven fertilization dose treatments that were repeated four times. The treatment consists of:

- A = control (without fertilizer)
- B = 100% recommended dose of N, P, K
- C = Consortium inoculant 10 L ha^{-1}
- D = Consortium inoculant 10 L ha^{-1} + 100% NPK
- E = Consortium inoculant 10 L ha^{-1} + 75% N, P, K
- F = Consortium inoculant 10 L ha^{-1} + 50% N, P, K
- G = Consortium inoculant 10 L ha^{-1} + 25% N, P, K

2.1. Land Preparation and Lettuce Nursery

The research location is located in Lembang, Bandung, West Java. The land of the Andisol order is cleared of remaining plants or existing grass, then land is cultivated. The top soil layer is processed to a depth of 20-30 cm, then the soil is mapped with an area of 1.5 mx 2 m totaling 28 plots, followed by making arcs between plots with a width of 40 cm and making planting holes by means of drilling with a distance of 20 cm x 25 cm. Silver black plastic mulch is also used in research to suppress weed growth in research fields. Lettuce seeds are sown in a plastic container size of 40 cm x 30 cm and its height of 5 cm which contains the seeding medium. The seeds were sown on the surface of the seeding medium in the form of a mixture of soil and cow manure a ratio of 1: 1 and a media height of 5 cm. Distribution of seed grooves at a distance of 10 cm. The 15 days old seedlings were used for the experimental.

Inoculation of the consortium inoculant on the experimental soil was carried out using the soil inoculation method in a mixture of cow dung compost. The application of the consortium inoculant was carried out by immersing as much as 10 l / h (3 ml / plot) into the experimental soil and stirring it homogeneously to a depth of 20 cm. Subsequently incubated for 7 days and then NPK application according to the treatment was carried out when the lettuce seedlings were planted. Lettuce seeds planted are 15day old seedlings. The land maintenance is carried out every day until the harvest phase, by watering it to maintain moisture and water adequacy.



FIG. 1. EXPERIMENT LAYOUT OF EACH TREATMENT ON LETTUCE PLANTS

3. RESULT AND DISCUSSION

3.1. *Azotobacter* spp. due to the Consortium's microbial inoculation

The results of observations and statistical analysis of the treatment of inoculant consortium combined with N, P, K showed a significant effect on the population of *Azotobacter* spp. (Table 1.).

Table 1. Effect of Consortium Biofertilizer combined with Fertilizer N, P, K on the population of *Azotobacter* spp.

Treatments	Population of <i>Azotobacter</i> spp. 10 ⁵ CFU g ⁻¹
A Control (without fertilizer)	7,28 a
B 100% recommended dose of N, P, K	5,92 a
C Consortium inoculant 10 L ha ⁻¹	13,77 ab
D Consortium inoculant 10 L ha ^{-1b} + 100% N, P, K	9,84 a
E Consortium inoculant 10 L ha ⁻¹ + 75% N, P, K	13,64 ab
F Consortium inoculant 10 L ha ⁻¹ + 50% N, P, K	18,73 b
G Consortium inoculant 10 L ha ⁻¹ + 25% N, P, K	12,4 ab

Note: The number followed by the same letter means the treatment has no significant effect on the response according to Duncan's multiple range test at the 5% significance level.

The results of the preliminary analysis showed that the total population of *Azotobacter* spp. indigenous of the experimental soil was 1.2 x 10⁵ CFU g⁻¹. The investigation results revealed that the population of *Azotobacter* spp. after application of each treatment combination of consortium with N, P, K increased to 7.28 x10⁵ - 18.73 x 10⁵ CFU g⁻¹. The population of *Azotobacter* spp. in the consortium treatment of 10 L ha⁻¹ + 50% doses of N, P, K showed significantly different from the control treatment, and was the highest population compared to other treatments. Population of *Azotobacter* spp. was increasing 93.5% compared with increasing population density before treatment condition. This indicated that consortium inoculant combined with N, P, K fertilizers supported the *Azotobacter* spp. population. However, an interesting thing happened, it was found that with the application of NPK 100%, the high NPK dose, tend decrease *Azotobacter* spp. population that to be lower than control treatment. This indicated that high doses of NPK tend to consistently suppress the growth of *Azotobacter* spp. This phenomenon can explain that by an excess of NPK content in soil can interfere with the development and population growth of *Azotobacter* spp. Because basically *Azotobacter* spp in the rhizosphere area actually has obtained an adequate source of nutrition for its growth and development comes from root exudates and they capable to fix N which lead to provide N for them self. So that with the presence of a high dose of NPK in the rhizosphere, it can actually be an inhibiting factor for growth for *Azotobacter* spp. The rhizosphere environment in this research is a suitable habitat for growing microbes, because in the rhizosphere zone, plant roots exudates which include sugars, amino acids and organic acids will be released and become stimulants that can increase bacterial activity and population. And root exudates are the most important source of nutrition for bacteria in the rhizosphere.

3.2. Nitrogen Uptake and N Content in Lettuce Plants

The results of statistical analysis on the observations of Nitrogen Uptake and N Content in Lettuce are presented in Table 2. The application of consortium inoculant combined with N, P, K can increase N uptake not significantly, and also the nitrogen content of lettuce plants does not increase significantly. The control treatment resulted in nitrogen uptake of 15.6 mg plant⁻¹. However, in general each consortium inoculant treatment can increase N absorption, although it does not increase insignificantly. The relatively high absorption of N nutrients was found in the inoculant treatment of the consortium 10 L ha⁻¹ + 25% N, P, K, which was 29.25 mg / plant. This shows that the application of consortium inoculant contributed in increasing the uptake of N by plants, however the increasing was not significant. The insignificant performance of this inoculant effect indicated that nutrient saturation occurs in the soil. From the experimental soil analysis, the available N and P are classified as high. The saturation condition of N and P nutrients resulted in decreased efficiency of N uptake and there was no increasing in plant productivity. Nutrient saturation resulted in the treatment having no effect on N uptake.

Table 2. Effect of Combination of inoculant Consortium with N, P, K Fertilizers on Nitrogen Uptake and Nitrogen Content of Lettuce Plants

Treatments	Nitrogen Uptake (mg plant ⁻¹)	Plant Nitrogen Content (%)
A Control (without fertilizer)	15,62	4,97
B 100% recommended dose of N, P, K	22,84	3,98
C Consortium inoculant 10 L ha ⁻¹	20,93	3,95
D Consortium inoculant 10 L ha ^{-1b} + 100% N, P, K	23,76	3,75
E Consortium inoculant 10 L ha ⁻¹ + 75% N, P, K	20,91	3,69
F Consortium inoculant 10 L ha ⁻¹ + 50% N, P, K	24,98	3,68
G Consortium inoculant 10 L ha ⁻¹ + 25% N, P, K	29,25	3,65

Note: The number followed by the same letter means the treatment has no significant effect on the response according to Duncan's multiple range test at the 5% significance level.

Another factor that influences the effectiveness of the inoculant is insufficient soil moisture at the time of research. Because it was noted that the climate when the research took place was in the dry season with high evaporation. So that the adequacy of water will affect the activity of inoculants in the soil, therefore water is an important factor for photosynthesis and the rate of nutrient uptake by plants. Water plays a role in nutrient translocation and photosynthesis [14].

The nutrients needed by plants are absorbed in the form of ions, 98.8% nitrogen is absorbed through the mass flow mechanism. There is a movement of nutrients towards the root surface along with the movement of water masses and nutrient solubility is influenced by groundwater conditions. For plants that have a water deficit, the translocation of photosynthate to the target organs will be inhibited, as evidenced by the results of this study, whereas the value of N nutrient uptake increased, but not significantly.

The results showed that the nitrogen content in the plants was sufficient, ranging from 3.68 to 4.97%. The N concentration of lettuce is sufficient if the value reaches 2.5-4.5% and insufficient level if the value is less than 2.5%. The N content in lettuce (*Lactuca sativa* L.) ranges from 3-3.5%, so the N content of plants is low, if the N content in plants ranges from 3.5-4.5% then the N content of the plant is classified as sufficient, and the N content of the plant shows a number greater than 4.5%, it includes the relatively high N content of plants. The N content in the lettuce used in this study was categorized as high enough.

3.3. Lettuce Crops Yield

Observation and statistical analysis on lettuce crop yields are shown in Table 3. Statistically, each treatment did not show any significant difference but in value showed that the percentage of fresh lettuce weight was increasing, higher than the control treatment.

Table 3. Effect of Consortium inoculant Combined with N, P, K Fertilizers on Lettuce Plant Yield

Treatment	Lettuce Weight (g) plant ⁻¹	fresh Lettuce fresh weight gain (%)
A Control (without fertilizer)	72,20	-
B 100% recommended dose of N, P, K	94,15	30.40
C Consortium inoculant 10 L ha ⁻¹	84,85	17.52
D Consortium inoculant 10 L ha ^{-1b} + 100% N, P, K	97,80	35.46
E Consortium inoculant 10 L ha ⁻¹ + 75% N, P, K	88,90	23.13
F Consortium inoculant 10 L ha ⁻¹ + 50% N, P, K	107,75	49.24

G	Consortium inoculant 10 L ha ⁻¹ + 25% N, P, K	102,55	42.04
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Note: The number followed by the same letter means the treatment has no significant effect on the response according to Duncan's multiple range test at the 5% significance level.

Lettuce crop yields are presented in Table 3. The application of consortium inoculant combined with N, P, K fertilizers can increase the yield of lettuce between 17.52 - 49.24% fresh weight. Fresh weight in control treatment is equivalent to 9.1ton ha⁻¹. This figure is in accordance with the description of the Grand Rapid variety of lettuce which has a production of 3-9 tones ha¹. Consortium inoculant treatment combined with N, P, K fertilizers in all treatments resulted in an increasing in fresh weight compared to the control treatment. This indicates that the inoculant of the *Azotobacter* spp. and phosphate solubilizing bacteria used contributed in facilitating the availability of N and P elements and sufficiency phytohormones for the growth of lettuce plant. The role of *Azotobacter* spp. is to fix N₂ from the air and convert to ammonia which is mediated by the nitrogenase enzyme, the amount of N₂ is converted into ammonia which is then used by plants. The results of the observations also showed that the application of NPK 50% combined with the consortium inoculant increased the highest fresh weight of lettuce by 49.24%, while the application of 100% NPK combined with the consortium inoculant gave a lower increase in fresh weight of lettuce, which was 35.46%, compared with the other treatment. This shows that high NPK in the soil will inhibit the effectiveness of *Azotobacter* spp. Nitrogen in high amounts in the soil increases the uptake of N by roots, but inhibits transcription of the nitrogenase enzyme, so that the nitrogenase activity of *Azotobacter* spp. becomes inhibited by Nitrogen excess in soil, as a result in this research revealed that the effectiveness and contribution of *Azotobacter* spp. to N uptake level by plant and lettuce fresh weight was increasing but nonsignificant. .

4. CONCLUSION

The application of consortium inoculant combined with NPK 50% increased *Azotobacter* spp. population during lettuce growth, the population was significantly higher compared to the control treatment and 100 NPK treatment. While the highest N absorption value was obtained as a result of the Consortium's inoculant treatment combined with 25% NPK. And the Consortium inoculant combined with 50% NPK treatment was increasing lettuce fresh weight in highest level, and the enhancement of fresh weight yield of lettuce was 49.24% compared to the control treatment. While 100% NPK treatment increased the fresh lettuce weight by 30%. The application of the consortium inoculant can reduce NPK requirements by 50%.

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