Journal Bionature
p-ISSN 1411-4720
e-ISSN 2654-5160
Vol. 23. No. 1. April 2023, p. 188-198
http://ojs.unm.ac.id/bionature

Vegetative Growth Response of Corn (*Zea mays*) to Actinomycetes, Azospirillum, and Azotobacter Isolated from the Rhizosphere of Neem (*Azadirachta indica*)

Yusminah Hala[1]*, A. Irma Suryan[1], Octavia Cisiliana Devi[1]

[1]Universitas Negeri Makassar, Indonesia

Correspondence E-mail: yushala12@gmail.com

Received: 17 - 02 - 2023 Published: 30 - 04 - 2023

Abstract

This research is an experimental study that aims to determine the effect of the application of N2-fixing bacteria Actinomycetes, Azospirillum, and Azotobacter on the vegetative growth parameters of corn plants and on the nitrogen content of corn plants. Parameters for measuring the growth of corn plants include: formation rate of radicle length, plant height, number of leaves, leaf length, fresh weight of plants, fresh weight of plant roots, dry weight of plants, dry weight of plant roots, total N content in plants, as well as physical and chemical qualities of the ground. Experiments on the application of N2 -fixing bacteria on corn plants on a laboratory scale were carried out to determine the in vitro effect of microbes on corn seeds. The pot test was carried out to determine the effect of N2-fixing bacteria on the vegetative growth of corn plants in vivo. Data were analyzed using ANOVA with Duncan's test. The results showed that corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter showed a significant different growth from corn plants without nitrogen fixing bacteria, namely formation rate of radicle length, plant height, number of leaves, leaf length, fresh weight, dry weight, total N content (%) of crops, and soil physical and chemical quality. It can be concluded that the inoculation of nitrogen-fixing bacteria in corn plants has a significant effect on plant vegetative growth parameters and plant nitrogen content..

Keywords: Nitrogen fixing bacteria, Actinomycetes, Azospirillum, Azotobacter, Plant vegetative growth.

INTRODUCTION

Indonesia is one of the countries with megadiversity and a high level of plant diversity, making it beneficial to increase the efficiency and production of food crops. As the population increases, the need for food production also rises. One of the efforts to increase food production is through the growth of the industrial sector, which encourages the emergence of modern agricultural systems with a high dependence on chemical fertilizers. However, increasingly advanced technological developments are starting to make people aware of the adverse effects of excessive chemical fertilizer use in agriculture. Therefore, it is necessary to continue developing alternative agricultural practices that are environmentally sound and sustainable. Utilization of microbes as potential biofertilizers that are environmentally friendly is a solution to overcome the adverse effects of excessive chemical fertilizer use. According to **Lekatompessy & Nurjanah (2019)**, the combination of biological and chemical fertilizers can help reduce dependence on chemical fertilizers gradually. Biofertilizers, which contain live microbes, can promote plant growth when applied to seeds, soil, or the surface of plant roots, and can also be used as biocontrol agents that are safe for the environment. Microbes that are often reported as biological fertilizers include rhizobium, which fixes air N_2 through a symbiotic process with legumes, as well as Azospirillum and Azotobacter, which fix air N_2 without having to be in a symbiotic relationship with plants.

Azotobacter has a complete mechanism as a potential microbe, which involves providing nitrogen, phytohormones, and antifungals. Azotobacter PGPR promotes plant growth through nitrogen fixation, production of phytohormones, and exopolysaccharides, which increase plant tolerance to drought and resistance to antimicrobials. Several studies have reported that Azotobacter also plays a role as a plant protector from pathogens because it produces anti-fungal compounds. The increase in plant height by Azotobacter is due to one or both of these mechanisms: acquisition of available nitrogen resulting from nitrogen fixation and increased levels of plant phytohormones derived from Azotobacter-produced phytohormones. Innoculation of plant with Azotobacter is more effective for increasing plant height because nitrogen and phytohormones that have been formed during the production of biological fertilizers are absorbed through leaf stomata and can enter the plant metabolic system more quickly for cell formation and enlargement during the vegetative phase (Hindersah et al., 2018).

Bacteria of *Azospirillum sp.* are one of the microorganisms that can fix nitrogen from the air. They are microaerobic and are able to associate with higher plants. In the atmospheric nitrogen fixation process, *Azospirillum sp*. binds free nitrogen and converts it into a network, which then provides some of the air nitrogen as available nitrogen to higher plants through weathering, ammonification, and nitrification processes (Erfin et al., 2016).

Actinomycetes are also bacteria that have many abilities, including dissolving phosphate, antagonizing plant pathogenic fungi, and promoting plant growth. They are also able to suppress excessive amounts of ethylene in plants. Actinomycetes can be used as a biofertilizer agent because they can increase plant growth and development, as well as exhibit antifungal abilities. Additionally, actinomycetes act as a plant growth booster by producing auxins, such as Indole Acetic Acid (IAA), gibberellins, and cytokinins (Anggraini. et al., 2018). Actinomycetes include rod-shaped, gram-positive, anaerobic, or facultative bacteria. They have a structure in the form of hyphae or mycelia, as found in fungi, and have conidia on upright hyphae. Actinomycetes reproduce by cell division, are susceptible to penicillin, but are resistant to antifungal agents **(Wulandari & Sulistyani, 2016)**.

Early research on Azotobacter, Azospirillum, and Actinomycetes bacteria has shown that they can be used as biofertilizers or biological fertilizers for upland rice (*Oryza sativa*). The results of this study indicated that upland rice plants inoculated with these bacteria showed significantly different growth compared to upland rice plants without nitrogen-fixing bacteria treatment, including speed of radicle formation and radicle length, plant height, number of tillers, fresh weight, dry weight, and total nitrogen content (%) of plants. It can be concluded that the inoculation of nitrogen-fixing bacteria to upland rice plants has a significant effect on plant vegetative growth parameters and plant nitrogen content (Gunawan, et al., 2021).

These nitrogen-fixing bacteria can be found and isolated from the plant rhizosphere. Information about the presence of nitrogen-fixing bacteria in the plant rhizosphere that can live in dry areas and a small amount of nutrients is rarely studied. One of the plants that can grow well in dry and barren soil conditions is the neem plant (*Azadirachta indica*). The neem plant is classified as a shrub plant which was first discovered in the Hindustan area, in Madhya Pradesh, India. Neem came or spread to Indonesia, estimated since 1500 BC. This plant grows in West Java, East Java and Madura at an altitude of up to 300 m above sea level. This plant grows in the tropics, in the lowlands, and grows in arid places periodically which are often found on the side of the road as a shady tree or in a sunny forest (Hala, 2020). Since 2001, the government has been promoting the Gema Palagung program (**Ge**rakan **Ma**ndiri **Pa**di, Kede**la**i, dan Ja**gung**). This program is quite effective, as evidenced by the increase in the amount of domestic corn production. However, it still unable to meet domestic demand. Hence, corn is still being imported. This condition may indicate that efforts to increase corn production still need to be carried out. Corn plants also require nutrients for survival. These nutrients consist of C, H, O, N, P, K, Ca, Mg, S, Fe, B, Cu, Zn, Mo, Mn, Cl, Si, Na, and Co. These nitrogen-fixing bacteria can be found and isolated from the plant rhizosphere. Information about the presence of nitrogen-fixing bacteria that can live in dry areas in the plant rhizosphere, as well as the study of the small amount of nutrients available, is rare. One of the plants that can grow well in dry and barren soil conditions is the neem plant (*Azadirachta indica*), which is classified as a shrub plant and was first discovered in the Hindustan area of Madhya Pradesh, India. Neem was estimated to have arrived in Indonesia around 1500 BC and grows in West Java, East Java, and Madura at an altitude of up to 300 m above sea level. The neem plant grows in the tropics, in the lowlands, and periodically in arid places, often found on the side of the road as a shady tree or in a sunny forest (Hala, 2020). Since 2001, the government has been promoting the Gema Palagung program (Gerakan Mandiri Padi, Kedelai, dan Jagung), which has been effective in increasing domestic corn production, but has not met domestic demand, indicating that efforts to increase corn production need to be continued.

Corn plants require nutrients that include C, H, O, N, P, K, Ca, Mg, S, Fe, B, Cu, Zn, Mo, Mn, Cl, Si, Na, and Co. These nutrients come from the weathering of rocks in the soil. However, the ability of the soil to provide nutrients for plants is very limited because the number of microorganisms involved in the weathering process differs between types and layers of soil. Therefore, biological fertilization is one way to provide the nutrients needed by plants. Biological fertilization can increase corn yields both qualitatively and quantitatively **(Ekowati & Mochamad, 2011)**.

Based on the description above, this research was carried out with the aim of minimizing the use of chemical fertilizers by designing the application of biological fertilizers (Azotobacter, Azospirillum, and Actynomycetes) from the neem plant (*Azadirachta indica*) to corn (*Zea mays*).

RESEARCH METHODS

The tools used include incubators, analytical balances, autoclaves, hot plates and stirrers, micro tubes, test tubes, petri dishes, Erlenmeyer, beakers, spreader rods, round loops, stirring rods, spirit lamps, centrifuges, Laminar Air Flow, spectrophotometers , digital calipers, rulers, sprayer bottles, material scales.

The materials used in this study were Actinomycetes, Azotobacter, and Azospirilum bacteria isolated from the rhizosphere of neem (*Azadirachta indica*), corn (*Zea mays*) seeds, Soluble Sodium Agar (SNA) Media, Nitrogen Free Brothymol Blue (NFB) Media, Alcohol, Spirits, Aquades, Soil, Sodium hypochlorite, Polybag, Plastic wrap, Aluminum foil, N2 fixing bacterial isolate culture.

The research was conducted at the Biology Laboratory of the Department of Biology FMIPA UNM and the Pot Test was carried out on a greenhouse scale. The research was conducted for 11 months from November 2020 - December 2021. Research procedures, Purification and Propagation of Nitrogen-fixing Bacteria; Compatibility test of N2 fixing bacteria with corn (*Zea mays*) seeds; Test the ability of garden scale N2 fixing bacteria on corn growth; Planting Media Processing; Maintenance of Corn Plants; Measurement of Vegetative Parameters of Corn Plants in the form of height of corn plants (cm), number of leaves, Biomass (dry weight) of roots and tops of corn plants (grams) (after harvest), and wet weight of roots and tops of corn plants (grams) (after harvest); Testing of Total N Content in Corn Plants and the Rhizosphere; Bacterial Population Measurement; Analysis of Soil Physical and Chemical Properties. The research design used was a randomized block design (RBD). The data obtained were analyzed using ANOVA and Duncan's test with a level of 5%.

RESULTS AND DISCUSSION

a. Results Effect of Inoculation of Actinomycetes, Azospirillum, and Azotobacter bacteria on the speed of radicle formation of corn plant seeds

Inoculation of Actinomycetes, Azospirillum and Azotobacter bacteria in corn seeds affected the speed of radicle formation and radicle length. The fastest radicle formation was seen in seeds treated with Azospirillum inoculation. Calculation of the speed of radicle formation and radicle length was carried out on the 5th day after inoculation. The results of radicle length measurement can be seen in Figure 1.

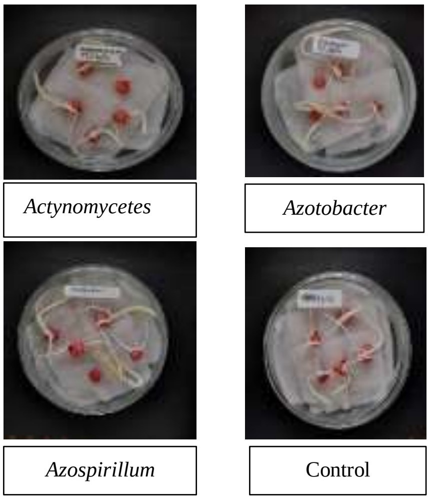

Figure 1. Corn seed radicle length on the 5th day after inoculation (DAI) of Actinomycetes, Azospirillum, and Azotobacter.

The speed of radicle formation in corn seeds can be seen on the 5th day after inoculation. The radicle on the 5th day after inoculation (DAI) was the longest in the treatment of *Azospirillum sp.* Table 1 shows that Azospirillum bacteria can stimulate the rate of radicle formation faster than other treatments. Duncan's further test showed that Actynomycetes and Azotobacter's treatment of Azospirillum was significantly different. Azospirillum stimulated the growth of corn seed radicle more quickly than Actynomycetes and Azotobacter, while Actynomycetes and Azotobacter were not significantly different. But the treatment of Nitrogen fixing bacteria (BPN) with the control was significantly different.

Table 1. Average radicle length of corn seeds after inoculation of Actinomycetes, Azospirillum, and Azotobacter bacteria

Treatment	Corn seed radicle length(mm) *5 DAI**			Mean
	R-I	R-II	R-III	
Control	15	10	5	**10,0^{a}**
Actynomycetes	20	45	30	**30,0^{b}**
Azospirillum	50	40	40	**43,3^{c}**
Azotobacter	30	20	30	**26,7^{b}**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Inoculation; R: Repetition*

b. Effect of Actinomycetes, Azospirillum and Azotobacter bacterial inoculation on corn plant height

The results of measuring the height of corn plants at 50 DAP (days after planting) showed that the height of corn plants treated with nitrogen-fixing bacteria was significantly different from the control. Treatment with Azotobacter bacteria gave the highest corn plant height compared to the 2 treatments with Actinomycetes bacteria and Azospirillum bacteria (Table 2).

The results of Duncan's test showed that the nitrogen fixing bacteria Actynomycetes, Azospirillum, and Azotobacter were not significantly different, but gave results that were significantly different from the control.

Table 2. Effect of Actinomycetes, Azospirillum, and Azotobacter inoculation on corn plant height.

Treatment	Plant Height (cm) 50 DAP*			Mean
	R-I	**R-II**	**R-III**	
Control	91	128	125	**115.00^{a}**
Actynomycetes	140	145	141	**142.00^{b}**
Azospirillum	147	148	140	**145.00^{b}**
Azotobacter	172	161	141	**158.00^{b}**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

c. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on the number of corn leaves

The leaves of corn plants began to form on the 14th day after planting. Measurement of the number of leaves was carried out at the age of 50 DAP. The measurement result of the number of leaves of corn plants at the age of 50 DAP showed that the highest value was in the treatment of Azotobacter bacteria. This value was also significantly different from the treatment of Actinomycetes and Azospirillum bacteria. Corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria had a significantly different number of leaves from the control (Table 3). The increase in the number of leaves of corn plants inoculated with Actinomycetes, Azospirillum, and Azotobacter bacteria at 50 DAP can be seen in Table 3.

Table 3. Effect of Actinomycetes, Azospirillum and Azotobacter bacterial inoculation on the number of leaves of corn plants.

Treatment	Number of leaves 50 DAP*			Mean
	R-I	**R-II**	**R-III**	
Control	8	9	9	**8.67^{a}**
Actynomycetes	10	11	11	**10.67^{b}**
Azospirillum	11	11	11	**11.00^{b}**
Azotobacter	13	12	12	**12.33^{c}**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

d. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on leaf length of corn plants

Long leaves of corn plants began to form 2 weeks after planting (WAP). Measurement of leaf length was carried out at the age of 50 DAP. The measurement result of corn leaf length at 50 DAP showed that the highest value was in the treatment of Azotobacter bacteria. This value was also significantly different from the treatment of Actinomycetes and Azospirillum bacteria. Corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria had leaf lengths that were significantly different from the control (Table 4). The increase in leaf length of corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria at the age of 50 DAP can be seen in Table 4.

Table 4. Effect of inoculation of Actinomycetes, Azospirillum and Azotobacter long-leaf corn plants.

Treatment	Leaf Length (cm)			Mean
	50 DAP*			
	R-I	**R-II**	**R-III**	
Control	55	60	62	**59.00[a]**
Actynomycetes	75	75	81	**77.00[b]**
Azospirillum	76	76	83	**78.33[b]**
Azotobacter	101	93	82	**92.00[c]**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

e. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on root wet weight

The wet weight of corn plant roots measured at 50 days after planting was highest in corn plants treated with Azotobacter bacteria, and the wet weight of corn plant roots was also significantly different from the two other bacterial treatments, namely Actinomycetes bacteria and Azospirillum bacteria, but in the Azospirillum and Actynomycetes are not significantly different. Corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria had root fresh weight of corn plants that were significantly different from the control (Table 5). Comparison of the wet weight of corn plant roots inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria at 50 DAP can be seen in Figure 5.

Table 5. Effect of Inoculation of Actinomycetes bacteria on the wet weight of corn plant roots

Treatment	Root Wet Weight (gr)			Mean
	50 DAP*			
	R-I	**R-II**	**R-III**	
Control	2,57	3,55	4,22	**3.00[a]**
Actynomycetes	13,88	7,24	14,19	**10.00[b]**
Azospirillum	13,43	9,44	8,65	**10.67[b]**
Azotobacter	12,48	12,14	8,97	**11.33[c]**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

f. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on plant wet weight

The wet weight of the corn plants measured at 50 DAP was highest in the corn plants treated with Azotobacter bacteria, and the wet weight of these corn plants was also significantly different from the two other bacterial treatments, namely Actinomycetes bacteria and Azospirillum bacteria. However, the wet weight of the Azospirillum and Actynomycetes were not significantly different from controls. Corn plants inoculated with Azotobacter had a wet weight of corn plants that were significantly different from the control (Table 6). Comparison of the wet weight of corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria at 50 DAP can be seen in Figure 6.

Table 6. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on wet weight of corn plants

Treatment	Plant Wet Weight (gr)			Mean
	50 DAP			
	R-I	**R-II**	**R-III**	
Control	44,75	10,50	11,01	**25,67[a]**
Actynomycetes	36,26	87,35	63,83	**62,00[b]**
Azospirillum	51,49	49,09	22,30	**40,67[b]**
Azotobacter	95,81	53,52	56,31	**68,00[c]**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

g. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on root dry weight

The dry weight of corn plant roots which were weighed at 50 DAP was highest in corn plants treated with Azotobacter bacteria, and the dry weight of corn plants was also significantly different from the two other bacterial treatments, namely Actinomycetes bacteria and Azospirillum bacteria. However, the root dry weight of the Azospirillum and Actynomycetes treatments were not significantly different from the control treatment. Corn plants inoculated with Azotobacter bacteria had significantly different root dry weight from the control (Table 7). Comparison of root dry weight of corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria at 50 DAP can be seen in Figure 7.

Table 7. Effect of inoculation of Actinomycetes, Azospirillum and Azotobacter bacteria on root dry weight of corn plant roots

Treatment	Root Dry Weight (gr) 50 DAP			Mean
	R-I	**R-II**	**R-III**	
Control	1,02	1,02	1,02	**1,02^{a}**
Actynomycetes	4,62	1,86	4,55	**3,00^{ab}**
Azospirillum	4,73	3,84	3,07	**3,33^{ab}**
Azotobacter	4,24	6,39	3,91	**4,33^{c}**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

h. Effect of inoculation of Actinomycetes, Azospirillum and Azotobacter bacteria on top dry weight of corn plants

The top dry weight of the corn plant which was weighed at the age of 50 DAP was highest in the corn plant treated with Azotobacter bacteria, but the top dry weight of the plant was not significantly different from the other two bacterial treatments, namely Actinomycetes bacteria and Azospirillum bacteria. Corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria had significantly different top dry weight from the control (Table 8). Comparison of the top dry weight of corn plants inoculated with Actinomycetes, Azospirillum and Azotobacter bacteria at the age of 50 DAP can be seen in Figure 8.

Table 8. Effect of inoculation of Actinomycetes, Azospirillum and Azotobacter bacteria on the top dry weight of corn plant

Treatment	Top dry weight of plant (gr) 50 DAP			Mean
	R-I	**R-II**	**R-III**	
Control	8,31	7,19	7,18	**7,33^{a}**
Actynomycetes	22,01	16,98	20,55	**19,33^{b}**
Azospirillum	25,41	20,11	22,29	**20,67^{b}**
Azotobacter	36,16	20,84	25,97	**27,00^{b}**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

1. Total N Content in Corn Plants

The total N content in corn plants is one of the parameters of the N content in corn plant tissues. Parameters of total N content in corn plants were analyzed from the top of the corn plant to the roots of the corn plant. To measure the effect of Actinomycetes, Azospirillum and

Azotobacter bacterial inoculation on total N corn plants, the total N in corn plant tissue was calculated at the age of 50 DAP. The highest DAP was in plants given the Azotobacter bacterial treatment, but the total N in corn plant tissue was not significantly different from the 2 bacterial treatments, namely Actynomycetes and Azospirillum. Corn plants inoculated with the Actynomycetes, Azospirillum, and Azotobacter bacterial treatments had significantly different total N from the control (Table 9). Comparison of the average total N in corn plant tissue inoculated with bacteria at 50 DAP is shown in the following table.

Table 9. Effect of Actinomycetes, Azospirillum and Azotobacter inoculation on total N in corn plants

Treatment	Total N of maize plant tissue (%) 50 DAP			Mean
	R-I	**R-II**	**R-III**	
Control	1,70	2,07	2,11	**1,96^{a}**
Actynomycetes	2,27	2,00	2,04	**2,09^{b}**
Azospirillum	2,19	2,21	2,16	**2.18^{b}**
Azotobacter	2,29	2,32	2,54	**2,38^{b}**

*Note: The same letters indicate "not significantly different" results based on Duncan's test results with a confidence level of α = 0.05; *Days After Planting; R: Repetition*

2. Analysis of Soil Physical and Chemical Properties

Analysis of the Physical and Chemical Properties of Soil is a supporting factor in the provision of nutrients (fertility) in the soil for the growth of corn plants. Parameter analysis of soil properties and characteristics is one of the conditions for the growth of corn plants. Analysis of the properties and characteristics of this soil is taken only from soils treated with nitrogen-fixing bacteria.

Table 10. Physical and chemical quality of soil

Treatment	**Texture**			**pH**		**Organic Material (N)**
	Sand(%)	**Dust(%)**	**Clay%)**	**H_2O**	**KCl**	
Control (-)	**36**	**37**	**27**	**4,43**	**3,93**	**0,33**
Control (+)	**39**	**39**	**22**	**4,75**	**3,97**	**0,33**
Actynomycetes	**38**	**38**	**24**	**5,10**	**4,03**	**0,32**
Azospirillum	**35**	**40**	**25**	**4,68**	**3,95**	**0,30**
Azotobacter	**39**	**39**	**22**	**4,49**	**3,96**	**0,26**

Treatment of free nitrogen-fixing bacteria on corn plants significantly affected the parameters of plant vegetative growth, even at the stages of seed growth, radicle formation, and plant growth. Parameters of vegetative growth of corn plants consisted of plant height, number of leaves, leaf length, as well as wet and dry weight of the roots and the top of the plant.

Corn plants treated with nitrogen-fixing bacteria namely Actinomycetes, Azospirillum and Azotobacter gave the highest average parameter measurement results in corn plants treated with Azotobacter, and the highest radicle elongation in treatment was Azospirillum. Corn plants inoculated with nitrogen fixing bacteria showed that nitrogen fixing bacteria namely Actinomycetes, Azospirillum and Azotobacter had compatibility with corn plants and made a significant contribution to providing the nutrients needed by corn plants. The ability of nitrogen-fixing bacteria to produce growth regulators (PGR) and stimulate root development caused corn plants inoculated with nitrogen-fixing bacteria to have more root biomass than plants without nitrogen-fixing bacteria inoculation. This will cause the corn plants to absorb nutrients far greater than plants without bacterial inoculation treatment (control). Other parameters such as the number of leaves, and the length of the leaves are more maximal if given the treatment of nitrogen fixing bacteria, because the innoculation of nitrogen fixing bacteria gives a positive response to the vegetative growth of corn plants.

The results showed that N-fixing microbes (*Azospirillum sp.*, *Azotobacter sp*.) have a dual ability in fixing free nitrogen from the air as well as stabilizing soil aggregates. The study concluded that several strains isolated and selected for N-fixing microbes (Azotobacter) had dual ability, i.e. to fix nitrogen from the air, as well as to produce growth regulators. Almost all isolates of N-fixing bacteria isolated from acid soils have been shown to be free nitrogen fixers from the air and capable of producing the phytohormones of Indole Acetic Acid (IAA) and Gibrelic Acid (Purnomo et al., 2017). According to Fitriatin et al (2019), the application of the biological fertilizers of the *Azotobacer chroococum* consortium, *Azospirilum sp.* able to increase plant height significantly at doses of NPK fertilizer 25-50% recommendation. This shows that the application of biological fertilizers can increase plant growth and can reduce the need for inorganic fertilizers. This also proves that biological fertilizers in the form of a consortium of phosphate solubilizing microbes and N fixing bacteria can increase plant growth. This is reinforced by the statement of Itelima et al (2018), that biological fertilizers can increase plant growth and land productivity in a sustainable manner. In the total N test results on corn plant tissue, the highest results were obtained by the treatment of *Azotobacter sp.*. Azotobacter bacteria are free nitrogen-fixing bacteria (N_2 from the air) which are capable of producing growth-promoting substances such as gibberellins, cytokinins, and indole acetic acid. Hence, they can stimulate root growth (Nosrati et al., 2014). Some bacteria that can fix N from the air freely or not freely, include Rhizobium, Azotobacter, and Azospirillum bacteria which help convert N_2 from the air into NH_3 using nitrogenase enzymes, then NH3 is converted into glutamine and alanine (Ward & Jensen 2014). Plant height growth is one of the plant responses in producing primary bodies where apical meristem tissue is the main key in producing cells for plants elongated growth. Therefore, the presence of the N element becomes an essential part in increasing plant height growth. The dry weight of a plant is the balance between photosynthesis and respiration. Photosynthesis results in an increase of plant dry weight due to CO_2 uptake, while respiration results in a decrease of dry weight due to CO_2 expenditure (Hala, 2020).

Plant dry weight reflects plant growth and the amount of nutrients absorbed per unit weight of the biomass produced. If the supply of N is sufficient, plant leaves will grow large and expand the surface available for photosynthesis. High nitrogen supply will accelerate the conversion of carbohydrates into proteins and are used to construct cell walls. The higher the water content in the plant, the lower the plant dry matter. One of the factors that affect plant dry matter is the availability of nutrients. Excessive N supply will cause high protoplasm formation, so that plants contain lots of water. Data on the results of the variation in wet weight measurement of corn plants with controls were significantly different in which the administration of *Azotobacter sp.* had an average value of 68.00, while the control treatment had an average value of 25.67. The results of the diversity of dry weight of corn plant tissue showed a significantly different value between *Azotobacter sp.* and the control. The average value of *Azotobacter sp.* is 27.00 while the average value of control is 7.33.

The increase in root dry weight is thought to be due to the role of bacteria as a plant growth promoter, namely the producer of growth hormones. Azotobacter, besides being able to bind N_2 in the air, also produces indole acetic acid (IAA) in an amount that is directly proportional to its population density. Azospirillum also has the ability to produce IAA (Lestari et al., 2007). The content of IAA is useful in stimulating root growth through increasing length or surface area, so that roots are able to bind water and increase wet weight. Bacteria from the genera of Azospirillum, Azotobacter, etc. are able to fix nitrogen, produce growth hormones and protect plants from disease. Application of biofertilizers based on growth-promoting bacteria from the *Bacillus sp.*, *Pseudomonas sp.*, *Azospirillum sp.* and *Azotobacter sp.* has been proven to stimulate the growth and production of rice and maize in greenhouses and fields (Hamim et al., 2008).

Danapriatna (2016), added that among the member of genus Azospirillum, in addition to being able to fix air N, certain isolate of *Azospirillum sp.* also produces growth hormone IAA. The potential of this microbe is used as a biological fertilizer because Azospirillum can increase plant growth, such as nitrogen fixation, phosphate solubilization and production of the phytohormones indole 3-acetic acid (IAA), cytokinins, abscisic acid (ABA), ethylene, gibberellic acid (GA3) and zeatin. Nitrogen (N) is the main component of chlorophyll. The N content of the leaves will remobilize N to the kernel, causing a longer photosynthetic process and helping the plant continuously fill the kernel. Nitrogen (N) is a very important nutrient for plant growth. The total nitrogen content of urea is around 45-46%. High nitrogen content is needed for the initial growth of corn plants (Aina et al., 2020). Soil analysis was conducted to determine the role of nitrogen fixing bacteria on soil quality. Soil samples were taken from soil that was previously treated with bacteria, with soil that had not been treated with bacteria. The results of soil physical and chemical tests on nitrogen-fixing bacteria treatment showed an increase in soil pH. The results of the difference in soil pH without the addition of anchoring bacteria to the type of soil treated with anchoring bacteria did not produce a significantly different pH, this is because the plants are exposed to rainwater. Hence, rainwater which has an acidic pH affects soil quality. Then, for organic matter, namely the N content, the type of soil that has been treated with bacteria, the N content in the soil is very small, this is because the N content in the soil is absorbed by plant tissues, and is lost through washing in rainwater.

CONCLUSION

Based on the study's results, inoculating corn plant (*Zea mays*) seeds with nitrogen-fixing bacteria significantly influenced all of the maize growth parameters. Inoculating corn plants (*Zea mays*) with nitrogen-fixing bacteria resulted in the highest plant nitrogen content.

ACKNOWLEDGEMENT

We would like to thank all those who have helped and contributed to this research.

REFERENCES

Aina, N., Hala, Y., Djawad, Y. A., Iriany, N., Makkulawu, A. T., Inubushi, K., & Jumadi, O. (2020). Response of corn plants (Zea mays L.) to application of zeolite coated urea as nitrogen slow release fertilizer. *In IOP Conference Series: Earth and Environmental Science*, *484*(1), . 012091.

Anggraini., Y. S., Tetty, M. L., & Wahyu, L. (2018). Seleksi Aktinomisetes dalam Menghasilkan Indole Acetic Acid dan Efektivitas Terhadap Perkecambahan Benih Cabai Merah (Capsicum annum L.). *Biospecies* , *11*(2), 115–122.

Danapriatna, N. N. (2016). Penjaringan Azotobacter Sp Dan Azospirillum Sp Dari Ekosistem Lahan Sawah Sebagai Sumber Isolat Pupuk Hayati Penambat Nitrogen. *Jurnal Agrotek Indonesia (Indonesian Journal of Agrotech)*, *1*(2).

Ekowati, D., & Mochamad, N. (2011). Pertumbuhan Tanaman Jagung (Zea Mays, L.) Varietas Bisi-2 Pada Pasir Reject Dan Pasir Asli Di Pantai Trisik Kulonprogo (The Growth of Maize Crop (Zea mays L.) . *BISI-2 Variety on Rejected and Non Rejected Sand at Pantai Trisik Kulon Progo), 18*(3), 220–231.

Erfin, Natsir, S., & Malesi, L. (2016). Identifikasi Bakteri Azospirillum Dan Azotobacter Pada Rhizosfer Asal Komba-Komba (Chromolaena Odorata). *JITRO*, *3*(2).

Fitriatin, B. N., Nabila, M. E., Turmuktini, T., Sofyan, E. T., & Yuniarti, A. (2019). Kontribusi Pupuk Hayati dan Pupuk Anorganik untuk Meningkatkan Pertumbuhan dan Hasil Tanaman Padi Gogo pada Ultisols Jatinangor. *Soilrens*, *17*(1).

Gunawan A, Hala Y, Ali A, Jumadi O, & Junda M. (2021). Vegetative growth response of upland rice to Actynomycetes, Azospirillum, and Azotobacter. terhadap Actinomycetes, Azospirillum dan Azotobacter. *Biology Dept, Math and Natural Science Faculty, Makassar University State, Indonesia. IOP Conf. Series: Earth and Environmental Science*, 012060.

Hala, Y. (2020). The Effect of Nitrogen-Fixing Bacteria Towards Upland Rice Plant Growth and Nitrogen Content. *In IOP Conference Series: Earth and Environmental Science*, 012086.

Hamim, R., Nisa, S., Ida, H. S., & Nani. (2008). *Pengaruh Pupuk Biologi Terhadap Pola Serapan Hara, Ketahanan Penyakit, Produksi Dan Kuautas Hasil Beberapa Komoditas Tanaman Pangan dan Sayuran Unggulan*.

Hindersah, R., Marthin, K., Abraham, T., & Yansen, L. (2018). Nitrogen Fixing Bacteria Azotobacter As Biofertilizer And Biocontrol In Long Bean. *AGRIC*, *30*(1), 25–32.

Itelima, J. U., Bang, W. J., Onyimba, I. A., Sila, M. D., & Egbere, O. J. (2018). Bio-Fertilizers as Key Player in Enhancing Soil Fertility and Crop Productivity: A Review. *Jornal of Agriculture and Food Science* , *6*(3), 73–83.

Lekatompessy, S. J., & Nurjanah, L. (2019). Effect of the Combination of Biological and Organic Fertilizers on Upland Rice Plants. *In Prosiding Seminar Nasional Masyarakat Biodiversitas Indonesia*, 222–227.

Lestari, P., Susilowati, D. N., & Riyanti, E. I. (2007). Pengaruh Hormon Asam Indol Asetat yang Dihasilkan Azospirillum sp. terhadap Perkembangan Akar Padi. *AgroBiogen*, *3*(2), 66–72.

Nosrati, R., Owlia, P., Saderi, H., Rasooli, I., & Malboobi, M. A. (2014). Phosphate Solubilization Characteristics of Efficient Nitrogen Fixing Soil Azotobacter Strains. *Iranian Journal of Microbiology*, *6*(4), 285.

Purnomo, A. J., Anggraeni, A., Astuti, R. K., Lestari, A. B., & Antasari, G. A. (2017). Potensi Bakteri Penambat Nitrogen dan Penghasil Hormon IAA Dari Sampel Rhizosfer Paku Epifit Di Mulut Gua Anjani, Kawasan Karst Menoreh. *Biotropic : The Journal of Tropical Biology*, *1*(2), 9–15. https://doi.org/10.29080/biotropic.2017.1.2.9-15

Wulandari, S., & Sulistyani, N. (2016). Pengaruh Media terhadap Pertumbuhan Isolat Actinomycetes Kode Al35 serta Optimasi Produksi Metabolit Antibakteri Berdasarkan Waktu Fermentasi dan pH. *Media Farmasi*, *13*(2), 186–198.