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Utilization of *Azotobacter* as bio-fertilizer to support sustainable agriculture

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Abstract

Biofertilizers are microbes or the result of microbial activities that can directly or indirectly increase plant production by increasing the amount and availability of nutrients, accelerating the decomposition process of organic matter, inhibiting the development of nuisance organisms, producing compounds that can accelerate and increase plant growth. , and break down toxic materials in the soil. *Azotobacter* is an example of a microbe classified in biological fertilizers which is well-known as an airborne N2-fixing microbe. *Azotobacter* is a gram-negative bacterium that can fix N2 freely (non-symbiotically) with an average fixation equivalent of 20 kg N/ha/year. *Azotobacter*'s ability to fix N2 can come from the nitrogenase enzyme produced by these bacteria, but this enzyme is very sensitive to the presence of oxygen. *Azotobacter* is a bacterium that is commonly found in soil, water, and sediments. The use of *Azotobacter* as a biological fertilizer is one solution to reduce the use of chemical fertilizers and pesticides, reduce the cost of chemical fertilization, and reduce environmental pollution caused by the use of chemical fertilizers and pesticides. This paper aims to identify and provide information about the role and use of *Azotobacter* as a biological fertilizer to support sustainable agriculture. In addition, this paper also contains information on the development of *Azotobacter* biofertilizers which have been commercialized in several countries, including India, China, and Indonesia.

Keywords: Bacteria, biofertilizer, environmental, enzyme, microbes.

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1. Introduction

National food needs are increasing in line with the increasing population, which if projected by 2035 it will reach 305.6 million people with a population growth of 1.40% [5]. One sector that plays an important role in meeting national food needs is the agricultural sector. The decline in production in the agricultural sector, especially in several main commodities, can lead to dependence on imports and pose a threat to national food security. Several studies have stated that land productivity for agricultural cultivation activities, especially food agriculture, is decreasing. [1] more than 73% of agricultural land in Indonesia, including paddy fields, had been degraded. This is caused by the excessive use of chemical fertilizers and not combined with the use of organic fertilizers, causing the soil to be damaged and the organic matter content of the soil to be low. The low content of soil organic matter can cause agricultural land to become sick so that it cannot produce optimally. The current condition of agricultural land is generally polluted by the excessive use of chemical pesticides so that it has an impact on decreasing agricultural land productivity. This condition certainly has an impact on national food production and the sustainability of the agricultural system.

One of the efforts that can be made to maintain and increase national food production is by restoring soil fertility and health using quality organic fertilizers and biological fertilizers to realize a sustainable agricultural system. In addition, the use of organic fertilizers and biological fertilizers also plays a role in reducing the amount of use of chemical fertilizers and chemical pesticides so as to save costs on chemical fertilizer subsidies, improve environmental conditions, produce higher quality and healthier agricultural production and become more efficient agricultural systems. Organic fertilizers are materials derived from plants or animals, either in the form of plant biomass or livestock manure. Biofertilizers are microbes or the result of microbial activities that can directly or indirectly increase plant production by increasing the amount and availability of

nutrients, accelerating the decomposition process of organic matter, inhibiting the development of nuisance organisms, producing compounds that can accelerate and increase plant growth., and break down toxic materials in the soil. Several types of biological fertilizers that have been developed in Indonesia according to [2] include: (1) N2-fixing microbes in the air; (2) phosphate solubilizing microbes; (3) potassium solubilizing microbes; (4) microbes that inhibit disease development (antagonists); (5) microbes that can remodel pollutant compounds in the soil; and (6) microbes that can break down organic matter (decomposers). *Azotobacter* is an example of a microbe classified in biological fertilizers which is well-known as an airborne N2-fixing microbe. However, several studies have shown that these bacteria not only help the process of fixing N2 in the air, but that these bacteria can also have other functions that can increase crop production so that the agricultural system becomes more efficient and sustainable.

This paper aims to identify and provide information about the role and use of *Azotobacter* as a biological fertilizer to support sustainable agriculture. In addition, this paper also contains information on the development of *Azotobacter* biofertilizers which have been commercialized in several countries, including India, China, and Indonesia.

2. Potential of *Azotobacter* as Biological Fertilizer

2.1. Characteristics

Azotobacter is a gram-negative bacterium that can fix N2 freely (non-symbiotically) with an average fixation equivalent of 20 kg N/ha/year [12]. This bacterium is classified as an active bacterium (has high motility), and visually has a greenish-yellow or brown pigment [3]. Chennapa et al (2013) stated that *Azotobacter* which had been isolated from rice rhizosphere was a gram-negative bacterium, rod-shaped, cyst forming, had brown to black-colored colonies, glistening, slimy, and smooth on Ashby's agar media. Biochemical tests carried out on these bacteria showed that these bacteria were capable of producing Indoleacetic acid and citric acid. The ability of these bacteria to tether N2 in the air is due to the presence of the nitrogenase enzyme possessed by these bacteria. The nitrogenase enzyme in *Azotobacter* sp. very sensitive to O2 which will reduce N2 fixation capacity. [15] stated that *Azotobacter* sp. It is known to use two mechanisms to protect the nitrogenase system against the presence of oxygen, namely: (1) high respiration resulting in infrequent cellular oxygen utilization activities that can prevent the diffusion of oxygen into cells and nitrogenase, (2) protection of enzyme conformation or switch off nitrogenase activity by shethna or FeSII protein. Recently, alginate formation was considered as a novel protective mechanism for nitrogenases against oxygen.

The size of these bacteria ranges from 2-10 x 1-2.5 m, young cells have peritrichous flagella and are used as locomotive organs. Cysts germinate under favorable conditions to form vegetative cells. This cyst also produces polysaccharides. *Azotobacter* is quite sensitive to acidic pH, high salt content and temperatures above 350C. Until now, there are eight species of *Azotobacter* sp. Those that have been successfully described include *Azotobacter*.

2.2. Potential of Azotobacter

Azotobacter is one of the bacteria that can be used as biological fertilizer because these bacteria can increase crop production in a sustainable manner. The use of *Azotobacter* as a biological fertilizer is one solution to reduce the use of chemical fertilizers and pesticides, reduce the cost of chemical fertilization, and reduce environmental pollution caused using chemical fertilizers and pesticides. Several studies have been carried out by several world researchers to determine the potential possessed by *Azotobacter* related to the use of these bacteria as biological fertilizers.

Azotobacter is a bacterium commonly found in soil, water, and sediment. These bacteria are known as bacteria that can bind N2 freely (non-symbiotically). These bacteria are known to have the ability to produce vitamins, amino acids, growth hormones, antifungal substances, hydrogen cyanide, and siderophores that can increase plant growth and protect plants from pathogen attack. In addition, this bacterium is a bacterium that is able to live in extreme environmental conditions such as high salt concentration (max NaCl 6%), environmental pH reaching 8.0, and ambient temperature reaching 45ºC [8].

2.3. N_2 fixation by Azotobacter

Nitrogen is an essential nutrient needed by plants in large amounts. Nitrogen is important for supporting plant growth and other soil microbial life. The atmosphere is composed of 78% N_2 gas, but nitrogen in the form of N_2 gas cannot be directly utilized by plants so that an auxiliary agent is needed that can fix N_2 to meet nitrogen needs for plants. The anchoring of N_2 can be done biologically and the mooring can be done by the fertilizer industry. Biological N_2 fixing process carried out by microbes is a very important way of providing nitrogen for plants. *Azotobacters* are microbes that can bind N_2 freely.

Azotobacter is a free-living N_2-fixing bacterium capable of fixing N_2 on average 20 kg N/ha/year. The presence of these bacteria can help increase plant growth and yield and increase nitrogen content in the soil through N_2 fixation. The use of *Azotobacter* for agricultural cultivation activities has several beneficial effects besides helping the N2 fixation process, including as a producer of growth

regulators, protecting plants from pathogens, increasing plant nutrient uptake, and being able to reduce the use of chemical fertilizers so as to improve environmental conditions and improve soil health [12]. The effect of *Azotobacter* on microbiological activity in the rhizosphere and the growth of hybrid maize in organic production systems. The results showed that there was an increase in plant yields in the treatment inoculated with *Azotobacter*. This is due to the presence of *Azotobacter* in this treatment, which is thought to stimulate the seed germination process, seed resistance to stress conditions, assist nitrogen fixation, and produce phytohormones [11].

A previous study conducted on [21] the application of *Azotobacter* and its combination with urea fertilizer on wheat plants. The results showed that the combination application of *Azotobacter* with urea fertilizer showed higher plant and root dry weight compared to the application of urea or *Azotobacter* alone (Table 1). This is due to the assistance of N2 fixing carried out by *Azotobacter* so that it can increase the availability and uptake of nitrogen nutrients for plants and obtain higher crop yields. This researcher said the use of *Azotobacter* could reduce the use of urea fertilizer by up to 20 kg/ha without reducing the yield of wheat crops. Inoculation of 5 kg/ha of *Azotobacter* on agricultural land could reduce the dose of chemical fertilizer application up to 50 kg/ha with an increase in crop yields of 5-10% [10]. *Azotobacter*'s ability to fix N_2 can come from the nitrogenase enzyme produced by these bacteria, but this enzyme is very sensitive to the presence of oxygen. The activity of nitrogenase in binding N_2 increased with the lower the amount of oxygen in the cell. [15] said that the rate of oxygen transfer into cells decreased with increasing alginate concentration during the *Azotobacter* vinelandii culture process. This phenomenon provides oxygen in the cell to be low so that the N_2 tethering process can take place optimally. Alginates are a family of linear copolymers consisting of a variable amount of (1-4)-β-D-*mannuronic acid* and its epimers such as -L-*guluronic acid*. Alginates are important in a variety of biotechnological and biomedical applications, for example to immobilize cells in pharmaceuticals or as stabilizers, thickeners, and gelling agents in food production.

2.4. Azotobacter as Plant Growth Promoting Rhizobacteria (PGPR)

Several studies have stated that apart from helping the N_2 fixing process, *Azotobacter* can also increase plant growth through its role as PGPR. The results of [22] showed that *Azotobacter* inoculation could significantly increase the content of gibberellins, kinetin, and Indoleacetic acid (IAA). The presence of growth regulators produced by *Azotobacter* can directly increase several plant growth parameters including increasing plant dry weight, root morphology development, increasing biomass yield, protein, nutrient uptake, and mineral content in the plant. Inoculation of plants with *Azotobacter* can increase the synthesis of several growth regulators such as auxin, cytokinin, and gibberellin acid which can control the increase in tomato plant growth and can stimulate root development [12]. *Azotobacter* can produce PGPR in the form of Indoleacetic acid (IAA) as much as 24.50 g/mL, and gibberellic acid as much as 15.2 g/25 mL. The presence of growth regulators produced by *Azotobacter* can directly affect the increase in plant growth [8]. *Azotobacter*s are a group of bacteria that can not only do N_2 fixation but can also produce phytohormones that have a positive impact on plant growth and yields including IAA, gibberellin acid, cytokinins, and vitamins. Inoculation of *Azotobacter chroococcum* could positively increase plant growth through the phytohormones produced by these two bacteria [10]. The results of [6] showed that *Azotobacter* was able to produce IAA of 34.4 g/mL in a medium given 5% pendimethalin (a type of pesticide commonly used for rice plants).

A previous study conducted on [21] on the application of *Azotobacter* and its combination with urea fertilizer on wheat plants showing that higher crop yields were obtained in the treatment containing *Azotobacter*. This is due to the influence of growth regulators, namely auxin (IAA), gibberellins and cytokinins produced by *Azotobacter* so that they can trigger root development and growth of wheat plants. The results of this study indicate that the *Azotobacter* strain studied can produce IAA hormone of 20.24 ppm. The IAA produced triggers root development and plant growth to obtain higher crop yields. Previously researchers [17] isolated *Azotobacter* from the rhizosphere of tomatoes, eggplant, and chilies in the Namakkal area, Tamil Nandu, India using Jenson's and Ashby's mannitol selective media. The isolates were identified based on their morphological and biochemical characteristics. The results of this study indicate that *Azotobacter* that has been isolated has various abilities which are presented in Table 2. In addition, some strains of these bacteria are also able to survive at concentrations of zinc and mercury up to 100-200 g/mL, and act as antifungals for several pathogenic fungi such as *Aspergillus flavus*, *Cercospora* sp, and *Fusarium oxysporum*. Table 2. shows that *Azotobacter* isolates can produce growth regulators, namely IAA, increase nitrogen availability, increase phosphate dissolution, produce cyanide acid, and produce siderophores. The ability of *Azotobacter* to produce IAA will increase if there is an attempt to add Tryptophan to a certain concentration. Based on the results of the study, the concentration of Tryptophan added to produce the highest IAA was 60 and 90 g/mL. The presence of biocontrol produced by *Azotobacter* in the form of growth regulators can be used to increase vegetative growth and increase plant yields. The results of another study showed that the production of IAA produced by *Azotobacter* in media fertilized with urea (18.28-35.54 ppm IAA) was lower than in media without urea fertilized (33.89-42.01 ppm IAA). The presence of the IAA hormone produced by *Azotobacter* can

stimulate root growth through increasing root length or surface area, so that the roots can bind water and increase root wet weight significantly [19].

2.5. Azotobacter as Phosphate Solvent

Another ability possessed by *Azotobacter* is the ability of *Azotobacter* to dissolve phosphate in both organic and inorganic forms. Phosphate is one of the essential nutrients needed in large quantities (macro) for plant growth. Phosphate has an important role in the process of photosynthesis, respiration, energy storage and transfer, cell formation and various other metabolic processes. In the soil, phosphate is relatively unavailable to plants because it is bound by binding agents such as Fe and Al (in acid soils), and Ca and Mg (in alkaline soils). This causes phosphate fertilization to be inefficient. One alternative solution to deal with this problem is to utilize phosphate solubilizing microbes. Several studies have stated that *Azotobacter* can dissolve phosphate so that it is relatively more readily available to plants to support their growth and increase the efficiency of phosphate fertilization, three *Azotobacter* species, namely *A. chroococcum, A. virelandii and A. beijerinckii* [4] [15] The results showed that most of the strains of the three species were able to dissolve phosphate by forming a clear zone in minimal media containing poorly soluble P. *A. virelandii* had the highest ability to form a clear zone with a diameter of 21.5 mm in the first two days of incubation. In addition, *Azotobacter* also has the ability to grow and reproduce in extreme environmental conditions, namely temperatures reaching 45ºC, high salt content (NaCl 5%), and able to live at pH 5 to 10 when dissolving P.

Researchers [18] isolated and tested the ability of these isolates to dissolve phosphate using NBRIP (National Botanical Research Institute's Phosphate) media on the rhizosphere of tomatoes, ginger, brinjal, and garlic. The results showed that *Azotobacter* obtained from the rhizosphere was able to dissolve phosphate derived from calcium phosphate by forming a clear zone. The formation of this clear zone occurs due to the dissolution of phosphate from the insoluble to the soluble which is mediated by organic acids around the media. Researcher [16] isolated and selected *Azotobacter* from soil by inserting a soil suspension into Jensen's media (containing Sucrose, K_2HPO_4, $MgSO_4$, NaCl, K_2SO_4, Na_2MoO_4) with a pH of 6.9, then incubated for one week. After the incubation period, the bacteria that grew were cultured on Ashby's media and incubated for one week and morphological characterization was carried out directly on the medium. To test the ability of rhizosphere bacteria to dissolve phosphate, Pikovskaya's agar test media was used with the addition of tricalcium phosphate (TCP) as a source of phosphate. The test medium was poured into a petri dish and holes were made with a cork hole with a diameter of 6 mm and filled with a suspension of the tested rhizosphere bacterial isolates. The test medium was incubated for 3 days in an incubation chamber at 28º C. The ability to dissolve phosphate in each isolate was evaluated qualitatively based on the formation of a clear zone (halo zone) around the hole containing the suspension of the test isolate. The results obtained indicate that *Azotobacter* can be used as a biological fertilizer because it produces positive IAA, has the ability to dissolve phosphate, is stable at pH 5 to 7, and is able to grow at a temperature of 40-50ºC. The ability of *Azotobacter* to dissolve phosphate is indicated by the formation of a halo zone. However, the halo zone formed had different sizes depending on the tested *Azotobacter* isolates (Table 3).

2.6. Azotobacter as Siderophore Producer

Several studies have shown that *Azotobacter* has been assessed as having the ability as a biocontrol agent to treat root pathogens such as Fusariurm sp., Alternaria sp., Phytophthora sp., Rhizoctonia sp., Colletotrichum sp. and Curvularia sp. through its ability to produce siderophores that can bind Fe^{3+} so that the iron is not available to pathogens. Researchers [14] tested the siderophores produced by *Azotobacter* which had been isolated from several soil samples around Chennai by growing the isolates on Chrome azurol sulfonate agar media. The results showed that *Azotobacter* started producing siderophores 10 hours after incubation with a maximum siderophore production of 65% at 30 hours after incubation, and the number decreased after 42 hours of incubation (Figure 1). In addition, the results of this study also showed that the siderophore production was most optimal at neutral pH, at pH below and above neutral the production of siderophores was lower than the siderophore production at neutral pH (Table 4).

2.7 Azotobacter is tolerant to pesticides

That 13 of the 14 *Azotobacter* strains that had been isolated from the rice rhizosphere were able to grow on media containing pesticides which are very commonly used for rice plants such as pendimethalin, glyphosate, chloropyrifos, and phorate [6]. However, there were five *Azotobacter* strains that were able to grow on media containing pesticides up to a concentration of 5% without affecting the growth and metabolism of these bacteria. In addition, *Azotobacter* was also able to produce an IAA of 34.4 g/mL in media that was given 5% pendimethalin.

2.8. Azotobacter as a producer of Cyanide Acid (HCN)

Biocontrol mechanism produced by *Azotobacter* to reduce the population of plant pathogens is by producing cyanide acid (HCN). *Azotobacter* is able to produce HCN (cyanide acid) by 77% which can protect plants from

pathogen attack. In addition, *Azotobacter* is also capable of producing antibiotic compounds similar to anisomycin which have been identified as compounds that can function as fungicidal antibiotics. Some fungi that can be affected by the presence of *Azotobacter* include *Alternaria*, *Fusarium*, *Collectotrichum*, *Rhyzoctonia*, *Microfomina*, *Diplodia*, *Batryiodiplodia*, *Cephalosporium*, *Curvularia*, *Helminthosporium* and *Aspergillus*. So far, research results show that there is no species of *Azotobacter* that has a negative effect on food [12].

2.9. Ability of Azotobacter as Petroleum Biodegradation Agent

Azotobacter vinelandii can be used as a bioremediation agent, biodegradation, and environmental cleaning process from petroleum waste contamination that pollutes the environment, especially soil through biosurfactants produced by these bacteria. Biosurfactants have a role as surface tension that can separate oil, water, solids from oil sludge [9]. Biosurfactant production began to increase in the exponential phase (propagation) reaching a maximum after 48 hours of incubation (9.81 g/L). The results of this study indicate that the maximum biosurfactant biosynthesis from glucose occurs mainly at the end of the exponential growth phase. The emulsification activity of broth-free cells increased to 90% in the first 24 hours of incubation, whereas surfactant accumulation increased during this period and began to decrease after reaching maximum synthesis. This may be due to the use of biosurfactants as a carbon source by *Azotobacter* vinelandii. In a study conducted by [20] by adding *Azotobacter* sp. on oil sludge produced by a company in Indonesia. The results showed that *Azotobacter* could be used as a biodegradation agent to reduce total petroleum hydrocarbons (TPH) up to 6.83%, 3.48%, and 2.11% for slurries from 1:1 ratio, 2:1 ratio, and 3:1 ratio in 35 days. This decrease was caused by the presence of biosurfactants produced by these bacteria so that they were able to release the surface tension between the oil and the oil sludge (Figure 2).

2.10 Azotobacter as Mercury Bioaccumulator

Mercury is the most toxic heavy metal compared to other heavy metals. Some bacteria are resistant to mercury. One genus of bacteria that is mercury resistant and capable of accumulating mercury is *Azotobacter*. *Azotobacter* is a bacterium that produces Exopolysaccharide (EPS) which can function as metal chelating agents. The results of research by [13] showed that there were several strains of *Azotobacter* that were able to live in media containing mercury up to a concentration of HgCl2 of 20 mg/L.

2.11 Other abilities of Azotobacter

Some *Azotobacter* strains have the ability to produce amino acids when grown on media. The results of biochemical analysis which included the content of chlorophyll, nitrogen, phosphorus, potassium, and protein were higher in the treatment inoculated with *Azotobacter* compared to the treatment not inoculated with *Azotobacter* [12].

3. *Azotobacter* Biodive Fertilizer Production

3.1. Azotobacter isolation and selection

Generally, *Azotobacter* can be isolated from the plant rhizosphere. *Azotobacter* isolation can be done by growing it on selective media including: NFM media (N-Free Medium), Jenson's selective media and Ashby's mannitol. The isolates were then identified based on their morphological and biochemical characteristics as well as ability tests such as their ability to produce phytohormones, siderophores, cyanide acid, the ability to dissolve phosphorus, as well as other required tests so that the isolates obtained for the development of biological fertilizers were isolates that were truly superior.

3.2. Carrier Formulation

The use of *Azotobacter* sp inoculants. as a biological fertilizer, it must pay attention to the effective bacterial cell population, the type of carrier material, and the resistance of the cells in the carrier material. The types of carriers that are often used as biological fertilizers are generally in solid and liquid forms. The use of *Azotobacter* sp inoculants. in the carrier material makes it easy to apply biofertilizers. The effect of carrier material, sterilization, and storage temperature regulation on the biological activity of *Azotobacter chroococcum* [7]. The carrier materials compared in this study include peat, a combination of peat with vermiculite (1:2), wheat bran (wheat bran), rice husk, clay, and sodium alginate. The sterilization treatments compared were: (1) sterilization using an autoclave at 121ºC for 20 minutes; (2) sterilization using gamma ray radiation; (3) without sterilization. The storage temperature was compared between storage at 8ºC and 30ºC. The biological activities observed included nitrogenase, phosphatase, potassium solubilization, and the ability of *Azotobacter chroococcum* to produce growth regulators such as IAA, gibberellin acid, and cytokinins. The results showed that in the sterilized treatment using autoclave and gamma rays there were no contaminants until the end of the 6-month incubation period at 8ºC and 30ºC storage, but in the untreated treatment there were a large number of fungi and bacteria and decreased *Azotobacter chroococcum* population at incubation period. In the sterilization treatment, the highest density was in the treatment which was sterilized using gamma rays. The carrier material that had the highest density was alginate reaching

11,905 log 10 CFU/g at both storage temperatures for a 6 month incubation period, followed by wheat bran, namely 10.81 log 10/CFU/g (gamma ray sterilization) and 10.68 log 10/CFU/ g (sterilization using autoclave) at a storage temperature of 30ºC. Based on the research results, alginate is one of the best carriers among the six types of carriers studied. However, the use of alginate as a carrier material is quite expensive, so in its development the carrier material that is more recommended is rice husk and clay which is sterilized using gamma rays.

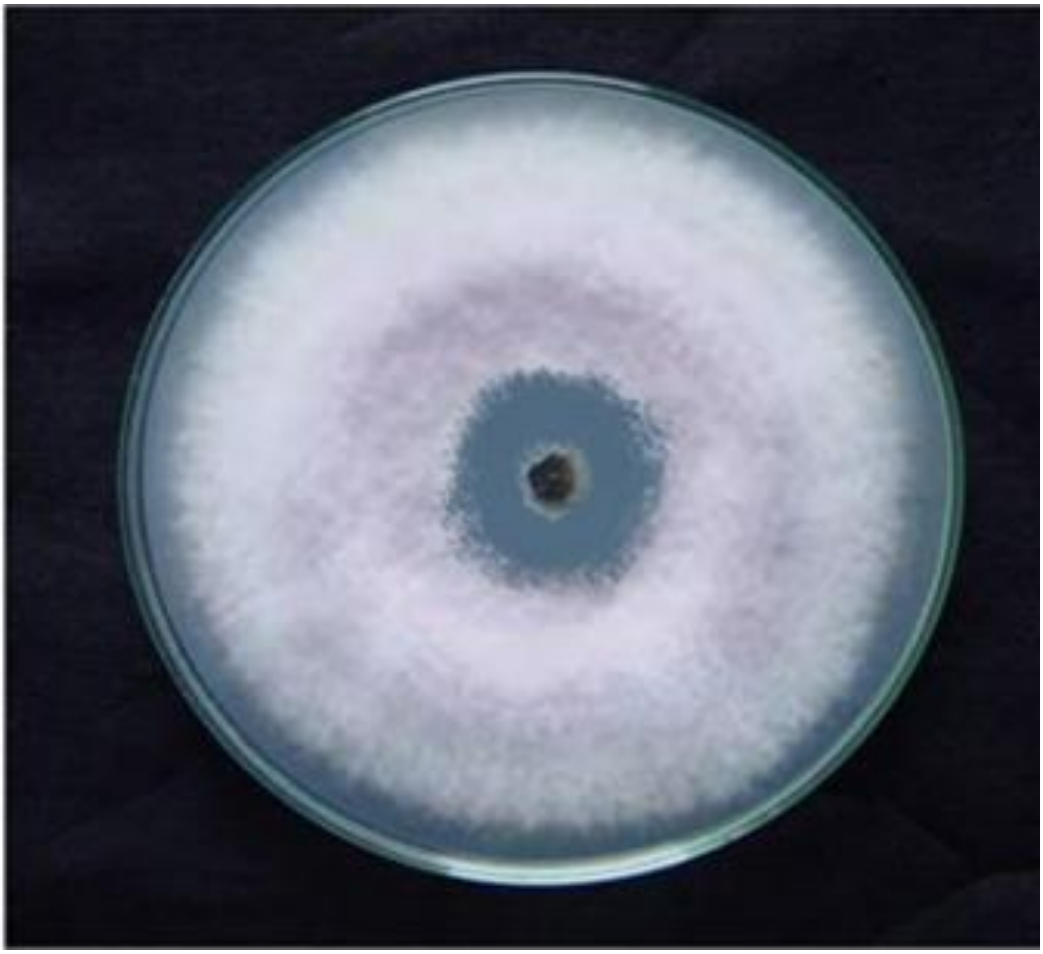

Fig. 1: Siderophore antagonistic activity against *Fusarium oxysporum* [14]

Fig. 2: The separation phase after mixing with biosurfactant and oil sludge at a ratio of 1:1 (left) and 2:1 (right).

Table 1: *Azotobacter* biofertilizer on wheat plant growth on dry land [21]

	Treatment	Plant dry weight (g)		Plant dry weight (g)	
		2008	**2009**	**2008**	**2009**
T1	Kontrol	24.20	25.80	6.60	7.40
T2	Azo-8	26.90	26.10	8.10	7.90
T3	Azo-8+20kg N/ha (Urea)	26.20	29.80	8.20	8.80
T4	Azo-8+40kg N/ha (Urea)	28.30	31.70	8.60	9.40
T5	Azo-8+60kg N/ha (Urea)	32.50	35.50	9.70	10.30
T6	Azo-8+60kg N/ha (Urea)+ 20 kg N/ha (FYM)	36.60	37.40	10.60	11.40
T7	Azo-8+60kg N/ha (Urea)+40 kg N/ha (FYM)	39.20	40.80	11.90	12.10
T8	120 kg N/ha (Urea)	38.90	36.10	11.80	11.20

Table 2: Ability of *Azotobacter* isolates [17]

***Azotobacter* isolates**	**Indole acetic acid production**	**Amonia production**	**Hydrogen cyanide production**	**Phosphate solubilization**	**Siderophore production**
AZT1	+++	+++	+++	+++	+++
AZT2	++	+	-	-	++
AZT3	+++	+++	+++	+++	+++
AZB4	+++	+++	+++	+++	+++
AZB5	+	+	-	++	-
AZC6	+++	+++	+++	+++	+++
AZC7	-	+	++	+	+
AZC8	+	-	++	+	+
AZC9	+	++	-	-	+
AZC10	-	+	-	++	-

Note: AZT – *Azotobacter* isolated from tomato rhizosphere; AZB- *Azotobacter* isolated from eggplant rhizosphere; AZC- *Azotobacter* isolated from chili rhizosphere. + low; ++ moderate; +++ high; - no color change.

Table 3: Isolation of *Azotobacter* indigenous, Optical Density (OD) value, IAA production, and Halo zone diameter (cm) from 21 isolates

Plant rhizosfer	Isolate code	OD value	IAA Production	Halo zone diameter (cm)
Padi gogo	MP3c	0,222	+	0,8 (+)
Padi sawah	KP6d	0,166	+	0,8 (+)
Tebu I	LT2d1	1,542	+	0,8 (+)
Ubi kayu I	LU2c1	0,169	+	0,8 (+)
K.Buncis	RB4c	0,169	+	0,8 (+)
Jagung I	LJ5e	0,347	+	0,8 (+)
Padi gogo	LP3c	0,152	+	0,8 (+)
Sorgum II	MS3f	0,128	+	0,8 (+)
Tebu	LT2d	0,122	+	0,8 (+)
Tebu III	LT2d3	0,111	+	0,8 (+)
Ubi kayu	LU2c	0,190	+	0,8 (+)
Rumput liar I	RR8b1	0,121	+	0,8 (+)
Gambas I	Rg4c	0,191	+	0,8 (+)
Padi gogo I	Mp1f	0,142	+	0,8 (+)
Rumput liar II	RR8b2	0,152	+	0,8 (+)
Tebu II	LT2b2	0,132	+	0,8 (+)
Lada	ML1j	0,919	+	0,8 (+)
Padi sawah	LP7a	0,661	+	1,2 (++)
Sorgum	MS3e	0,566	+	0,8 (+)
Rumput liar	RR8a	0,174	+	0,8 (+)
Ubi jalar	KU6e	0,765	+	1,2 (++)

Table 4: Production of siderophores at different pH levels

Sr. No.	pH	Siderophore unit
1	4	Trace
2	5	29
3	6	56
4	7	62.8
5	8	58
6	9	28
7	10	Trace

4. Conclusions

The use of *Azotobacter* as a biological fertilizer is an alternative solution to reduce the use of chemical fertilizers and pesticides without reducing crop production so as to create a more efficient and sustainable agricultural system and higher quality crop yields. *Azotobacter* biofertilizers have various beneficial benefits, including helping the N_2 fixation process, producing growth regulators (IAA, Gibberellins, cytokinins), protecting plants from pathogens by producing siderophores and cyanide acid, helping to dissolve phosphate from an unavailable form to become available to plants. , increasing the availability and uptake of plant nutrients, able to reduce the use of chemical fertilizers so as to improve environmental conditions and improve soil health, as agents of bioremediation, biodegradation, and the process of cleaning the environment from waste contamination.

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