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Azotobacter as a Possible Bio-fertilizer for Managing Soil and Plant Health: A Review

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Review Article

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ABSTRACT

Azotobacter is one kind of bio-fertilizer that is sprayed onto the soil's surface or distributed through seeds. It is a living organism that helps colonise the rhizosphere, or the internal portions of plants, and it also promotes development by making more primary nutrients available to plants. A free-living, gram-negative bacterium with an oval or spherical form is called an *Azotobacter*. It is a significant bio-fertilizer that increases soil fertility by fixing nitrogen, which in turn increases crop productivity by allowing plants to absorb biologically active chemicals through the process of biosynthesis. In comparison to chemical fertilisers, it is more environmentally benign because it contributes significantly to nutrient cycle and boosts nutrient availability. According to study, using *Azotobacter* also boosts some crops *Azotobacter* yields. An increasing body of research has shown that using bio-fertilizer, either on its own or in conjunction with other fertilisers or pesticides, can produce positive outcomes when it comes to saving natural resources. Thus, it is preferable to

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utilise bio-fertilizers in order to slow down the rate at which chemicals are being used up on the soil. It is therefore crucial to become more knowledgeable about the significance and applications of the specific bio-fertilizer before putting it to use.

Keywords: Azotobacter; growth; yield; agriculture; bio-fertilizers; soil fertility; nitrogen fixation and microbes.

1. INTRODUCTION

Bio-fertilizers, often called bio-inoculants, are organically manufactured fertilisers that contain microorganisms that provide agriculture outcome with nutrients, especially N and P (Chatterjee and Bandyopadhyay, 2017). When utilised as a seed treatment, bio-fertilizers (Singh et al., 2016) or applied in soil (Wani et al., 2016) they grow quickly in order to increase the population in the rhizosphere (Sharma et al., 2007). Because they are nontoxic, nonhazardous, and environmentally friendly, bio fertilisers are becoming more and more important in agriculture (Sudhkar et al., 2000). Bio fertilizers including *Azotobacter* (Tripathiet al., 2008), Blue green algae (Mehnaz, 2015), Azospirillum (Sadhana, 2014), mycorrhizae (Kalayu, 2019) and psolubilizing microbes (Selvakumar et al., 2009) as the advanced tools are being used and impart various benefits to the agriculture sector (Gandotraet al., 1998). *Azotobacter* species are aerobic soil-dwelling, free-living, gram-negative bacteria with an oval or spherical form (Kloepper, 1978; Kaviyarasan et al., 2020). *Azotobacter* was first discovered by a Dutch microbiologist and botanist Beijerinck in 1901(Martyniuk and Martynuik, 2003). The genus *Azotobacter* has 7 different species which includes *Azotobacter* croococcum, A. armeniacus, A. beijerinckii, A. paspali A. salinestris A. nigricansand A. vinelandii(Garrityet al., 2015; Alhia and Hassan et al., 2015). Their size ranges from 1-2 µm wide and 2-10 µm long (Yates and Jones, 1974).

These bacteria use the nitrogen gas in the atmosphere to synthesise their cell proteins (Bishop et al., 1986). After the *Azotobacter* cell dies, this cell protein will mineralise and contribute to the crop plants' available nitrogen (Tchan et al., 1983). It is discovered that the *Azotobacter* spp. are susceptible to high salt concentrations, acidic pH, and temperatures exceeding 35°C (Baral and Adhikari, 2013). This bacterium stimulates rhizospheric microorganisms, which benefits crop growth and productivity (Parmarand Dufresne, 2011; Chen, 2006), bio-synthesizing the active substance and producing phyto-pathogenic inhibitors (Lenart, 2012; Franche et al., 2009).

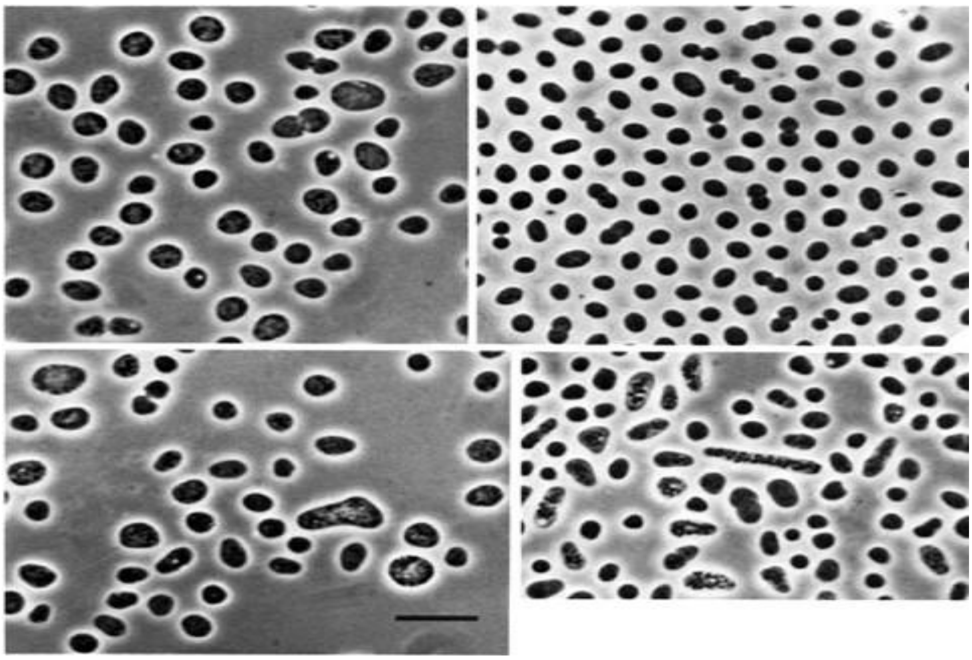

Fig. 1. *Azotobacter* species cells

2. *Azotobacter* AS A BIO INOCULANT AND BIO FERTILIZER

The use of *Azotobacter* as bio fertilizer was introduced by Gerlach and Voel (1902) for providing soil with nitrogen, which is biologically fixed N2 as one of this microbe's functions (Tienet al., 1979). This bacterium is involved in many different aspects of plant growth stimulation. In addition to fixing atmospheric N2, it also aids in the production of PGRs like auxins, cytokinins, gibberellins, amino acids, and vitamins, as well as phosphate solubilisation (Barea and Brown, 1974; Apte and Shende, 1981; Abbass and Okon, 1993; Damiret al., 2011; Chahal and Chahal, 1988). A report from (Apte and Shende, 1981; Abbass and Okon, 1993) demonstrates that this bacterium has a high acetylene reduction assay (ARA) and a wide range of N2 fixation, with 2–15 mg of N fixed per gramme of glucose ingested. *Azotobacter* chroococcum contributes to a 48% reduction in nematode infection, with Azospirillum (4%) and Pseudomonas (11%) following suit (Mishra et al., 2013).

3. *Azotobacter* IN SOIL FERTILITY

As chemical fertilizers are costly and have large production costs, they also negatively impact the microbial population and soil health (Lenart, 2012), the best option for preserving soil fertility in such a scenario is bio-fertilizer (Bhardwaj et al., 2014; Kour et al., 2020). Bio fertilizers being environmentally friendly and economic (Nagananda et al., 2010), they are found to be very useful for better crop production and yield (Yousefi et al., 2017). *Azotobacter* species in soils provide numerous advantages for plant growth, including enhancing seed germination (Sobariu et al., 2017; Wani et al., 2013) and also has positive response on Crop Growth Rate (CGR) (Kizilkaya, 2009), additionally, a large number of soil physico-chemicals (such as organic matter, pH, soil moisture, and soil temperature) and microbiological characteristics are positively correlated with the abundance of these bacteria (Vojinoviv, 1961). According to the soil profile depth, the abundance also varies (Hamilton et al., 2011). Nitrogen fixation turns out to be the most important microbial activity (Vojinoviv, 1961) and biological processes (Vance and Graham, 1995) happening on the earth surface right after photosynthesis. Biological nitrogen fixation plays a critical function in preserving soil fertility (Hakeem et al., 2016). Because *Azotobacter* grows quickly and has high nitrogen fixation levels, it can be utilized to study nitrogen fixing and plant inoculation (Robson and Postgate, 1980; Prajapati et al., 2008). *Azotobacter* is able to convert nitrogen into ammonia, which plants can subsequently absorb (Shokri and Emtiazi, 2010). As *Azotobacter* spp. being non-symbiotic (Hajnal et al., 2004) and heterotrophic bacteria can fix 20 kg of nitrogen per hectare annually, which can be utilized to produce crops (Gosal et al., 2012). Bacterization helps in improving growth of plants (Bali et al., 1992) and boosts soil nitrogen by using carbon for its metabolism through nitrogen fixation (Monib et al., 1979; Kukreja et al., 2004).

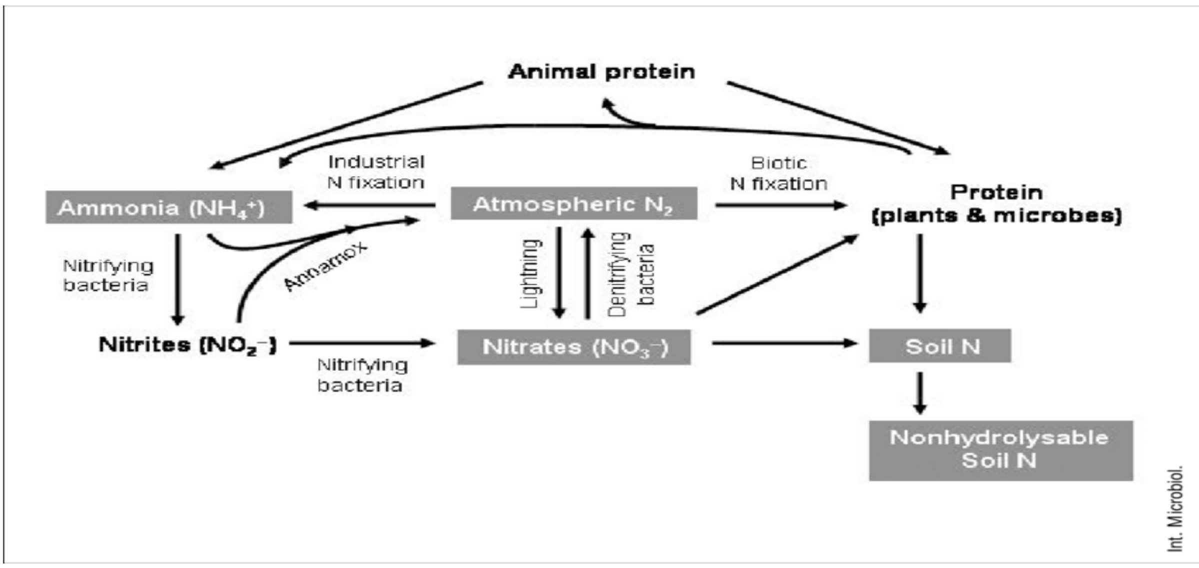

Fig. 2. Nitrogen Fixation by *Azotobacter* spp. (Sharma et al., 2007)

4. *Azotobacter* IN CROP PRODUCTION

Azotobacter species produce physiologically active compounds, which have numerous positive effects on crop growth (Jnawali et al., 2015) generating rhizospheric microbial stimulation and phytopathogenic inhibitors (Levai et al., 2008). Additionally, *Azotobacter* increases the availability of some nutrients, such as carbon, phosphorus, nitrogen, and sulfur, by speeding up the mineralization of organic wastes in soil(Sharma et al., 2007) while preventing the absorption of several heavy metals (Joshi et al., 2006). Because *Azotobacter* can supply nitrogen in the form of ammonium, amino acids, and nitrate without causing overdosing, it has emerged as a significant substitute for artificial fertilizers (Bhattacharjee and Dey, 1974). When used in farming operations, *Azotobacter*, a nitrogen biofertilizer, enhances crop growth and yield (Barea and Brown, 1974) Table 1.

Table 1. *Azotobacter* effects on crop yield

Sr. No.	Crop	Yield increased over yield obtained from chemical fertilizers (%)
1	Rice	5
2	Sorghum	15-20
3	Wheat	8-10
4	Maize	15-20
5	Potato	13
6	Tomato	2-24
7	Carrot	16
8	Cauliflower	40
9	Cotton	7.27
10	Sugarcane	9-24

Source: Bhattacherjee and Dey, 2014

5. *Azotobacter* IN GROWTH AND YIELD OF CROPS

Plant growth regulators and fixed nitrogen trigger the development of branching, flowering, roots, leaf creation, and fruiting, all of which are aided by *Azotobacter* (Zena and Peru, 1974). Additionally, this bacterium increases plants' resistance to drought in unfavorable weather circumstances. A report says that the yield of potato has been greatly increased after using *Azotobacter* spp. by 33.3% (Singh and Dutta, 2006). There is also a report that shows a significant increase in yield of mustard (var. Yella) and Rapeseed (7.86q ha^{-1}) after inoculation with *Azotobacter* (Das and Saha, 2007). From a report of Das and Saha 2007; Sandeep et al., 2011), the combination inoculation of diazotrophs, *Azotobacter*, and Azospirillum increases rice grain and straw production by 4.5 to 8.5 kg ha-1. Compared to non-infected plants, plants inoculated with *Azotobacter* exhibit superior crop yields and a favorable response to maize grain yield (Moriri et al., 2015). The grain yield increased while using *Azotobacter* in three different maize hybrids is shown in Table 2.

6. *Azotobacter* IN NUTRIENT CYCLING

Azotobacter prevents the absorption of various heavy metals while enabling the availability of specific nutrients such as carbon, sulfur, phosphorus, and nitrogen through the mineralization process of organic wastes in soil (Joshi et al., 2006). Since *Azotobacter* can convert nitrogen into ammonia, amino acids, and nitrate without overloading the plants, it has emerged as one of the most significant substitutes for artificial fertilizers in modern times (Bhattacharjee and Dey, 2014). In addition to assisting in the uptake of macro and micronutrients for optimal utilization of plant root exudates, the bacterium supports plant growth and yield even in situations when the soil has low levels of phosphate (Abdiev et al., 2019).

Table 2. Effect of *Azotobacter* spp. in maize yield

Variant	Maize hybrids			
	ZP555 su	620 k	NS 609b	NS 6030
Control	12.27	4.27	8.88	10.59
100 ml A. chroococcum	13.32	4.97	8.39	10.9
75 ml A. chroococcum	13.24	4.89	8.87	10.75
50 ml A. chroococcum	13.31	4.3	8.92	10.96

Source: Jafari et al., 2012

7. *Azotobacter* IN SEED INOCULATION AND NUTRIENT UPTAKE

Azotobacter aids in the uptake of macronutrients like N and P as well as certain micronutrients like Fe and Zn when it is injected into seeds (Naseri and Mirzaei, 2010; Naseri et al., 2013). Additionally, these bacterial strains are employed to enhance the nutritional value of maize, wheat, and rice (Sahoo et al., 2014; Gholami et al., 2009). Because *Azotobacter* aids in providing nitrogen to standing crops, crop yields are significantly boosted (Kizilkaya, 2009). In a greenhouse experiment, it was discovered that *Azotobacter*-inoculated seeds boosted the protein and carbohydrate content of two maize types (Inra260 and Inra210) (Chen, 2006). The biomass of the maize crop increases when manure and *Azotobacter* are applied together (Yasari and Patwardhan, 2007).

8. EFFECTS OF *Azotobacter* COMBINED WITH CHEMICAL FERTILIZERS

Compared to chemical fertilizers alone, *Azotobacter* has some effects on plant growth, branch count, height, dry weight, and safflower freshness when treated in conjunction with 50% chemical fertilizers, such as N and P (Soleimanzadeh and Gooshchi, 2013). The same is true for organic fertilizers; applying them with half a dose of chemical fertilizers and *Azotobacter* bi-phosphate increases the safflower's economic yield (Saribay, 2003). With increased N levels, the efficiency of *Azotobacter* is found to be decreasing (Ojaghloo et al., 2007). The same is true for organic fertilizers; applying them with half a dose of chemical fertilizers and *Azotobacter* bi-phosphate increases the safflower's economic yield (Balajee and Mahadevan, 1990; Mandal et al., 2008).

9. EFFECTS OF *Azotobacter* COMBINED WITH PESTICIDES

Azotobacter croococcum uses the herbicide 2, 4-D and its byproducts, p-chlorophenol and p-chlorophenoxy-acetic acid, as carbon, which then activates the nitrogenase enzyme (Kanungo et al., 1995). According to a research study, the pesticide carbofuran aids in promoting the nitrogenase enzyme's activity (Martinez-Toledo et al., 1991). According to a report, neither growth nor dialyzed or sterilized soil media nor Azotobacter croococcum growth are impacted by the herbicide simazine (Schenckzu Schweinsberg-Mickan and Muller, 2009). Azotobacter can be cultivated with cells that have more ATP when simazine is present.

10. STRESS TOLERANCE CHARACTERISTICS OF *Azotobacter*

Heavy metals make up the majority of the significant contaminants that irrigation causes in agricultural soils (Say et al., 2001). These heavy metals build up over time, which delays plant growth and eventually reduces production (Gauri et al., 2012). *Azotobacter* exopolysaccharides are very effective at immobilizing heavy metals (Weppen and Hornburg, 1995). High absorptive nature of EPS removes heavy metals from the soil (Gauri et al., 2011; Gauri et al., 2012). In contaminated soils, heavy metals like Cr and Cd can be immediately absorbed and bound by *Azotobacter*'s extracellular polymeric substance (EPS) (Joshi and Juwarkar, 2009; Otero and Vincenzini, 2003). The breakdown of *Azotobacter* EPS can also provide macronutrients and micronutrients (Zhang and Miller, 1994). Through altering the magnitude and affinity between hydrocarbons and microbial soils, the EPS-based *Azotobacter* aids in boosting the aqueous dispersion of some poorly soluble chemicals (Barkay et al., 1999); Santruckova et al., 1999). The biological processes of compacted and non-compacted soils can be distinguished by their microbial activity.

11. CONCLUSION

Gram-negative *Azotobacter* species can fix 20 kg of nitrogen per hectare annually. This bacteria, known as PGPR, aids in the synthesis of growth chemicals and plays a significant role in promoting development and growth while preventing the proliferation of phytopathogens through the secretion of inhibitors. According to additional research, *Azotobacter* species contribute to soil fertility, germination rate, and crop growth rate, all of which increase yield and promote healthy growth. *Azotobacter* species can also be employed in combination with chemical pesticides and fertilizers to increase economic yield. Because this bacterium can also make EPS, it can withstand more stress. To learn more about the positive traits of *Azotobacter* species, more research is needed.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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