

Review Article

Azotobacter as a possible bio-fertilizer for managing soil and plant health: A review

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 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

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* (Tripathi *et 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 (Gandotra *et 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 Martyniuk, 2003). The genus *Azotobacter* has 7 different species which includes *Azotobacter croococcum*, *A. armeniacus*, *A. beijerinckii*, *A. paspali*, *A. salinestrus*, *A. nigricans* and *A. vinelandii* (Garrity *et 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).

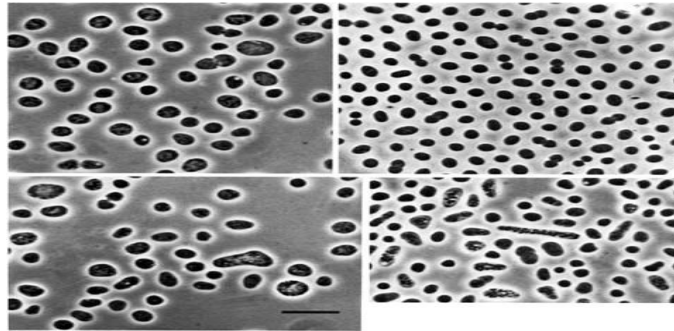


Figure-1: *Azotobacter* species cells

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 (Parmar and Dufresne, 2011; Chen, 2006), bio-synthesizing the active substance and producing phyto-pathogenic inhibitors (Lenart, 2012; Franche *et al.*, 2009).

***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 *et 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 *et 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).

***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

(Vojinovic, 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 (Vojinovic, 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).

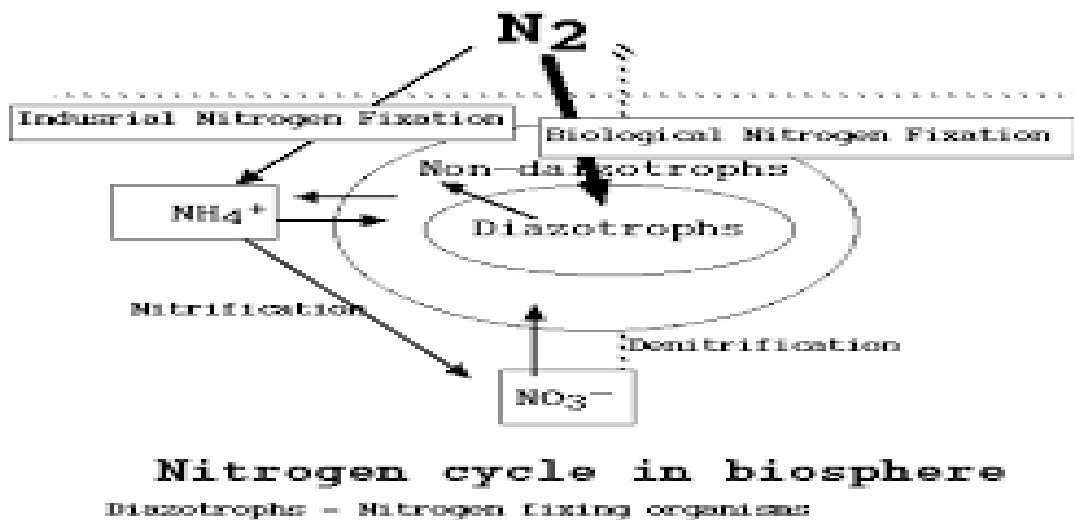


Figure 2: Nitrogen Fixation by *Azotobacter* spp. (Sharma *et al.*, 2007).

***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 (%)
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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: Bhattacharjee and Dey, 2014

***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.

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 <i>A. chroococcum</i>	13.32	4.97	8.39	10.9
75 ml <i>A. chroococcum</i>	13.24	4.89	8.87	10.75
50 ml <i>A. chroococcum</i>	13.31	4.3	8.92	10.96

Source: Jafari *et al.*, 2012

***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).

***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).

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).

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.

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.

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.

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