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 products 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 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(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).

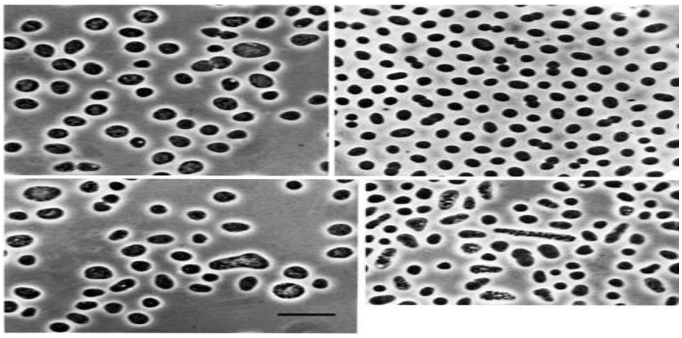

Figure-1: *Azotobacter*species cells

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 andDufresne, 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 supplying N to soil which is biologically fixed N2 as one of the activities for this microbe (Tien*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 andShende, 1981; Abbass and Okon, 1993; Damir*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 quite expensive and give high cost of production which also have adverse effects on microbial population as well as soil health (Lenart, 2012), in such a situation, bio fertilizer becomes the best alternative for maintaining soil fertility (Bhardwaj*et al*., 2014; Kour*et al*., 2020). Bio fertilizers being environmentally friendly and economic (Naganandaa*et al*., 2010), they are found to be very useful for better crop production and yield (Yousefi*et al*., 2017). *Azotobacter* spp. in soils has so many benefits on growth of plants, helps in improving germination of seeds (Sobariu*et al*., 2017; Wani*et al*., 2013) and also has positive response on Crop Growth Rate (CGR) (Kizilkaya, 2009), also the abundant presence of these bacteria has positive relation to many of the soil physico-chemicals (e.g. organic matter, pH, soil moisture and temperature of the soil) and microbiological properties 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. The role of biological nitrogen fixation is very important in maintaining fertility of the soil (Hakeem *et al*., 2016). *Azotobacter* can be used for the study of nitrogen fixation as well as plant inoculation because of its rapid growth and having high levels of nitrogen fixation (Robson and Postgate, 1980; Prajapati*et al*., 2008). *Azotobacter* has the capability for conversing nitrogen into ammonia which later on taken up by the plants (Shokri and Emtiazi, 2010). As *Azotobacter* spp. being non-symbiotic (Hajnal*et al*., 2004) and heterotrophic bacteria, they have the capability of fixing 20 kg N per hectare per year which can be used for crop production (Gosal*et al*., 2012). Bacterization helps in improving growth of plants (Bali *et al*., 1992) and increases soil nitrogen by utilizing carbon through nitrogen fixation for its metabolism (Monib*et al*., 1979; Kukreja*et al*., 2004).

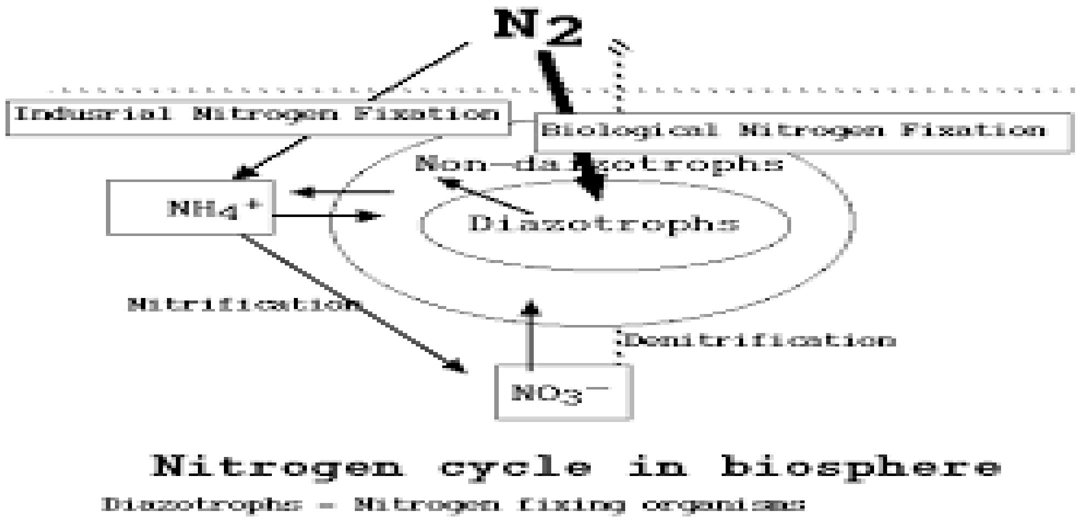

Figure 2: Nitrogen Fixation by *Azotobacter* spp. (Sharma *et al*., 2007).

Azotobacter IN CROP PRODUCTION

Azotobacter spp. have so many beneficial effects on growth of crops through the process of biosynthesis of biologically active substances (Jnawali*et al*., 2015) producing phytopathogenic inhibitors and stimulation of rhizospheric microbes (Levai*et al*., 2008). *Azotobacter* also makes the availability of some nutrients like carbon, phosphorus, nitrogen and sulphur through the process of accelerating the mineralized organic residues in soil (Sharma *et al*., 2007) while avoiding the uptake of different heavy metals (Joshi *et al*., 2006). *Azotobacter* has become an important alternative for chemical fertilizers as it can provide nitrogen in ammonium form, amino acids and nitrate without leaving the situation over dosage (Bhattacharjee and Dey, 1974). *Azotobacter* as a nitrogen bio fertilizer helps in improving the growth and yield of different crops in field conditions (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

Azotobacter IN GROWTH AND YIELD OF CROPS

Azotobacter helps in stimulating the development of branching, flowering rooting foliage formation and fruiting which is initiated by plant growth regulator and fixed nitrogen (Zena and Peru, 1974). This bacterium also helps plants in increasing tolerance to lack of water supply under adverse climatic conditions. 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 combined inoculation of *Azotobacter* with Azospirillum as well as diazotrophshelps in increasing the straw and grain yield of rice by 4.5 to 8.5 kg ha^{-1}. Plants inoculated with *Azotobacter* have better crop yield as compared to those with non-inoculated plants and have a positive response on grain yield of maize (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 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

Azotobacter IN NUTRIENT CYCLING

Azotobacter allows certain nutrients available like carbon,sulphur, phosphorus and nitrogen through the mineralization process of organic residues in soil while avoiding the uptake of different heavy metals (Joshi *et al*., 2006). Nowadays *Azotobacter* has become one of the most important alternatives for chemical fertilizers as it can provide nitrogen into ammonia, amino acids and nitrate without overdosing the plants (Bhattacharjee and Dey, 2014). The bacterium helps in sustaining the growth of plants and yield even when the soil content is low in phosphate and also helps in taking up the macro and micro nutrients for better utilizing the plant root exudates (Abdiev*et al*., 2019).

Azotobacter IN SEED INOCULATION AND NUTRIENT UPTAKE

When seeds are inoculated with *Azotobacter,* the bacterium helps in the uptake of macronutrients like N and P along with some micronutrients like Fe and Zn (Naseri and Mirzaei, 2010; Naseri*et al*., 2013). These bacteria strains are also used for improving the nutrition of rice, wheat and maize (Sahoo*et al*., 2014; Gholami*et al*., 2009).The yield of crops is profoundly increased as the *Azotobacter* helps in supplying nitrogen in standing crops (Kizilkaya, 2009). *Azotobacter* inoculated seeds are found to be increased in protein and carbohydrate content of two varieties of corn (Inra260 and Inra210) in an experiment done in a greenhouse (Chen, 2006). The combined application of manure with *Azotobacter* gives increase in biomass of maize crop (Yasari and Patwardhan, 2007).

EFFECTS OF *Azotobacter* COMBINED WITH CHEMICAL FERTILIZERS

Azotobacter when applied in combination with 50% chemical fertilizers i.e., N and P, has some effects on growth of plants, number of branches, height, dry weight and freshness of safflower as compared to chemical fertilizers only (Soleimanzadeh and Gooshchi, 2013). Same goes for the organic fertilizers as well when applied with *Azotobacter*biphosphate along with half dose of chemical fertilizers helps in increasing the economic yield of safflower (Saribay, 2003). With increased N levels, the efficiency of *Azotobacter* is found to be decreasing (Ojaghloo*et al*., 2007). The best combination turns out to be with NH4Cl @ 0.1 g/L while the combination with copper and *Azotobacter* are found to be toxic even when applied in low concentration (Balajee and Mahadevan, 1990; Mandal*et al*., 2008).

EFFECTS OF *Azotobacter* COMBINED WITH PESTICIDES

The herbicide 2, 4-D along with its products p-chlorophenol and p-chlorophenoxy-acetic acid are utilized by *Azotobacter*croococcum in the form of carbon, which later on stimulate nitrogenase enzyme (Kanungo*et al*., 1995). Result found from a report that the insecticide carbofuran helps in stimulating the activity of nitrogenase enzyme (Martinez-Toledo *et al*., 1991). A report says that the herbicide simazine has no effect on growth of *Azotobacter*croococcum, neither growth nor sterilized or dialyzed soil medium (SchenckzuSchweinsberg-Mickan and Muller, 2009). With the presence of simazine, the *Azotobacter* can be grown with cells having higher ATP content.

STRESS TOLERANCE CHARACTERISTICS OF *Azotobacter*

Most of the important pollutants through irrigation in agricultural soils are the heavy metals (Say *et al*., 2001). The consecutive accumulation of these heavy metals lead to retardation of plant growth later on affects the yield (Gauri*et al*., 2012). Exopolysaccharides of *Azotobacter* have a great role in 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). The EPS of *Azotobacter* can directly uptake and bind the heavy metals like Cr and Cd in contaminated soils (Joshi and Juwarkar, 2009; Otero and Vincenzini, 2003).

Macronutrients and micronutrients can also be supplied through the decomposition of EPS from *Azotobacter*(Zhang and Miller, 1994). The EPS based *Azotobacter* helps in increasing the aqueous dispersion of some poorly soluble compounds by changing the affinity and magnitude between hydrocarbons and microbial soils (Barkay*et al*., 1999); Santruckova*et al*., 1999). The microbial activity can differentiate the biological process between compacted soil and non-compacted soils.

CONCLUSION

Azotobacter spp. are gram negative and capable of fixing 20 kg N per hectare per year. This bacterium is regarded as PGPR which helps in synthesizing growth substances and takes a great role in enhancing growth, development, and inhibiting phytopathogenic growth as they secrete inhibitors. From more research, *Azotobacter* spp. helps in increasing soil fertility, germination rate and has a positive response on Crop growth rate which results in more yield and healthy growth. *Azotobacter* spp. can also be used combining with chemical fertilizers as well as pesticides which help in increasing the economic yield. More stress can be tolerated by this bacterium as they can also produce EPS. More research is required so as to get more good qualities of the *Azotobacter* spp.

REFERENCES

Abbass, Z. and Okon, Y., (1993). Plant growth promotion by *Azotobacter*paspali in the rhizosphere.*Soil Biology and Biochemistry*, **25**(8), pp.1075-1083.

Abdiev, A., Khaitov, B., Toderich, K. and Park, K.W., (2019). Growth, nutrient uptake and yield parameters of chickpea (*Cicerarietinum* L.) enhance by Rhizobium and *Azotobacter* inoculations in saline soil. *Journal of Plant Nutrition*, **42**(20), pp.2703-2714.

Alhia, Basel Mohammed Hassan (2010).The Effect of *Azotobacter*Chrococcum as Nitrogen Biofertilizer on the Growth and Yield of CucumisSativus.*The islamic university*. http://hdl.handle.net/20.500.12358/21551

Apte, R. and Shende, S.T., 1981. Studies on *Azotobacter*chroococcum: I. Morphological, biochemical and physiological characteristics of *Azotobacter*chroococcum. ZentralblattfürBakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. ZweiteNaturwissenschaftlicheAbteilung: Mikrobiologie der Landwirtschaft, der *Technologie und des Umweltschutzes*, **136**(7), pp.548-554.

Balajee, S. and Mahadevan, A., (1990). Influence of chloroaromatic substances on the biological activity of *Azotobacterchroococcum*. *Chemosphere*, **21**(1-2), pp.51-56.

Bali, A., Blanco, G., Hill, S. and Kennedy, C., (1992).Excretion of ammonium by a nifL mutant of *Azotobacter*vinelandii fixing nitrogen.*Applied and Environmental Microbiology*, **58**(5), pp.1711-1718

Baral, B.R. and Adhikari, P., (2013).Effect of *Azotobacter* on growth and yield of maize.SAARC *Journal of Agriculture*, **11**(2), pp.141-147.

Barea, J.M. and Brown, M.E., (1974). Effects on plant growth produced by *Azotobacter*paspali related to synthesis of plant growth regulating substances. *Journal of Applied Bacteriology*, **37**(4), pp.583-593.

Barkay, T., Navon-Venezia, S., Ron, E.Z. and Rosenberg, E., (1999).Enhancement of solubilization and biodegradation of polyaromatic hydrocarbons by the bioemulsifieralasan.*Applied and environmental microbiology*, **65**(6), pp.2697-2702.

Bhardwaj, D., Ansari, M.W., Sahoo, R.K. and Tuteja, N., (2014). Bio fertilizers function as key player in sustainable agriculture by improving soil fertility, plant tolerance and crop productivity. *Microbial cell factories*, **13**(1), pp.110.

Bhattacharjee, R. and Dey, U., (2014). Bio fertilizer, a way towards organic agriculture: A review. *African Journal of Microbiology Research*, **8**(24), pp.2332-2343.

Bishop, P.E., Premakumar, R., Dean, D.R., Jacobson, M.R., Chisnell, J.R., Rizzo, T.M. and Kopczynski, J., (1986). Nitrogen fixation by *Azotobacter*vinelandii strains having deletions in structural genes for nitrogenase.*Science*, **232**(4746), pp.92-94.

Chahal, P.P.K. and Chahal, V.P.S., (1988). Biological control of root-knot nematode of brinjal (Solanummelongena L.) with *Azotobacter*chroococcum.In US-Pakistan International Workshop on Plant Nematology, Karachi (Pakistan), 6-8 Apr 1986.NNRC.

Chatterjee, R. and Bandyopadhyay, S., (2017). Effect of boron, molybdenum and Bio fertilizers on growth and yield of cowpea (Vignaunguiculata L. Walp.) in acid soil of eastern Himalayan region.*Journal of the Saudi Society of Agricultural Sciences*, **16**(4), pp.332-336.

Chen, J.H., (2006), October. The combined use of chemical and organic fertilizers and/or bio fertilizer for crop growth and soil fertility. In International workshop on sustained management of the soil-rhizosphere system for efficient crop production and fertilizer use (Vol. 16, No. 20, pp. 1-11). Land Development Department Bangkok Thailand.

Damir, O., Mladen, P., Božidar, S. and Srñan, N., (2011).Cultivation of the bacterium *Azotobacter*chroococcum for preparation of Bio fertilizers.*African Journal of Biotechnology*, **10**(16), pp.3104-3111.

Das, A.C. and Saha, D., (2007). Effect of diazotrophs on the mineralization of organic nitrogen in the rhizosphere soils of rice (*Oryza sativa*).*J Crop Weed*, 3, pp.47-51.

Franche, C., Lindström, K. and Elmerich, C., (2009). Nitrogen-fixing bacteria associated with leguminous and non-leguminous plants. *Plant and soil*, **321**(1), pp.35-59.

Gandotra, V., Gupta, R.D. and Bhardwaj, K.K.R., (1998). Abundance of *Azotobacter* in great soil groups of NorthWest Himalayas. *Journal of the Indian Society of Soil Science*, **46**(3), pp.379-383.

Garrity, G.M., Bell, J.A. and Lilburn, T., (2015).Proteobacteriaphyl.nov.Bergey's Manual of Systematics of Archaea and Bacteria, pp.1-1.

Gauri, S.S., Archanaa, S., Mondal, K.C., Pati, B.R., Mandal, S.M. and Dey, S., (2011). Removal of arsenic from aqueous solution using pottery granules coated with cyst of *Azotobacter* and portland cement: Characterization, kinetics and modeling. *Bioresource technology*, **102**(10), pp.6308-6312.

Gauri, S.S., Mandal, S.M. and Pati, B.R., (2012).Impact of *Azotobacter*exopolysaccharides on sustainable agriculture.*Applied microbiology and biotechnology*, **95**(2), pp.331-338.

Gholami, A., Shahsavani, S. and Nezarat, S., (2009).The effect of plant growth promoting rhizobacteria (PGPR) on germination, seedling growth and yield of maize.World Academy of Science, *Engineering and Technology*, **49**, pp.1924.

Gosal, S.K., Kalia, A., Uppal, S.K., Kumar, R., Walia, S.S., Singh, K. and Singh, H., (2012). Assessing the benefits of *Azotobacter*bacterization in sugarcane: a field appraisal.Sugar Tech, **14**(1), pp.61-67.

Hajnal, T., Jarak, M. and Milosevic, N., (2004).Bacterization of maize: yield response of maize to inoculation. In Book of abstracts of the 10th international symposium on microbial ecology: isme-10, cancun, mexico (Vol. 207).

Hakeem, K.R., Akhtar, M.S. and Abdullah, S.N.A. eds., 2016. Plant, soil and microbes: volume 1: implications in crop science. *Springer*.

Hamilton, T.L., Ludwig, M., Dixon, R., Boyd, E.S., Dos Santos, P.C., Setubal, J.C., Bryant, D.A., Dean, D.R. and Peters, J.W., (2011).Transcriptional profiling of nitrogen fixation in *Azotobacter*vinelandii.*Journal of bacteriology*, **193**(17), pp.4477-4486.

Jafari TH, Latkovic D, Duric S, Mrkovacki N, Najdenovska O (2012) *Research. J AgricSci***44**(2)

Jnawali, A.D., Ojha, R.B. and Marahatta, S., (2015). Role of *Azotobacter* in soil fertility and sustainability–A Review.*Adv. Plants Agric. Res*, **2**(6), pp.1-5.

Joshi, K.K., Kumar, V., Dubey, R.C., Maheshwari, D.K., Bajpai, V.K. and Kang, S.C., (2006). Effect of Chemical Fertilizeradaptive Variants, Pseudomonas aeruginosa GRC 2 and *Azotobacter*chroococcum AC 1, on Macrophominaphaseolina Causing Charcoal Rot of *Brassica juncea*.*Korean Journal of Environmental Agriculture*, **25**(3), pp.228-235.

Joshi, P.M. and Juwarkar, A.A., (2009).In vivo studies to elucidate the role of extracellular polymeric substances from *Azotobacter* in immobilization of heavy metals.Environmental science & technology, 43(15), pp.5884-5889.

Kalayu, G., (2019). Phosphate solubilizing microorganisms: promising approach as Bio fertilizers. International Journal of Agronomy, 2019.

Kanungo, P.K., Adhya, T.K. and Rao, V.R., (1995). Influence of repeated applications of carbofuran on nitrogenase activity and nitrogen-fixing bacteria associated with rhizosphere of tropical rice. Chemosphere, 31(5), pp.32493257.

Kaviyarasan, G.P., Shricharan, S.P. and Kathiravan, R.P., (2020). Studies on isolation, biochemical characterization and nitrogen fixing ability of *Azotobacter* sp. isolated from agricultural soils. International Journal of Scientific Engineering and Applied Science (IJSEAS) – 6, 11.118-125

Kizilkaya, R., (2009). Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J. Environ. Biol, 30(1), pp.73-82.

Kizilkaya, R., (2009). Nitrogen fixation capacity of *Azotobacter* spp. strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. J. Environ. Biol, 30(1), pp.73-82.

Kloepper, J.W., (1978). Plant growth-promoting rhizobacteria on radishes.In Proc. of the 4th Internet.Conf. on Plant Pathogenic Bacter, Station de PathologieVegetaleetPhytobacteriologie, INRA, Angers, France, 1978 (Vol. 2, pp. 879-882).

Kour, D., Rana, K.L., Yadav, A.N., Yadav, N., Kumar, M., Kumar, V., Vyas, P., Dhaliwal, H.S. and Saxena, A.K., (2020). Microbial Bio fertilizers: Bioresources and eco-friendly technologies for agricultural and environmental sustainability. Biocatalysis and Agricultural Biotechnology, 23, p.101487.

Kukreja, K., Suneja, S., Goyal, S. and Narula, N., (2004). Phytohormone production by *Azotobacter*–a review.Agricultural Reviews, 25(1), pp.70-75.

Lenart, A., (2012). Occurrence, Characteristics, and Genetic Diversity of *Azotobacter*chroococcum in Various Soils of Southern Poland.Polish Journal of Environmental Studies, 21(2).

Lévai, L., Veres, S., Bákonyi, N. and Gajdos, É., (2008). Can Wood Ash and Bio-fertilizer Play a Role in Organic Agriculture?.Agronomskiglasnik: GlasiloHrvatskogagronomskogdruštva, 70(3), pp.263-272.

Mandal, S.M., Pati, B.R., Das, A.K. and Ghosh, A.K., (2008). Characterization of a symbiotically effective Rhizobium resistant to arsenic: isolated from the root nodules of Vignamungo (L.) Hepper grown in an arsenic-contaminated field. The Journal of general and applied microbiology, 54(2), pp.93-99.

Martinez-Toledo, M.V., Salmeron, V. and Gonzalez-Lopez, J., (1991).Effect of simazine on the biological activity of *Azotobacter*chroococcum.Soil Science, 151(6), pp.459-467.

Martyniuk, S. and Martyniuk, M., (2003).Occurrence of *Azotobacter* spp. in some Polish soils.Polish Journal of Environmental Studies, 12(3), pp.371-374.

Mehnaz, S., (2015). Azospirillum: a bio fertilizer for every crop. In Plant microbes symbiosis: Applied facets (pp. 297314). Springer, New Delhi.

Mishra, D., Rajvir, S., Mishra, U. and Kumar, S.S., (2013). Role of bio-fertilizer in organic agriculture: a review. Research Journal of Recent Sciences ISSN, 2277, p.2502.

Monib, M., Abd-El-Malek, Y., Hosny, I. and Fayez, M., (1979).Seed inoculation with *Azotobacter*chroococcum in sand cultures and its effect on nitrogen balance.ZentralblattfürBakteriologie, Parasitenkunde, Infektionskrankheiten und Hygiene. ZweiteNaturwissenschaftlicheAbteilung: Mikrobiologie der Landwirtschaft, der Technologie und des Umweltschutzes, 134(3), pp.243-248.

Moriri S, Owoeye LG, Mariga IK (2015). Role of *Azotobacter* in Soil Fertility and Sustainability–A Review.Adv Plants Agric Res 2(6): 00069. DOI: 10.15406/apar.2015.02.00069.

Nagananda, G.S., Das, A., Bhattacharya, S. and Kalpana, T., (2010). In vitro Studies on the Effects of Bio fertilizers(*Azotobacter* and Rhizobium) on Seed Germination and Development of Trigonellafoenum-graecum L. using a Novel Glass Marble containing Liquid Medium. International Journal of Botany, 6(4), pp.394-403.

Naseri, R. and Mirzaei, A., (2010). Response of yield and yield components of Safflower (Carthamustinctorius L.) to seed inoculation with *Azotobacter* and Azospirillum and different nitrogen levels under dry land condition. Agric. Environ. Sci, 9, pp.445-449.

Naseri, R., Moghadam, A., Darabi, F., Hatami, A. and Tahmasebei, G.R., (2013). The effect of deficit irrigation and *Azotobacter*chroococcum and Azospirillum brasilense on grain yield, yield components of maize (SC 704) as a second cropping in western Iran. Bull EnvPharmacol Life Sci, 2, pp.104-112.

Ojaghloo, F., Farahvash, F., Hassan-Zadeh, A. and Pour-Yusef, M., (2007).Effect of inoculation with *Azotobacter* and barvar phosphate Bio fertilizers on yield of safflower (Carthamustinctorius L.).Journal of Agricultural Sciences, Islamic Azad University, Tabriz Branch, 3, pp.25-30.

Otero, A. and Vincenzini, M., (2003). Extracellular polysaccharide synthesis by Nostoc strains as affected by N source and light intensity. Journal of Biotechnology, 102(2), pp.143-152.

Parmar, N. and Dufresne, J., (2011). Beneficial interactions of plant growth promoting rhizosphere microorganisms.In Bioaugmentation, biostimulation and biocontrol (pp. 27-42).Springer, Berlin, Heidelberg.

Prajapati, K., Yami, K.D. and Singh, A., (2008). Plant growth promotional effect of *Azotobacter*chroococcum, Piriformosporaindica and vermicompost on rice plant.Nepal Journal of Science and Technology, 9, pp.85-90.

Robson, R.L. and Postgate, J.R., (1980). Oxygen and hydrogen in biological nitrogen fixation.Annual Reviews in Microbiology, 34(1), pp.183-207.

Sadhana, B., (2014). ArbuscularMycorrhizal Fungi (AMF) as a bio fertilizer-a review. Int J CurrMicrobiol App Sci, 3(4), pp.384-400.

Sahoo, R.K., Ansari, M.W., Dangar, T.K., Mohanty, S. and Tuteja, N., (2014). Phenotypic and molecular characterisation of efficient nitrogen-fixing *Azotobacter* strains from rice fields for crop improvement.Protoplasma, 251(3), pp.511-523.

Sandeep, C., Rashmi, S.N., Sharmila, V., Surekha, R., Tejaswini, R. and Suresh, C.K., 2011. Growth response of Amaranthusgangeticus to *Azotobacter*chroococcum isolated from different agroclimatic zones of Karnataka. Journal of Phytology, 3(7).

Šantrůčková, H., Heinemeyer, O. and Kaiser, E.A., (1993).The influence of soil compaction on microbial biomass and organic carbon turnover in micro-and macroaggregates.In Soil Structure/Soil Biota Interrelationships (pp. 587-598).Elsevier.

Sarıbay, G.F., (2003). Growth and nitrogen fixation dynamic of *Azotobacter*chroococcum in nitrogen-free and omw containing medium (Master's thesis).

Say, R., Denizli, A. and Arıca, M.Y., (2001). Biosorption of cadmium (II), lead (II) and copper (II) with the filamentous fungus Phanerochaetechrysosporium. Bioresource technology, 76(1), pp.67-70.

SchenckzuSchweinsberg-Mickan, M. and Müller, T., (2009).Impact of effective microorganisms and other Bio fertilizers on soil microbial characteristics, organic-matter decomposition, and plant growth.Journal of plant nutrition and soil science, 172(5), pp.704-712.

Selvakumar, G., Lenin, M., Thamizhiniyan, P. and Ravimycin, T., (2009).Response of Bio fertilizers on the growth and yield of blackgram (Vignamungo L.). Recent Research in Science and Technology, 1(4), pp.169-175

Sharma, K., Dak, G., Agrawal, A., Bhatnagar, M. and Sharma, R., (2007).Effect of phosphate solubilizing bacteria on the germination of Cicerarietinum seeds and seedling growth.Journal of Herbal Medicine and Toxicology, 1(1), pp.61-63.

Shokri, D. and Emtiazi, G., (2010). Indole-3-acetic acid (IAA) production in symbiotic and non-symbiotic nitrogenfixing bacteria and its optimization by Taguchi design. Current microbiology, 61(3), pp.217-225.

Singh, M., Dotaniya, M.L., Mishra, A., Dotaniya, C.K., Regar, K.L. and Lata, M., (2016).Role of Bio fertilizers in conservation agriculture.In Conservation Agriculture (pp. 113-134).Springer, Singapore.

Singh, M.S. and Dutta, S., (2006). Mustard and rapeseed response to *Azotobacter*-A review.Agricultural Reviews, 27(3), pp.232-234.

Sobariu, D.L., Fertu, D.I.T., Diaconu, M., Pavel, L.V., Hlihor, R.M., Drăgoi, E.N., Curteanu, S., Lenz, M., Corvini, P.F.X. and Gavrilescu, M., (2017). Rhizobacteria and plant symbiosis in heavy metal uptake and its implications for soil bioremediation.New biotechnology, 39, pp.125-134.

Soleimanzadeh, H. and Gooshchi, F., (2013).Effects of *Azotobacter* and nitrogen chemical fertilizer on yield and yield components of wheat (Triticumaestivum L.).World Applied Sciences Journal, 21(8), pp.1176-1180.

Sudhakar, P., Chattopadhyay, G.N., Gangwar, S.K. and Ghosh, J.K., (2000). Effect of foliar application of *Azotobacter*, Azospirillum and Beijerinckia on leaf yield and quality of mulberry (Morusalba).The Journal of Agricultural Science, 134(2), pp.227-234.

Tchan, Y.T., Wyszomirska-Dreher, Z., New, P.B. and Zhou, J.C., (1983).Taxonomy of the *Azotobacter*aceae determined by using immunoelectrophoresis.International Journal of Systematic and Evolutionary Microbiology, 33(2), pp.147-156.

Tien, T.M., Gaskins, M.H. and Hubbell, D., 1979. Plant growth substances produced by Azospirillum brasilense and their effect on the growth of pearl millet (Pennisetumamericanum L.). Applied and environmental microbiology, 37(5), pp.1016-1024.

Tripathi, R.D., Dwivedi, S., Shukla, M.K., Mishra, S., Srivastava, S., Singh, R., Rai, U.N. and Gupta, D.K., (2008). Role of blue green algae bio fertilizer in ameliorating the nitrogen demand and fly-ash stress to the growth and yield of rice (Oryza sativa L.) plants.Chemosphere, 70(10), pp.1919-1929.

Vance, C.P. and Graham, P.H., (1995). Nitrogen fixation in agriculture: application and perspectives. In Nitrogen fixation: Fundamentals and applications (pp. 77-86). Springer, Dordrecht.

Vojinoviv, Z., (1961). Microbiological properties of main types soil in Serbia for nitrogen cycling. Journal for Scientific Agricultural Research, 43, pp.3-25.

Wani, S.A., Chand, S. and Ali, T., (2013). Potential use of *Azotobacter*chroococcum in crop production: an overview. CurrAgric Res J, 1(1), pp.35-38.

Wani, Sartaj A., Subhash Chand, Muneeb A. Wani, M. Ramzan, and Khalid Rehman Hakeem. "*Azotobacter*chroococcum–a potential bio fertilizer in agriculture: an overview." Soil science: agricultural and environmental prospective (2016): 333-348.

Weppen, P. and Hornburg, A., (1995). Calorimetric studies on interactions of divalent cations and microorganisms or microbial envelopes. Thermochimicaacta, 269, pp.393-404.

Yasari, E. and Patwardhan, A.M., (2007). Effects of (*Azotobacter* and Azosprillium) inoculants and chemical fertilizers on growth and productivity of canola (Brassica napus L.).Asian Journal of Plant Sciences.

Yates, M.G. and Jones, C.W., (1974). Respiration and nitrogen fixation in *Azotobacter*.In Advances in Microbial Physiology (Vol. 11, pp. 97-135).Academic Press.

Yousefi, S., Kartoolinejad, D., Bahmani, M. and Naghdi, R., (2017).Effect of Azospirillum lipoferum and *Azotobacter*chroococcum on germination and early growth of hopbush shrub (Dodonaeaviscosa L.) under salinity stress.Journal of Sustainable Forestry, 36(2), pp.107-120.

Zena, G.G. and Peru, C., (1986). Effect of different rates of *Azotobacter* and frequency of application of Agrispon on yield and quality in the growing of onion (Allium cepa L.) in Cajamarca.National University of Cajamarca Faculty of Agriculture Sciences and Forestry.

Zhang, Y. and Miller, R.M., (1994). Effect of a Pseudomonas rhamnolipidbiosurfactant on cell hydrophobicity and biodegradation of octadecane.Applied and Environmental Microbiology, 60(6), pp.2101-2106.