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AZOTOBACTER SPECIES AND OTHER SOIL MICROORGANISMS ENHANCE PLANT HEALTH –A PERSPECTIVE ANALYSIS

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ABSTRACT
In the present literature, the best alternative of chemical fertilizer is necessary because of its adverse effects on the soil fertility. There are several alternatives available to enhance the soil fertility. Among the plant growth promoting Rhizobacteria (PGPR), Azotobacter spp. are considered to improve the plant health. It helps in synthesis of growth regulating substances like auxins, cytokinin and gibberlic acid. Application of this bacteria has also become helpful in the reclamation of soil suggesting to be a putative agent which can be used in the transformation of virgin land to fertile one. In addition, it stimulates rhizospheric microbes, protects the plants from phyto-pathogens, improves nutrient uptake and ultimately boost up biological nitrogen fixation. The potential of variety of soil microorganisms to exert beneficial effects on various crops is now well established. Rhizosphere bacteria may promote plant growth directly by providing nutrients or growth factors or indirectly by antagonising soil borne phyto-pathogens through secondary metabolites. The present review enlightened on the biological nitrogen fixation by Azotobacter species and other soil microorganisms.

KEYWORDS: PGPR, Rhizobacteria, Azotobacter species and nitrogen fixation.

INTRODUCTION

Azotobacterspecies are gram negative, free living, aerobic, non-symbiotic nitrogen fixing bacterium increases fertility of soils. Lohnis and Smith (1923) described Azotobacter having a complex life cycle. The morphology of Azotobacter in pure culture is remarkably variable. It is bluntly rod shaped or oval cells measuring roughly 2x4µ (Winogradsky, 1930; 1938). Resting cells called cysts are spherical, rounded and metabolically dormant (Hitchins and Sadoff, 1970; 1973). Around six species in the genus Azotobacter have been reported, some of which are motile by means of peritrichous flagella while others are non-motile (Martyniuk and Martyniuk 2003). The genus Azotobacter was recognized in 1901 by Dutch microbiologists, botanist and founder of environmental Microbiology –Beijerinck and his co-workers. Research on *Azotobacterchroococcum*in crop production has shown its importance in improving plant nutrition and amelioration of soil fertility (Kurrey et al, 2018). Several strains of Azotobacter are found to be able to produce amino acids when grown in culture media supplemented with various carbon and nitrogen sources (Gonzalez- Lopez etal, 2005). Such substances produced by these rhizobacteria are implicated in several processes thus leading to plant growth promotion (Inawali et al; 2015). The scope of utilizing *Azotobacterchroococcum* in research experiments as microbial inoculant through release of growth substances and their impact on the plant has markedly improved crop production in agriculture. (Gothandapani et al, 2017).

Soil Fertility and Azotobacter

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. Azotobacter species in soils has so many benefits on growth of plants, helps in improving germinaton of seeds and also has positive response on crop growth rate (CGR), also the abundant presence of these bacteria has positive relation to many of the soil physico – chemicals (e.g. organic matter, P^H, soil moisture andtemperature of the soil) and microbiological properties. According to the soil profile depth, the abundance also varies.

Interaction between *Azotobacter* and other soil microorganism

The various interactions between microorganisms those occur in soil and rhizosphere have been discussed by Parker *et al.* (1977). There are reports where favourable interaction has been observed in soil as a result of addition of energy source such as glucose (Chowdhary, 1977). *Azotobacterchroococcum* growth and its nitrogen fixation were inhibited by common soil inhabitant *Cephalosporium*sp. (Iswaran and Subba Rao, 1966).

Cellulolytic microorganism which degrade plant residues in soil are known to encourage the proliferation of *Azotobacter* in soil (Mishustin and Shilnikova, 1969)(Chan *et al.* 1970) reported beneficial effect of *Bacillus megaterium, Pseudomonas, Radiobacter*and many species of actinomycetes on the growth and nitrogen fixation of *Azotobacter* whereas *Bacillus subtilis*, B. mesentricus and *Pseudomonas putida* cause harmful effects on the growth of *Azotobacter*. Ostwal and Bhide (1972) found that *Pseudomonas fluorescens* exhibited inhibition phenomenon in between *Azotobacterchroococcum* and *Rhizobium* sp. Lakshmikumari *et al.* (1972) reported antifungal activity of *Azotobacterchroococcum*against *Fusariummoniliforme*. Shende*et al*. (1973) also studied interaction between *Azotobacterchroococcum, Bacillus subtilisvar. phosphaticum* and *Rhizobium* sp. *Azotobacterchroococcum* growth and phosphate solubilization by the *Bacillus megaterium*var. phosphaticum was affected. Bagyaraj and Menge (1978) reported synergism of *Azotobacter*and vesicular mycorrihizal (VAM) fungi and their effect on rhizospheremicroflora and plant growth. Ocampo*et al.* (1975) studied interaction of *Azotobacter* with phosphobacteria and lavender plants (*Lavandulaspica*L.). They observed more *Azotobacter* and Phosphobacteria in the rhizosphere after mixed inoculation as compared to single and results in more plant growth. Meshram (1984) reported suppressive effect of *Azotobacterchroococcum* on *Rhizoctoniasolani*infestation of potato Sharma *et al.* (1986) noted that the growth of *Pseudomonas putida, Bacillus subtilis*and *Xanthomonas*was suppressed by *Azotobacterchroococcum.* Page and Dale (1980) observed stimulation of *Agrobacterium tumerfaciens* growth by *Azotobactervinelandii*ferrisiderophore.

Pandey and Kumar (1990) showed inhibitory effect of *Azotobacterchroococcum* and *Azospirillumbrasilense*on growth of 14 rhizospheric fungi including 9 pathogens. *A. brasilense*strain were found to be fungistatic towards 7 fungi while a *Azotobacterchroococcum*s train showed this trend towards these 7 and 6 additional fungi (Meshram*et. al.*, 1993).

Meshram*et al.* (1993) showed that seed germination of cereal can be improved by combined application of pesticide with *Azotobacter* inoculation. Sharma *et al.* (1994) conducted experiments with *Azotobacter* and *Azospirillum*biofertilizer to observe their effect on incidence of majority mulberry disease such as leaf spot, powdery mildew, leaf rust, leaf blight and bacterial blight under graded level of nitrogen. Maximum disease reduction was noticed when *Azotobacter* and *Azosphirillum* were applied in combination with 225 kg N/ha/year.

EL Shanshoury *et. al*(1994) reported inhibitory action of *Azotobacterchroococcum* and *Streptomyces atroolivaceus*extracted metabolites on *Xanthomonascompestris*pvmalvacearum. Suneja *et al.* (1994) reported antagonistic action of three siderophore positive (Sid$^+$) culture and one negative (Sid$^-$) mutant of *Azotobacterchroococcum* to *Sclerotiniasclerotiorum* and *Xanthomonascampestris* and other pathogen.

Saikia*et al.* (1995) reported inhibition of some plant pathogenic fungi by strains of nitrogen fixing *Azotobacter* RRLJ 203producing Siderophere isolated from acid p^H (5.0) and iron rich (10% iron) soil. Arya *et al.* (1998) reported significant reductions of flag smut incidence in wheat by seed treatment with *Azotobacterchroococcum.*

Loveless *et al.* (1999) identified genes unique to Mo independent nitrogenase systems in diverse diazotrophs. A number of N_2 fixing bacteria were screened using PCR for genes (Vnif G and an fG) unique to the V – containing nitrogenase (Vnif) and the Fe only nitrogenase (anif) system. Products with sequences similar to that of vnfG were obtained from *Azotobacterpaspali* and *A. salinestris*genomic DNAS and products with sequences similar to that of an fG were obtained from *Azomonasmacrocytogems, Rhodospirulumrubrum* and *A. paspali* DNAS.

Pecina *et al.* (1999) purified alginate lyase enzyme from *Azotobacterchroococcum.* The alginate lyase encoding gene (alg L) of *Azotobacterchroococcum* was localized to a 3.1 kb ECORIDNA fragment that revealed on open reading frame 1.116 bp.

The various interactions between microorganisms those occur in soil and rhizosphere have been discussed by Parker et-al (1977). There are reports where favourable interaction has been observed in soil as a result of addition of energy source such as glucose (Chowdhary, 1977). *Azotobacterchroococcum* growth and its nitrogen fixation were inhibited by common soil inhabitant Cephalosporium species (Iswaran and Subbarao, 1966).

Many soil microorganisms possess multiple beneficial traits of nutrient mobilization, production of plant growth promoting substances and biocontrol ability (Gutterson et-al; 2008). PGPR, a group of root associated bacteria, intimately interact with the plant roots and consequently influence plant health and soil fertility. They offer an excellent combination of traits useful in disease control and plant growth promotion. Amongst the PGPRs, *Pseudomonas fluorescence* and *Bacillus subtilis* produce highly potent broad spectrum anti-fungal molecules against various phyto-pathogens, thus acting as effective biocontrol agents (Defago et-al; 2010).

Azotobacter species is a free living nitrogen fixing bacterium, it can successfully grow in the rhizospheric zone of wheat, maize, rice, cotton, tomato, bhendi and many others and fix 10-20kgN/ ha cropping per season (Jadhav et al; 1987). Azotobacter synthesizes and secretes, considerable amounts of biologically active substance like B vitamin, nicotinic acid, pentothanic acid, biotin, heteroauxins and gibberlins etc., which enhance root growth of plants. Azotobacter species has the ability to produce antifungal antibiotics and fungi static compounds against pathogens like Fusarium species, Alternaria sp. Trichoderma sp. (Witter et al 1996). All these factors combined together produce positive effects on crop yield.

Role of Azotobacter in plant disease management

In addition to its beneficial impact on plant growth promotion Azotobacter is also known to be associated with the suppression of pathogenic diseases of plant, Maheshwari et al (2012) demonstrated that the strain TRA2 of *A.chrooccocum* which is an isolate of wheat rhizosphere showed strong antagonistic activity against root rot fungus *Macrophominaphaseolina* and *Fusariumoxysporum* in addition to improving plant growth of wheat which might be due to ameliorated plant health. Azotobacter provided good protection to the plants by agressively colonizing the roots of wheat crops. Akram et al (2016) found that disease incidence by root knot nematode Meloidogneinconita was significantly reduced when *A.chrooccocum* was applied to chickpea plants. Several mechanisms can be implicated behind the management strategies used by the bacteria for the control of plant diseases. Azotobacter is reported to produce an antibiotic having similar structure as that of anisomycin, which is well established fungicidal antibiotic. Some ex.of the pathogens that have been managed by the use of Azotobacter as a bioinoculant includes Alternaria, Fusarium, Rhizoctonia, Macrophomina, Curvularia, Helminthosporium and Aspergillus (Inawali et al, 2015).

Interaction between *A.chroococcum* and certain rhizospheremicrofungi indicated that *Trichodermaviride*, *Fusariumsemitectum* and *Alternariasolani* were more antagonistic to *A. chroococcum*. Interaction between various types of soil microorganisms is well known (Wierenga, 1963; Gangawane and Salve, 1987). In this investigation *Trichodermaviride* is well known biological control agent while *Fusariumsemitectum* and *Alternariasolani* are potential pathogens (Sanford and Broadfoot, 1931; Garrett, 1965). Pande and Kumar (1990) showed inhibitory effect of *A. chroococcum* and *Azospirilliumbrasilense* on growth of 14 rhizospheric fungi including 9 pathogens. While Sharma *et al.* (1994) conducted experiments with *Azotobacter* and *Azospirillum* biofertilizer to observe their effect on mulberry diseases like leaf spot; powdery mildew, leaf rust, leaf blight and bacterial blight. However, Vincent (1965) showed that growth of *Azotobacterchroococcum* may be inhibited by other microorganisms in the rhizosphere of crop plants. Unidentified antagonistic principles secreted by these fungi might be responsible to inhibit *A. chroococcum* in this investigation.

Table 1: Interaction of *Azotobacterchroococcum*with other microbes.

Bacteria	Interaction with *A. chroococcum*		
	Neutral	Stimulatory Zone (mm)	Antagonistic
S. aureus	-	2.5	-
Proteus sp.	-	2.0	-
Bacillus	-	2.5	-
E. coli	-	2.8	-
Pigmented (red)	-	2.3	-
Pigmented (yellow)	-	2.2	-
Irregular colony	-	2.6	-

Table 2: Interaction of rhizosphere fungi with *Azotobacterchroococcum*.

Fungi	Interaction with *A. chroococcum*		
	Neutral	Stimulatory Zone (mm)	Antagonistic
Peicilliumcenirocum	+	-	-
Aspergillustereus	+	-	-
Aspergillus spp.	+	-	-
Fusariumoxysporum	+	-	-
Fusariumsemitectum	-	-	3.6
Rhizopusstoronices	-	-	3.0
Trichodermaviride	-	-	3.2
Alternariasolani	-	-	3.4
Helminthosporiumtetramera	-	-	-

CONCLUSION

Azotobacter sp. are free living, non-symbiotic, heterotrophic bacteria capable of fixing an average of 20kgN/haper year. These bacteria are regarded as plant growth promoting rhizobacteria (PGPR) which synthesize growth substances that enhances plant growth and development and inhibit phytopathogenic growth by secreting inhibitors. It also helps in nutrient uptake and produces some biochemical substances such as protein, amino acids etc. Azotobacter improves seed germination and has beneficiary response on crop growth rate. It helps to increase nutrient availability and to restore soil fertility for better crop response. It is an important component of integrated nutrient management, system due to its significant role in soil sustainability.

Interaction between *A.chrooccocum and* rhizospheremicroflora of tomato was studied by agar well technique. It was seen that some of the fungi were inhibitory. Among fungi *Trichodermaviride, Fusariumsemitectum and Alternariasolani* were more antagonistic (P.N.Jadhav, L.V.Gangawane, 2004, Table 1 and 2).

More research is necessary in future to explore the potentiality of Azotobacter in soil fertility, and interaction between Azotobacter sp. with soil microorganisms

Declaration of competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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