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Role of *Azotobacter* sp. Isolates as a Plant Growth Promoting Agent and their Antagonistic Potentiality against Soil Borne Pathogen (*Rhizoctonia solani*) under *in vitro* Condition

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A B S T R A C T

The overdependence of chemical fertilizers in agriculture leads to decrease the fertility of soil and increase the chemical pollution. Thus, it is essential to adopt a new technology which is eco-friendly, cost-effective and reliable. Biofertilizer (*Azotobacter*, *Azospirillum*, etc) have been identified as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. Therefore the present experiment with different isolates of *Azotobacter* sp. was carried out to find out effective isolate for plant growth promoting activities and biological control. In case of *in vitro* screening for plant growth promoting efficacy, it was found that chilli seeds bacterized with AZT8 exhibited the highest per cent seed germination (98%) which was followed by AZT6 (86 %) and AZT4 (81%). Root length (4.18 cm) and shoot length of chilli seedlings (6.5 cm) were found maximum for the isolate AZT8. The calculated vigour index based on germination percentage, root length and shoot length was also recorded maximum for the isolate AZT8 (1105.445) followed by AZT6 (937.4) and AZT4 (805.95). In case of *in vitro* antagonistic potentiality test, AZT3 was found the most active isolate against *Rhizoctonia solani* giving an inhibition zone of 1.1 cm and also 72.2% growth inhibition. Another isolate showing better result, was AZT4, exhibiting zone of inhibition of 0.7 cm and mycelia inhibition of 61.1%. Therefore it may be concluded from the present experiment that AZT8 as plant growth promoting activities and AZT3 as bio control agent can be used in West Bengal.

Introduction

Agricultural production has increased in developing countries due to use of high-yielding varieties and enhanced consumption of high analysis chemical fertilizers after the era of green revolution. But the excessive use of chemical fertilizers has generated several environmental problems including the decreasing of soil fertility, greenhouse effect, ozone layer depletion and acidification of water. Therefore bio fertilizer is adopted as an alternative to chemical fertilizer to increase soil fertility and crop production in sustainable farming. For the last one decade, biofertilizers are used extensively as an eco-friendly approach to minimize the use of chemical fertilizers, improve soil fertility status and for enhancement of crop production by their biological activity in the rhizosphere. Biofertilizers are mainly the nitrogen fixing, phosphate solubilizing and

plant growth-promoting microorganisms. They are *Azotobacter*, *Azospirillum*, blue green algae, *Azolla*, P-solubilizing microorganisms, mycorrhizae and sinorhizobium (Selvakumar *et al.,* 2009).They significantly contribute to nitrogen fixation accounting for about 65% of the total annual N fixation to sustain life on earth (Newton, 1996). Amongst them, *Azotobacter* strains play a key role in harnessing the atmospheric nitrogen through its fixation in the roots. They have been also reported to improve fertility condition of the soil. Aerobic bacteria belonging to the genus *Azotobacter* represent a diverse group of free living diazotrophic (with the ability to use N_2 as the sole nitrogen source) microorganisms commonly occurring in soil. In Indian soils, the population of *Azotobacter* is not more than 10^5/gram of soil. The population of *Azotobacter* is mostly influenced by other microorganisms and soil biotic / abiotic factors. *Azotobacter* also produces some substances, thereby antagonises plant pathogens such as *Alterneria, Fusarium* and *Helminthosporium* etc. Hence, *Azotobacter* also acts as a biological control agent for management of different plant diseases. *Azotobacter* isolated from rhizosphere of certain plants was adopted to survive in the root zone of different plants such as rice, wheat, sugarcane, tea, maize, turmeric, vegetables, fruits, flowers and forest trees. They not only fix atmospheric nitrogen but also provide nitrogen compounds, growth promoting substance during process of nitrogen fixation. The beneficial effects of *Azotobacter* are not only due to its ability to fix atmospheric nitrogen, but also to secrete growth substances and antifungal antibiotics, which improve plant stands in inoculated fields by inhibition root pathogens. In fact, *Azotobacter*has beneficial effects on plant yields, due to their ability of fixing nitrogen (Tejera *et al.,* 2005), solubilizing phosphates (Hayat *et al.,* 2010 and Farajzadeh *et al.,* 2012) and to the microbial secretion of stimulating phytohormones, like gibberellins, auxins and cytokinins (Hayat *et al.,* 2010; Farajzadeh *et al.,* 2012). *Azotobacter*also produces antifungal antibiotics i.e. fungistatic substances with a broad active spectrum and inhibits the growth of *Fusarium, Aspergillus, Helminthosporium, Alterneria, Cephalo-sporium, Rhizoctonia and Sclerotium rolfsii*. Therefore the present experiment was carried out with the following objectives to find out the effective isolate of *Azotobacter* sp. as a plant growth promoting (PGP) agent as well as bio control agent from *in vitro* screening and antagonistic activity in West Bengal.

Materials and Methods

Roving survey was conducted in wheat fields in different districts of West Bengal including Jalpaiguri, Nadia, Hooghly, Murshidabad situated at different agro-ecological sub regions of West Bengal (AESR). Rhizospheric soils were collected in sterile polythene bags using a sterile soil digger to a depth of 20 cm of 3 of each of the five subplots of a field. Each soil sample of a field was mixed thoroughly *in situ* to prepare composite sample. All aseptic measures were taken during collection of samples to avoid contamination. Each composite sample was brought to the laboratory and subdivided into two parts of 200 g each, one for enumeration of bacterial population, another for nematode population and rest for physico-chemical property analysis. The samples were either undergone to instant biological analysis or dried at room temperature and sieved through a 50 mesh sieve and undergone to physicochemical property analysis using standard methods (Jackson, 1973 and Subbiah and Asija, 1956). For *in vitro* screening of bacterial isolates for their plant growth promoting (PGP) activities, the method is followed as described by (Shende *et al.,* 1977) and (Elliot and Lynch,1984) with few

modifications. Seeds were surface sterilized with 0.1% $HgCl_2$ for 1 minute followed by successive washing with sterile water and the water was decanted. Seeds were then added to cultures grown in liquid medium for 48 hours containing 10^6 cells/ml. After 10 minutes the medium was decanted and 0.8% sterile water agar was poured into the Petri plates and then seeds were kept on soft agar plates and left for incubation at 30ºC for 5-6 days. Three replicates were kept for each treatment. Seeds treated with sterilized water alone were placed on control plates. After 6 days, germination percentage, root length and shoot length were recorded.

The antagonistic effects of the *Azotobacter* sp. isolates were observed against *Rhizoctonia solani* by dual culture technique. The Petri plates are poured with 20 ml PDA (without antibiotic) and the fresh bacterial loopful culture was streaked linearly leaving 1 cm from the margin. The pathogens are placed as 4 mm disc from the 3 days old culture at the centre of each Petri plate and plates were incubated at 28^0C for 3days. The distance between the fungal growth and the bacterial colonies was recorded.

Results and Discussion

Result of this experiment for selection of effective isolate from *in vitro* screening and antagonistic potentiality test of *Azotobacter* sp. isolates as plant growth promoting agent as well as bio control agent is presented here.

In vitro plant growth promoting efficacy of the native *Azotobacter* isolates

Chilli seeds were treated with *Azotobacter* isolates and after six days of seed bacterization, the germination percentage, root length and shoot length of seedlings were monitored. Analysis of the observed data revealed that chilli expressed the best performance in plant growth promoting activity associated with seed germinability, root length and shoot length as well as vigour index in all treatments over check. Among the ten bacterial isolates, the isolate AZT8 was found to be best plant growth promoter compared to other rhizospheric bacteria. From the data in the Table 1, it was found that seeds bacterized with AZT8 exhibited the highest per cent seed germination (98%) in chilli, which was followed by AZT6 and AZT4. Root length and shoot length of chilli seedlings were found maximum for the isolate AZT8.

The calculated vigour index based on germination percentage, root length and shoot length was also recorded maximum for the isolate AZT8 (1105.44) followed by AZT6 (937.40) andAZT4 (805.95) (Table 1 and Fig. 1). Similar observation was also recorded by (Soleimanzadeh *et al.,* 2010) who reported that *Azotobacter* when applied to seeds which improved seed germination to a considerable extent. The *Azotobacter* isolates were able to dissolve inorganic and organic phosphate compounds (Farajzadeh *et al.,* 2012). *Azotobacter* has significant role in plant growth promotion including production of growth regulators, protection from root pathogens, and modification of nutrient uptake by the plant (Tchan, 1988).

In vitro antagonistic potentiality of the native putative *Azotobacter* isolates against soil borne pathogen *Rhizoctonia solani*

Perusal of the data in Table 2 indicated that two *Azotobacter* isolates showed antagonistic effect against *Rhizoctonia solani* under *in vitro* condition and inhibited the vegetative growth of the fungus at varied level.

Table.1 *In vitro* plant growth promoting efficacy of the native putative *Azotobacter* sp. isolates

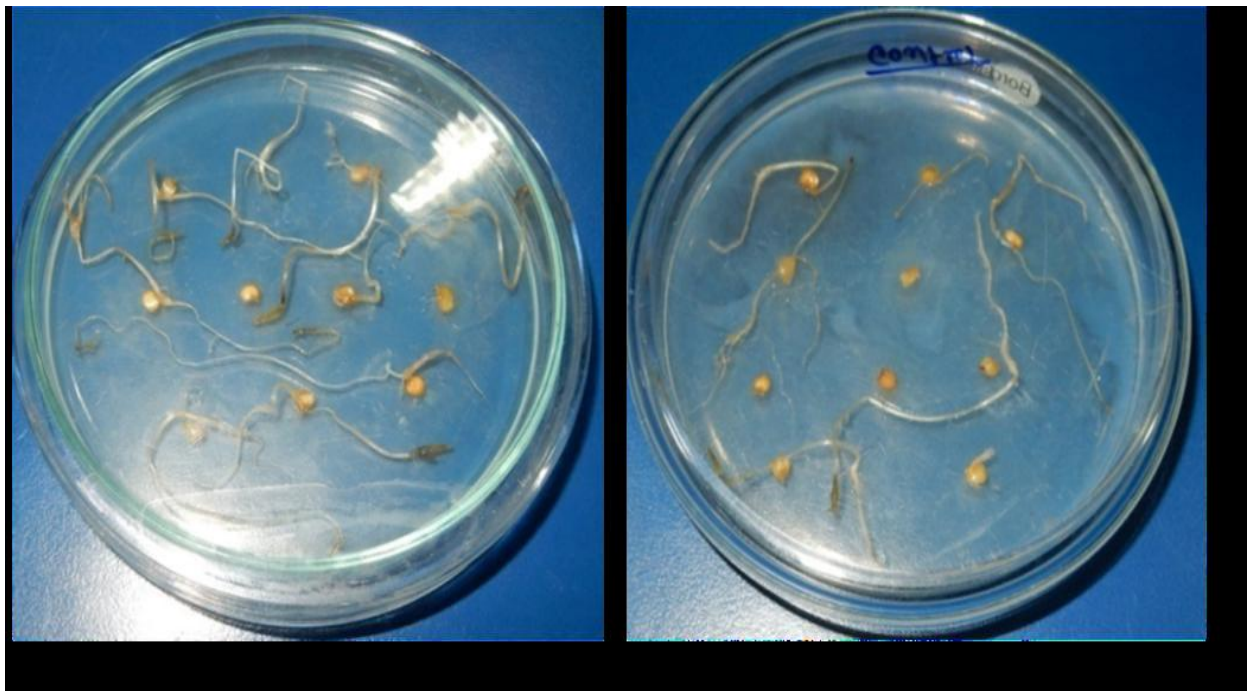
Sl. No.	Isolate No.	Percentage seed germination	Root length(cm)	Shoot length(cm)	Vigor Index	Avg. Fresh wt. (mg)
1	AZT 1	65(53.7)	3.60^{d}	5.16^{cd}	569.40	28.18
2	AZT 2	65(53.7)	4.12^{c}	6.22^{a}	672.10	28.58
3	AZT 3	72(58.1)	4.24^{bc}	5.48^{b}	699.84	31.30
4	AZT 4	81(64.2)	4.55^{abc}	5.40^{bc}	805.95	32.58
5	AZT 5	64(53.1)	3.32^{d}	4.78^{e}	518.40	21.16
6	AZT 6	86(68.0)	4.66^{ab}	6.24^{a}	937.40	32.72
7	AZT 7	74(59.3)	4.34^{abc}	4.88d^{e}	682.28	29.36
8	AZT 8	**98(81.9)**	**4.78^{a}**	**6.50^{a}**	**1105.44**	**33.33**
9	AZT 9	76(60.7)	4.52^{abc}	5.40^{bc}	753.92	32.00
10	AZT 10	64(53.1)	3.52^{d}	4.14^{f}	490.24	20.75
11	Control	62(51.9)	2.36^{e}	3.88^{f}	386.88	12.71
12	SEM±	**1.00**	**0.14**	**0.10**		
13	CD	**2.95**	**0.40**	**0.29**		

[Values are mean of three replications. Figures in parentheses are angular transformed values. A common letter means they are not significantly different (p= 0.05) by DMRT.]

Table.2 *In vitro* antagonistic potentiality of the native putative *Azotobacter* isolates against soil borne pathogen *Rhizoctonia solani*

Sl. No.	Isolate No.	% Inhibition	Zone of inhibition(cm)
1	AZT 1	2.2(8.6)^{i}	0
2	AZT 2	13.3(21.4)^{g}	0
3	AZT 3	**72.2(58.2)^{a}**	**1.1**
4	AZT 4	61.1(51.4)^{b}	0.7
5	AZT 5	27.8(31.8)^{e}	0
6	AZT 6	5.6(13.6)^{h}	0
7	AZT 7	0.0(0.0)^{j}	0
8	AZT 8	22.2(28.1)**f**	0
9	AZT 9	57.8(49.5)^{c}	0
10	AZT 10	53.3(46.9)^{d}	0
11	SEM±	**0.35**	
12	CD	**1.05**	

Fig.1 *In vitro* plant growth promoting potentiality of the native putative *Azotobacter* sp. isolates



Chilli seed treated with isolate AZT 8 Control (Untreated)

Fig.2a, 2b *In vitro* antagonistic efficacy of the soil native putative *Azotobacter* isolates against soil borne pathogen *Rhizoctonia solani*

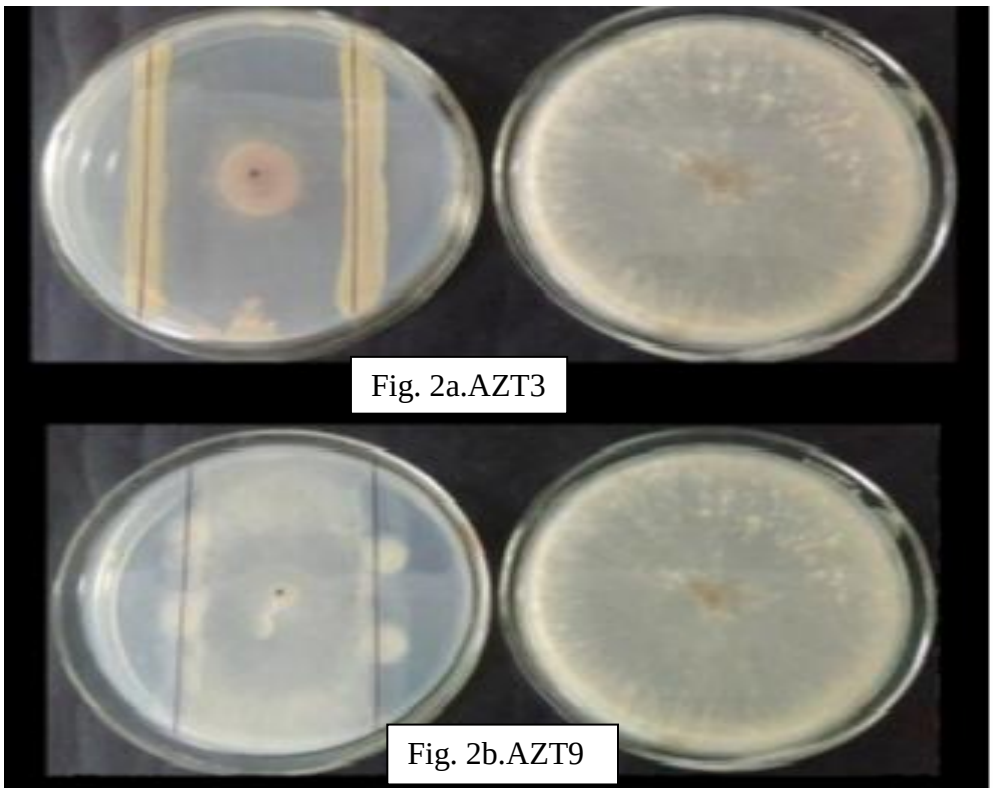
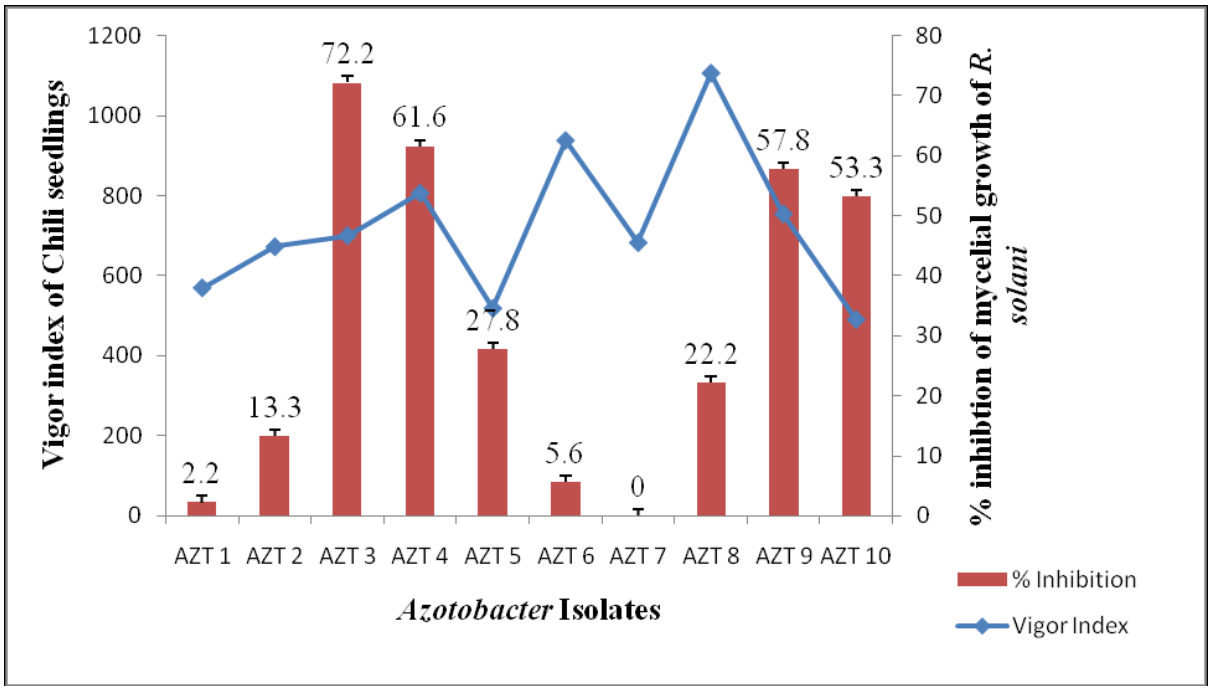


Fig.3 *In vitro* percent inhibition of mycelial growth of soil borne pathogen *R. solani* and vigor index of chili seedlings by different native *Azotobacter* isolates



Among all the isolates, AZT3 was found to be the most active isolate giving an inhibition zone of 1.1 cm and also 72.2% growth inhibition (Table 2, Figure 2 and 3). Another isolate showing better result, was AZT4, exhibiting zone of inhibition of 0.7 cm and mycelia inhibition of 61.1%. Similar observation was also reported by (Mishustin and Shilnikova, 1969), indicating that *Azotobacter* can produce an antifungal antibiotics which inhibits *Rhizoctonia solani* growth. *Azotobacter* sp. can also produce antifungal compounds to fight against many plant pathogens (Jen-Hshuan, 2006).

In this experiment it was found that chilli seeds inoculated with AZT8 exhibited the highest per cent seed germination (98%). Root length and shoot length of chilli seedlings were also found maximum for the isolate AZT8 and AZT3 was found to be the most active isolate giving highest growth inhibition (72.2%) of *Rhizoctonia solani* than other isolate. Therefore it may be concluded that AZT8 as plant growth promoting and AZT3 as bio control agent can be used in West Bengal.

References

Elliott, L.F. and Lynch, J.M., 1984. *Pseudomonads* as a factor in the growth of winter wheat (*Triticum aestivum* L.).*Soil Biol. Biochem.*16: 69-71.

Farajzadeh, D., Yakhchali, B., Aliasgharzad, N., Sokhandan-Bashir, N. and Farajzadeh, M. 2012.Plant growth promoting characterization of indigenous *Azotobacteria* Isolated from Soils in Iran. *Curr Microbiol*. 64:397-403.

Hayat, R., Ali, S., Amara, U., Khalid, R. and Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Ann Microbiol*. 60: 579-598.

Jackson, M.L. 1973. Soil Chemical Analysis. Prentice Hall of India Private Limited,

New Delhi, 30: 106-109.

Jen-Hshuan, C. 2006.The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. International workshop on Sustained Management of the Soil Rhizosphere System for Efficient Crop Production and Fertilizer Use. 20:1-10.

Mishustin, E.N. and Shilnikova, V.K.1969. Free – living nitrogen fixing bacteria of the genus *Azotobacter*, In soil Biology, Reviews of Research, UNESCO Publication. Paris. 82-109

Newton, W.E. 1996.Kish-Othmer Encyclopedia of Chemical Technology (EdsJohn Wiley and Sons) New York.172–204.

Selvakumar, G., Lenin, M., Thamizhiniyan, P. and Ravimycin, T. 2009. Response of biofertilizers on the growth and yield of blackgram (*Vigna mungo* L.). *Recent Research in Science and Technology*. 1(4): 169-175.

Shende, S.T., Apte, R.G. and Singh, T. 1977. Influence of *Azotobacter* on germination of rice and cotton seed. *Curr Sci*.46:675

Soleimanzadeh, H., Habibi, D., Ardakani, M.R., Paknejad, F., Rejali, F. and American Eurasian, J. 2010.Plant Growth Promotional Effect of *Azotobacter chroococcum*. *AgriEnv Sci*. 7(3): 265-268.

Subbiah, B.V. and Asija, G.L. 1956. A rapid procedure for estimation of available nitrogen in soils. *CurrSci*, 25: 259-260.

Tchan, Y.T. 1988. Some aspects of non-rhizobial diazotrophs: Their past and their future. In Microbiology in Action (Eds. Murrell WG and Kennedy IR) Research Studies Press/Wiley, Chichester, UK.193-207.

Tejera, N., Lluch, C., Martinez-Toledo, M.V., Gonzalez-Lopez, J. 2005.Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant Soil*.270:223–232.

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