Germination and growth stimulation of *Triticum aestivum* L. seedlings by consortium treatment of *Azotobacter chroococcum* and *Pseudomonas putida*

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Abstract:

The microorganisms with the aim of improving nutrients availability for plants are an important practice and necessity of agriculture. During the past decades, plant growth-promoting rhizobacteria (PGPR) had started replacing the use of chemical fertilizers in agriculture, horticulture and environmental cleanup strategies. To increase the yield of the *Triticum aestivum* L. (wheat) the present study was focused on the growth effect of two PGPR strains viz. *Azotobacter chroococcum* (MTCC) and *Pseudomonas putida* (MTCC) on wheat. Promising results were obtained for the consortium study based on the mixture of both bacterial inocula, which enhanced the growth as compared to the single treatment as well as with control. Seed germination after 5 days was observed higher in W1 (9.000 + 0.57a) compared to lowest in control (4.667 + 0.88b) and W3 (7.333 + 0.88a). The Seed Vigor Index (SVI) showed higher when treated with both bacterial strain i.e., W1 (2184.26 $\pm$ 127.05a) followed with single treatment of *P. putida*, W3 (1814.43 $\pm$ 266.10a) and least SVI was recorded in control with 1112.83 $\pm$ 228.79b.

Index terms- *Triticum aestivum L., Azotobacter chroococcum, Pseudomonas putida,* SVI, etc.

1 INTRODUCTION

Wheat is the major cereal grain that sustains humanity. Wheat is grown in temperate climate and it is staple food for almost 35% of world's population. On other hand, it provides more calories and protein in the diet than any other crop. Scientifically, classified as, *Triticum aestivum* L., it

belongs to family Poaceae, and is cultivated worldwide. In 2016, 749 million tonnes was the total production of wheat globally[3]. It provides protein about 12%, much higher to other major cereals and crops [1, 2]. India is ranked third in wheat production after European Union and China. Still, it is needed to increase the productivity of the wheat as per demand [3].

The soil productivity is dependent upon its nutritional threshold, which it provides to the standing crop. Soil micro flora in this regard, particularly the PGPRs and VAM, etc. play a pivotal role. Rhizobacteria enhances the seed germination, seedling vigor, emergence, plant stand, root and shoot growth, total biomass of the plants, seed weight, grains, fodder and fruit yields etc. [4, 5]. Extracellular plant growth promoting rhizobacteria (e-PGPR) that may exist in the rhizosphere, on the rhizoplane or in the spaces between the cells of root cortex such as *Azotobacter, Azospirillum, Bacillus, Pseudomonas,* etc. [6]. PGPR showed positive effect on plant by various mechanisms. The mode of action of PGPR that promotes plant growth includes; abiotic stress tolerance in plants; nutrient fixation for easy uptake by plant; plant growth regulation; production of siderophores; biosynthesis of volatile organic compounds; and synthesis of proteolytic enzyme such as chitinase, glucanase, and ACC-deaminase for the prevention of plant diseases [7, 8]. They also have the ability to increase the uptake of nutrient by solubilizing the NPK inorganic compound into ready to absorb, organic matter in the rhizospheric region and prevent leaching out [7]. As an example, nitrogen, which is needed for the synthesis of amino acids and proteins, is the most limiting nutrient for plants. *Azotobacter chroococcum*, free living microbe helps in nitrogen fixation in soil associated with cereals and rice crops [9, 10, 11, 12].

Pseudomonas species is ubiquitous bacteria in agriculture soil and has many traits that make them well suited as PGPR. Most effective strains are *P. fluorescens, P. putida and P. aeruginosa.* The strains of *Pseudomonas* are able to solubilize phosphorous in soil and increase its availability to plant [13]. *P. putida* can utilize the heterologous siderophore produced by rhizosphere microorganisms to enhance the level of iron available to it in natural habitat [14]. *P. putida* shows biocontrol potential against phytopathogenic fungi *in vivo* and *in vitro* conditions from Chickpea rhizosphere. *P. putida* has potential for the biocontrol of root-rot disease complex of chickpea and other crops by showing antifungal activity [15].

Azotobacter species are Gram negative, free-living, aerobic soil microbes which are oval to spherical having thick walled cyst with peritrichous flagella [16, 17, 18]. *A. chroococcum* was

the first known aerobic free-living nitrogen fixer [19]. These bacteria utilize atmospheric nitrogen gas for their cell protein synthesis. After the death of *Azotobacter* cells, protein discharged in soil can be mineralized to provide nitrogen to the crop plant. Acidic pH, high salts, and temperature have influences on *Azotobacter spp.* [20]. By biosynthesis of biologically active substances, stimulation of rhizospheric microbes and by the production of phytopathogenic inhibitors, positive effects on crop growth and yield increases by *A. chroococcum* [21, 22]. *A. chroococcum* modify the nutrient uptake and ultimately enhance biological nitrogen fixation [23]. The presence of *Azotobacter sp.* in soils has beneficial effects on plants, but the abundance of these bacteria is related to many factors, soil physico-chemical (e.g. organic matter, pH, temperature, soil moisture) and microbiological properties [24]. Its abundance varies as per the depth of the soil profile [25, 26, 27, 28, 29]. *Azotobacter spp.* is much more abundant in the rhizosphere of plants than that in the surrounding soil and abundance also depends on the crop species [30, 31]. *Azotobacter* species are non-symbiotic heterotrophic bacteria capable of fixing an average 20 kg N/ha/per year [24]. Use of bacterial inoculum helps to improve plant growth and to increase soil nitrogen through nitrogen fixation by utilizing carbon for its metabolism [32]. A seed of corn and wheat plant treated with *Azotobacter* help in uptake of N, P as well as some micronutrients such as Fe and Zn and thus helps in improvement of crop nutrients [23, 33].There is an increment in productivity of maize, with the application of manure and *Azotobacter* [34].

Seeds of wheat (*Triticum aestivum* L.) were inoculated with 11 bacterial strains of *A. chroococcum*, which resulted that all *A. chroococcum* strains had positive effect on the yield and N concentrations of wheat [35].

On the basis of previous literary works, the present study was carried out with two promising PGPR bacterial strains which enhances the soil fertility as well as provide the NPK utility and other major growth regulators i.e. IAA, etc. to the growing plant.

2 METHODOLOGY

2.1 Soil and seed sterilization: Soil was collected from Rasoolabad Ghat, Teliarganj, Allahabad and analysis of soil sample was conducted at IFFCO, Phoolpur, Allahabad. After sieving process, the soil was mix with coarse sand with ratio 1:3 (Soil: Sand). The sandy soil was sterilized with four different ways:

(a) Acid washed with 10% HCl and washed 3 times with running tap water.

(b) Further, soil was surface sterilized with 10% Formalin for 24 hours and washed with tap water and sterilized ionized water.

(c) Soil was then wet sterilized two times by using Autoclave (121.5°C, 15 bar, 20 minutes).

(d) Further, soil was put in the Hot Air Oven (at 200°C) for 24 hours.

Seed was procured from NBPGR, New Delhi and were sterilized with 2% $HgCl_2$ and washed with 3 times with deionized water.

2.2 Inoculum preparation: Two PGPR strain were selected *viz., Azotobacter chroococcum* (MTCC No.7724) and *Pseudomonas putida* (MTCC No.1259) procured from MTCC, IMTECH, Chandigarh, Punjab (India).

The inoculum was prepared by comparing with 0.5 McFarland Standard Solution at 397 nm using Spectramax Plus 384.

2.3 Competitive inhibitory test (Antibiosis) of *A. chroococcum* and *P. putida*: The two bacterial strains were incubated together in single Petri plate to study their inhibitory effect against each other.

2.4 Biochemical test of two bacterial strains: Plant growth promoting assay was done on both strain *viz.,* IAA, PSB, KSB, Urea degradation, Citrate utilization and Catalase activity.

2.5 Pot Experiment: The seeds were soaked in an inoculum of *A. chroococcum* and *P. putida* separately as well as in mixed inocula for 12 hours.

The plastic cups (200 gm capacity) were sterilized by UV light for 24 hours. Cup was filled with sterilized sandy-soil. In each cup, 10 seeds were sown in triplicate with the test bacterial inoculum.

The seedlings were placed in growth chamber under photosynthetically active radiation (PAR) of 150 µmol photons $m^{-2}s^{-1}$ with 16:8 h day and night regime and 55%$\pm$ 5 % relative humidity at 25°C $\pm$1°C for 15 days after sowing. After 5 days of sowing, germination percentage was recorded and after 15 days of sowing shoot and root length were taken and SVI and Standard error and deviation was taken by using SPSS 16.

3 RESULTS AND DISCUSSION

The Soil sample was analyzed at IFFCO, Phoolpur, Allahabad.

Table 1: Soil analysis of sample collected from Rasoolabad at CORDET, IFFCO, Phoolpur, Allahabad.

The Soil was analyzed with high metallic concentration of Boron, Copper, Iron, Manganese, Sulphur and Zinc. It was also recorded with higher concentration of Phosphorus and Potassium

with pH 8.3 which is slightly alkaline. The Electrical Conductivity of Soil was measured with 0.18 and organic carbon was found to be low 0.12. Thus, analysis clearly indicated that the soil had lost its fertility and thereby not suitable for the agricultural practices.

Figure 1: Sterilized sandy soil for the pot experiment (sand + soil mixed in a ratio of 3:1)

Soil was mixed with sand in the ratio 1:3 and sterilized by acid wash, surface, wet and dry technique to assure that no microbes were left in the soil.

Figure 2: Revived cultures on Nutrient Agar **A.** *A. chroococcum* **B**. *P. putida*

Figure 3: Antibiosis assay of *A. chroococcum* and *P. putida* on NA plates.

3.1 Antibiosis Assay: Antibiosis assay was done with both the test bacteria against each other. No inhibitory effect was showed by the test bacteria against each other and grows smoothly in culture. So, both of the bacteria grown mutually in the same culture and help in growth parameter of the plants. The antibiosis effect was not found between all rhizobacteria. They clearly flourish the growth without affecting the growth of other bacterial inoculum.

3.2 Biochemical Test: Biochemical assay of both rhizobacterial strains showed more activity on IAA production, Potassium Solubilizing activity and catalase activity. In additional *A. chroococcum* showed high activity on Phosphate Solubilizing and citrate utilization and low activity was observed in Urea degradation, whereas *P. putida* showed higher in Urea degradation but low activity was observed in citrate utilization and Phosphate Solubilizing activity.

Table 2: Biochemical test of procured bacteria *A. chroococcum* and *P. putida*.

Figure 4: Biochemical test of *A. chroococcum* and *P. putida* (**A**. Simmon Citrate; **B**. Indole Acetic Acid; **C**. Urease; **D**. Phosphate Solubilizing).

Figure 5: Biochemical test of *A. chroococcum* and *P. putida* (A. Potassium Solubilizing activity; B. Catalase activity).

3.3 Pot Experiment: Consortium study was done on sterilized (surface) seeds of Wheat treated with *A. chroococcum* and *P. putida* and all observations were taken in 3 days and 15 days after sowing.

3.4 Seed Germination Percentage: Treatment with bacterial inocula of consortium showed higher germination rate with much healthier shoot and root length (W1) with 90 %. Least was recorded in control (40.66 %) and single treatment of *P. putida* with 70.33 % (W3).

Table 3: Seed Germination Percentage of wheat treated with bacteria *A. chroococcum* and *P. putida*.

Figure 6: Pot experiment of *A. chroococcum* and *P. putida* on seed germination of wheat. **A**: after 3 days of treatment. **B**: after 15 days of treatment.

Graph 1: Seed germination percentage; **A**) after 5 days of sowing and Shoot and root length and; **B**) after 15 days of sowing.

3.5 Shoot- Root Length (in cm): The shoot and root length was observed highest in (W3) seeds were treated with *A. chroococcum* and *P. putida*. Lowest was observed in seeds treated with *P. putida* and control.

Table 4: Shoot and root length of wheat treated with bacteria *A. chroococcum* and *P. putida*

Table 5: Seed Vigor Index of wheat treated with bacteria *A. chroococcum* and *P. putida*

Figure 7: Shoot and root length (in mm) measured after 15 days of sowing under standard condition in Plant growth chamber. (Control- seeds+ Deionized water; W1- seeds+ *A. chroococcum*+ *P. putida*; W2- seeds + *A. chroococcum*; W3- seeds+ *P. putida*.)

Graph 2: Seed Vigor Index after 15 days of sowing.

4 CONCLUSION

The present study revealed the promising result as the test bacteria *viz.*, *Azotobacter chroococcum* and *Pseudomonas putida* showed higher germination rate as well as high Seed Vigor Index on wheat plant when consortium treatment was subjected. This can be used as a biofertilizer which can fulfill/supplement the requirement of NPK for the growing crop as well as many micro and macro nutrient with other plant growth promoting activity.

5 ACKNOWLEDGEMENTS

Thanks are due to Head, Department of Botany, University of Allahabad, Prayagraj for providing the lab facility; to IFFCO, Phoolpur for soil analysis and UGC for financial assistance.

6 FIGURES, GRAPHS AND TABLES

Figure 1: Sterilized sandy soil for the pot experiment (sand + soil mixed in a ratio of 3:1)

A. **B.**

Figure 2: Revived cultures on Nutreint Agar. **A.** *A. chroococcum* **B.** *P. putida*.

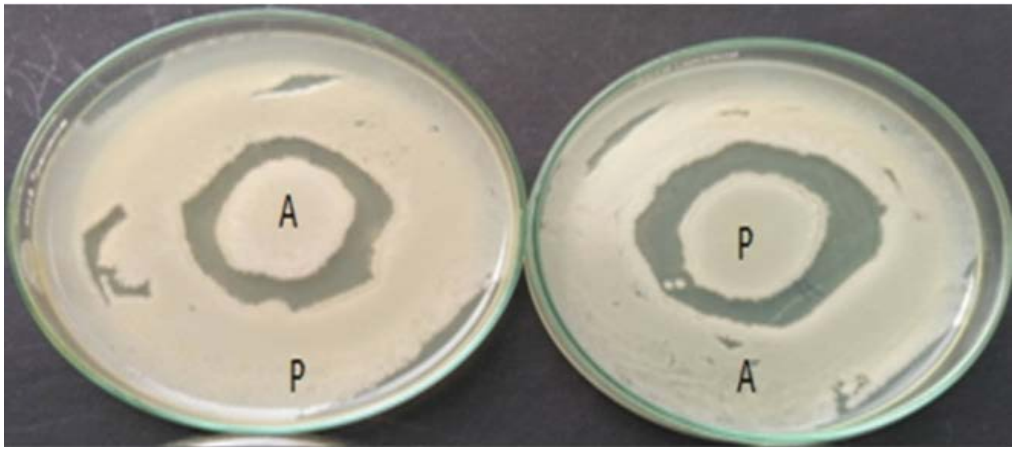
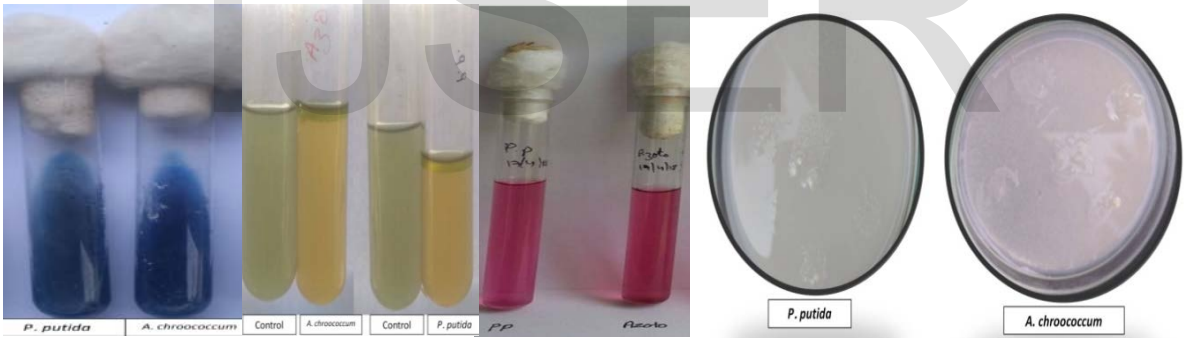
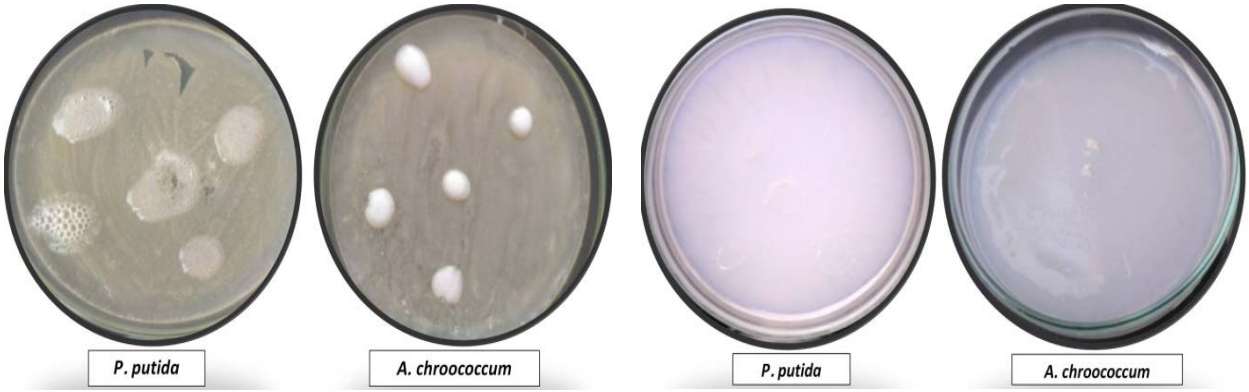


Figure 3: Antibiosis assay of *A. chroococcum* and *P. putida* on NA plates.



A **B** **C** **D**

Figure 4: Biochemical test of *A. chroococcum* and *P. putida* (**A**. Simmon Citrate; **B**. Indole Acetic Acid; **C**. Urease; **D**. Phosphate Solubilizing).



A **B**

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A **B**

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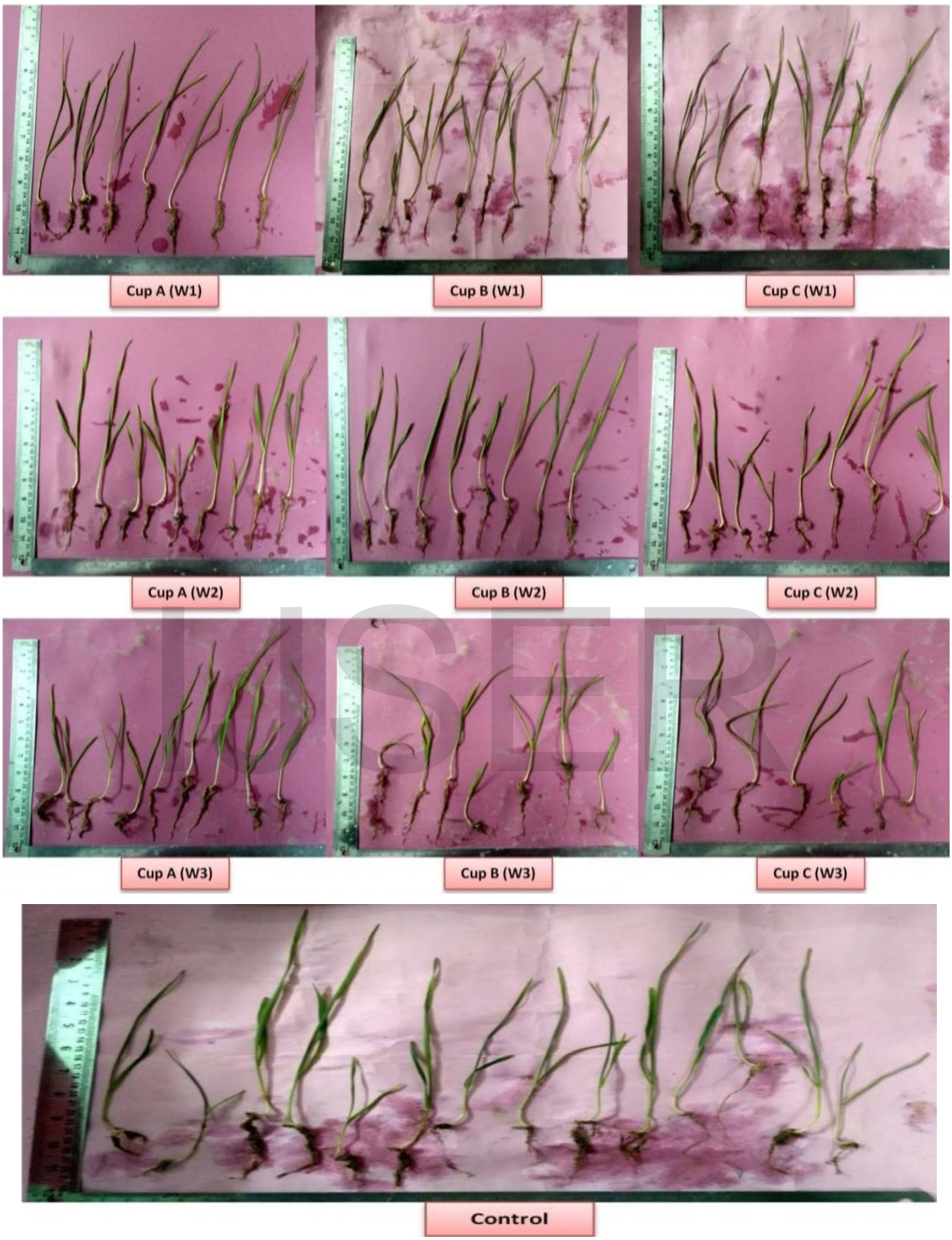
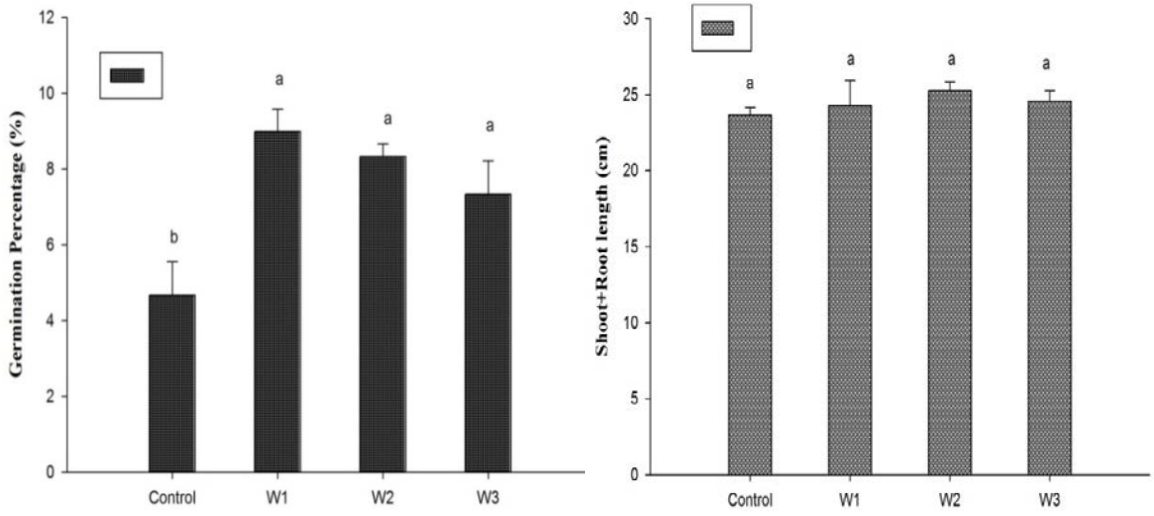
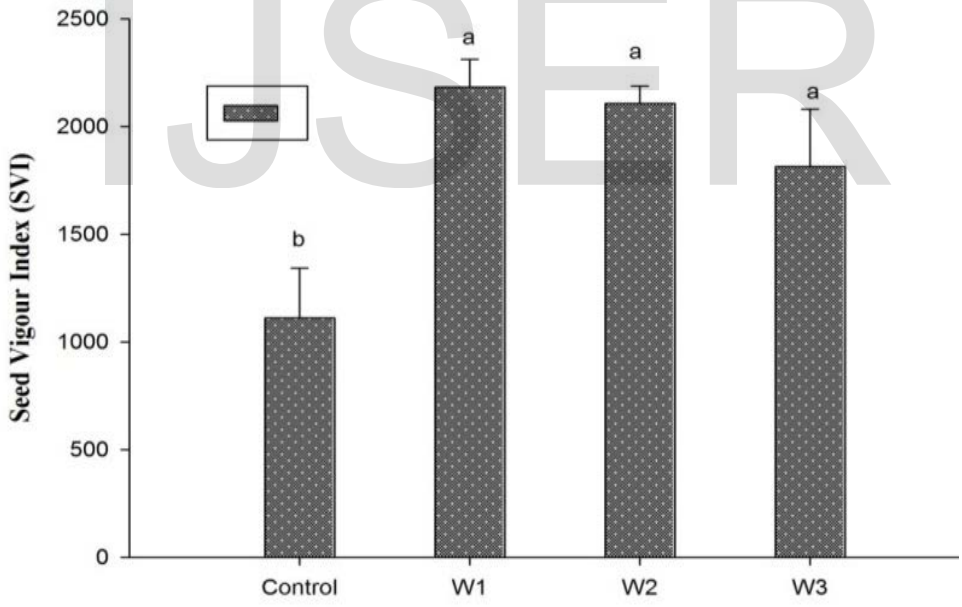


Figure 7: Shoot and root length (in mm) measured after 15 days of sowing under standard condition in Plant growth chamber. (Control- seeds+ Deionized water; W1- seeds+ *A. chroococcum*+ *P. putida*; W2- seeds + *A. chroococcum*; W3- seeds+ *P. putida*.)



A B

Graph 1: Seed germination percentage; **A**) after 5 days of sowing and Shoot and root length and; **B**) after 15 days of sowing.



Graph 2: Seed Vigor Index after 15 days of sowing.

Table 1: Soil analysis of sample collected from Rasoolabad at CORDET, IFFCO, Phoolpur, Allahabad.

Lab Ref.	Soil Analysis													
	Boron		Copper		Iron		Manganese		Sulphur		Zinc		pH	
	value	level	Value	level	value	level	value	level	value	level	value	level	value	level
	1.3	H	1.44	H	12.4	H	10	H	10	H	2	H	8.3	Alk

38146												
	Elec. Cond.		Organic Carbon				Phosphorus			Potassium		
	value	level	BTV	TV	value	level	MR	value	level	MR	value	level
	0.18	LS	20	19.2	0.12	L	91	15	L	14	157	M

Value: Concentration in ppm; Level: **H**= High, **M**= Medium, **L**=Low; Electrical Conductivity: **LS**= Low Salinity; pH: **Alk**= Alkaline; Organic Carbon: **BTV**=Blank Titration Value, **TV**= Titration Value, ; Phosphorus and Potassium: **MR**=Resilient Modulus value.

Table 2: Biochemical test of procured bacteria *A. chroococcum* and *P. putida*.

Name	Citrate test	IAA test	Urea test	PSB test	KSB test	Catalase test	Gram staining
A. chroococcum	+++	+++	++	+++	+++	+++	negative, oval
P. putida	++	+++	+++	++	+++	+++	negative, oval

+= good, ++= moderate, +++= high, - = no activity

Table 3: Seed Germination Percentage of wheat treated with bacteria *A. chroococcum* and *P. putida*.

S. No.	Treatment	Mean ± Error
1	Control	$4.667 + 0.88^{b}$
2	W1	$9.000 + 0.57^{a}$
3	W2	$8.333 + 0.33^{a}$
4	W3	$7.333 + 0.88^{a}$

Table 4: Shoot and root length of wheat treated with bacteria *A. chroococcum* and *P. putida*

S. No.	Treatment	Mean ± Error
1	Control	23.66 ± 0.48^{a}
2	W1	24.27 ± 1.66^{a}
3	W2	25.29 ± 0.56^{a}
4	W3	24.60 ± 0.67^{a}

Table 5: Seed Vigor Index of wheat treated with bacteria *A. chroococcum* and *P. putida*

S. No.	Treatment	Mean ± Error
1	Control	1112.83 ± 228.79^{b}
2	W1	2184.26 ± 127.05^{a}
3	W2	2106.16 ± 82.17^{a}
4	W3	1814.43 ± 266.10^{a}

REFERENCES

1. Seilmeier, W., Belitz, H.D. and Wieser, H., Separation and quantitative determination of high-molecular-weight subunits of glutenin from different wheat varieties and genetic variants of the variety Sicco. Zeitschrift fur Lebensmittel-Untersuchung und Forschung, 1991. 192(2): p. 124-129.
2. Halford, N.G., Field, J.M., Blair, H., Urwin, P., Moore, K., Robert, L., Thompson, R., Flavell, R.B., Tatham, A.S. and Shewry, P.R., Analysis of HMW glutenin subunits encoded by chromosome 1A of bread wheat (*Triticum aestivum* L.) indicates quantitative effects on grain quality. Theoretical and Appl. Gen., 1992. 83(3): p. 373-378.
3. Commodity Profile of Wheat for January. 2018.
4. Van Loon, L.C., Bakker, P.A. and Pieterse, C.M.J., Systemic resistance induced by Rhizosphere bacteria. Annu. Rev. Phytopathol., 1998. 36: p. 453-483.
5. Ramamoorthy, V., Viswanathan, R., Raghuchander, T., Prakasam, V. and Samiyappan, R., Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. Crop Protec., 2011. 20: p. 1-11.
6. Figueiredo, M.V.B., Seldin, L., Araujo, F. and Mariano, R.L.R., Plant growth promoting rhizobacteria: fundamentals and applications. Plant Growth and Health Promoting Bacteria, 2011. p. 21-43.
7. Choudhary, D.K., Sharma, K.P. and Gaur, R.K., Biotechnological perspectives of microbes in agro-ecosystems. Biotechnol. Letters, 2011. 33(10): p. 1905-1910.
8. García-Fraile, P., Menéndez, E. and Rivas, R., Role of bacterial biofertilizers in agriculture and forestry. AIMS Bioeng, 2015. 2: p. 183-205.
9. Rajaee, S., Alikhani, H.A. and Raiesi, F., Effect of Plant Growth Promoting Potentials of *Azotobacter chroococcum* Native Strains on Growth, Yield and Uptake of Nutrients in Wheat. Agris. Records, 2007. 11(41): p. 285-297
10. Lloret, L. and Martinez-Romero, E., Evolution and phylogeny of rhizobia. Rev. Latinoam Microbiol., 2005. 47(1-2): p. 43-60.
11. Raymond, J., Siefert, J.L. and Staples, C.R., The natural history of nitrogen fixation. Mol. Biol. Evol., 2004. 21: p. 541-554.
12. Tejera, N., Lluch, C. and Martínez, T.M.V., Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. Plant Soil, 2005. 270(1): p. 223-232.

13. Sundara, B., Natarajan, V. and Hari, K., Influence of Phosphorous solubilizing bacteria on the changes in soil available phosphorous and sugarcane and sugar yields. Field Crop Res., 2002. 77: p. 43-49. http://dx.doi.org//0.1016/50378-4290 (02)00048-5.
14. Loper, J.E. and Henkels, M.D., Utilization of Heterologous siderophores enhances levels of Iron available to *Pseudomonas putida* in the rhizosphere. Appl. Environ. Microbiol., 1999. 65(12): p. 5357-5363.
15. Saraf, M., Thakker, A. and Patel, B.V., Biocontrol activity of different species of *Pseudomonas* against phytopathogenic fungi *invivo* and *invitro* conditions. Intl. Jour. Biotechnol. Biochem., 2008. 4(3-4): p. 11-18.
16. Gandora, V., Gupta, R.D. and Bhardwaj, K.K.R., Abundance of *Azotobacter* in great soil groups of North-West Himalayas. Jour. Indian Soc. Soil Sci., 1998. 46(3): p. 379-383.
17. Salhia, B., The Effect of *Azotobacter chroococcum* as Nitrogen biofertilizer on the growth and yield of *Cucumis sativus*. The Islamic University Gaza, Deanery of Higher Education Faculty of Science, Master of Biol. Sci. Botany, 2013.
18. Martyniuk, S. and Martyniuk, M., Occurrence of *Azotobacter spp.* in some polish soils. Polish Jour. Environ. Stud., 2003. 12(3): p. 371-374.
19. Beijerinck, M.W., Ueber, O.M., Zentralblattfiir, B., Parasitenkunde, Infektionskrankheiten and Hygiene, Abteilung, 1901. 2(7): p. 561-582.
20. Tchan, Y.T. and New, P.B., Azotobacteraceae. In: Holt JG, Williams & Wilkins (Eds.) Bergey's Manual of Systematic Bacteriol. Baltimore, USA, 1989. 1: p. 220-229.
21. Chen, J., The combined use of chemical and organic fertilizers and/or biofertilizer for crop growth and soil fertility. Intl. workshop on Sustained Management of the Soil-Rhizosphere System for Efficient Crop Production and Fertilizer Use, Thailand, 2006. 1: p. 10.
22. Lenart, A., Occurrence, Characteristics and Genetic Diversity of *Azotobacter chroococcum* in Various Soils of Southern Poland. Pol. Jour. Environ. Stud., 2012. 21(2): p. 415-424.
23. Somers, E., Vanderleyden, J. and Srinivasan, M., Rhizosphere bacterial signaling: A love parade beneath our feet. Crit. Rev. Microbiol., 2004. 30(4): p. 205-240.
24. Kizilkaya, R., Nitrogen fixation capacity of *Azotobacter spp.* Strains isolated from soils in different ecosystems and relationship between them and the microbiological properties of soils. Jour. Environ. Biol., 2009. 30(1): p. 73-82.

25. Vojinoviv, Z., Microbiological properties of main types soil in Serbia for nitrogen cycling. Jour. for Sci. Agri. Res., 1961. 43: p. 3-25.
26. Sariv, Z., Biogenic levels of the horizons of calcerous chernozem in Vojvodina. Contemporary Agriculture, 1969a. 17: p. 819-825.
27. Sariv, Z., Biogenity of limeless chernozem in Vojvodina. Annals of Scientific Work at the Inst. for Res. in Agri. Novi Sad, 1969b. 7: p. 145-151.
28. Malek, A.Y., Hosny, I. and Shawky, B.T., Nitrogen-fixing capacity of *Azotobacter* as affected by the type and depth of substrate. ZentralblBakteriolNaturwiss, 1979. 134(5): p. 390-397.
29. Kalaigandhi, V., Kannapiran, E., Harimuraleedharan, M.A., Sivakumar, T. and Arusa, V.T., *Azotobacter* population in Rhizosphere and Non-Rhizosphere sediments of Tondi Coast. Intl. Jour. of Biol. Technol., 2010. 1(1): p. 63-65.
30. Sariv, Z. and Ragoviv, B., The influence of the maize on the dynamic of *Azotobacter* in the soil. Soil Plant, 1963a. 13: p. 273-277.
31. Sariv, Z. and Ragoviv, B., The effect of some plants on the dynamics of *Azotobacter* in the soil. Annals of Scientific Work at the Faculty of Agriculture Novi Sad, 1963b. 7: p. 1-11.
32. Monib, M., Hosny, I. and Fayez, M., Effect of *Azotobacter* inoculation on plant growth and soil nitrogen. Zentralbl Bakteriol Naturwiss, 1979. 134(2): p. 140-148.
33. Kizilog, I.U., Bilen, F.T. and Ataplu, N., Effect of Inoculation Eight *Azotobacter chroococcum* and Nitrogen Fertilizer on Plant Growth of Corn (*Zea mays*) Carbohydrate and Protein Contents. ZiraatFakultesiDergisi Ataturk Universitasi, 2001. 32: p. 215-221.
34. Meshram, S.U. and Shende, S.T., Response of Maize to *Azotobacter chroococcum*. Plant and Soil, 1982. 69: p. 265-273.
35. Kizilkaya, R., Yield response and nitrogen concentrations of spring wheat (*Triticum aestivum*) inoculated with *Azotobacter chroococcum* strains. Ecol. Eng., 2008. 33(2): p. 150-156.