

Evaluation of PGPR Isolates for Plant Growth Promotion and Yield Enhancement of Groundnut Crop

Arti Thakur^{1*} and Samir C. Parikh²

¹Ph.D. Student, ²Principal,

Smt. S. M. Panchal, Science College, Talod,

Affiliated to Hemchandracharya North Gujarat University, Patan, Gujarat, India

*Corresponding Author E-mail: arti.thakur@rediffmail.com

Received: 17.03.2018 | Revised: 26.04.2018 | Accepted: 3.05.2018

ABSTRACT

The study was carried out to evaluate the five previously isolated and PGPR screened; efficient and multitrait rhizobacterial isolates GSP 4, GST 3, GSB 13, GSH 1 and GSV 3 under pot study and field application. Promising results were obtained under pot application of five efficient isolates in terms of increased shoot length (21.50 cm to 14.22 cm), root length (9.70 cm to 13.32 cm) and dry weight (13.10 g to 18.52 g) of groundnut plants compared to control. Further the field application of three most promising isolates GSH 1, GSB 13 and GST 3, singly and in combination also showed a significant enhancement of the groundnut crop growth and yield. Maximum increase in the plant growth was observed with treatment T 4, which is the consortium of three efficient isolates. On 16s rRNA sequence analysis the three efficient isolates were identified as *Enterobacter cloacae* (GST 3), *Burkholderia cepacia* (GSH 1) and *Burkholderia cenocepacia* (GSB 13). The study showed the exploitation of the groundnut plant associated rhizobacteria as biofertilizer. Further this study illustrated the advantage of co-inoculation technique involving the synergism over single inoculation for better performance under field application to bring enhanced improvement in crop productivity with sustainability.

Key words: Rhizosphere, Plant growth promoting rhizobacteria, Co-inoculation, Enhanced crop production.

INTRODUCTION

Concern to the environmental problem, lead to resurgence the use of biofriendly practice to achieve productive yield at low production cost and enhanced environmental sustainability. In this context the plant growth promoting microorganisms serve a crucial role. The microorganisms also found to control

plant diseases thereby offering an attractive alternative way for synthetic chemicals¹. Plants inoculation with plant growth promoting microorganism (PGPM) has been practiced about since the early 20th century, when patent on a product containing *Rhizobium* sp. was reported².

Cite this article: Thakur, A. and Parikh, S.C., Evaluation of PGPR Isolates for Plant Growth Promotion and Yield Enhancement of Groundnut Crop, *Int. J. Pure App. Biosci.* 6(6): 644-652 (2018). doi: <http://dx.doi.org/10.18782/2320-7051.6345>

Afterward a wide numbers of PGPM strains have been screened and evaluated in the laboratory, greenhouse and field conditions all over the world³. Enhanced plant growth and yield among various crops employing the PGPR have been reported for the various crops such as in rice, sunflower and wheat^{4,5,6,7}. Selection of PGPR with multiple plant growth-promoting attributes or co-inoculation of efficient microorganisms heaving different plant growth promoting attributes proved as more significant compare to using a single inoculant. The different plant growth promoting traits found to work additively as was suggested in the “additive hypothesis,” that multiple mechanisms, such as phosphate solubilization, dinitrogen fixation, ACC deaminase, antifungal activity, IAA and siderophore biosynthesis etc. are additively responsible for the plant growth promotion and increased yield⁸.

Synergistic influence of *Pseudomonas* and nitrogen-fixing bacteria has also been reported for several crops, such as synergism of *Pseudomonas*, *Mesorhizobium ciceri* and *Azotobacter chroococcum* in chickpea⁹, *Pseudomonas fluorescens* with *Azospirillum lipoferum* in rice¹⁰, *Pseudomonas* and *Rhizobium* in maize¹¹ and *Bacillus* sp., *Pseudomonas* sp. and *Sinorhizobium meliloti* in alfalfa¹². Therefore the selection of PGPR with multiple plant growth-promoting attributes has been advocated for the development of microbial inoculants with better consistency in field.

Groundnut (*Arachis hypogea*) is one of the principal economic crops of the world, ranks 13th among the most important food crop and 4th among most important oilseed crop of the world. Though some study have been done to explore the groundnut rhizosphere in other parts of the country and world, but the whole groundnut cultivating area of district “Sabarkantha”, in Gujarat, India remain unexplored from microbiological research point till now. Thereby this study has endeavored the exploration of novel PGPR rhizobacterial species of groundnut

rhizosphere and their evaluation to enhance the groundnut crop productivity.

MATERIAL AND METHODS

Evaluation for plant growth and yield enhancement

In our study the previously isolated and screened, five efficient and multitrait rhizobacterial isolates GSP 4, GST 3, GSB 13, GSH 1 and GSV 3 were checked for their potential to enhance the plant growth by studying their effect under pot and field condition.

Pot Study

Pot experiment was carried out in the pots of 20 cm diameter and filled with the mixture of sterilized sand and soil in 1:2 wt/wt ratios. Healthy groundnut seeds of variety G-20 was collected from, “Agriculture Research Station, Talod”. All selected seeds were surface sterilized and mixed with each selected inoculums singly, using sterilized vermicompost as carrier and three seeds were sown in each pot. Three replicates were used for each treatment. Two plants of each treatment (2 plants/ replicate/ treatment) were uprooted at 20th day of growth and measurement was done for shoot length, root length and plant dry weight compared to control. The plants dry weight was measured after oven-drying the whole plants at 70 °C for three consecutive days.

Field Trials

Field experiment was done in agricultural farms of the “Agriculture Research Station” of “Dantivada Agriculture University” located at Talod. Field evaluation of each selected potential plant growth promoting rhizobacterial isolates was done singly and in combination on the groundnut (*Arachis hypogea*) crops of variety G-20. The experiments were conducted in the plot of size 60 × 30 m² during the rainy seasons of the year 2015. The plot was then demarked into the different areas of size 3 × 2 m², each treatment was replicated in thrice. Application of inoculums was done by seed treatment and repeat application in field after 30 days of

growth after sowing. The whole experiment was conducted in following steps:

a. Inoculum Preparation

Actively growing culture (10^9 cells/ ml) of each inoculum in NA broth were harvested and mixed with the pre-prepared, sterilized and cooled vermicompost as carrier.

b. Carrier Preparation

Vermicompost was dried in sunlight and made to dried powder form. Then sterilized continuously up to 3 hours by intermitted steaming to ensure the complete sterilization followed by cooling and made ready to use as carrier.

Mixing of the Inoculum with Carrier

300 ml of each selected inoculum was mixed with one kg of carrier separately, to make the cell count of the carrier mixed culture of 10^8 cfu/g. All the process was done under aseptic conditions. After inoculation mixture were kept for curing in room temperature for up to 3 days and used for application in field.

Seed Inoculation

Seeds were mixed with 10% sugar solution to make the proper adhesion with inoculum and then mixed with vermiculite-based inocula for each selected microorganism. After shade drying for up to 30 minutes, sowing was done within 24 hours.

Field Application

The whole experimental field was divided into the demarcated randomized block of size 3×2 m² each. The five treatments of inoculum were formulated as: T1 – GSH 1 + Vermicompost, T2 – GSB 13 + Vermicompost, T3 – GST 3 + Vermicompost, T4 – GSH 1 + GSB 13 + GST

3 + Vermicompost, T5 – Untreated seeds (control) and sown in field experimental area in triplicate.

Data Acquisition

Sampling was done on day 40 and day 80 after sowing. Six plants from each plot were picked and root length, shoot length, plant biomass, number of seeds were measured.

Statistical Analysis

Results of the measurements were subjected to analysis of variance using window SPSS 16.0, 2007 software. Mean, standard error, standard deviation and significance were calculated by comparing the mean by applying one sample t-test and Kruskal Wallis test.

Genotypic Characterization

The three most efficient isolates GST 3, GSB 13 and GSH 1 were identified by 16S rRNA sequencing at Gujarat state biotechnology mission (GSBTM), Gandhinagar, Gujarat, India and deposited in their gene bank repository.

RESULT

Evaluation for Plant Growth and Yield

Pot Experiment

Pot experiment results after 20 days of growth recorded in table no. 1 and the experimental setup shown in figure 1. As per the result recorded in table 1, all the five isolates showed a significant effect for the enhancement of groundnut plants shoot height, root length and plant dry weight than control. Statistically there was no significant difference across all value of growth parameter among all categories of bacterial isolates; it was normally distributed with mean and standard deviation.

Table 1. Effect of application of selected efficient rhizobacterial isolates on plant growth in pot study after 20 days of sowing

Inoculum	Shoot length (cm/plant)	% Increase in shoot length over control	Root length (cm/plant)	% Increase in root length over control	Plant dry weight (g/plant)	% Increase in plant dry weight over control
GSP 4	14.22 ± .32	47.82%	9.70 ± .60	56.44%	13.10 ± .34	39.00%
GST 3	13.42 ± .49	39.50%	13.32 ± .87	114.84%	15.12 ± .25	60.00%
GSB 13	19.74 ± .70	105.20%	12.42 ± .38	100.31%	17.47 ± 1.43	84.87%
GSH 1	21.50 ± .89	123.30%	13.07 ± .09	110.81%	18.52 ± .65	95.98%
GSV 3	16.22 ± .45	68.61%	12.60 ± .64	103.23%	14.00 ± .82	48.13%
Control	9.62 ± .37		6.2 ± .54		9.45 ± .62	
Over all						
Mean	15.79		11.22		14.60	
SD	435		2.77		3.26	
SE (± mean)	1.78		1.14		1.33	
Sig. at 95% CI	1.0		1.0		1.0	

Values are the mean of three replicates ± standard error of the mean (% SE). Sig.- significance, CI - confidence interval.

a. Shoot height

A significant increase in height was obtained for treated plants than un-inoculated control. Maximum increase in plant height of 123.30% (21.50 cm) over un-inoculated control (9.62 cm) was recorded for the strain GSH 1 followed by GSB 13 (19.74 cm) of 105.20%, GSV 3 (16.22 cm) of 68.61%, GSP 4 (14.22 cm) of 47.82% and for GST 3 (13.42 cm) of 39.50%.

b. Root length

Growth in the root length of groundnut plants showed a significant increase ranged from 56.44% to 114.84% (table 1) on bacterial inoculation over the control. Isolate GST 3 and GSH 1 showed maximum increase in root length of 13.32 cm (114.84%) and 13.07 cm

(110.81%) followed by GSV 3 (103.23%), GSB 13 (100.31%) and GSP 4 (56.44%) of 12.60 cm, 12.42 cm and 9.70 cm over the un-inoculated control (6.20 cm).

c. Plant dry weight

The dry weights of plants were also showing a significant increase on inoculation with selected strains compared to control (table 1). The maximum increase in shoot dry weight of 95.98% and 84.87% over the control was recorded for the plant treated with isolates GSH 1 (18.52 g) and GSB 13 (17.47 g), followed by GST 3 (15.12 g), GSV 3 (14.00 g) and the least increase was shown by isolate GSP 4 (13.10 g) of 60%, whereas the control (9.45 g/ plant) was with least effect.



Figure 1. (A-E): Influence of efficient rhizobacterial isolates on plant growth of Groundnut plants in pot experiment after 20 days of sowing: (A) GSH 1, (B) GSB 13, (C) GSV 3, (D) GSP 4 (E) GST 3, (F) Control.

Field Experiment Results

Based on the efficiency of the five PGPR under pot experiment, three best PGPR: GST 3, GSH 1 and GSB 13 were selected for further study under field application. Result of the field inoculation of PGPR on the growth of plants shoot length, root length, plant dry weight and seed yield on 40 day and 80 day

after sowing recorded in table 2, shown by bar diagram in figure 2, 3 and the experimental setup shown in figure 4. A significant result was obtained for the enhancement of the groundnut plants growth in terms of its shoot length, root length, plant dry weight and seed yield in comparison to un-inoculated control.

Table 2. Effect of the five treatments (T1, T2, T3, T4 and T5) of potent isolates singly and in combination on plant growth of groundnut (*Arachis hypogaea*) var. G 20 crop on day 40 and day 80 after sowing, under field condition

Treatment	Shoot length (cm)		Root length (cm)		Plant biomass (g plant ⁻¹)		Yield (seed/ plant)
	day 40	day 80	day 40	day 80	day 40	day 80	day 80
T1 (GSH 1)	22.66 ± 0.068	33.26 ± 0.066	11.34 ± 0.204	15.32 ± 0.233	12.22 ± 0.246	41.31 ± 0.321	73
T2 (GSB 13)	21.57 ± 0.088	31.35 ± 0.085	10.35 ± 0.275	13.85 ± 0.565	11.35 ± 0.314	39.38 ± 0.216	65
T3 (GST 3)	21.87 ± 0.392	31.19 ± 0.043	9.87 ± 0.154	11.93 ± 0.183	11.08 ± 0.144	39.13 ± 0.187	69
T4 (GSH 1 + GSB 13 + GST 3)	24.63 ± 0.105	35.74 ± 0.045	14.07 ± 0.234	17.81 ± 0.232	14.95 ± 0.283	43.68 ± 0.355	89
T5 (Control)	11.27 ± 0.833	22.51 ± 0.919	7.09 ± 0.224	9.92 ± 0.407	7.17 ± 0.185	19.30 ± 0.287	42
Overall							
Mean	20.40	30.80	10.53	13.77	11.33	36.55	67.60
SD	5.33	4.98	2.51	3.04	2.80	9.82	16.95
SE (± mean)	2.33	2.22	1.13	1.36	1.24	4.38	7.59
P _{≥.05}	5.29	0.670	0.530	0.537	0.513	0.609	0.783

Values are the mean ± SE

x. Effect on Plant Growth and Plant Dry Weight

Results recorded in table 2, and figure 2, showed that the maximum increase in shoot length (24.63 cm, 35.74 cm), root length (14.07 cm, 17.81 cm) and dry weight (14.95 g, 43.68 g) was shown by the treatment – T 4, which was the consortium of three strains GSH 1 + GSB 3 + GST 3 followed by GSH 1, GSB 13, GST 3 and then control. In single treatment the isolate GSH 1 showed the most

efficient results, to increase the shoot height (22.66 cm, 33.26 cm), root length (11.34 cm, 15.32 cm) and plant dry weight (12.22 g, 41.31 g) whereas the isolate GST 3 was the least efficient of all treatments except control. Statistically there was no significant difference across all value of growth parameter among all categories of treatment and control; it was normally distributed with mean and standard deviation.

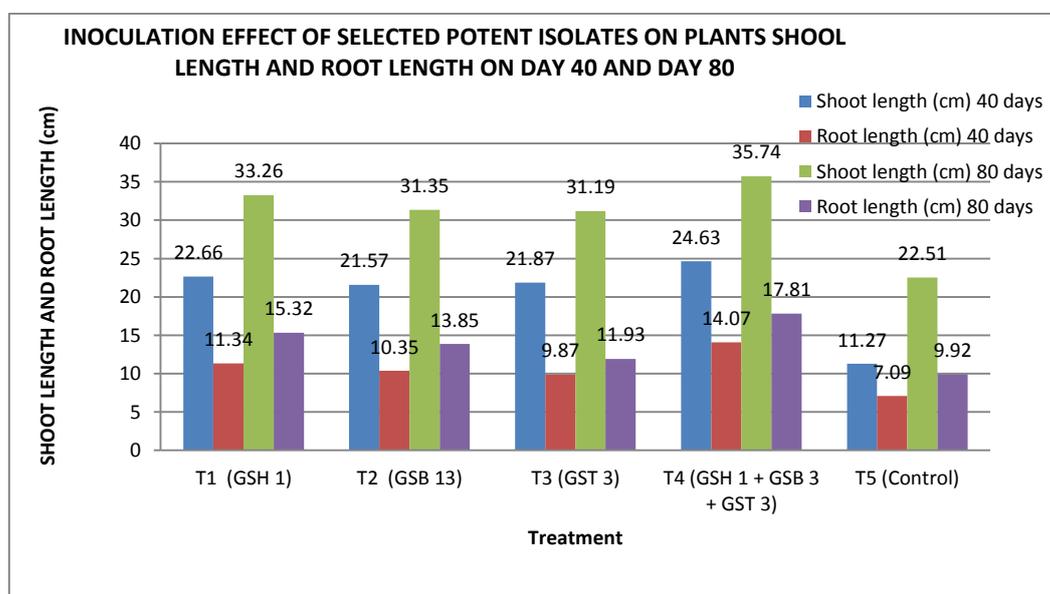


Figure 2. Bar diagram showing the inoculation effect of five treatments (T1, T2, T3, T4 and T5) of potent isolates singly and in combination on shoot length, root length of groundnut plants on day 40 and day 80 after sowing in field study.

y. Seed yield

As per the table 2 and figure 3, treatment T4 the mixed inoculums of all three bacterial strains showed the maximum seed yield of 89

seeds/ plants for followed by treatment T1 consisted of strain GSH – 1 (73 seeds/ plants). The isolate GSB 3 (treatment – T2) showed seed yield of 65 seeds/ plants followed by GST

3 (treatment – T3) gave seed yield of 69 seeds/plants while the lowest seed yield 42 seeds/plants was noted for un-inoculated controls, the treatment –T5. Statistically there was no

significant difference of the seed yield among all categories of treatment and control there was a normal distribution through mean and standard deviation.

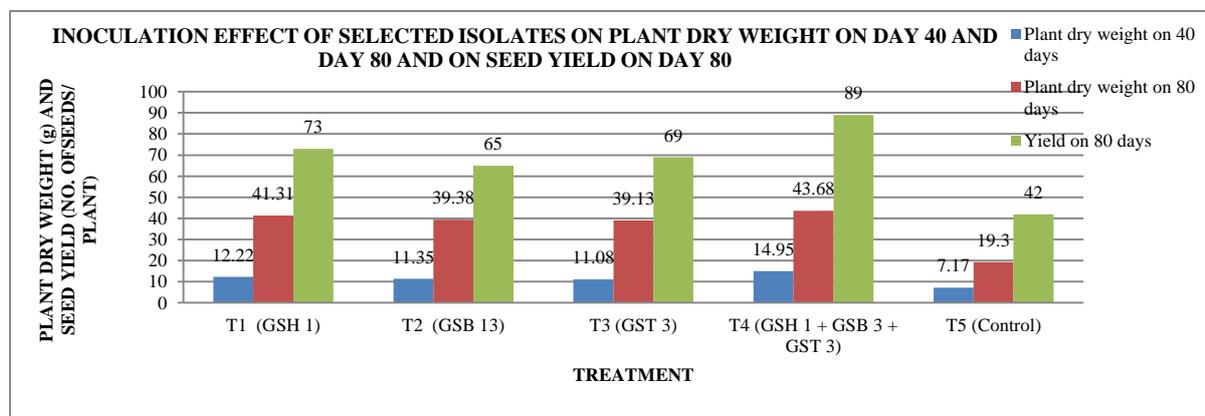


Figure 3. Bar diagram showing the inoculation effect of selected potent isolates on plant dry weight and seed yield in field study.



Figure 4. Field inoculation of efficient rhizobacterial treatment in Groundnut (var. G20) crop at Agriculture Research Station of "Dantivad Agriculture University" experimental farms located at Talod.

Genotypic Identification

Genotypic identity of the three novel strains: GST 3, GSB 13 and GSH 1, their gene bank and NCBI accession number shown in table 3. The results obtained after 16S rRNA gene sequencing showed that the isolates have 99% of similarity with *Enterobacter cloacae*,

Burkholderia cenocepacia and *Burkholderia cepacia* sequences present in National Centre for Biotechnology Information data (web site: www.ncbi.nlm.nih.gov) and assigned with the accession number BAB 4883 - KT462763, BAB 4884 - KT462742 and BAB 4887 - KT462764.

Table 3. Gene bank ID and blast results of 16S rRNA gene sequences of rhizobacteria submitted with NCBI GenBank

Isolates code	Bio gene ID	Organism identified	Accession No.	Identity
GST 3	BAB-4883	<i>Enterobacter cloacae</i>	KT462763	99%
GSB 13	BAB-4884	<i>Burkholderia cenocepacia</i>	KT462742	99%
GSH 1	BAB-4887	<i>Burkholderia cepacia</i>	KT462764	99%

DISCUSSION

A vast variety of rhizosphere microorganisms are of immense importance in many way and plant growth-promoting bacteria are the one of the most importance among them. PGPB have opened the avenue towards the potent source of biofertilizer and biocontrol agents for sustainable development. Therefore in this study we tried to exploit the promising candidate of multiple plants growth-promoting attributes for consistent performance under field application. Effect of the five PGPR isolates on growth of groundnut plants under pot and field conditions are discussed under following heads:

Pot Experiment

Significant enhancement in the groundnut plants shoot length, root length and plant dry weight on inoculation with five efficient rhizobacterial isolates over un-inoculated control exhibited their plant growth promoting potential (tables 2). Similar finding have also reported for the enhanced plant growth of wheat plants by *Pseudomonas* strains with highest multiple plant growth-promoting attributes under greenhouse and field conditions¹³. However increment in plant growth by these five selected strains was not in accordance to their PGPR attributes indicated the synergistic involvement of multiple plant growth-promoting attributes. Similar finding for synergistic involvement of multiple plant growth-promoting attributes was also reported by various other workers^{13, 14, 15}. Thereby screening of rhizobacteria with multiple plant growth-promoting attributes for growth promotion through pot culture experiment appears to be a good strategy in the primary selection of potential PGPR strains for further study for their field evaluation.

Field Experiment

The field evaluation of three most efficient isolates *Enterobacter cloacae* (GST 3), *Burkholderia cepacia* (GSH 1) and *Burkholderia cenocepacia* (GSB 13) on the basis of their multiple plant growth-promoting attributes and pot study was undertaken to test the selected isolates for improving crop productivity in groundnut crop under natural environmental conditions.

Vermicompost was selected as carrier because it has been found to increase the efficiency of biofertilizer by maintaining the longer shelf - life of microorganism for up to 8 month to more than one year¹⁶. Beside it is cheaper in cost, easily available and found with higher nutritional quality which also improving the physical, chemical and biological properties of soil.

Significant results was obtained for the enhancement of the plant shoot length, root length, plant biomass and seed yield as per observation taken on day 40 and day 80 of plant growth (tables 3). However the combined treatment - T4 consisted of all three selected strains GST 3, GSH 1 and GSB 13 showed the maximum enhancement in plant growth parameter recorded on day 40 and day 80 of growth than the application of the inoculants singly and control. Beside among the individual application, the treatment T1 consisted of the strain *Burkholderia cepacia* (GSH 1) showed the maximum enhancement in plant growth followed by treatment T2 - *Enterobacter cloacae* (GST 3) and treatment T3 - *Burkholderia cenocepacia* (GSB 13), corroborating the results with pot study. Significant enhancement in the plant growth parameter with the treatment T4 (GSH 1 + GSB 13 + GST 3), the combined application of microbial inoculants over the individual applications showed involvement of synergism between the three isolates *Enterobacter cloacae*, *Burkholderia cepacia* and *Burkholderia cenocepacia* in improving the groundnut crop productivity. Previously similar mechanism of synergistic influence of *Serratia*, *Trichoderma* for enhanced growth and yield of peanut crop has also reported¹⁷.

CONCLUSION

Current study have illustrated the practical implication of the selection of multitrait plant growth promoting rhizobacteria in developing plant growth-promoting microbial inoculants with greater consistency under field application. The results of the field study demonstrated the advantage of synergistic inoculations of *Enterobacter cloacae*,

Burkholderia cepacia and *Burkholderia cenocepacia* over single inoculation for improving crop productivity in groundnut crop as the best alternate to reduce and stop chemical fertilizer application.

Acknowledgements

We beholden to “Agricultural Research Station”, Talod, for proving us research facility for pot and field experiment. Further we feel privileged to express our deep sense of regards to the Director “Gujarat State Biotechnology Mission (GSBTM)”, for permitting us to utilize their lab facility for this research work.

REFERENCES

1. Roberts, D.P., Lohrke, S.M., Meyer, S.L.F., Buyer, J.S., Bowers, J.H., Baker, C.J.Li.W., Souza, J.T., Lewis, J.A. and Chung, S., Biocontrol agents applied individually and in combination for suppression of soil-borne disease of cucumber, *Crop Prot.* **24**: 141-1559 (2005).
2. Nobbe, F. and Hiltner, L., Inoculation of the soil for cultivating leguminous plants, U.S. Patent. 570 - 813 (1896).
3. Zehnder, G.W., Murphy, J.F., Sikora, E.J. and Kloepper, J.W., Application of rhizobacteria for induced resistance, *Eur. J. Plant Pathol.* **107**: 39–50 (2001).
4. Lucas, J.A., Solano, B.R., Montes, F., Ojeda, J., Megias, M. and Manero, F.J.G., Use of two PGPR strains in the integrated management of blast disease in rice (*Oryza sativa*) in Southern Spain, *Field Crops Res.* **114**: 404-410 (2009).
5. Cassan, F., Perrig, D., Sgroy, V., Masciarelli, O., Penna, C., and Luna, C., *Azospirillum brasilense* Az39 and *Bradyrhizobium japonicum* E109, inoculated singly or in combination, promote seed germination and early seedling growth in corn (*Zea mays* L.) and soybean (*Glycine max* L.), *Eur J Soil Biol.* **45**: 28–35 (2009).
6. Shahid, M., Hameed, S., Imran, A., Ali, S., and van Elsas, J.D., Root colonization and growth promotion of sunflower (*Helianthus annuus* L.) by phosphate solubilizing *Enterobacter* sp. Fs-11, *World J Microbiol Biotechnol.* **28**: 2749-2758 (2012).
7. Majeed, A., Abbasi, M.K., Hameed, S., Imran, A. and Rahim, N., Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion, *Front Microbiol.* **6**: 198 (2015).
8. Bashan, Y. and Holguin, G., *Azospirillum*-plant relationships: environmental and physiological advances (1990-1996), *Can J Microbiol.* **43**: 103-121 (1997).
9. Wani, P.A., Khan, M.S., Zaidi, A., Effect of metal tolerant plant growth promoting *Bradyrhizobium* sp. (vigna) on growth, symbiosis, seed yield and metal uptake by green gram plants, *Chemosphere.* **70**: 36–45 (2007).
10. Raja, P., Una, S., Gopal, H., Govindarajan, K., Impact of BioInoculants consortium on rice root exudates, biological nitrogen fixation and plant growth, *J Biol Sci* **6**: 815–823 (2006).
11. Bano, A. and Fatima, M., Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*, *Biol. Fertility Soils.* **45**: 405-413 (2009).
12. Guinazu, L., Andres, J., Del-Papa, M., Pistorio, M. and Rosas, S., Response of alfalfa (*Medicago sativa* L.) to single and mixed inoculation with phosphate solubilizing bacteria and *Sinorhizobium meliloti*. *Biol. Fertil. Soils.* **46(2)**: 185–190 (2010).
13. Zabihi, H.R., Savaghebi, G.R., Khavazi, K., Ganjali, A., and Miransari, M., *Pseudomonas* Bacteria and Phosphorous Fertilization, Affecting Wheat (*Triticum aestivum* L.) Yield and P Uptake under Greenhouse and Field Conditions, *Acta Physiol Plant.* **33**: 145-152 (2011).
14. Zahir, A.Z., Ghani, U., Naveed, M., Nadeem, S.M., Asghar, H.N., Comparative effectiveness of *Pseudomonas* and *Serratia* sp. containing ACC-deaminase

- for improving growth and yield of wheat (*Triticum aestivum* L.) under salt stressed conditions, *Arch Microbiol.* **191**: 415-424 (2009).
15. Ahmad, M., Zahir, Z.A., Asghar, H.N. and Asghar, M., Inducing salt tolerance in mung bean through co-inoculation with *Rhizobium* and PGPR containing ACC-deaminase, *Can J Microbiol.* **57**: 578-589 (2011).
16. Vidhyasekaran, P., and Muthamilan, M., Development of formulations of *Pseudomonas fluorescens* for control of chickpea wilt, *Plant Dis.* **79**: 782–786 (1995).
17. Badawi, F.S.H.F. Biomy, A.M.M. and Desoky, A.H., Peanut plant growth and yield as influenced by co-inoculation with *Bradyrhizobium* and some rhizomicroorganisms under sandy loam soil conditions, *Ann. Agric. Sci.* **56**: 17 – 25 (2011).