INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 8, No. 5, p. 112-121, 2016

RESEARCH PAPER OPEN ACCESS

Effect of *Rhizobium* and phosphate solubilizing bacteria at different levels of phosphorus applied on nodulation, growth and yield of peas (*Pisum sativum*)

Kulsoom Abid[1], Tariq Sultan[1], Muhammad Zahid Kiani[2*], Shahbaz Ahmad[1], Tauseef Tabassam[1], Mahmood-ul-Hassan[1]

[1]*Land Resources Research Institute, National Agricultural Research Center, Islamabad, Pakistan*

[2]*Department of Environmental Sciences PMAS-Arid Agriculture University, Rawalpindi, Pakistan*

Key words: *Rhizobium,* Phosphate Solubilizing Bacteria, Peas.

http://dx.doi.org/10.12692/ijb/8.5.112-5 Article published on May 18, 2016

Abstract

Bacterial inoculation of Rhizobium species tend to improve the root architecture of legume crop through their potential of colonization and nitrogen fixing ability. Co-inoculation of Rhizobium and phosphate solubilizing bacteria (PSB) strain will further improve the nutrient availability to plant. This study was conducted to evaluate the response of peas to Rhizobium and PSB inoculation individually and together at 20 and 40 mg kg^{-1} phosphorus (P) application *in-vitro* and pot culture experiment. Recommended fertilizer dose was an additional treatment. The co-inoculation of Rhizobium + PSB at 40 mg P kg^{-1} soil significantly increased shoot and root length, number of flowers, pod, and nodules per plant, root and shoot dry weight, 100 grain weight and number of grains per pod up to 37, 25, 60, 220, 25, 125, 34, 19 and 20%, respectively over 20 mg kg^{-1} P application. Similarly, N and P concentration of straw and grain as well as soil N and P contents after harvesting was also significantly increase by the co-inoculation of nitrogen fixing and PSB compared to single inoculation at 20 mg kg^{-1} P application. Results showed that co-inoculation at 20 and 40 mg kg P application can improve the nodulation, growth parameters, yield and nutrient concentrations and uptake.

* **Corresponding Author:** Muhammad Zahid Kiani ✉ zahidkiani55@gmail.com

Introduction

A system producing 3Mg ha^{-1} both of wheat and rice could take out 148 kg N ha^{-1}, 45.4 kg P_2O_5 and 207 kg K_2O ha^{-1} (Zia *et al.*, 2000) apart from considerable quantity of various micro and secondary nutrients. This cause a major depletion of available resources as well as damage to soil. As a grain legume, field peas are able to fix most of the required nitrogen from environment in the presence of *rhizobia* and not only to support its own growth without nitrogen fertilizer application but also spare some of its biologically fixed N in the soil for subsequent crop (Shah *et al.*, 2003). Legume-rhizobia symbiosis actively fixes nitrogen and is important to agriculture crop production (Peppe,r 2000). Peas form a extremely distinctive relationship with its rhizobial microsymbiont (Ahemad and Khan, 2010).This symbiosis performs a vital role in increasing the sustainability of yields with minimal non-renewable external inputs (Vance 2001). Pea (*Pisum sativum*, dry pea) is an important legume of North America, West Europe, Pakistan, Australia, India and South America (Nisar *et al.*, 2008). In Pakistan, peas are cultivated at an area of 10000 hectares with total production of 80,000 tonns approximately. It contains 23% crude protein, 46% starch, and 1.4% oil (Hickling, 2003). Continuous cereal system is a nutrient exhaustive system and drawing out the soil nutrients rapidly. Many researchers have stated considerable effect of *Rhizobium* application on nodulation, growth and yield of different legumes (van Rhijn *et al.,* 2001; Huang and Erickson, 2007). Phosphorus is the major essential macro nutrient. Its deficiency is common in agricultural crops grown on alkaline calcareous soils of Pakistan as large amount of total phosphorus exist in the soil is not accessible to plants either as a result of formation of sparingly soluble compounds with calcium in soil or its fixation by calcium carbonate (Kosar *et al.,* 2002). Nearly 75 to 90% of applied phosphorus fertilizer is fixed by calcium, aluminum and iron complexes present in the soils (Turan *et al.,* 2006) which leads to repeated and excessive application of phosphorus fertilizer to crop land. Phosphorus supply through biological means is a worthwhile alternative. Phosphate solubilizing microorganisms (PSM) could be a solution for sustaining the provision of available phosphorus to plants as they are able to convert both organically and inorganically bounded forms of phosphorus (Chen *et al.,* 2002). The phosphate solubilizing bacteria (PSB), phosphate solubilizing fungi (PSF) and actinomycetes have been reported to be effective in transformation of fixed phosphorus to available forms of phosphorus by many investigators (Chabot *et al.*,1996; Pal 1998) and increase the yield of crops (Adesemoye and Kloepper, 2009). However, the combined inoculation with PSB and nitrogen fixing bacteria can be more efficient than individual inoculation for supplying a more balanced nutrition for plants (Belimov *et al.,* 1995). In this perspective, the combined application of phosphate solubilizing microorganisms and arbuscular mycorrhizal (AM) fungi (Zaidi *et al.*, 2003) and *Rhizobium* and PSM (Perveen *et al.,* 2002) have been reported to enhance growth of plants more than individual inoculation of microbes in certain conditions when the soil have inadequate phosphorus. The present research work was undertaken to determine the response of pea to single and co-inoculation in the presence of phosphorus fertilizer application. Objectives of the work were to determine cumulative effects of *Rhizobium* and Phosphorus Solubilizing Bacteria (PSB) on pea yield and compare the nodulation ability, N and P uptake of pea with increased phosphorus availability.

Materials and methods

Growth chamber study was conducted in laboratory using control, *Rhizobium* inoculation and PSB inoculation of seeds in sand whereas pot culture experiment was conducted under greenhouse conditions and Complete Randomized Design (CRD) was followed with four replications. The original soil was analyzed for its physical and chemical characteristics. Plant data was collected at flowering and maturity stage. After harvest, soil was analyzed for nitrate-nitrogen and available phosphorus. The bacterial cultures were prepared in respective media viz Yeast Manitol Broth for *Rhizobium* (Somasegaran and Hoben, 1985) & Pikovskaya for PSB (Pikovskaya, 1948). The pH of media was maintained at 6.8-7.0 at the time of its preparation.

Growth Chamber study

The response of seeds germination to *Rhizobium* (lathyrus) and PSB (WPS-8) were tested in the growth chamber. Single strains were compared with control without culture. *Rhizobium* and PSB were compared with control. Inoculation of seeds was done by dipping surface sterilized seeds in respective cultures for fifteen minutes. Inoculated seeds were sown in the small sterilized pots (1kg) filled with sterilized sand and tri-replicated by applying Complete Randomized Design (CRD). The shoot and root length, shoot and root dry weight was recorded. *Rhizobium* and PSB strains were used from microbial gene bank of Soil Biology & Biochemistry lab, National Agricultural Research Center Islamabad.

Green House study

To evaluate the response of pea to *Rhizobium* and phosphate solubilizing bacteria and chemical fertilizers, a pot experiment was conducted under greenhouse conditions. Treatments were distributed with 20 mg P/kg and 40 mg P/kg with and without inoculation of Rhizobium and PSB strains. Recommended dose was labeled as control treatment. Nitrogen was applied at 15mg N kg^{-1} soil as basal dose to all treatments.

Soil physico-chemical analyses

Soil analyses were performed before and after harvesting. Particle size distributions, pH and saturation percentage were determined (Page,1982;McLean 1982) and soil texture class was determined by ISSS triangle (Klute, 1986) (Table:1).

Nitrate – Nitrogen (NO_3– N) in soil was determined by Salicylic acid method (Vendrell and Zupancic, 1990). Standards were also prepared. Readings for samples and standards were noted using spectrophotometer at 410 nm. Soil available phosphorus was determined by spectrophotometer at 880 nm method described by Olsen and Sommers (Page, 1982). Soil extract was obtained by AB-DTPA extraction method and K was determined using Flame Photometer (Ryan *et al.,* 2007). Total organic carbon (TOC) was observed spectrophotometer at 610 nm (Heanes, 1984) (Table:1).

Crop parameters

Crop parameters were taken at flowering and maturity. Crop was observed for shoot length, root length, number of nodules per plant, number of flowers per plant, number of pods per plant, shoot and root dry biomass, total number of grains per pod,100 grain weight, total grain weight, total nitrogen uptake (mg/plant) ant total phosphorus uptake. Measured (mg/plant).

Plant analyses

Plant samples were dried out in oven at 65 °C for 48 hrs and was ground in a Wiley Machine and saved in plastic packs. These samples were analyzed for total nitrogen and phosphorus. Digestion of samples was done colorimetrically as described Anderson and Ingram, 1993. Total nitrogen was measured by Kjeldhal method (Winkleman *et al.*, 1984). Nitrogen percentage was calculated by equation:

% N = 14.0067* (mL of titrate for sample - mL of titrate for blank)-* N of acid/ weight of sample (grams) *10.

Plant phosphorus was observed by the method described by (Ryan *et al.* 2007). The readings were noted on spectrophotometer at 470 nm.

Statistical analysis

The data was analyzed statistically by Analysis of Variance (ANOVA) technique at 5% level of significance and means were evaluated by using Fisher`s Least Significant Difference (LSD) Test (Steel *et al.* 1980).

Results

Two groups, the nitrogen fixing bacteria (*Rhizobia*) and phosphate solubilizing bacteria were used in a lab and a pot study to verify the effect of their single and co-inoculation on peas at two phosphorus levels (@20 and 40 mg kg^{-1}).

Table 1. Chemical analysis of the soil samples from experimental site.

Parameters	Amounts/class
Organic matter	1.6%
Total nitrogen	0.35%
NO_3–N	1.13 µg g^{-1}
Available P	5.8 mg kg^{-1}
Available K (extractable)	95 mg kg^{-1}
Electrical Conductivity (EC)	0.15dS m^{-1}
pH	7.9
Particle size analysis (textural class)	Silty clay loam
Saturation percentage	34%

Effect of Rhizobium and PSB on Growth of Peas in Lab Experiment

The response of pea plants to *Rhizobium* and PSB inoculation was studied in vitro. *Rhizobium* and PSB showed statistically significant response over control (Table: 2). Maximum shoot length was observed with the inoculation of PSB that was 47% higher over un-inoculated control. The inoculation of *Rhizobium* increased the shoot length up to 35% over control. Inoculation of both *Rhizobium* and PSB increased the root length (Table: 2) that was 58% and 55% higher over control.

Table 2. Effect of Rhizobium and PSB on Growth of Peas in Lab Experiment.

Treatments	Shoot length (cm)	Root Length (cm)	Shoot Dry Weight (g)	Dry Weight (g)
Control	8**c**	8.5**b**	0.73**b**	0.5**b**
WPS-8	11.9**a**	13.3**a**	1.13**a**	0.7**a**
Lathyrus	10.8**b**	13.5**a**	0.97**ab**	0.6**ab**

There was no difference between the effect of *Rhizobium* and PSB with respect to root length. Highest shoot dry weight was observed in case of inoculation with PSB that was 40% higher over control. The inoculation of *Rhizobium* increased the shoot fresh weight up to 20% over control. However the difference between the effects of *rhizobium* and PSB as well as *Rhizobium* and control was non-significant. Highest root dry weight was observed in case of inoculation of PSB that was 57% higher over control without inoculation.

This was followed by the inoculation of *Rhizobium* that was 43% higher over control (Table: 2) However, again the difference between the effects of *Rhizobium* and PSB as well as *Rhizobium* and control was non-significant.

Table 3. Effect of Rhizobium and PSB on Growth, Nodulation and Yield of Peas in Pot Experiment.

Treatments	Nodules /plant	Flowers/ Plant	Shoot Length (cm)	Root Length (cm)	Shoot Dry weight (g)	Root Dry weight (g)	No. of pods/ plant	No. of Grains/ pod	No. of Grains/plant	Grain Weight/ plant (g)	Hundred Grain Weight (g/plant)
Control-I (P20)	3b	4d	48.8e	16.7d	4.0d	0.20d	3d	3.4e	10.2c	1.4e	13.5d
(P20+Rhiz)	12b	5c	56.4cd	19.9c	6.0c	0.29bc	4b-d	4.0cd	16.1b	2.8c	16.3bc
(P20+PSB)	11b	5c	68.7ab	19.7cd	6.1c	0.24cd	5bc	4.1b-d	14.7bc	2.7cd	16.2bc
(P20+Rhiz+PSB)	17a	6bc	63.2abc	20.7bc	7.1ab	0.32b	5b	4.5a-c	23.3a	4.0a	16.6bc
Control-II (P40)	5cd	5c	51.0de	20.1c	5.8c	0.24cd	3cd	3.9c-e	13.9bc	2.3cd	15.7c
(P40+Rhiz)	10b	5c	61.2c	21.7bc	6.3bc	0.32b	5bc	4.1b-d	18.1b	3.6ab	17.7ab
(P40+PSB)	11b	6b	62.2bc	23.5ab	6.4bc	0.30b	5bc	4.3a-c	17.2b	3.4b	17.2a-c
(P40+Rhiz+PSB)	16a	8a	70.2a	25.8a	7.8a	0.53a	7a	4.7a	22.9a	4.1a	18.7a
(Rec NP)	6c	6bc	49.7de	19.4cd	6.4bc	0.29b	4cd	3.7de	15.4b	2.3d	16.5bc

Effect of Rhizobium and PSB on Growth, Nodulation and Yield of Peas in Pot Experiment

In pot experiment, co-inoculation with phosphorus 40 mg kg^{-1} performed better than the single inoculation, un-inoculated control and recommended NP. Number of flowers plant¹ showed 60% increase with simultaneous application of *rhizobium* and PSB and phosphorus (40 mg P kg^{-1}) over control-II (40 mg P kg^{-1}). This treatment was also 33% higher compared to recommend NP (Table: 3). Nodulation was more in treatment receiving both nitrogen fixing and phosphate solubilizing bacteria along with phosphorus fertilizer (40 mg kg^{-1}) by 220% when compared to control-II (40 mg P kg^{-1}).

Table 4. Post harvest soil and plant analyses.

Treatments	Soil NO_3 Nitrogen (ppm)	Soil Phosphorus (ppm)	Grain Total Nitrogen (%)	Straw Total Nitrogen (%)	Straw Phosphorus(%P)	Grain Phosphorus(%P)	Total N uptake (mg/plant)	Total P uptake (mg/plant)
Control-I (P20)	0.9d	4.1d	2.989d	0.790c	0.029c	0.245de	71.03e	4.94f
(P20+Rhiz)	3.3b	4.5cd	3.270ab	0.909bc	0.035bc	0.263bc	140.59cd	9.02c-e
(P20+PSB)	3.1b	5.8b	3.164b-d	0.898bc	0.037b	0.258cd	129.41d	8.74de
(P20+Rhiz+PSB)	3.5b	6.2b	3.230bc	1.064ab	0.039b	0.271bc	203.58b	12.95b
Control-II (P40)	0.9d	5.8b	3.062cd	0.911bc	0.029c	0.246de	118.72b	7.19ef
(P40+Rhiz)	3.4b	5.9b	3.300ab	0.986bc	0.038b	0.242e	168.15bc	10.10cd
(P40+PSB)	3.2b	6.2b	3.128b-d	0.890bc	0.039ab	0.274b	149.55cd	11.26bc
(P40+Rhiz+PSB)	4.2a	6.9a	3.462a	1.279a	0.046a	0.309a	244.94a	15.83a
(Rec NP)	1.5d	5.0c	3.134b-d	0.876bc	0.037b	0.264bc	121.48d	7.93de

This treatment also showed 167% increase over recommended NP respectively. Other growth parameters i.e. number of flowers plant^{-1}, shoot and root length, shoot and root dry weight increased significantly with simultaneous application of phosphate solubilizing bacteria and *Rhizobium* and phosphorus application (40 mg P kg^{-1}) by 50, 37, 25, 34 and 120% when compared to control-II (40 mg P kg^{-1}) (Table: 3). When this treatment was compared with recommended NP it showed 33, 41, 33, 22 and 83% increase respectively. Co-inoculation with reduced dose of phosphorus (20 mg P kg^{-1}) was also 60, 30, 33, 22 and 83% higher in above mentioned parameters over control-I (20 mg P kg^{-1}) respectively. Simultaneous application of *Rhizobium* and phosphate solubilizing bacteria with phosphorus (40 mg P kg^{-1}) also showed significant increase in yield and yield contributing parameters i. e. number of pod plant^{-1}, number of grains pod^{-1}, number of grains plant^{-1}, grain weight plant^{-1} and hundred grain weight up to 133, 20, 34, 78 and 19% over control-II (40 mg P kg^{-1}). This treatment was also 75, 27, 51, 78 and 13% higher when compared to recommend NP. Dual inoculation with slighter dose of phosphorus (40 mg P kg^{-1}) was also 67, 32, 68, 186 and 23% higher over control-I (40 mg P kg^{-1}) in all above parameters respectively (Table: 3).

Post harvest soil and plant analyses

Nutrient uptake by plants increased due to simultaneous application of nitrogen fixing and phosphate solubilizing bacteria along with phosphorus fertilizer (40 mg P kg^{-1}). This treatment showed 46, 14, 59, 24, 106 and 110% increase in nitrogen content in straw and grain, phosphorus content in straw and grain and total N and P uptake respectively compared to control-II (40 mg P kg^{-1}) (Table: 4). This treatment was also 46, 10, 24, 19, 102 and 100% higher when compared with recommended NP respectively. In case of dual inoculation with reduced dose of phosphorus (20 mg kg^{-1}) 35, 8, 34, 12, 186 and 12% increase was observed in above mentioned parameters respectively over control-I (20 mg P kg^{-1}). Nutrient status in soil i. e. nitrogen and phosphorus content in soil also improved with dual inoculation of nitrogen fixing and phosphate solubilizing bacteria along with phosphorus fertilizer (40 mg P kg^{-1}) up to 367 and 19% respectively over control-II (P @ 40 mg kg^{-1}) (Table: 4). When this treatment was compared with recommended NP it showed 180 and 41% increase respectively. Combined inoculation with slighter dose of phosphorus (20 mg P kg^{-1}) was 289 and 51% higher in above mentioned parameters respectively over control-I (20 mg P kg^{-1}) (Table: 4).

Discussion

Nutrients must be available in adequate and balanced quantities for optimum growth and yield of crops. Soil has natural reserves of plant nutrients except nitrogen which is low in soil. However, these reserves are mostly in unavailable forms for plants. The most constraining nutrients for plant development and production are nitrogen and phosphorus. During the last few decades, the use of phosphorus fertilizers has increased more than fourfold and nitrogen nearly ninefold (Vance, 2001). The excessive use of chemical fertilizers in agriculture land is posing momentous threats to the environment (Yu *et al.,* 2009). Soil microbes play a significant role in providing nutrients for plant growth and yield production (Adesemoye *et al.*, 2008; Berg 2009). Use of microbial inoculants can be a biological alternative to compensate synthetic fertilizers and to sustain environment friendly crop production (Dobbelaere *et al.,* 2003). Combined use of chemical fertilizers and microbial inoculants is gaining attention, more nutrients can be harvested from the soil and fertilizer doses can be reduced. Combined inoculation of microbes is more effective than single inoculation. Effect of simultaneous application of microbes on growth and development of legumes compared to either single inoculations or un-inoculated control (Rudresh *et al.,* 2005). Inoculation had a significant influence on shoot length, root length, shoot fresh weight and root fresh weight compared to un-inoculated control. *Rhizobium* and PSB also improves growth and biomass of legumes (Rugheim and Abdelgani, 2009) as was observed in this study. Combined use of nitrogen fixing and phosphate solubilizing microorganisms was more effective than the single inoculation. Inoculation either single or combined resulted in increased growth and nodulation of peas compared to un-inoculated control.

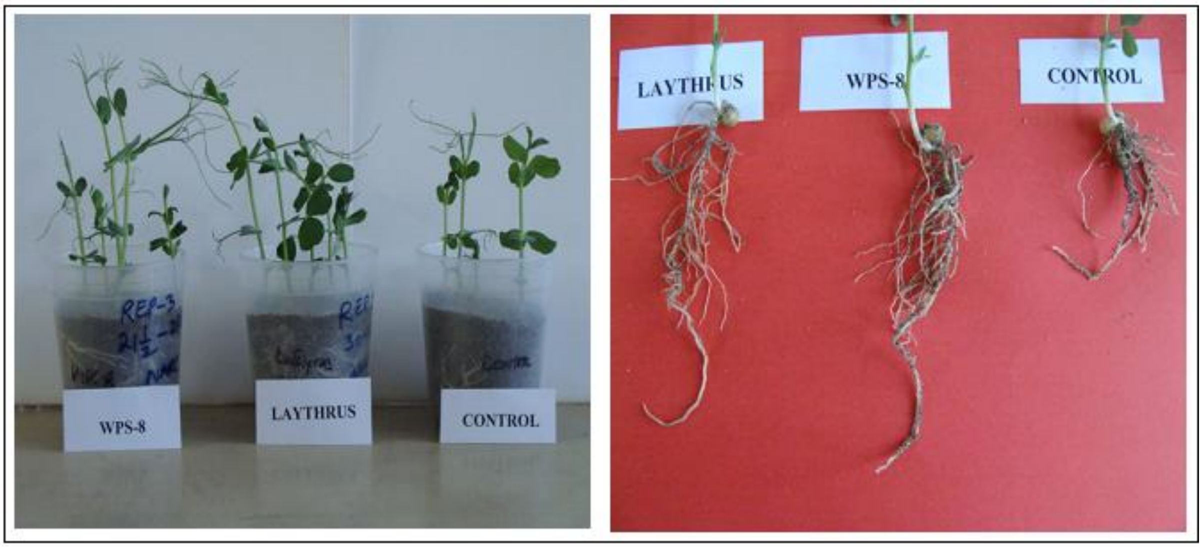

Fig. 1. Response of peas to inoculation in lab experiment & effect on root growth of peas in lab experiment.

Nodulation, shoot length, root length, number of flowers $plant^{-1}$,root weight and shoot weight increased significantly due to the individual and simultaneous application of *Rhizobium* and phosphate solubilizing bacteria compared to un-inoculated control. Combined use of nitrogen fixing and phosphate solubilizing microorganisms was more effective than the single inoculation. There is a significant effect of simultaneous application of microbes on growth and development of legumes compared to either single inoculations or un-inoculated control (Khurana and Sharma 2000,; Elhassan *et al.,* 2010) as shown by results. In the present research besides, better nodulation and growth parameters, co-inoculation also significantly increased yield and yield contributing parameters i.e. number of grains pod^{-1}, number of pods per plant^{-1}, number of grains plant^{-1}, 100-grain weight and grain weight plant^{-1} of peas over non inoculated control. These results are favored by the outcomes of other researchers who observed the effects of inoculation on growth and yield of various legume crops (Mirza *et al., 2007,; Yadegari et al.,; 2008, Zahir et al. 2010).* Measurement of nitrogen and phosphorus content in plant can be a direct criterion to verify the efficiency of nitrogen fixing and phosphate solubilizing microbes, because the increment of fixed nitrogen and increased available phosphorus leads to accumulation of nitrogen and phosphorus in plant. Plant nutrient uptake i.e. phosphorus and nitrogen content in straw and grain were also enhanced by inoculation of *Rhizobium* and phosphate solubilizing bacteria. These findings are in agreement with those of Rudresh *et al.*, 2005 who reported that the simultaneous inoculation of *Rhizobium* and phosphate solubilizing bacteria gave a higher nutrient uptake compared to single inoculation and un-inoculated control. Enhancement of nutrient uptake by plants with inoculation has also reported by many researchers (Wani *et al.*, 2007, Rokhzadi and Toashih, 2011) compared to control without inoculation. In addition, nutrient status i.e. nitrogen and phosphorus content of soil was improved by the simultaneous application of phosphate solubilizing and nitrogen fixing bacteria compared to single inoculation and un-inoculated control. Same results were observed by (Zaidi *et al.,* 2003,; Linu *et al.*, 2009). The co-inoculation of *Rhizobium* and PSB can significantly increase the yield/production of peas even using lower doses of nitrogen and phosphate fertilizers and then can contribute in the uplift of farmer's economic status and ultimately in the national economy. It can also improve the nutrient status/quality of peas.

Conclusions

Simultaneous application of nitrogen fixing and phosphate solubilizing bacteria with phosphorus (40 mg P kg^{-1}) proved to be the better than single inoculation and recommended NP in increasing all growth and yield parameters, nutrient uptake by plants and nutrient status of soil. Combined inoculation of nitrogen fixing and phosphate solubilizing bacteria even with reduced dose of phosphorus (20 mg P kg^{-1}) also showed significant increase in all above said parameters compared to single inoculation and recommended NP. Food Security is one of the major concerns in the developing world and sustainable development of agriculture sector as well as soil reclamation can play vital role in feeding the world.

This study gives a thorough insight of the importance of bacterial inoculation for growth and development of vegetables as well as provides natural reclamation of the soil resources. Inoculants production and application can play an imperative role in improving our food system. Thus, it is concluded that the technology of co-inoculation of N-fixing and PSB for peas crop needs demonstrations in the field for recommendation.

Acknowledgments

Soil Biology & Biochemistry lab, Land Resources Research Institute, National Agricultural Research Center is acknowledged for its resource utilization and financial support of this research.

References:

Anderson JM, Ingram JSI. 1993. Tropical soil biology and Fertility: A hand Book of method, CAB international, Wallingford, UK.

Adesemoye A, Torbert H, Kloepper J. 2008. Enhanced plant nutrient use efficiency with PGPR and AMF in an integrated nutrient management system. Canadian journal of microbiology **54,** 876-886.
http://dx.doi.org/10.1139/w08-081

Adesemoye AO, Kloepper JW. 2009. Plant–microbes interactions in enhanced fertilizer-use efficiency. Applied microbiology and biotechnology **85,** 1-12.
http://dx.doi.org/10.1007/s00253-009-2196-0.

Ahemad M, Khan MS. 2010. Comparative toxicity of selected insecticides to pea plants and growth promotion in response to insecticide-tolerant and plant growth promoting Rhizobium leguminosarum. Crop protection **29,** 325-329. http://dx.doi.org/10.1016/j.cropro.2010.01.005

Belimov A, Kojemiakov A, Chuvarliyeva CN. 1995. Interaction between barley and mixed cultures of nitrogen fixing and phosphate-solubilizing bacteria. Plant and Soil **173,** 29-37.

Berg G. 2009. Plant–microbe interactions promoting plant growth and health: perspectives for controlled use of microorganisms in agriculture. Applied Microbiology and Biotechnology **84,** 11-18. http://dx.doi.org/10.1007s00253-009-2092-7

Chabot R, Antoun H, Cescas MP. 1996. Growth promotion of maize and lettuce by phosphate-solubilizing Rhizobium leguminosarum biovar. phaseoli. Plant and Soil **184,** 311-321.

Chen C, Condron L, Davis M, Sherlock R. 2002. Phosphorus dynamics in the rhizosphere of perennial ryegrass, Lolium perenne L. and radiata pine, Pinus radiata. Soil Biology and Biochemistry **34,** 487-499. http://dx.doi.org/10.1104/pp.111.175448

Dobbelaere S, Vanderleyden J, Okon Y. 2003. Plant growth-promoting effects of diazotrophs in the rhizosphere. Critical Reviews in Plant Sciences **22,** 107-149. http://dx.doi.org/ 10.1080/713610853

Elhassan GA, Abdelgani ME, Osman AG, Mohamed SS, Abdelgadir BS. 2010. Potential production and application of biofertilizers in Sudan. Pakistan Journal of Nutrition **9,** 926-934.

Heanes D. 1984. Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. Communications in Soil Science & Plant Analysis **15,** 1191-1213. http://dx.doi.org/10.1080/00103628409367551

Hickling D. 2003. Canadian feed peas industry guide. Pulse Canada, Winipeg, MB, Canada, 35 p.

Huang H, Erickson R. 2007. Effect of Seed Treatment with Rhizobium leguminosarum on Pythium Damping-off, Seedling Height, Root Nodulation, Root Biomass, Shoot Biomass, and Seed Yield of Pea and Lentil. Journal of phytopathology **155,** 31-37. http://dx.doi.org/10.1111/j.1439-0434.2006.01189.

Khurana A, Sharma P. 2000. Effect of dual inoculation of phosphate solubilizing bacteria, Bradyrhizobium sp.(cicer) and phosphorus on nitrogen fixation and yield of chickpea. Indian Journal of Pulses Research **13,** 66-67.

Klute A. 1986. Methods of soil analysis. Part 1. Physical and mineralogical methods, American Society of Agronomy, Inc.

Kosar HS, Gill M, Aziz T, Akhtar M, Imran M. 2002. Solublization of tri-calcium phosphate by different wheat genotypes. Pakistan Journal of Agricultural Sciences **39,** 273-277.

Linu M, Stephen J, Jisha M. 2009. Phosphate solubilizing Gluconacetobacter sp., Burkholderia sp. and their potential interaction with cowpea (Vigna unguiculata (L.) Walp.). International Journal of Agricultural Research **4,** 79-87.

McLean E. 1982. Soil pH and lime requirement. Methods of soil analysis. Part 2. Chemical and microbiological properties, 199-224.

Mirza BS, Mirza MS, Bano A, Malik KA. 2007. Coinoculation of chickpea with Rhizobium isolates from roots and nodules and phytohormone-producing Enterobacter strains. Animal Production Science **47,** 1008-1015. http://dx.doi.org/10.1071/EA06151

Nisar M, Ghafoor A, Ahmad H. 2008. Evaluation of genetic diversity of pea germplasm through phenotypic trait analysis. Pak. J. Bot **40,** 2081-2086.

Page AL. 1982. Methods of soil analysis. Part 2. Chemical and microbiological properties, American Society of Agronomy, Soil Science Society of America.

Pal SS. 1998. Interactions of an acid tolerant strain of phosphate solubilizing bacteria with a few acid tolerant crops. Plant and soil **198,** 169-177.

Pepper I. 2000. Beneficial and pathogenic microbes in agriculture. Environmental Microbiology, 425-446.

Perveen S, Saghir Khan M, Zaidi A. 2002. Effect of rhizospheric micro-organisms on growth and yield of greengram (Phaseolus radiatus). Indian journal of agricultural science **72,** 421-423.

Pikovskaya R. 1948. Mobilization of phosphorus in soil in connection with vital activity of some microbial species. Mikrobiologiya **17,** 362-370.

Rokhzadi A, Toashih. 2011. Nutrient uptake and yield of chickpea (Cicer arietinum L.) inoculated with plant growthpromoting rhizobacteria. Aust J Crop Sci 5(1):44–48

Rudresh D, Shivaprakash M, Prasad R. 2005. Effect of combined application of Rhizobium, phosphate solubilizing bacterium and Trichoderma spp. on growth, nutrient uptake and yield of Chickpea aritenium L. Applied Soil Ecology **28,** 139-146. http://dx.doi.org/ 10.1016/j.apsoil.2004.07.005

Rugheim AME, Abdelgani ME. 2009. Effects of Rhizobium and Bacillus Megatherium var. Phosphaticum strains and chemical fertilizers on symbiotic properties and yield of faba bean (Vicia Faba L.). Advances in Environmental Biology **3,** 337-346.

Ryan J, Estefan G, Rashid A. 2007. Soil and plant analysis laboratory manual, ICARDA.

Shah Z, Shah S, Peoples M, Schwenke G, Herridge D. 2003. Crop residue and fertiliser N effects on nitrogen fixation and yields of legume–cereal rotations and soil organic fertility. Field Crops Research **83,** 1-11.

Somasegaran P, Hoben HJ. 1985. Methods in legume-Rhizobium technology, University of Hawaii NifTAL Project and MIRCEN, Department of Agronomy and Soil Science, Hawaii Institute of Tropical Agriculture and Human Resources, College of Tropical Agriculture and Human Resources.

Steel RG, Torrie JH, Dickey DA. 1980. Principles and procedures of statistics: a biometrical approach. McGraw-Hill, New York, 168.

Turan M, Ataoğlu N, Şahın F. 2006. Evaluation of the capacity of phosphate solubilizing bacteria and fungi on different forms of phosphorus in liquid culture. Journal of Sustainable Agriculture **28,** 99-108.

Van Rhijn P, Fujishige NA, Lim PO, Hirsch AM. 2001. Sugar-binding activity of pea lectin enhances heterologous infection of transgenic alfalfa plants by Rhizobium leguminosarum biovar viciae. Plant physiology **126,** 133-144.

Vance CP. 2001. Symbiotic nitrogen fixation and phosphorus acquisition. Plant nutrition in a world of declining renewable resources. Plant physiology **127,** 390-397.

Vendrell PF, Zupancic J. 1990. Determination of soil nitrate by transnitration of salicylic acid. Communications in Soil Science and Plant Analysis **21,** 1705-1713.

Wani P, Khan M, Zaidi A. 2007. Co-inoculation of nitrogen-fixing and phosphate-solubilizing bacteria to promote growth, yield and nutrient uptake in Chickpea. Acta Agronomica Hungarica **55,** 315-323. DOI: 10.1556AAgr.55.2007.3.7

Yadegari M, Rahmani H, Noormohammadi G, Ayneband A. 2008. Evaluation of bean (Phaseolus vulgaris) seeds inoculation with Rhizobium phaseoli and plant growth promoting rhizobacteria on yield and yield components. Pak J Biol Sci **11,** 1935-1939 http://dx.doi.org/10.1007/978-3642-1969-7.

Yu Y, Chu X, Pang G, Xiang Y, Fang H. 2009. Effects of repeated applications of fungicide carbendazim on its persistence and microbial community in soil. Journal of Environmental Sciences **21,** 179-185.

Zahir A, Yasin H, Naveed M, Anjum M, Khalid M. 2010. L-Tryptophan application enhances the effectiveness of Rhizobium inoculation for improving growth and yield of mungbean (Vigna Radiata L. Wilczek). Pak J Bot **42,** 1771-1780.

Zaidi A, Khan MS, Amil M. 2003. Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea, Cicer arietinum L. European Journal of Agronomy **19,** 15-21. http://dx.doi.org/10.1016/ SI 161-0301(02)00015-1

Zia MS, Aslam M, Baig M, Ali A. 2000. Fertility issues and fertilizer management in rice–wheat system: A review. Quart. Sci. Vision **5,** 59-73.