American-Eurasian J. Agric. & Environ. Sci., 1 (1): 42-47, 2006
ISSN 1818-6769
© IDOSI Publications, 2006

Bacterial and Fungal Solubulization of *Ogun* Rock Phosphate for Sustainable Phosphorus Availability in Soil

[1]*L.B. Taiwo and* [2]*M.O. Ogundiya*

[1]Institute of Agricultural Research and Training, Obafemi Awolowo University,
Moor Plantation, P. M. B. 5029, Ibadan, Nigeria
[2]Department of Science Laboratory Technology (Biology Unit),
The Polytechnic, Ibadan, Nigeria

Abstract: Laboratory and soil incubation studies were carried out to evaluate the potential of soybean and cowpea rhizobia as well as *Bacillus subtilis* and *Aspergillus niger* to release phosphorus (P) from *Ogun* Rockphosphate (ORP) both in broth and in soil. In the broth, the quantum of P released increased as the rhizobial inoculum rates increased. The potential for P release declined (r^2=0.72 at day 1 to r^2=0.45 at day 15) as the solubulization time increased. In the soil however, the potential to release P was strong at 10 days after inoculation. Mixed culture of *Bacillus subtilis* and *Aspergillus niger* in broth increased P solubulization earlier than cowpea and soybean rhizobia. There was, however, a decline in the potential of the 2 microorganisms to solubulize ORP as the microbial cells aged (r^2 of 0.58 at day 5 to r^2=0.16 at day 15). In the soil, the potential of the mixed culture of *Bacillus* and *Aspergillus niger* to solubulize ORP became stronger at day 5 and 10 with the respective r^2 values of 0.309 and 0.420 from its initial value of 0.01 at day 1. Generally, *Ogun* rock phosphate solubulization was optimum between the 5th and 15th day after microbial inoculation. Legumes will benefit from this process because the period marks the beginning of nodule initiation, nodule formation and nitrogen fixation.

Keywords: *Aspergillus niger* · *Bacillus subtilis* · Bradyrhizobium · *Ogun* rockphosphate · soil · solubilization

INTRODUCTION

Acidulation process, using mineral acid or through the biological method will yield phosphate but in different quantities depending on the process. Although acidulation process using mineral acid is faster, microbial solubilization is natural and environment-friendly. Generally, acidulation of rock phosphate (RP) before use had been found to be beneficial to crops. Paulraj and Velayudham [1] worked on direct and residual effect of Mussorie RP, organic manure and phosphorus bacteria in rice and found that rice yield although highest when single superphosphate was used, did not significantly differ from yield of rice fertilized with MRP enriched with manure and bacteria. Singh and Kapoor [2] assessed the effect of inoculation of phosphate solubilizing microorganisms (PSM), *Bacillus circulans* and *Cladosporium herbarum* and Arbuscular (AM) fungus, *Glomus fasciculatum* with or without MRP in a phosphorus deficient natural non-disinfected sandy soil on *Vigna radiate*. Results showed that AM levels increased following the addition of MPR or inoculation with PSM or *G. fasciculatum*. Improved nitrogen (N) and phosphorus (P) uptake was recorded after treatment with combination of *B. circulans*, *C. herbarum* and *G. fasciculatum* in the presence of MRP.

Similarly, Manjaiah *et al.*, [3] reported highest pod yield of 1.98 t ha^{-1} of groundnut when MRP, FYM were mixed with *Aspergillus awamori* when compared with SSP and MRP alone. The authors studied the influence of MRP and organic amendment with or without P solubilizer bacteria (*Pseudomonas straita*) and fungus (*A. awamori*) on the nutrient status of an acid sandy soil and found that a combination of organic amendments and P solubilizer with MRP made N, P, K and S available. Kole and Hajra [4] reported increased availability of P_2O_5,

Corresponding Author: Dr. L.B. Taiwo, Institute of Agricultural Research and Training, Obafemi Awolowo University, Moor Plantation, P. M. B. 5029, Ibadan, Nigeria

dry matter yield of *Phaseolus aureous* and total P uptake in an acid terai soil when compared with the un-inoculated control.

Preliminary studies indicated that *Ogun* Rock Phosphate (ORP), similarly enhanced plant growth and crop yield [5]. Various application methods such as direct application and pre-application acidulation gave varying results. There is therefore, the need to evaluate P yield *in-vitro* and within the soil environment as influenced by soil microorganinsms to enable the adoption of a better approach in ORP application for enhanced crop production.

This study therefore, examined microbial solubilization of ORP prior to application to soil and *in-situ* soil solubilization of the phosphate using some microbes.

MATERIAL AND METHODS

Laboratory and soil incubation studies were carried out to measure the amount of phosphorus (P) released from ORP. In the laboratory, *Bradyrhizobium japonicum*, IRj 284 and a cowpea rhizobium IRc 252 were used as mixed culture to solubilize ORP in one of the laboratory experiments. Ammonium sulphate-yeast extract glucose (AYG) medium [6] was used with ORP powder ~240 mesh size) as a source of insoluble phosphate. The medium contained: glucose, 20.0 g; $(NH4)_2SO_4$, 1.0 g; $MgSO_4.7H_2O$, 0.5 g; yeast extract, 0.2 g; $FeCl_3$, 2.0 mg; $MnSO_4.H_2O$, 4.0 mg in 1 litre of distilled water. After dissolving the materials, the pH of the medium was adjusted to 6.8 using standard buffer (pH 7) before dispensing into a number of 200 mL flasks at 100 mL per flask. Following autoclaving, at 121°C for 15 minutes, the flasks were inoculated with the individual isolates in each case and incubated for 3 days on a rotary shaker at 28°C. Three flasks were used for each microbial isolate and three for un-inoculated control. The contents of the culture flasks at the end of incubation period were centrifuged at 15000 g for 20 minutes to remove biomass and un-solubulized matter. Samples were taken at 1, 5, 10, 15 and 20 days after inoculation. The soluble phosphates, expressed as equivalent phosphorus (P), were determined as a measure of the extent of solubulization of rock phosphate [6].

In the second laboratory study, a mixed culture of *Bacillus subtilis* and *Apergillus niger* were used. The broth was carefully formulated to accommodate the nutritional requirements of the 2 microorganisms and ORP was used as the main source of P. Thus, 1 g mL^{-1} of distilled water, 2 g of yeast extract and 5 g each of peptone and sodium chloride per L litre were combined with 4 g of potato extract and 20 g of dextrose and were dissolved in 1 L of water. The cultures were similarly centrifuged after the incubation periods and P determined on a spectro-20 electro photometer following the procedure described by Jackson [7].

Table 1: Pre-cropping soil physico-chemical analysis

Parameters	Values
Sand %	82.00
Silt %	12.00
Clay %	6.00
% N	0.11
% C	1.28
Ca (meq 100^{-1})	2.60
Mg	1.73
K	0.21
Na	0.66
H+	0.11
CEC	5.31
Base (%)	98.00
Av. P (mg kg^{-1})	1.46
pH	5.5
Microbial population (CFU)	7.2×10^8

In the soil incubation study, soil samples for the incubation experiment were analyzed for their nutrient status, microbial population and results are presented in Table 1.

The mixed culture was mixed with ORP before application to 1 kg soil at the rate of 45 kg P_2O_5 ha^{-1} (18 mg $P_2O_5Pot^{-1}$). Sampling was also done at 1, 5, 10, 15 and 20 days after inoculation. Microbial inoculation in the broth was between 0 and 5% while soil inoculation was made at 0 to 20%.

All data generated were subjected to regression analysis.

RESULTS AND DISCUSSION

Microorganisms bring about a number of transformations of the chemical element such as P in soil. One of these transformations is the oxidation or reduction of inorganic phosphorus compounds. In the process, some amounts of P in excess of the required amount are released into the soil environment. They convert considerable amount of the element for their use by the process of immobilization. This process reduces availability of such element for plant use. The efficiency of the process depends on the population of the organisms, time of introduction of such compounds and other environmental factors. In this study and as shown in Fig. 1, the phosphorus (P) in broth increased as the inoculum size of the mixed culture of IRj 284 and IRc 252 increased. The quantum at each point of release may be

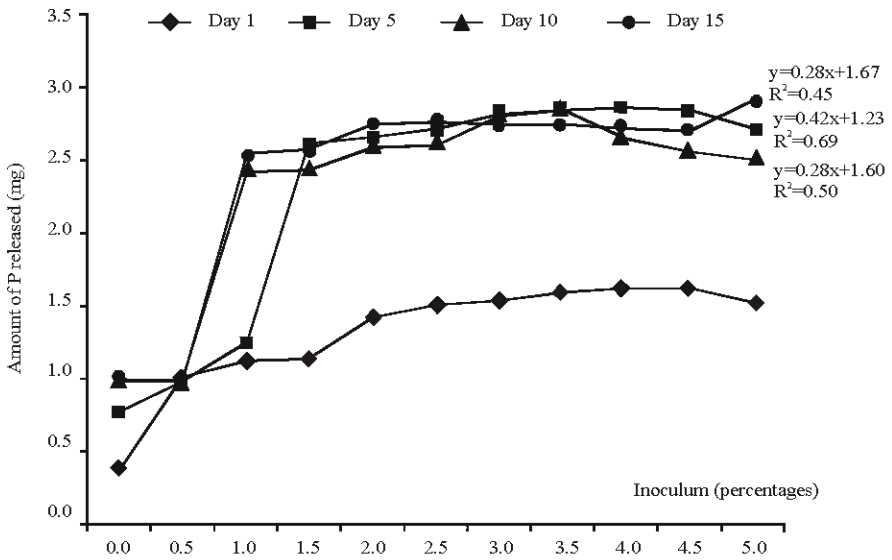

Fig. 1: Amount of phosphorus (mg) released into broth as influenced by varying rates of rhizobium inoculum

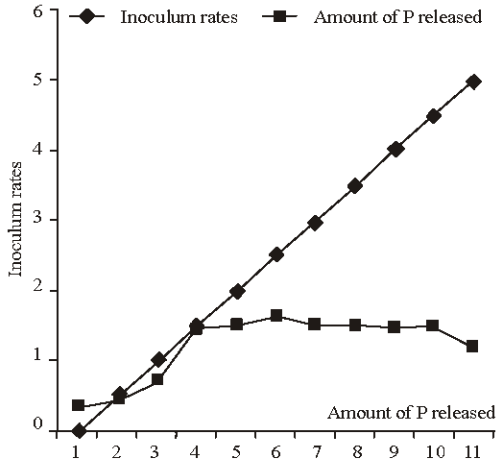

Fig. 2: Amount of P released in response to increase in inoculum rates

regarded as an excess of the specific amount required for microbial cell formation and growth. The percentage of P released at day 1 was expectedly lower than the values obtained at later dates (Fig. 1). The highest P value was obtained at the 15th day after inoculation, the significant correlation coefficient (r^2= 0.45) at that period was, however, the lowest indicating that the potential to release P through microbial solubulization process at that period had become reduced when compared with strong P release potentials at 1st, 5th and 10th day after inoculation. Generally, as the inoculum volumes increased, there was a concomitant increase in P released from ORP (Fig. 2). However, at more than 2.5% inoculum volume, a decline in P release was observed suggesting that a maximum value of between 2.5 and 3.0% might be an ideal value for optimum P release in the broth.

In the soil incubation study, the highest P release was obtained a day after inoculation when 12.5% inoculum rate was used (Fig. 3). There was a general decline in soil P availability at 5th, 10th, 15th and 20th day after inoculation. The coefficient of correlation (r^2) at these periods (0.28, 0.59, 0.37 and 0.20, respectively) were, however stronger than the value obtained at day 1 (r^2=0.03). This observation suggests that although, there was immobilization of P as ORP stayed longer in the soil, there might be some consistency in P release through microbial process as the rock phosphate got older in soil. Some leguminous crops such as cowpea (*Vigna unguiculata*) and Soybean (*Glycine max*) will undoubtedly benefit from the P flux at these periods since the time mark the beginning of nodule formation and symbiotic nitrogen fixation. Substantial amount of P released might however, not be available for crop uptake because of its immobilization. This was expected in view of the complex nature of soil environment, principal of which is the capacities of both clay minerals and organic compounds (humic and fuvic acids) to fix-up P in soil solution. In the soil used, the initial P value was low (1.46 mg kg^{-1}) (Table 1) indicating that considerable amount of P is required to meet the soil biological and crop P demands.

In the broth containing *Bacillus subtilis* and *Aspergillus niger*, the peak of P release was attained when the volumes of inoculum were between 2.5 and 3%

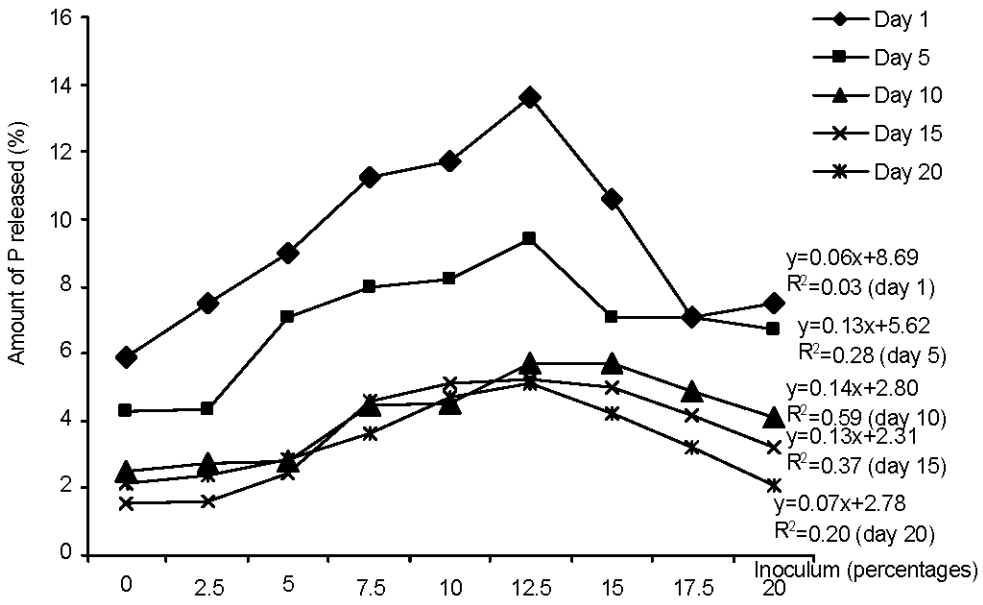

Fig. 3: Amount of P released into soil from *Ogun* RP at various days after inoculation with *Rhizobium inoculum*

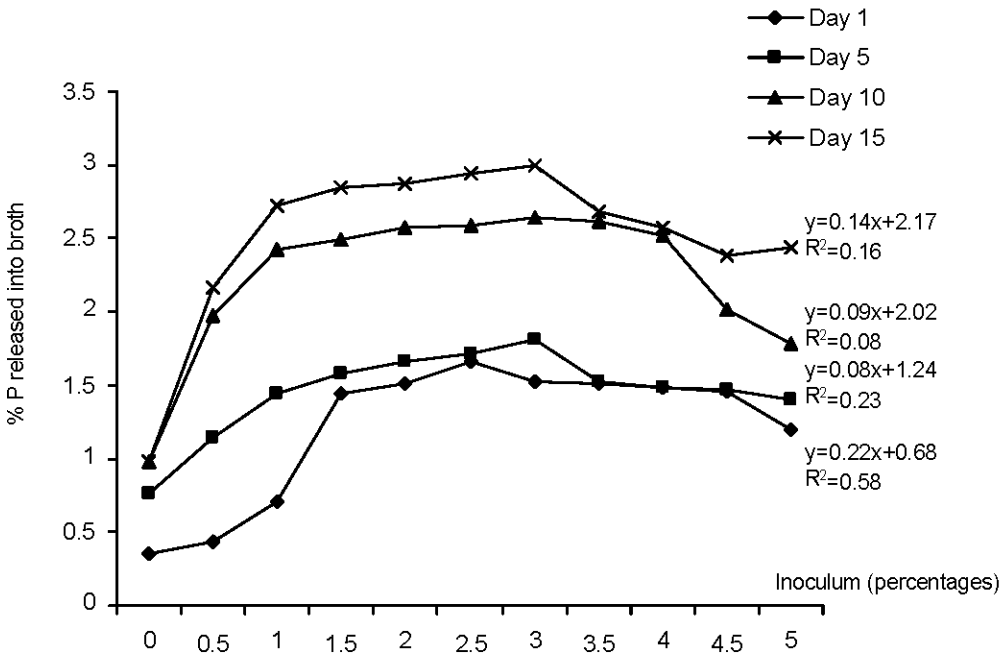

Fig. 4: Amount of P released into broth from *Ogun* RP by a mixed culture of *Bacillus* sp and *Aspergillus niger* at various days of incubation

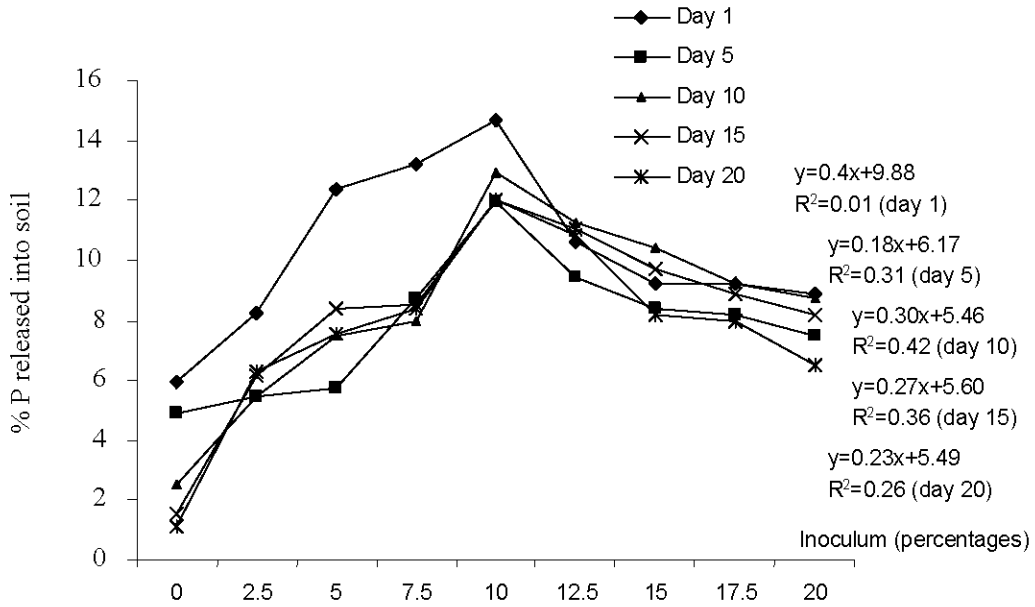

Fig. 5: Amount of P released into soil from *Ogun* RP at various sampling period by combined *Bacillus* sp. and *Aspergillus niger*

(Fig. 4), subsequent P release at higher rates were lower than the values of P obtained at the peak. A similar observation was made when rhizobia were used in the laboratory study. The results showed that as the microbial population increased and the cells got older, there were declining releases at the 5th and 10th day after inoculation. This observation suggests that there was microbial immobilization of P at these periods of time. Several reasons may be adduced to this observation. The first is that biochemical alterations in the growth medium might have altered P availability, reduction in population of viable cells and inability of the aging cells to effectively and efficiently carry out their biochemical functions of P recycling. Sarhan [8] obtained a relationship between the rate of solubulization of rock phosphate and the extra-cellular exudates including organic acids, alkaline and acid phosphatases in the growing medium. It should be noted that solubulization of ORP with mixed culture of these microorganisms may not be a rational option of producing P from ORP. This argument, does not however, defeat the fact that bulk-soil microbes do impact markedly and positively on P release either from the added RP or from the less-available indigenous P sources as observed in this study. This is corroborated by the report of Toro *et al.*, [9] that found phosphate-solubulizing rhizobacteria, *Enterobacter* sp and *Bacillus subtilis* influencing in a positive way, the biochemical P cycling. Similarly,. Reyes [10] obtained positive increase in maize yield when a bacterium and *Penicillum rugulosum* strains were used on rock phosphate. The reports of Burford *et al.*, [11], Didiek *et al.*, [12], Taiwo and Oso [13] and Gull *et al.*, [14] are consistent with this positive effect of microbes in rock phosphate solubulization.

In the soil, inoculation with *Bacillus subtilis* and *Aspergillus niger* was best when the inoculum volume was about 10% (Fig. 5). There was a general reduction in P available in soil when the inoculum rate was more than that rate. The P release at day 1 might be considered to be spontaneous (r^2=0.01) and not as a result of inoculation but possibly in response to soil pH (5.9) which tends to acidity. Although, there was reduction in P release as the cells aged, there might be some consistency in solubulization process as long as ORP was available. From this study, the optimum P yield from microbial process was at day 10 (r^2=0.42). Considerable amount of P might also be obtained at day 15 and 20, but this would depend on other environmental factors.

Generally, microbial inoculation of rock phosphate is dependent on time of inoculation to the soil. Late application in the crop life cycle may not be of considerable benefit to the crops but P release from phosphate rock is generally enhanced by the microbes.

CONCLUSIONS

In this study, a general increase in P solubilized from ORP by all the microorganisms was observed. However, the consistency in phosphate rock solubulization

declined as the microbial cells aged. *Ogun* rock phosphate solubulization was optimum between the 5th and 15th day after inoculation. Solubulization of ORP in the soil using *Bacillus subtilis* and *A. niger*, may be a rational option of P production from ORP especially before it is 20th day old in soil. Legumes will no doubt, benefit from this process because the period mark the beginning of nodule initiation, nodule formation and nitrogen fixation.

ACKNOWLEDGEMENTS

The authors acknowledge the assistance of Dauda, O.R., Adegoke, O.A. and Durojaye, O. of Ibadan polytechnic, Ibadan, Nigeria for their assistance in the incubation experiment and laboratory analysis of all the samples. We also thank the management of Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan for allowing the publication of this work.

REFERENCES

1. Paulraj, N.S. and K. Velayudham, 1995. Direct and residual effect of Mussorie rock phosphate, organic manures and phosphor-bacteria in rice-black grain system. Madras Agric. J., 3: 220-221.
2. Singh, S. and K.K. Kapoor, 1998. Effect of inoculation of phosphate solubilizing microorganisms and an Arbuscular mycorrhizal fungus on mungbean grown under natural soil conditions. Mycorrhiza, 7: 297-299.
3. Manjaiah, K.M., H.T. Channal and T. Satyanarayana, 1996. Influence of rock phosphate on nutrient dynamics in the presence of organic amendment and P solubilizer at different stages of growth of groundnut. Karnataka J. Agric. Sci., 9: 21-26.
4. Kole, S.C. and J.N. Hajira, 1999. Effects of P solubilizers on the availability of rock phosphate by green grain (*Phaseolus aureus*) in an acidic terai soil. Karnataka J. Agric. Sci., 3: 97-105.
5. Akande, M.O., E.A. Aduayi, R.A. Sobulo and A. Olayinka, 1998. Efficiency of Rock Phosphate as Fertilizer source in South-western Nigeria. J. Plant Nutr., 21: 1339-1353.
6. Halder, A.K., A.K. Mishra, P. Brattacharyya and Y. Chaknabartlty, 1990. Solubilization of rock phosphate by rhizobium and Bradyrhizobium. J. General Applied Microbiol., 36: 81-92
7. Jackson, M.L., 1958. Soil Chemical Analysis. Prentice Hall Inc., Englewood Cliffs, New Jersey, pp: 498.
8. Sarham, M.M., 1999. Influence of nutritional requirements and heavy metals on the solubilization of rock phosphate by *Aspergillus niger*. J. Union Arab Biol., 8: 39-52.
9. Toro, M., R. Azcon and J.M. Barea, 1997. Improvement of Arbuscular Mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (pp: 32) and nutrient cycling. Applied Environ. Microbiol., 63: 4408-4412.
10. Reyes, I., L. Bernier and H. Antoun, 2002. Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of *Penicillium rugulosum*. Microbial. Ecol., 44: 39-48.
11. Burford, E.P., M. Fomina and G.M. Gadd, 2003. Fungal involvement in bioweathering and biotransformation of rocks and minerals. Mineralogical magazine, 6: 1127-1155.
12. Didiek, H.G., K. Siswanto and S. Yudho, 2000. Bioactivation of Poorly Soluble Phosphate Rocks with a Phosphorus-Solubilizing Fungus. Soil Science of American J., 64: 927-932.
13. Taiwo, L.B. and B.A. Oso, 1997. The Influence of some pesticides on soil microbial flora in relation to changes in nutrient level, rock phosphate solubilization and P release under laboratory conditions. Agriculture, Ecosystems and Environment, 65: 59-68.
14. Gull, M., F.Y. Hafeez, M. Saleem and K.A. Malik, 2000. Phosphorus uptake and growth promotion of chickpea by co-inoculation of mineral phosphate solubilizing bacteria and a mixed rhizobial culture. Australian J. Experimental Agric. 44: 623-628.