Environment Conservation Journal 12(1&2) 59-63, 2011
(ISSN 0972-3099) Abstracted and Indexed

Vesicular arbuscular mycorrhiza (VAM) mediated solubilization of phosphorus in clayey soil

Aparna Asokan[1], Snehita Chauhan[1]✉ and Prem Kishor Kumar[2]

Received: 12.12.2010 **Accepted: 29.02.2011**

Abstract

One of the major essential macronutrient for plant is phosphorous and is applied to soil in the form of chemical phosphatic fertilizers which is immobilized rapidly and becomes unavailable to plants. Microorganisms are involved in the transformation of soil P and is thus an integral part of the soil P cycle. In particular, soil microorganisms are effective in releasing P from inorganic and organic pools of total soil P through solubilization and mineralization. P-solubilization ability of the microorganisms is considered to be one of the most important properties. The adverse impact of chemical fertilizers on the environment and the less cost effectiveness stimulates the exploration of Phosphate solubilisers. 2/3rd of phosphate fertilizer is unavailable within a very short period of its application due to fixation in the soil complex. To overcome the problem of phosphorus solubilisation and to raise its concentration in soil, the present work was undertaken which deals with the isolation and inoculation of VAM spores from four sets of soil sample mainly clayey textured soil as classified on the basis of its morphological characteristics done through particle size analysis. The result of the present study showed that AM symbiosis associated with plant roots and soil aggregates optimizes the phosphorus solubilization and it is confirmed by the physico-chemical and biochemical estimations along with the mineralogical studies, where the results are within expectations.

Keywords: *Clayey soil, VAM spores, Solubilisation, Phosphorus, Biochemical*

Introduction

The solubilization of mineral phosphates to low molecular weight organic acids contributes in phosphorous solubilisation (Halder *et al.*, 1990). The presence of hydroxyl and carboxyl groups in these organic acids results in chelating the cations bound to phosphate, thereby converting it into soluble forms. Microbial solubilization of inorganic and organic phosphatic compounds has been extensively studied under Indian conditions. Therefore, one of the approaches would be to increase the number and activity of efficient Phosphate Solubilizing Microorganisms (PSM) in the root zone of plants by use of microbial inoculants for increasing phosphorus availability to the plants from the soil as well as added phosphate. It is estimated that India alone has about 140 million tones of rock phosphate deposits, most of which are low grade and contain impurities. Only high grade rock phosphates, free from impurities are utilized for the manufacture of phosphatic fertilizers. Direct use of even low-grade rock phosphate as fertilizer is feasible in neutral to alkaline soil if PSM are used as inoculants. Mycorrhizal symbiosis play an important role in nutrient cycling in agricultural and natural ecosystems. VAM fungi colonize the root cortical region cells of plants and develop an extrametrical hyphae network that could absorb nutrients from the soil. In ecosystem they play a vital role and their association improves the capacity and longevity of root uptake of growth nutrients, enhanced the absorption of remotely available nutritional elements especially phosphorus and reduces the susceptibility of plants to soil-borne pathogens (Rokni *et al*., 2010). Phosphorus is an important plant nutrient classed as a major plant nutrient element. It is associated with several vital functions and is responsible for several characteristics of plant growth such as utilization of sugar and starch, photosynthesis, nucleus formation and cell division, fat and albumin formation, cell organization and the transfer of heredity (Saha and Biswas, 2009). As reported by Khiari and Parent (2005), VAM plays a major role in converting the complex organic phosphorus into inorganic

Author's Address
[1]Department of Microbiology, Career College, Bhopal, India
[2] Boston College for Professional Studies, Gwalior, India
E-mail: apaso@rediffmail.com,
snehita.chouhan@rediffmail.com

solubilized form through phosphatase enzyme which varies with pH. Its mechanism at initial level of its presence in complex form gradually converted into the soluble form but for a short period and automatically get converted into more stable form and in a fraction of time become unavailable to the soil system for uptake by plants (Khan *et al.*, 2009).

Materials and Method

Sample collection: Freshly soil samples were collected from four different locations within the Bhopal city, India from depth of 5-10 cms by adopting conventional coning and quartering method for property evaluation. The soil samples were named as SS1, SS2, SS3 and SS4 under two categories *i.e.*, VAM (+) and VAM (-). The soil samples were brought to the laboratory in polybags and were kept under ice cold conditions for initial level studies. These soil samples were then transferred in to earthen pots for further experimental analysis.

Physico-chemical and biological properties: The samples were tested in terms of physical properties like pH, conductivity, water holding capacity, density, porosity and texture, adopting the conventional experimental details of Page *et al.*, (1982), mineralogical analysis was done by X- ray diffract meter (PW-1710), the technique applied to identify different phases present in initial soil and in treated with VAM inoculants soil after 90 days; available phosphorous was estimated by Bray and Kurtz method (1945); Phosphatase enzyme activity was estimated by Tabatabai and Bremner (1969).

Isolation of Spores (**Gerdemann and Nicolson, 1963):** The top soil was sieved through a series of sieves of maximum size 500 microns and minimum of 50 microns. VAM spores were collected on the 50 microns sieve. Generally the soil in the top sieve is washed 3 times.

Proliferation and inoculation of VAM Spores in Test Soil: Starter inoculums (spores) of VAM fungi were isolated from soil by Wet sieving and decantation technique. Spores were transferred to the sterile soil (in duplicate) and mixed well. Now, seeds of *Zea mays* and VAM spores were added in sterilized soil kept at 37 ºC for incubation.

Isolation and Screening of Phosphate Solubilizing Bacteria: Soil samples were collected from the rhizosphere of different soils. A total of 9-10 composite soil samples (pH 6.0-9.0), were used for the isolation of phosphate solubilizing bacteria. These bacteria were isolated from soil sample by serial dilution technique (Sharma, 2005) on Pikovskaya agar medium plates. Pikovskaya medium was used for the isolation, cultivation and maintenance of phosphate solubilizing bacteria (Gaur, 1990). All the flasks were maintained at 30˚C for 14 days with intermittent shaking twice a day. Un-inoculated medium served as control and each experiment was done in triplicate set.

Identification of Phosphate solubilising bacteria (Mac Faddin, 1980): Pure cultures were identified on the basis of their morphological, cultural and biochemical reactions. Isolates were spot inoculated on Pikovskaya medium (Pikovskaya, 1948) for detection of their phosphate solubilizing ability and incubated at 37 ºC for 48 hours of their phosphate solubilizing ability and incubated at 37 ºC for 48 hours. Halo surrounding the colonies were measured and the solubilizing efficiency (SE) was calculated by the following formula:

$$SE = \frac{\text{Solubilization diameter X 100}}{\text{Growth diameter}}$$

Results and Discussion

Spores from four sets of soil sample are isolated and identified as *Glomus aggregatum.* This genus could grow/multiply in a wide range of pH tolerance (pH 6.0-9.0). Most spores are isolated from the top soil (5-20 cm). Phosphorus is one of the major elements utilized by the plant largely used in membrane, cell division, nucleic acid and high energy compound. It is considered as important plant growth limiting factor because of many abiotic and biotic properties which restricts its mobility in soils. Table–1 incorporates the results of physico-chemical and biological properties at initial and final level of soil genesis for 90 days. The texture of the soil is clayey. The results showed view of various parameters which played an important role in enhancing soil properties. The pH is found to decrease with number of sampling at 14 days interval for a total period of 90 days, the water holding capacity of clayey soils is noted to be increase around 10-18% with raised conductance from 350 to 455 µmhos/cm, the porosity value ranges from 34 % at

initial level to 48% in the final soil. An increase in the available phosphorus concentration is noted in all the samples upto third sampling. Fig.1 shows the pattern of pH noted during the study of 90 days, where the initial level of pH begins at 8.2 and reduced to 5.8 *via* 6.2. Fig.2 indicates available phosphorous in ppm in test and control soil against number of samplings. An initial increase in the concentration with gradual decrease at later stages is observed. The possible explanation for such pattern is the production of organic acids in soil which solubilises the phosphorous into available form but due to its unstable nature, if not utilized by the plants immediately again revert to its unavailable form. In sandy soil an easy source for the uptake of phosphate by ions specifically Ca^{2+} and Mg^{2+} in alkaline pH range and Al^{3+} and Fe^{3+} in acidic range, is reported by Harley and Smith (1983), the same pattern along with noted depletion of available phosphorus in the test soils is measured and this observation confirmed the solubilization of the non labile to labile form of phosphorus. Arbuscular mycorrhizal produce a range of phosphatase enzyme and through these enzymes the phosphatase are taken up into cells and incorporate into nucleic acid, phospholipids which are stored as polyphosphates in vacuoles.

Table -1: Physico-chemical and biological properties of treated and untreated soil

Soils	pH	Conductivity (µmhos/cm)	WHC (%)	Porosity (%)	Av. PO_4 (ppm)	Phosphatase enzyme (µmoles/ml)
SS -1	8.2	340	38	22	0.424	0.600
SS 1+	6.4	445	44	36	1.042	0.297
SS -2	8.2	340	38	22	0.423	0.533
SS 2+	6.4	445	44	36	1.042	0.299
SS -3	8.2	340	38	22	0.439	0.625
SS 3+	6.4	445	44	36	1.042	0.319
SS -4	8.2	340	38	22	0.400	0.609
SS 4+	6.4	445	44	36	1.042	0.297

SS- : Soil Sample minus VAM spores
SS+: Soil Sample plus VAM spores

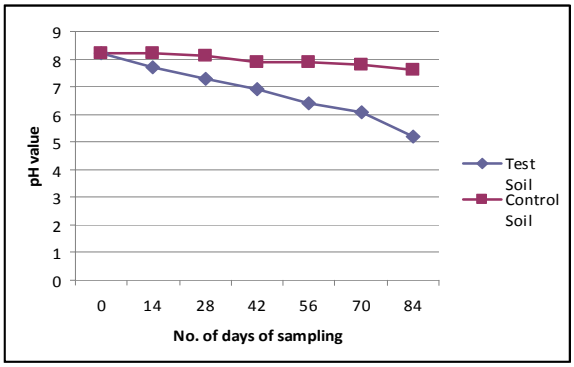

Fig.- 1: pH range in control soil (-VAM) and test soil (+VAM)

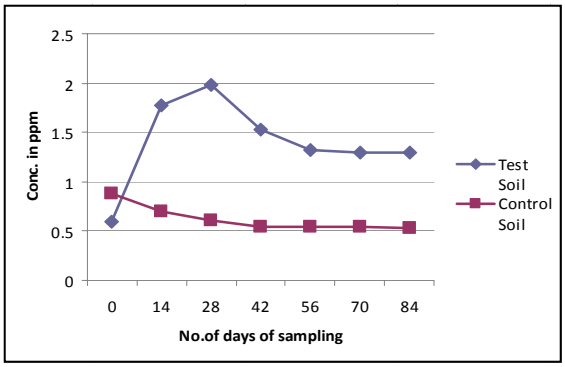

Fig.- 2: Available phosphorus range in control soil (-VAM) and test soil (+VAM)

The phosphatase enzyme activity in test soils is measured at 14 days interval after each spore inoculation in soil Fig. 3, where the enzyme activity is increased gradually and contributed in the formation of organic acid which is an indication of the formation of inorganic (soluble) phosphorus with phosphatase enzyme activity. The state has reached to saturation due to which no further increase in the concentration of phosphatase activity is observed. After sixth sampling, the

amount of available phosphorous as well as phosphatase enzyme activity is noted to be constant. The constant value of available phosphorus and phosphatase enzyme activity in soil confer to the presence of channel of phosphorus in soluble form transported through hyphae and the transfer of phosphorus across the host-fungus interface. Along with these observations, during the process of solubilization of phosphorus in soil the pH of the soil more importantly is taken in to account. The pH changes from 5.8 to 8.2 in the entire sampling of test soils. It is imperative from the observation that all these parameters are interdependent. The results of pH shows a decline from initial to the final level while in control where no spores are inoculated the pH is slightly fluctuated. Fankem *et al.,* 2006, reported the same experimental results with pH and could strengthen our studies.

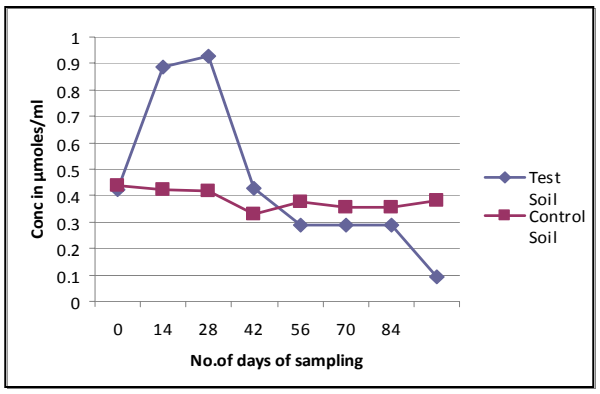

Fig.-3: Phosphatase enzyme activity in control (-VAM) and test soil (+VAM)

Phosphate dissolving microbial consortium are developed in Pikovskaya liquid medium from the soil. On screening the consortium, numerous colonies are noticed on the plates, which gave a zone of clearing. Two different bacterial colonies and a fungus are picked up from the plates of Pikovskaya agar showing the maximum zone of the clearing around these colonies. These two bacterial colonies and a fungus are purified and identified as *Pseudomonas striata*, *Bacillus* sps, through Probablistic identification of bacteria (PIB) (Bryant, 2003) and *Aspergillus niger* through its lawn and spore morphology microscopically. Table – 2 shows different mineral phases present in initial and treated soil detected by X ray diffractometer. It indicates that most of the elements were present in their oxide and silicate form. VAM spores inoculation is favored by certain factor which leads to release of phosphorus as elements in the ionic state after undergoing various chemical reactions as discussed here. The phosphate mineral reported in the table was lazulite along with different other minerals.

Table -2: Mineralogical Phases of Soils identified by XRD

Minerals	SS1	SS2	SS3	SS4
Quartz	Present	Present	Present	Present
Albite	Present	Present	Present	Present
Augite	Present	Present	Present	Present
Mizzonite	Present	Present	Present	Present
Lazulite	Present	Present	Present	Present
Meta- Present **aluminite**	-	Present	Present	
Magnetite	Present	Present	-	Present
Tenorite	Present	Present	Present	-

SS- : Soil Sample minus VAM spores
SS+: Soil Sample plus VAM spores
SS1, SS2, SS3, SS4 are soil samples in four replications

Conclusion

The overall results showed that the mycorrhizal inoculation could help in effective utilization of rock phosphate by changing it into available form, which is later taken up by the plants for their better growth and development.

Acknowledgement

Authors are thankful to the Principal of the Career College, BHEL, Bhopal, India for giving permission to publish the present work.

References

Bray R.H. and Kurtz L.T., 1945. Determination of total, organic, and available forms of phosphorus in soils, ***Soil Science*** 59, 39-45.

Bryant, T. N., 2003. *Probablistic Identification of bacteria, PIB computer kit, medical statistics and computing* University Southampton, S094 XYUK.

Fankem, H., Nwaga, D., Deubel, A., Dieng, L., Merbach, W. and Etoa, F. X., 2006. Occurrence and functioning of phosphate solubilizing microorganisms from oil palm tree (*Elaeis guineensis*) rhizosphere in Cameroon. ***African J. Biotech.*** 5, 2450-2460.

Gaur, A.C., 1990. *In "Phosphate solubilizing microorganisms as biofertilizers*." Omega Scientific Publisher.

Gerdemann, J. W. and Nicolson, T. H., 1963. *Spores of mycorrhizal Endogone extracted from soil by wet sieving and decanting* British Mycological Society Published by Elsevier Ltd 46(2), 235-244.

Halder, A.K., Mishra, A.K., Bhattacharya, P. and Chakrabarty, P.K., 1990. Solubilization of inorganic phosphate by *Rhizobium*. ***Indian J. Microbiol.***, 30, 311-314.

Harley, J. L. and Smith, S. E., 1983 *Mycorrhizal Symbiosis*. Academic Press, Toronto.

Khan, A. A., Jilani, G., Akhtar, M. S., Naqvi, S. M. S. and Rasheed, M., 2009. Phosphorus Solubilizing Bacteria: Occurrence, Mechanisms and their Role in Crop Production ***J. Agric. Biol. Sci***. 1(1), 48-58.

Khiari, L. and Parent, L.E. 2005. Phosphorus transformations in acid light-textured soils treat with dry swine manure. ***Can. J. Soil Sci***. 85, 75-87.

Mac Faddin, F. J. 1980. *Biochemical tests for identification of medical bacteria*. Williams and Wilkins *Co.* Baltsimore Vol. 1, USA.

Page, A.L., Miller, R.H. and Keeney, D.R., 1982. *Salicylic acid thiosulphate modification of Kjeldhal method to include nitrate and nitrite*, In: Methods of soil analysis. Part 2 Agronomy, 621-622.

Pikovskaya, R.I., 1948. Mobilization of phosphorus in soil in connection with vital capacity of source microbial species. ***Microbiologia***, 17, 362-370.

Rokni, N., Goltapeh, M. E. and Alizadeh, A., 2010. Some new recorded species of Arbuscular mycorrhizae fungi associated with sugarcane crop in Iran. ***J of Agricultural technology***, 6(1), 67-68.

Saha, N. and Biswas, S., 2009. Mineral Phosphate solubilising bacterial community ***African journal of biotechnology*** 8(20), 6863-6870.

Sharma, K., 2005. *In: Manual of Microbiology. Isolation, Purification and Identification of Bacteria*. Ane Books Pub. New Delhi, 41.

Tabatabai M.A. and Bremner, J.M., 1969. Use of p-nitrophenyl phosphate for assay of phosphatase activity. ***Soil biology and Biochemistry*** 1, 301-307.