Evaluation of Phosphate Solubilizing Fungi From Mangrove Soil as Biofertilizer

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Abstract: ***Phosphates are one of the major nutrients, second only to nitrogen in requirement for plants. A greater part of soil phosphorus, approximately 95-99% is present in the form of insoluble phosphates and cannot be utilized by plants. Most agricultural soils contain large reserves of phosphorus, a considerable part of which accumulates as a consequence of regular applications of phosphate fertilizers. In the present study fungal strains were isolated from mangrove soils, having potential to solubilize inorganic phosphates were characterized. Totally fifteen fungal strains were isolated, two fungal isolates were more potential activity for the phosphate solubilization assay. Two fungal strains such as Aspergillus niger and Penicillium sp. which are the most common fungi capable of phosphate solubilization. For a sustainable agricultural system, microbial solubilization of rock phosphate and its use in agriculture is receiving greater attention. Many soil fungi can solubilize inorganic phosphate in to soluble forms through the process of acidification, chelation, exchange reactions and production of organic acids. Application of phosphate solubilizing fungi in the field has been reported to increase crop yield.***

Keywords: Phosphate solubilizing fungi, mangrove soils, Biofertilizer, *Aspergillus niger*, *Penicillium* sp.

1. Introduction

Agriculture, occupies a unique position both conventional as well as in alternative. Phosphorus is one of the essential nutrients for plant growth. It is an integral part of the cellular activities of living organisms. Phosphorus, in Indian agriculture, it plays an important role in plants in many physiological activities such as cell division, photosynthesis and development of good root system and utilization of phosphate solubilizing microorganisms. Phosphorus is plant macronutrients that play a significant role in plant metabolisms, ultimately reflected on crop yields. Phosphate Solubilizing Microorganisms (PSM) have been well studied and their isolation and role in crop production have been recorded. Studies on the distribution of these organisms have been conducted in several soils. It is well known that the majority of phosphates in the sediments are present as insoluble organic and inorganic forms. The phosphate concentration in water depends upon various factors, of which the bottom deposits play a major role (Promod and Dhevendaran, 1987).

Microorganisms play an important role for transformation of phosphorus in water and sediments and the phosphate ions are reported to be strongly adsorbed by sediments with a high content of silt and clay (Seshadri *et al.,* 2002). The solubilization of phosphorus compounds may also be brought about by acids and enzymes of microbial origin (Alexander, 1961; Skujins, 1968). A few reports are available on the occurrence and distribution of phosphate solubilizing microbes in the marine environments (Ayyakkannu and Chandramohan, 1971; Venkateswaram and Natarajan, 1983; De Souza *et al.,* 2000; Seshadri *et al.,* 2002).

Phosphorus plays a significant role in plant growth and metabolism by supplying energy needed for metabolic processes (Lal, 2002) and is considered obligatory for the synthesis of nucleic acids molecules. Moreover, in soil the main problem with phosphorus for plant uptake is its availability in very minute quantity. Phosphorus containing chemical fertilizers after their application to agricultural soils either get fixed or precipitated in soils. A number of phosphate solubilizing bacteria and fungi act as plant growth promoters because of their ability to release IAA (Souchie *et al.,* 2007).

Soil microorganisms play an important role in making the phosphorus available to plants by mineralizing the organic phosphorus in the soil. These microorganisms have been isolated from a number of different soils in India. Several varieties of phosphate solubilizing microorganisms (PSMs) have been isolated from the rhizospheric soils of crops. They may be increased in yield of several crops, such as cereals, legumes, fibers, vegetables, oils, and other crop plants.

Phosphate fertilizers might increase phosphate availability initially, but will promote the formation of insoluble phosphate compounds making phosphorus unavailable to plants. Therefore, phosphate solubilizing microorganisms may be a choice for maintaining the steady supply of plant available phosphate (Xie, 2008). Root exudates are important for microbial attraction and fungal establishment on roots rhizosphere. Some of the metabolites known to excrete as the exudates from plants are the sugars and the aminoacids which serve as required nutrients for growth of microorganisms (Odunfa, 1979).

2. Materials and Method

Collection of Samples

Soil sample were collected from *Avicennia marina* rhizosphere soil sample from Muthukuda mangroves. Samples were collected from 2-3 cm deep pits dug in the area to be sampled. The samples were collected with a surface sterilized trowel. Soil was scraped along the walls of the pits and collected in polythene bags. Soil from 3-5 pits was pooled together and mixed in the same polythene bag.

Pot Culture Experiments

Crops selected for the Experiments *Vigna mungo* and *V. unguiculata* L. *V.mungo* and *V unguiculata* (Linn.) Hepper syn. *Phaseolus radiates* Roxb., non *vigna*; *P.mungo* Linn., non Roxb. auct. An erect, hairy plant, varying in height from 30 to 90 cm., sometimes long and twining, cultivated as a pulse crop nearly throughout India. Leaves trifoliate; leaf lets entry, ovate to rhombi- ovate in outline, acuminate, 5-10cm long flowers small, yellow on short but later elongating peduncles; pods cylindrical, erect or spreading, somewhat hairy, with long hairs and very short, hooked beak, 3.75 - 4.35cm. long; seeds usually 4, but may be reduced to 1 in a pod, oblong with square ends, 3mm long, generally black with a white hilum protruding from the seed, but concave in the middle, appearing therefore with two protruding ridges.

The seeds of *Vigna mungo* and *V. unguiculata* were purchased from local market in Thanjavur. Selected the healthy seeds were surfaced sterilized in 0.1% mercuric chloride for 10 minutes, then washed with distilled water, and again immersed the seeds in 70% ethanol for 1 minute, again washed with running water. The fungal isolates stored in RB broth and tested with surface sterilized seeds. The seeds were sowing with individual pots. The pots contain mangrove soil.

On *V. mungo*

A.niger as bioinoculant

Experiment I : Unsterilized + Uninoculated
Experiment II : Sterilized + Uninoculated
Experiment III : Unsterilized + *A. niger* inoculated
Experiment IV : Sterilized + *A. niger* inoculated

P. oxalicum as bioinoculant

Experiment I : Unsterilized + Uninoculated
Experiment II : Sterilized + Uninoculated
Experiment III : Unsterilized + *P.oxalicum* inoculated
Experiment IV : Sterilized +*P.oxalicum.* inoculated

On *V. ungiculata*

A. niger as bioinoculant

Experiment I : Unsterilized + Uninoculated
Experiment II : Sterilized + Uninoculated
Experiment III : Unsterilized + *A.niger* inoculated
Experiment IV : Sterilized + *A.niger* inoculated

P. oxalicum as bioinoculant

Experiment I : Unsterilized + Uninoculated
Experiment II : Sterilized + Uninoculated
Experiment III : Unsterilized + *P.oxalicum* inoculated
Experiment IV : Sterilized +*P.oxalicum.* inoculated

Five seeds were buried in each pot at 1cm deep, allowing enough space between the seeds. After that, the pots were kept under sunlight and water was sprinkled every day to maintain the moisture content. Seed germination was observed on the 3rd day in all the parts.

3. Results

The soil was collected from *Avicennia marina* rhizosphere soil samples in three different sampling sites. The fungal strains were isolated and identified and solubilizing the phosphate. Better phosphorus solubilization was recorded *Aspergillus niger* and *A. flavus.*

Morphometric analysis of seed treated with *A. niger* (7th day)

Black gram

The black gram seeds were treated with *A.niger*, the 7th day showed positive influence on the shoot length, root length, plant height, no. of leaf, leaf length and leaf width. The unsterilized inoculated pot measured 14.2, 7.1, 21.3, 2 numbers, 1.6 and 1.2 (cm) and sterilized inoculated pot was 11.1, 5.6, 16.7, 2 numbers, 1.1 and 0.7 (cm) respectively. While unsterilized uninoculated was 5.3, 2.8, 8.1, 2 numbers, 0.8 and 0.3 (cm) and sterilized uninoculated 4.9, 3.1, 8.0, 2 numbers, 0. 0.5 and 0.2 (cm) was measured (Table 1and Plate I).

Cow pea

The cow pea seeds were treated with *A.niger*, the 7th day showed positive influence on the shoot length, root length, plant height, no. of leaf, leaf length and leaf width. The unsterilized inoculated pot contains 20.0, 7.6, 27.6, 6 numbers.0, 2.5 and 1.9 (cm) then sterilized inoculated 11.1, 5.1, 21.3, 3numbers.0, 1.9 and 1.3 (cm) correspondingly. Unsterilized uninoculated 14.5, 5.4, 19.8, 2 numbers.0, 0.9 and 0.4 (cm) whereas sterilized and inoculated was 4.6, 8.6, 13.2, 2numbers.0, 0.5 and 0.2 (cm) respectively (Table 2 and Plate II).

Table 1 Morphometric analysis of seed treated with *A. niger* (7th day)

S.No	Parameters	*Vigna mungo*				*V. unguiculata*			
		A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	Shoot length (cm)	14.2	11.1	5.3	4.9	20.0	11.1	14.5	4.6
2	Root length (cm)	7.1	5.6	2.8	3.1	7.6	5.1	5.4	8.6
3	Plant height (cm)	21.3	16.7	8.1	8.0	27.6	21.3	19.8	13.2
5	No of leaf (Ns)	2	2	2	2	6	3	2	2
6	Leaf length (cm)	1.6	1.1	0.8	0.5	2.5	1.9	0.9	0.5
7	Leaf width (cm)	1.2	0.7	0.3	0.2	1.9	1.3	0.4	0.2

Note: A- Unsterilized inoculated *A.niger*, B- Sterilized inoculated *A.niger*, C- unsterilized uninoculated, D- sterilized uninoculated

Table 2: Morphometric analysis of seed treated with *P. oxalicum* (7th day)

S.No	Parameters	*Vigna mungo*				*V. unguiculata*			
		A	**B**	**C**	**D**	**A**	**B**	**C**	**D**
1	Shoot length (cm)	19.4	16.0	14.0	12.3	16.8	16.0	6.4	10.5
2	Root length (cm)	10.1	2.5	8.5	7.2	9.5	2.5	9.2	1.9
3	Plant height (cm)	29.5	18.5	22.5	19.8	26.3	18.5	15.6	12.4
5	No of leaf (Ns)	2	2	2	2	6	3	3	3
6	Leaf length (cm)	2.6	1.0	2.7	1.7	2.5	2.6	1.9	0.7
7	Leaf width (cm)	1.2	0.6	0.3	0.7	0.8	1.2	0.6	0.4

Note:A - Unsterilized inoculated *P.oxalicum*, B - Sterilized inoculated *P.oxalicum*, C- unsterilized uninoculated , D- sterilized uninoculated

Plate I: Growth of *V. mungo* in response to *Aspergillus niger* as bioinoculant

Plate II: Growth of *V.mungo* in response to *Penicillium oxalicum* as bioinoculant

4. Discussion

In the present investigation black gram and cow pea seed treated with *A.niger* and *Penicillium* sp, to analysis the 7th day morphometric effect the cowpea plant are greater results taken in both the species treated pot.

The effectiveness of *Aspergillus niger* and *Penicillium notatum* in TCP amended soils to enhance the growth of ground nut and improve the physicochemical characteristics of the soil (Malviya *et al*, 2011). The fungal strains, separately; improved the height and dry weight of plant. Dual inoculation of the fungal strains significantly increased the height up to 81% and the dry weight of plant up to 105%, respectively, compared to the non - inoculated TCP soil or that amended with super-P Significant increase in number of pods/plant and the weight of 50 seeds were also recorded with the application of single or dual inoculation of the tested strains. Thus the application of P solubilizing fungi is recommended as a sustainable way for increasing crop yield, under all experimental conditions. Many reports had shown the improvement in plant growth using P-solubilizing fungi.

In this study, *Aspergillus niger* was most efficient *in vitro* for reduction of *Fusarium oxysporum* growth, but it had low phosphate solubilizing ability. In previous investigation, *Aspergillus niger* was ineffective to inhibit *Fusarium oxysporum*. However, *Trichoderma* was not found in the soil sample in this study. If the interaction between pathogens and antagonistic fungi is site specific, *Aspergillus niger is* also a promising candidate for the development of biocontrol agent for organic production.

5. Acknowledgement

The authors acknowledge the UGC, (SERO) Hyderabad for the financial support by providing Minor Research Project.

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