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Role of Phosphate Solubilizing Fungi and Microbes for sustainable Agriculture and Agro forestry

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ABSTRACT

Phosphorus is an important nutrient required for plant growth, yield and adaptability to different ecological conditions. Many Indian soils are deficient in phosphorus. The organic form of available phosphorus is meagre and mineral phosphorus such as rock phosphate is found in large quantities. The mineral phosphorus needs to be solubilized by varied groups of microorganisms, thus making 'P' available for crop plants and forestry. This review presents information on role of phosphate solubilizing fungi and microbes for sustainable agriculture and agro-forestry.

KEYWORDS: Agriculture, agroforestry, fungi, microbes, phosphorus, rock phosphate.

INTRODUCTION

Phosphorus is a macro nutrient required for plant growth. The phosphate form of phosphorus is one of the least soluble mineral nutrients in soil. Available phosphorus in soil is seldom sufficient for optimum growth and yield of crops where no phosphatic fertilizers are applied. However, the application of organic matter and humus to soil improves the availability of phosphorus to the plants. Modern agriculture which is characterised by intensive cultivation method is totally dependent on regular input of numerous types of inorganic fertilizers. Shortage of raw materials compelled with energy crisis has forced to accept the challenge under such circumstances for increased production of crops, by economic and direct use of indigenous rock phosphate. As plant root grows through the soil, it introduces change to its surroundings. The affected zone is known as rhizosphere which differs biologically, chemically and physically from non-rhizosphere soils (Katznelson and Bose, 1959). The essential growth requirements are obtained by plant roots. Hence, though the soil-root interface of a root exerts a degree of influence over its growth environment. One such rhizosphere effect concerns changes in phosphate availability from organic and inorganic sources.

'P' STATUS IN INDIAN SOILS

The records of Indian soils have shown that 70-90% of soils have a low or medium available P-status (Ghosh and Hasan, 1979). The availability of phosphorus can be increased by adding water soluble Phosphatic-fertilizers, enzymatic decomposition or mineralization of the organic form of phosphorus in the soil and solubilisation of inorganic phosphates by phosphate solubilising microbes.

Phosphatic fertilizers such as single super phosphate and triple super phosphate are applied nearly to half of the area in India. But the efficiency of water soluble phosphatic fertilizer does not exceed 30% (Kapoor *et al*., 1989). Further, there is a greater shortage of phosphorus fertilizers and these are also very expensive. The high cost of these fertilizers is a major constraint for Indian farmers. Rock phosphate deposits are available in Uttar Pradesh, Rajasthan, Madhya Pradesh, Bihar, West Bengal, Tamil Nadu, Orissa and Andhra Pradesh. However, the available deposits contain low grade phosphorus.

The recent research in crop production has shown that Rock phosphate is found to be agronomically inferior to super phosphate when applied at the same ratio. Therefore it is always better to inoculate the soil or seeds of crop plants with phosphate solubilising organisms.

ROLE OF SOIL MICROBES AND FUNGI IN 'P' SOLUBILIZATION

Microorganisms play an important role in the solubilisation of various insoluble organic and inorganic phosphates present in the soil (Kapoor *et al.*, 1989). Such application of microbes results in increased availability of phosphorus for plant growth (Tomar *et al*., 1993). Systematic studies on microbial solubilisation of inorganic phosphates were reported by Pikovskaya (1948). Since then voluminous literature has accumulated reporting the ability of different groups of microbes to solubilize the insoluble phosphates in soil and in culture. The organisms includes bacteria, cyanobacteria, actinomycetes and fungi (**Table-1**). *Arthobacter, Azotobacter, Bacillus, Bradyrhizobium, Brevibacterium, Corynebacterium, Enterobacter, Escherichia, Flavobacterium, Micrococcus, Pseudomonos, Rhizobium, Anabaena, Nostoc, Scytonema, Tolypothrix, Micromonospora, Nocardia, Streptomyces, Candida, Alternaria, Aspergillus, Curvularia, Cephalosporium, Chaetomium, Humicola, Myrothecium, Paecelomyces, Penicillium, Phoma* and *Trichoderma* have been reported as phosphate solubilizing microbes (Bardiya and Gaur, 1972; Gaur *et al.*, 1973; Halder *et al.*, 1991; Katznelson *et al.*, 1962; Nateshan and Shanmugasundaram, 1989; Sardina *et al.*, 1986; Subba Rao and Bajpai, 1965; Surange, 1985).

Number of soil factors like pH, moisture, organic matter and NPK were found to influence microbial solubilization (Thakkar *et al.*, 1993; Gaur, 1990). Generally Pikovskaya agar medium is used for isolation of phosphate solubilising microbes. However, the presence of soluble 'P' in culture medium has been found to have reducing effect on the solubilisation of $Ca_3(PO_4)_2$ by some fungi (Chhonkar and Subba Rao, 1967). Phosphate solubilising microbes differ in

their ability to solubilize different types of phosphates like Aluminium phosphate, Ferrous phosphate, Calcium phosphate and others. Solubilization of rock phosphate has been found to depend on the physical and chemical nature of the rock phosphate and on the type of microbes used (Gaur, 1990). Fungi in general have been found to have solubilising ability of rock phosphate. The concentration of Rock phosphate also influences the phosphate solubilization by microbes (Venkateshwarlu *et al.*, 1984). There have been several research papers published on the mechanism of inorganic phosphate solubilisation. Yet the mechanism is not completely understood. Production of organic acids by microbes appear to be a major factor involved. Though, other factors like humic substances, H_2S production, H_2SO_4 formation, CO_2 production and enzymes are also reported to play a role in the solubilisation of inorganic phosphates (Gaur, 1990; Kapoor *et al.*, 1989). Several workers have also reported that it is not possible to equate or correlate that drop in pH of the medium with the amount of phosphate solubilized. A large number of pure aliphatic and aromatic acids can solubilize insoluble phosphates (Halder *et al.*, 1990). H+ ions extrusion by microorganisms seems to explain phosphate solubilization by some fungi. There is also possibility of involving the release of H+ ions from the microbial cytoplasm to the outer surface with the help of H+ translocating ATPase enzyme and it uses the energy of ATP hydrolysis (Beever and Burns, 1980).

Some phosphate solubilising microbes which seem to be effective in solubilizing phosphates in broth were found to be inactive when inoculated into soils (Gaur, 1990). Inoculation of phosphate solubilizing bacteria were found to increase the acid extractable P_2O_5 level of sterile or unsterile bone meal treated and untreated soils. *Bacillus megabacterium* was found to solubilize rock phosphate in sterile soils. Gaur (1990) reported that species of *Pseudomonas, Penicillium* and *Aspergillus* seem to have greater capacities to solubilize phosphate. The amendment of organic matter in sterile soil along with phosphate solubilizing bacteria seems to have increased the availability of phosphorus level (Bajpai and Sudararao, 1971). The effect of phosphate solubilizing fungi have been worked out on the plant growth and crop yield. Gerretsen (1948) was the first to report phosphate solubilisation by microbes resulting in greater uptake of phosphorus. Since then several workers have reported the beneficial effects of using phosphate solubilising microbes as seed inoculants, soil inoculants, etc. From the above literature, it can be concluded that the phosphate solubilising microbes are potential bio fertilizers in improving the available phosphorus in acidic, neutral and alkaline soils. Further it can also enhance plant growth along with other benefits.

ASPECTS AND PROSPECTS OF 'P' SOLUBILIZATION BY MICROBES AND FUNGI

Plant growth promotion by phosphate solubilising fungi: Phosphorus is abundant in soils nevertheless, it is not available to plants. Thus soil becomes phosphorus (P)-deficient, making 'P' one of the most important nutrient elements limiting crop productivity. To circumvent the ' P' deficiency, phosphate-solubilizing microorganisms could play an important role in making P available for plants by dissolving insoluble P. The solubilization of inorganic 'P' by microbial communities including fungi is though common under *in vitro* conditions, the performance of phosphate-solubilizing microbes *in situ* has been contradictory to some extent. Fungi exhibit traits such as mineral solubilization, biological control, and production of secondary metabolites. Thus, their potential to enhance plant growth is clear. The challenge is now to make use of such biological resources to maintain soil health while increasing the crop productivity by providing 'P' to plants through the application of phosphate solubilizing fungi or other biological means.

I. Deficiency of available phosphorus to plant is considered as a major limiting factor to crop production in many agricultural soils. Mineral 'P' resources are essential to restore soil phosphorus content. In rock soils where conventional fertilizers are not used due to cost limitations, local sources of rock phosphate are being increasingly used as an alternative to phosphorus fertilizers. In some cases, the phosphate released is often unable to supply phosphorus for crop uptake. Plant and microbial mediated mechanisms of low cost and appropriate technologies are essential to enhance the solubilization, thus increases the agronomic effectiveness of phosphate rock. Mechanisms of phosphate rock dissolution includes proton and organic acid production, which need to be evaluated extensively.

II. Phosphorus (P) deficiencies have been limiting crop production in many crop soils, where conventional fertilizers are inaccessible. Of total soil P, only 1 to 5% is in a soluble state and is in available form (Molla and Chowdhury, 1984). In tropical soils, 'P' deficiency is considered to be one of the main biophysical constraints to food production and deficiency is a result of low inherent P fertility due to weathering and nutrient extracting agricultural practices (Sanchez *et al.,* 1997). Diffusion of 'P' to plant roots is too low to meet the needs of the crop if soils have low 'P' solubility and/or a high 'P' fixation capacity (Hoberg *et al.,* 2005). The use of conventional 'P' fertilizers is highly limited in developing countries due to cost and is prohibited for use by organic farmers. Microbiological mechanisms that promote PR solubilization are recommended (Gyaneshwar *et al.,* 2002; Richardson, 2001 and Trolove *et al.,* 2003).

III. The soil 'P' cycle involves the transformation of 'P' by geochemical and biological processes. Plant available 'P' occurs in the soil solution as orthophosphate anions, H_2PO_4. Solid inorganic and organic forms of 'P' are found in labile and poorly soluble forms in the soil.

Plant available 'P' is in equilibrium with a relatively labile fraction of 'P' that is adsorbed to aluminum or ferric hydrous oxides, clays, calcium carbonates and organic matter (Whitelaw, 2000). Solution 'P' is easily replenished in response to plant uptake through desorption of 'P' from the labile solid fraction (Whitelaw, 2000). Small fraction of P in the solid phase remains in a labile form, as it can be adsorbed to the soil or participate in precipitation reactions. The effects of 'P' precipitation are significant in acidic soils, where twice

the amount of added 'P' per unit surface area is fixed compared to neutral or calcareous soils (Whitelaw, 2000). The organic 'P' pool constitutes 30 - 80% of the total soil 'P' (Oberson *et al.*, 1996) and a labile 'P' fraction that may supply 'P' to plants through mineralization by the microbial biomass. 'P' loss through crop removal can reduce the soluble and labile P in the soil and decrease total soil 'P' without external inputs. The addition of PR will increase total soil P and plant available P. Plant and microbial mechanisms that effectively extract P from PR and release it into the soil solution or into the labile fraction of the soil, PR resources may provide a viable alternative for 'P' fertilization.

IV. PR may originate from igneous, sedimentary, metamorphic, and biogenic sources, with sedimentary being the most widespread (Van Straaten, 2002). High Carbonate substituted forms of apatite (francolite) will solubilize more readily than pure forms of fluorapatite, releasing more 'P' for plant use (Anderson *et al.,* 1985). In addition to PR source, the major influences on PR solubility are soil properties, crop species, and management practices (Chien and Menon, 1995). The above factors have various influences on the equilibrium of the dissolution reaction of a given apatite mineral. Engelstad *et al.* (1974) found that with the lower soil pH, more of the 'P' from PR becomes available. The apatite dissolution releases $Ca2^{+}$ soils high in calcium do not support PR dissolution, in accordance with the mass action law. Similarly, the dissolution of PR will be favoured, if $Ca2^{+}$ is removed from soil solution.

V. Certain plant species exhibit mechanisms localized in the rhizosphere that allow for the efficient use of 'P' through the dissolution of PR. Bolan *et al.* (1997) found that PR dissolved more efficiently in the presence of plants than in the absence of plants. In addition, it is well known that the effect of PR on plant growth varies among species. Non mycorrhizal plants, including those in the *Brassicaceae* family have been found to utilize 'P' from PR particularly when soils are deficient in 'P' or in response to Al toxicity. Certain microorganisms are capable of solubilizing PR and are collectively termed phosphate solubilizing microorganisms (PSM). Rhizobacteria, such as *Pseudomonas, Bacillus*, and *Rhizobium*, are among the most powerful phosphate solubilizing bacteria (Rodriguez and Fraga 1999). It has been found that certain filamentous fungi, predominantly *Penicillium* and *Aspergillus* species, have greater phosphate solubilising ability than many bacteria (Gaur *et al.,* 1973). Mycorrhizal fungi have also been shown to increase P uptake (Barea *et al.,* 2005).

VI. Ectomycorrhizal fungi associated with *Pinus* plantations, woody plants and others were found to be efficient transporters of soil phosphorus through their network. Synthetic mineral phosphates, crystalline or amorphous, were differentially solubilized by ectomycorrhizal fungi. Natural phosphates do not seem to be solubilized by fungi under similar experimental conditions existing in the field. *Paxillus involutus* seems to solubilize calcium phosphates using either ammonium or nitrate nitrogen, but the different isolates were able to effectively solubilize phosphate in the presence of ammonium regarding the possible mechanism used to solubilize phosphate by these isolates. The type of crystals depends on the phosphate source and on the fungal strain. The importance of phosphate solubilising activity to plant growth stimulation needs to be determined by field trials. *Pisolithus tinctorius* has been found to be efficient ectomycorrhizal fungus for 'P' transport in *Casuarina* and *Eucalyptus (*Manoharachary, 2007).

VII. Plants that can mobilize P from PR can be used as green manures by incorporating the plant biomass into the soil. Mineralization of the plant residue may help in replenishing both labile and soil solution P, thus making the direct application of PR more agronomically effective. Optimizing microbial culture conditions, in a controlled environment for organic acid production, may enhance PR dissolution prior to field application. The use of organic acid producing PSM in a PR pre-treatment process, under controlled conditions, prior to field application, represents a promising low input and appropriate technology for increasing the agronomic effectiveness of PR.

VIII. Genetic engineering: The knowledge on the genetics of phosphate solubilization is still scanty, and the studies at the molecular level in order to understand how precisely the PSM brings out the solubilization of insoluble 'P' are inconclusive (Rodriguez *et al.,* 2006). Some genes involved in mineral and organic phosphate solubilization have been identified and characterized, followed by their expression in selected rhizobacterial strains opened a promising perspective for obtaining PSM strains with enhanced phosphate solubilizing capacity. The initial achievement in cloning of gene involved in 'P' solubilization from the Gram negative bacteria *Erwinia herbicola* was achieved by Goldstein and Liu (1987). An increase in extracellular phosphatase activity of the recombinant strain was also achieved (Fraga *et al.,* 2001).

IX. Despite their association with different ecological niches and multiple functional properties, P-solubilizing microorganisms have yet to fulfil their promise as commercial bio-inoculants. Recent developments in our understanding of the functional diversity, rhizosphere colonizing ability, mode of actions and judicious application are likely to facilitate their use as reliable components in the management of sustainable agricultural systems. However, the required technologies remain in its infancy. The use of PSM is one of the newly emerging options for meeting agricultural challenges imposed by the still-growing demand for food. Thus PSM biotechnology provides an excellent opportunity to develop environment-friendly phosphorus biofertilizer to be used as supplements and/or alternatives to chemical fertilizers.

X. Mechanism of phosphate solubilization by fungi: The use of phosphate-solubilizing fungi is a promising biotechnological strategy in the management of phosphorus (P) fertilization, as it allows the utilization of rock phosphates (RP) or the recovery of P fixed in soil particles. *Aspergillus niger, Penicillium canescens, Eupenicillium ludwigii* and *Penicillium islandicum* were able to solubilize all 'P' sources. Medium acidification was an effective solubilization

mechanism, particularly for $Ca_3(PO_4)_2$. The other P sources were mainly solubilized through organic acids produced by the fungi.

XI. Role of Phosphate solubilizing microbes in sustainable agriculture: Phosphorus is the second important key element after nitrogen as an essential mineral nutrient in terms of quantitative plant requirement. Although 'P' is abundant in soils, in both organic and inorganic forms, its availability is restricted as it occurs mostly in insoluble forms. The P content in average soil is about 0.05% (w/w) but only 0.1% of the total P is made available to plant because of poor solubility and its fixation in soil (Illmer and Schinner, 1995). The supply of phosphorus in early phases of plant development is important. It helps in seed formation and in early maturation of crops like cereals and legumes. Poor availability or deficiency of phosphorus (P) markedly reduces plant size and growth. Phosphorus accounts for about 0.2 - 0.8% of the plant dry weight. 'P' is usually added to the soil as chemical P fertilizer, but synthesis of chemical P fertilizer is highly energy intensive process, and has long term impact on the environment in terms of eutrophication, soil fertilility depletion, carbon footprint, etc. Plants can use only a small amount of this 'P 'since 75-90% of the added P is precipitated by metal-cation complexes, which rapidly becomes fixed in the soil. Several bacterial (*Pseudomonas* and *Bacilli*) and fungal strains (*Aspergilli* and *Penicillium*) have been identified as PSM, their performance under *in situ* conditions is not reliable and therefore needs to be improved by using either genetically modified strains or co-inoculation techniques. Several fungi have been reported to possess greater ability to solubilize insoluble phosphate than bacteria (Nahas, 1996)

XII. Rock Phosphate Solubilizing Fungi as Potential Biofertilizer: Rock Phosphate is the cheapest and abundant phosphatic fertilizer available but due to its sparse solubility it is not always agronomically effective. Combined application of rock phosphate with phosphate solubilising microorganisms has emerged as a logical solution to this issue. Thirty different fungal strains were chosen for rock phosphate solubilization in 5% senegal rock phosphate as a source of phosphorus in Pikovskayas's medium. Out of these, three isolates exhibited maximum rock phosphate solubilization i.e., 92 ppm, 381 ppm and 297 ppm in seven days with a considerable decrease in pH. Bio-fertilizer activity of these isolates in combination with rock phosphate was also individually tested on *Pennisetum glaucum* (bajra) in pots under natural environmental conditions. The results indicate positive effect of co-application of rock phosphate with phosphate solubilising fungi on plant growth.

XIII. Rhizosphere Fungi - Phosphate Solubilization Potential: Soil microorganisms play a critical role in natural phosphorus cycle and recently microbe-based approaches have been proposed to improve the agronomic value of RP. Utilization of microbe mediated RP has definite advantages such as (1) microbial products are considered safer than chemical fertilizers; (2) neither toxic substances nor microbes themselves will be accumulated in the food chain; and (3) self-replication of microbes circumvents the need for repeated application. Inoculation with appropriate phosphate solubilising microorganisms along with rock phosphate could be another strategy to improve the physicochemical and biological properties of the soil that help in improving crop production.

AUTHORS' DATA:

1. Surveyed phosphate solubilizing fungi in inland and seashore soils supporting *Casuarina equisetifolia*, Forst. Plantations located in Visakapatnam and Anakapally of Andhra Pradesh state, respectively.

2. Identified phosphate solubilizing species and studied their growth on agar media amending Tri Calcium Phosphate $Ca_3(PO_4)_2$ Aluminium Phosphate (Al PO_4) and Rock Phosphate (RP).

3. Solubilization of phosphates [$Ca_3(PO_4)_2$, AL PO_4 and RP] in liquid media has been worked out on selected phosphate solubilising fungi.

4. Screened phosphate solubilizing fungi as soil inoculants on the plant growth.

Casuarina can be used for many purposes, from amenity planting, land reclamation, shelter-belts and dune stabilization to the production of shingles, particle board, tannin, timber and roundwood, and perhaps paper pulp. The wood makes outstanding fuel. Therefore this plant has been selected.

MATERIALS AND METHODS:

The dilution plate technique (Waksman, 1952 and Johnson and Curl, 1972), soil plate technique (Warcup, 1950), root grinding and direct root pieces plating techniques (Stover and White, 1953) were employed to study the fungi of root region in the present investigations. Estimation of available Phosphorus was done as suggested by Olsen *et al.* (1954). Inorganic phosphate solubilizers are routinely isolated and screened by plate assay method using Pikovskaya agar medium (Pikovskaya, 1948). The amount of soluble phosphates was estimated by Durge and Palival (1961) method.

RESULTS AND DISCUSSION:

The 'P' solubilizing microbes and fungi are listed in the **Table 1** given below.

Table 1. 'P' Solubilizing Microbes and Fungi

Bacteria	Actinobacteria	Fungi	Mycorrhiza	Endophytes
Bacillus megaterium, *B. circulans*, *B. subtilis*, *B. polymyxa*, *B. sircalmous*, *Pseudomonas striata*, *Enterobacter* sp. *Beggiatoa*, *Thiomargarita* *Leifsonia xyli* *Burkholderia cenocepacia* *Burkholderia caribensis* *Burkholderia ferrariae*	*Actinobispora yunnanensis*, *Actinomodura citrea*, *Microtetrospora astidiosa*, *Micromonospora echinospora*, *Sacchromonospora viridis*, *Saccharopolyspora hirsute*, *Streptomyces albus*, *Streptoverticillium album*, *Streptomyces cyaneus*, *Thermonospora mesophila*69.	*Aspergillus* (*A. awamori*) *Penicillium* (*P. bilaii*).	*Glomus*, *Funneliformis*, *Pisolithus*, *Paxillus*, *Rhizophagus*, *Sclerocystis*, *Claroideoglomus*, *Gigaspora*, *Scutellospora*, *Racocetra*, *Acaulospora*, *Entrophospora*, *Pacispora*, *Diversispora*, *Otospora*, *Paraglomus*, *Geosiphon*, *Ambispora*, *Archaeospora* sp.	**Bacteria**: *AchromobacterAcinetobacter*, *Enterobacter cloacae*, *Pantoea agglomerans*, *Pseudomonas* sp. **Fungi:** *Piriformospora indica.*

Data regarding the number of physico-chemical factors and fungal species supporting *Casuarina equisetifolia* in sampling areas is given in **Table 2**.

Table 2. Fungal numbers in relation to physico-chemical factors of soils supporting *Casuarina equisetifolia*.

Sl. No	Sampling month	Fungal No.	pH	Temp (ºC)	% moisture	N in ppm	P in ppm	K in ppm	% Organic carbon
		S1 S2	S1 S2	S1 S2	S1 S2	S1 S2	S1 S2	S1 S2	S1 S2
1.	Jan	14 17	7.8 7.2	28.0 33.0	2.0 2,5	0.01 0.04	0.002 0.007	0.04 0.05	0.6 1.3
2.	Feb	21 13	7.8 7.0	30.0 34.5	2.8 2.6	0.02 0.04	0.002 0.006	0.03 0.05	0.6 1.3
3.	March	17 35	8.2 7.3	29.0 33.0	2.5 2.0	0.01 0.03	0.002 0.009	0.05 0.06	0.6 1.3
4.	April	12 22	8.2 7.4	30.5 35.0	2.0 1.8	0.02 0.03	0.003 0.008	0.05 0.06	0.7 1.4
5.	May	14 28	8.6 7.5	30.0 34.0	1.5 1.0	0.02 0.04	0.003 0.009	0.05 0.06	0.7 1.3
6.	June	14 24	8.6 7.4	24.0 30.0	4.0 3.6	0.02 0.05	0.002 0.001	0.03 0.05	0.6 1.2
7.	July	18 26	8.6 7.3	24.5 31.0	4.2 3.8	0.01 0.06	0.002 0.01	0.04 0.05	0.5 1.4
8.	August	8 27	8.4 7.2	23.5 30.5	3.6 3.5	0.02 0.06	0.003 0.009	0.03 0.05	0.5 1.4
9.	Sept.	10 15	8.4 7.0	23.0 30.0	3.5 3.2	0.02 0.06	0.003 0.01	0.05 0.05	0.5 1.3
10.	Oct.	10 6	8.6 7.2	22.0 27.0	3.0 2.6	0.02 0.08	0.002 0.01	0.04 0.06	0.5 1.3
11.	Nov.	6 13	8.6 7.3	22.5 28.0	3.0 2.4	0.02 0.07	0.002 0.01	0.04 0.06	0.5 1.4
12.	Dec.	5 17	7.8 7.0	28.0 36.5	3.5 2.9	0.01 0.03	0.002 0.007	0.04 0.05	0.8 1.3
13.	"r" value		0.1013-0.0085	0.4665 0.3374	0.1428 0.1056	0.1025-0.24	0.0004- 0.45	0.002-0.04	0.32 0.3004
14.	"t" value		0.3219-0.026	1.6676- 1.3333	0.4561-0.3357	0.3268-0.7369	0.6916- 1.59	0.006 -0.0126	0.01-0.9953

Altogether 28 fungal species have been isolated. There is not much difference in species composition among the non-rhizosphere and in the rhizoplane regions of the both areas i.e. sea shore and inland areas. It is evident that one or the other species got associated with three ecological niches. It is also evident that *Cladosporium cladosporioides*, *Cunninghamella blacksleeana* and *Stachybotrys cylindrospora* are mainly associated with the Inland areas. *Humicola grisea* and *Sclerotium oryzae* are the fungi associated with the sea shore soils. The qualitative account of fungi supporting *Casuarina equisetifolia* is given in **Table 3**.

Table 3. Fungal species composition of soils supporting *Casuarina equisetifolia*.

Sl. No.	Fungal Species	Sea Shore (Visakapatnam)			Inland (Anakapally)		
		NRS	RS	RP	NRS	RS	RP
1	*Aspergillus candidus* Link	+	-	+	+	-	+
2	*A. flavipes* (Bain & Srt) Thom & Church	+	+	-	+	-	+
3	*A. flavus* Link	+	+	+	+	+	+
4	*A. fumigates* Fresenius	+	+	+	+	+	+
5	*A. nidulans* (Eidem) Wint	+	+	-	+	+	-
6	*A.niger* Van Tieghum	+	+	+	+	+	+
7	*A.niveus* Blochwitz	+	+	-	+	+	-
8	*A.sydowii* (Bain & Srt) Thom & Church	+	+	+	+	+	+
9	*Aspergillus* sp.	+	+	-	-	+	+
10	*A.terreus* Thom & Turresson	+	+	+	+	+	+
11	*A.versicolor* (Vull) Tirabosch	-	+	+	+	-	-
12	*Chaetomium* sp.	+	+	-	+	+	-
13	*Cladosporium cladosporoides* (Fres) de vries	-	-	-	+	+	+
14	*Cunninghamella blacksleana* Lendner	-	-	+	-	+	+
15	*Curvularia lunata* (Walker) Boedijn	+	+	+	+	+	+
16	*Fusarium oxysporum* Schleehtentah	+	+	+	+	+	+
17	*Humicola grisea* Traaen	+	+	+	-	-	-
18	*Mucor racemosus* Fresenius	-	+	+	+	+	+
19	*Myrothecium leucotrichum* (Pock) Tulloch	-	+	+	+	+	+
20	*Penicillium citrinum* Thom	+	+	+	+	+	+
21	*P. lilacinum* Thom	-	+	-	+	+	+
22	*P. purpurogenum* Stoll	+	+	+	+	+	+
23	*Phoma nebulosa* Pers ex. Fr.	-	+	+	-	+	-
24	*Rhizopus nigricans* Ehrenberg	-	+	+	-	+	+
25	*Sclerotium oryzae* Catt.	-	+	+	-	-	-
26	*Stachybotrys cylindrospora* Jensen	-	-	-	+	+	+
27	*Syncephalastrum recemosum* (Cohn) Schroetes	+	+	+	+	+	-
28	*Trichoderm viride* Pers. ex. Fr.	+	+	+	+	+	+
Note: NRS= Non Rhizosphere Soil, RS= Rhizosphere Soil, RP= Rhizoplane.							

So as to make observations, soil samples were collected from 3 years old plantations. It was observed that the fungal numbers of rhizosphere soils are more than the normal soil samples in areas. The rhizosphere soil fungal numbers of seashore area are smaller than the fungal numbers of inland rhizosphere soil samples (**Table 4**).

The rhizoplane region quantitatively has shown similar/more

Table 4. Fungl numbers of **r**hizosphere soils in relation to physical factors of soils supporting *Casuarina equisetifolia*.

Sl. No.	Sampling Month	Fungal Numbers		pH		Moisture%	
		S1	S2	S1	S2	S1	S2
1.	Jan	19	19	7.8	7.2	2.0	2.5
1.	Feb	30	42	7.8	7.0	2.8	2.6
2.	March	48	38	8.2	7.3	2.5	2.0
3.	April	25	51	8.2	7.4	2.0	1.8
4.	May	28	30	8.6	7.5	1.5	1.0
5.	June	22	32	8.6	7.4	4.0	3.6
6.	July	20	42	8.6	7.3	4.2	3.8
7.	August	19	28	8.4	7.2	3.6	3.5
8.	September	32	15	8.4	7.0	3.5	3.2
9.	October	32	13	8.6	7.2	3.0	2.6
10.	November	22	16	8.6	7.3	3.0	2.4
11.	December	11	21	7.8	7.0	3.5	2.9
Note: S1= Sea shore soil (Visakapatnam), S2= Inland soil (Anakapally)							

fungal numbers than non-rhizosphere soil in case of Inland soil. In case of seashore soil, the rhizoplane region of *Casuarina equisetifolia* has shown more fungal population

Table 5. Fungal numbers of rhizoplane regions of *Casuarina equisetifolia*.

Sr. No.	Smpling Month	Fungal Numbers	
		S1	S2
1	Jan	34	22
2	Feb	28	46
3	March	55	19
4	April	55	16
5	May	28	9
6	June	26	31
7	July	30	41
8	August	28	27
9	September	22	17
10	October	40	25
11	November	25	18
12	December	28	12
Note: S1= Sea shore soil (Visakapatnam), S2= Inland soil (Anakapally)			

than rhizosphere and non-rhizosphere soil samples (**Table 5**).

Pot experiment

Both *Trichoderma viride* and *Penicillium lilacinum* were identified as phosphate solubilising fungi. $Ca_3(PO_4)_2$ which was added as a source of phosphate has been solubilized to greater extent by *Trichoderma viride* and *Penicillium lilacinum* resulting in the supply of such

Table 6. Role of Phosphate and microbe amendment on the plant growth of *Casuarina equisetifolia*.

Treatment	Hight (Cms)		Fresh Wt. (gms)		Dry Wt. (gms)	
	Shoot	Root	Shoot	Root	Shoot	Root
Soil+ Plant (Control)	37.5	24.5	9.0098	1.8199	2.9138	0.7061
Soil+ TCP+Plant	59	28	12.346	12.0873	4.0882	0.9020
Soil+*Trichoderma viride*+ Plant (Control)	50.1	23	14.6532	2.0041	4.6879	0.7410
Soil+*Trichoderma viride*+ TCP+Plant	64	34	15.6632	2.3150	4.9031	0.8411
Soil+*Penicillium lilacinum*+ Plant (Control)	52	26.5	10.6818	1.6369	3.4698	0.7344
Soil+ *Penicillium lilacinum* + TCP+Plant	66	33	15.6071	2.6232	5.3754	1.0410
Note: TCP= $Ca_3(PO_4)_2$						

nutrients to the plants and helping the plant in its growth increments (**Table 6**).

Radial growth:

All the fungi tested seem to possess phosphate solubilising capacity over control, thus playing a key role in the solubilisation of insoluble phosphorus sources or untapped phosphorus sources (**Table 7**).

Table 7. Radial growth (cms) of seven fungi on Pikovskaya medium including in Tri calcium Phosphate amended medium.

Fungal Species		Days of Incubation									
		1	2	3	4	5	6	7	8	9	10
Aspergillus flavus	T	0	0.8	2.1	3	4	5.2	7	8.2	9	-
	C	0	0.5	1.5	2.2	3	-	-	-	-	-
A. niger	T	0	0.7	1.5	3.9	5.5	6.2	7.4	7.8	8.4	9
	C	0	0.6	1.1	2.5	-	-	-	-	-	-
A. terreus	T	0	0.3	0.9	1.5	2	3	4.3	5	6.2	7
	C	0	0	0.4	1	1.5	-	-	-	-	-
Cladosporium cladosporoides	T	0	0.6	1.5	3	3.5	4.1	4.8	6	7	7.8
	C	0	0.2	0.9	1.8	2	2.2	-	-	-	-
Curvularia lunata	T	0	1	2.5	3.8	5.5	5.8	6.5	6.8	7.2	9
	C	0	0.3	0.9	1.4	-	-	-	-	-	-
Penicillium lilacinum	T	0	0.4	1	1.5	2	3.4	4.8	5.6	6.8	7.5
	C	0	0	0.6	0.9	1.4	-	-	-	-	-
Trichoderma viride	T	0	0.5	1.8	3	6	9	-	-	-	-
	C	0	0.4	1	1.2	2	-	-	-	-	-

Note: T= Treatment with $Ca_3(PO_4)_2$, C= Control

During the experimentation it was observed that all the fungi could solubilize significant amount of $Ca_3(PO_4)_2$ followed by $AlPO_4$ and Rock phosphate with some variations. Each 30 ml of Pikovskya medium has 6000 µg of $Ca_3(PO_4)_2$ 7600 µg of $AlPO_4$ and 5100 µg of Rock phosphate. The phosphate solubilized has been calculated as percentage at the end of 12 days of incubation. The percentage of phosphate source solubilized has been calculated over control (**Table 8**).

The values given are an average of three replicates. Among the fungi tested. *Aspergillus niger, A. terreus, Curvularia lunata, Fusarium oxysporum, Penicillium citrinum, P. lilacinum, Sclerotium oryzae* and *Trichoderma viride* have solubilized all the three phosphate sources.

$Ca_3(PO_4)_2$ was solubililized up to 94% by *Trichoderma viride* followed by *Penicillium lilacinum* with 81% and *Curvularia lunata* with 69%. $Ca_3(PO_4)_2$ seems to be solubilized slowly by *Aspergillus niger* $AlPO_4$ has been solubilized more rapidly by *A. niger* with 70%.

Table 8. Screening of Fungi for their ability to solubilize various types of insoluble inorganic phosphates in Pikovskaya Broth.

Sl. No.	Fungus Name	Treatment	% Phosphate solubilized	pH	Dry weight (in mg)
1	*Aspergillus niger*	TCP	56	3.16	0.203
		AP	70	2.94	0.190
		RP	42	3.20	0.047
2	*A. terreus*	TCP	75	5.98	0.147
		AP	50	3.12	0.167
		RP	92	4.66	0.143
3	*Curvularia lunata*	TCP	69	5.92	0.212
		AP	51	3.32	0.184
		RP	75	5.92	0.081
4	*Fusarium oxysporum*	TCP	67	5.80	0.219
		AP	37	3.50	0.209
		RP	51	4.54	0.162
5	*Penicillium citrinum*	TCP	68	6.20	0.132
		AP	47	3.58	0.200
		RP	74	6.41	1.083
6	*P. lilacinum*	TCP	81	6.41	0.143
		AP	51	3.41	0.473
		RP	80	5.82	0.431
7	*Sclerotium oryzae*	TCP	60	6.18	0.165
		AP	47	6.64	0.185
		RP	75	5.70	0.101
8	*Trichoderma viride*	TCP	94	5.41	0.210
		AP	45	2.80	0.103
		RP	84	5.64	0.108

Note: TCP=[$Ca_3(PO_4)_2$], AP=$ALPO_4$, RP= Rock Phosphate

92% solubilisation of Rock phosphate was observed with *A. terreus* followed by *Trichoderma viride* with 84%. However the data of mycelium dry weight clearly indicated the poor growth of *Curvularia lunata Aspergillus terreus* and *Sclerotium oryzae* in broth medium amended with Tri calcium phosphate. Aluminium phosphate and Rock phosphate, respectively. However *Pennicillium citrinum* showed luxuriant growth in Pikovskaya broth amended with Rock phosphate.

ABOUT LATE PROF. S.B. SAKSENA

Dr. S. B. Saksena started his research career (1947-48) at a time when there was a dearth of taxonomists in the country who could identify soil saprophytes with confidence. Dr. Saksena's devotion and persiverance so on brought fruits and he came out with a very interesting new genus of the order *Mucorales*, *Saksenia vasiformis* which he named after his guide and mentor Dr. Ram Kumar Saksena. He has been known by this monotypic genus because of its interesting morphology and the thoroughness with which it was worked out. The other two or three genera he described were of the Hyphomycetes and these have stood the test of time. He has guided 25 or more students for Ph.D and published numerous research papers. Dr. S.B. Saksena served as Head, Departmnt of Botany, Sagar University, Sagar (M.P). Later, during 1958-59 he came in contact with Dr. S.D. Garrett, F.R.S. of the Botany School, Cambridge. On his return from Cambridge he introduced in his laboratory studies on root infecting fungi of the soil including the control of *Phytophthora* blight of *Piper betle.*

Besides his academic attainments, his amiable temperament and helpful attitude towards his students and colleagues endeared him to all who came in his contact. The Scientific community of the country bestowed on him several honours, such as Fellow of the Indian Botanical Society, Indian Phytopathological Society, Indian Academy of Sciences, National Academy of Sciences, President of the Indian Science Congress and Indian Phytopathological Society. Prof. S.B. Saksena breathed his last on March 21, 1988. We really need more mycologists and humane like him to fulfil the vacuum created.

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