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Effect of Phosphate Solubilizing Fungi on Growth and Nutrient Uptake of Ground nut (*Arachis hypogaea*) Plants

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ABSTRACT
Phosphate-solubilizing Fungi (PSF) function in soil phosphorus cycle, increasing the bioavailability of soil phosphorus for plants. Aspergillus niger and Penicillium notatum were tested for their efficacy to solubilize tri-calcium-phosphate (TCP) in vitro as well as their effect in vivo to promote the growth of ground nut (Arachis hypogaea) plants grown in soil amended with TCP. The results showed high solubilizing index in agar plates. Also, they effectively solubilized TCP in Pikovskaya's liquid medium (PVK) and released considerable amounts of P into medium. Pot experiment showed that the dual inoculation of phosphate-solubilizing fungi (A. niger and P. notatum) significantly increased dry matter and yield of groundnut plants as compared to the control soil. Significant increment in percentage of protein and oil was also recorded. There was an increase in the percentage of N and P content of the plant. It was significantly resulted with N levels of groundnut plants but this increase was non-significant with the percentage of total phosphorus, under the experimental conditions. Soil analysis showed that the available P, organic carbon levels were significantly increased when compared to the initial soil. The pH was also lowered compared to the initial pH of the soil.
***Key words**: PSF, TCP, Aspergillus, Penicillium, Arachis hypogaea , Protein and oil*

INTRODUCTION

Fungicideshas been an impediment to development of sustainable agriculture. Employment of biofertilizers and biopesticides may be able to side-step some of the deleterious effects caused by chemical fertilizers. Root associated bacteria and fungi have been known to benefit plants and are hence referred to as plant growth promoting rhizobacteria (PGPR) [1] and plant growth promoting fungi (PGPF). It is reported that PGPF may make use of one of the several mechanisms to promote plant growth like production of phytohormones,solubilization of minerals and antagonism to phytopathogens . Phosphorus (P) is a vital plant nutrient, available to plant roots only in soluble forms that are in short supply in the soil. Adding phosphate based fertilizers to increase agricultural yields is a widely used practice; however, the bioavailability of P remains low due to chemical transformations of P into insoluble forms. Thus, phosphate solubilizing bacteria (PSB) play an important role in reducing P deficiency in soil [12]. Fungi are important components of soil microbiota typically constituting more of the soil biomass than bacteria, depending on soil depth and nutrient conditions. A wide range of soil fungi are reported to solubilize insoluble phosphorous. Strains of *Aspergillus niger* and *Penicillium* are the most common fungi capable of phosphate solubilization. Phosphorus is added in the form of phosphatidic fertilizers, part of which is utilized by plants and the remainder converted into soluble fixed forms. To circumvent phosphorus deficiency, phosphate-solubilizing microorganisms (PSM) could play an important role in supplying phosphate to plants in a more environmentally-friendly and sustainable manner [7].

This ability is generally associated to the release of organic acids, decreasing the pH. Moreover, these organic acids can increase considerably P in the soil solution through the quelation of Ca, Fe and Al, change reactions and solubilization of low soluble salts]. The inoculation of P-solubilizing microorganisms is a promising technique because it can increase P availability in soils fertilized with rock phosphates. Inoculation of phosphate solubilizing fungi and mycorrhizal fungi improves the physio-chemical, biochemical and biological properties of rock-P amended soil. Beyond the phosphate solubilization, many P-solubilizing microorganisms increase the mycorrhizal root colonization by production of specific metabolites as vitamins, amino acids and hormones [8]. It has also been reported that the available P and aggregate stability levels, higher soil C levels, enzyme activities and lower soil pH were also reported due to inoculation of these fungi .

The objective of this work was to evaluate solubilization ability of insoluble phosphates by several fungal isolates and their effect upon growth of groundnut plants grown in soil amended with tri-calciumphosphate.

MATERIALS AND METHODS

Fungal Strains, Isolation and Identification: Fungal strains were isolated from the soil and rhizosphere of roots of plants growing in fields, from various districts of Madhya Pradesh, after serial dilution of soil solution on potato dextrose agar (PDA) plates. Distinct colonies present on the plates were selected, purified by repeated culturing and maintained on PDA slants at 4ºC.

Screening for Phosphorus Solubilizing Activity

Screening for primary phosphate solublizing activity of the isolates was carried out by allowing the fungi to grow in selective media, i.e., Pikovskaya's agar for media for 7 to 10 days at 25°C. The appearance of a transparent halo zone around the fungal colony indicated the phosphate solublizing activity of the fungus [2].

$$\text{Solubilizing index} = \frac{\text{Colony diameter} + \text{clearing zone}}{\text{Colony diameter}}.$$

Soil Experiment: Seeds of ground nut were surface sterilized[33], rinsed 6 times with sterile water and dried. The surface disinfected seeds were coated by soaking seeds in liquid culture medium for 2 h using 10% gum Arabic as adhesive to deliver 108 cells seed-1 *Bradyrhizobium.* It was added as a biofertilizer in all treatments except the control. Spore suspensions (4 ml) of 2 × 106 ml-1 of *A. niger* and *P. notatum* was added to soils 48 h before sowing. The uninoculated seeds served as a control treatment for comparison. Tri-2 5 calcium phosphate (27.0%, P O), were obtained from MP Agro fertilizers Pvt. Ltd. It was added as phosphatidic P (20 mg kg-1) to the soil before seeding and was common in all treatments, except the control, which had 20 mg kg-1N (urea) and 40 mg kg-1 P (single super phosphate). The inoculated seeds were sown in earthen pots (10 seeds pot-1) having 10 kg of unsterilized sandy soil collected from different district of Madhya Pradesh. The soil contains 78% sand, 10% silt, and 12% clay, pH 7.6, EC mmohs/cm 0.12, organic matter % 0.60, calcium carbonate % 3.5, total N ppm 72.0, and available P ppm 4.0. Seedlings were thinned to 3 plants per pot after 5 days of emergence. The plants were irrigated with tap water as and when required. The pots with different treatments were arranged in a randomized complete block design with triplicates of each treatment. The treatments were as follows: control (uninoculated seeds); plant + *Aspergillus niger*; plant + *Penicillium notatum* and plant + *Aspergillus niger* + *Penicillium notatum* [9]. At 90 days plant age, a random sample was taken from each treatment to determine some growth parameters as follows: plant height (cm), dry weight/plant (g). At harvesting time the yield components were estimated as follows: number of pods/plant and 50-seed weight (g). Nitrogen (N) was extracted from plants with sulfuric acid using the semimicro Kjeldahl method. Phosphorus (P) was extracted by nitric-perchloric acid digestion and measured using the vanadono-molybdophosphoric colorimetric method.The protein content of plant tissues were estimated according to Bradford. Oil % in dry seeds was extracted with petroleum ether using Soxhlet apparatus according to A.O.A.C..

Statistical Analysis: The statistical analysis done by using SPSS program (Statistical Package for the Sciences System). The Variables were subjected to ANOVA (significance was set at $*P<0.05$ and $**P<0.01$).

RESULTS AND DISCUSSION

Fungal isolates showed phosphate Solubilizing activities as detected in Pikovskaya's agar medium by the appearance of halos around the inoculum on the medium. isolates of *Aspergillus* and *Penicillium* isolated from agricultural soil showed maximum level of phosphate solubilization activity *in vitro* when liquid medium was supplemented with both tricalcium phosphate and rock phosphate separately .

Pot Experiment: A pot experiment was undertaken to evaluate the effectiveness of *Aspergillus niger* and/or *Penicillium notatum* in TCP amended soils to enhance the growth of ground nut and improve the physicochemical characteristics of the soil. Table (1) showed that inoculation with the fungal strains, separately; improve 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. A [11]. 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 .

The increase in protein and oil percentage of ground nut plants was mainly attributed to the beneficial effect of inoculation with the two experimental fungal strains to the TCP amended soil, particularly in sand soil lacking enough nutrients (Table 1).

Table 1. Effect of Co- inoculation with phosphate Solubilizing fungi on some growth parameters ,yield ,p and N percentage in ground nut plants.

Treatments	Height (cm)	Dry weight of plant (g)	Number of pods/plant	Seeds weight (g)	N %	P %	Oil %	Protein %
Control	61.2	11.2	16.6	1.02	7.2	0.41	20.3	35.9
Soil +TCP	75.6	16.2	26.3	1.57	6.9	0.51	24.2	42.6
Soil +TCP +Aspergillus niger	98.4	19.0	32.5	1.79	7.6	0.55	26.1	43.9
Soil +TCP +Penicillium notatum	90.1	16.7	33.6	1.63	7.4	0.50	25.6	42.6
Soil +TCP +Aspergillus niger + Penicillium notatum	113.6	22.0	35.8	2.00	8.0	0.59	26.3	45.8
Mean	94.245	17.2	28.96	1.602	30.811	26.081	21.801	21.59

This significant increase reached 57.5% and 29.5% for protein and oil contents, respectively, comparing to the control. In this study, there was an increase in the percentages of N and P in plant. It was found that organic acids added to the soils increased the plant uptake of P from a water soluble P . Also, the release of organic acids that both sequester cations and acidify the microenvironment near the roots is thought to be a major mechanism of P-solubilization, as well as Mn, Fe and Zn by plants and non-vesicular mycorrhizal fungi [5]. An increase significantly resulted in N levels of ground nut plants but was non-significant with the percentage of total phosphorus, under the experimental conditions. Mechanisms such as production of phyto- hormones, vitamins or amino acid can be involved in the P-solubilizing micro-organisms effect [10]. The soil properties were also improved after inoculation of the tested fungi. The available P levels and organic carbon were significantly improved in all treatments compared to the initial values. Also this application reported a drop in pH values of the soil compared to the control. They reported higher available P, soil total carbohydrates, water soluble C and lower soil pH compared to control soils.

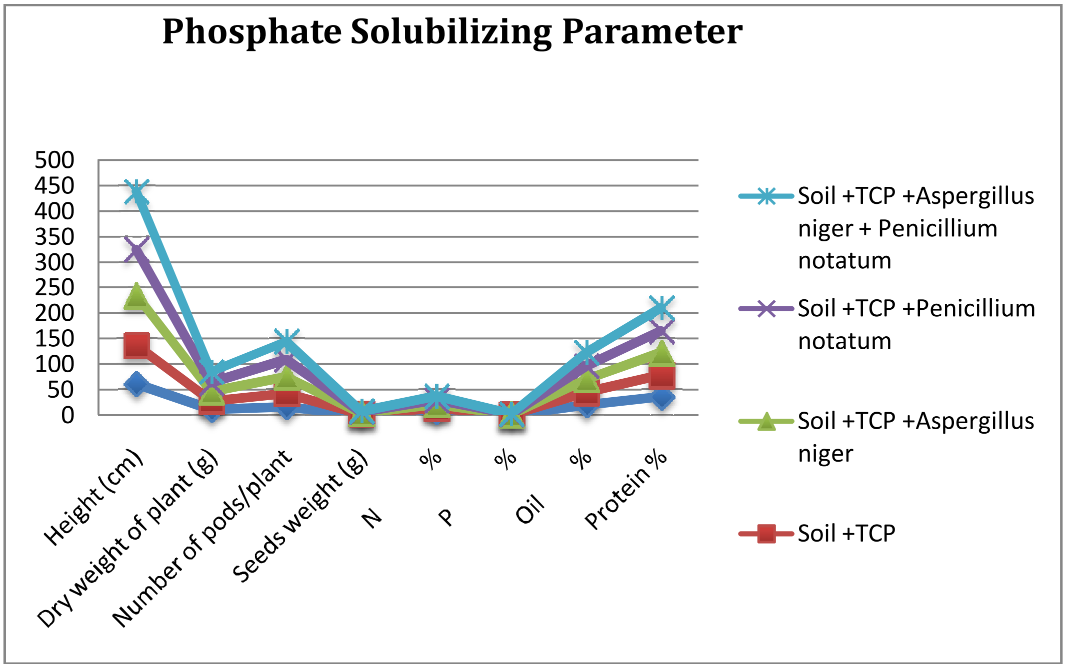

Fig 1 Growth Parameter phosphate Solubilizing fungi on some growth parameters ,yield ,p and N percentage in ground nut plants.

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