Biological Forum – An International Journal **15(5): 1466-1481(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Field Evaluation of Phosphatase and Phytase Producing Fungi under Clusterbean and Pearl Millet in arid Ecosystems

Amita Gharu Dhariwal[1], Indira Rathore[2] and J.C. Tarafdar[3*]
[1]Assistant Professor, Department of Chemistry,
Jai Narain Vyas University, Jodhpur (Rajasthan), India.
[2]Senior Scientist, Nano Research Lab,
IFFCO, Aonla unit, Bareilly (Uttar Pradesh), India.
[3]Former Principal Scientist and National Fellow,
ICAR-Central Arid Zone Research Institute, Jodhpur (Rajasthan), India.

(Corresponding author: J.C. Tarafdar)*
(Received: 08 March 2023; Revised: 12 April 2023; Accepted: 18 April 2023; Published: 20 May 2023)
(Published by Research Trend)

ABSTRACT: Four effective phosphatase and phytase-producing fungi, *Aspergillus candidus, Aspergillus ustus, Curvularia lunata,* and *Phoma species,* were selected for their native P mobilisation under field conditions, using clusterbean and pearl millet as test crops. The main aim was to increase agro ecosystem productivity and get rid of the negative effects of chemical fertilizers. The inoculation of selected fungi resulted in a significant enhancement in the grain yield (16–28%), plant biomass (10–57%), straw yield (19–38%), and plant P absorption (2–10%) in clusterbean. There was between 6 and 61 % more in acid phosphatase activity, between 9 and 66 % higher in alkaline phosphatase activity and between 13 and 50 % additional phytase activity under inoculated plots of clusterbean as compared to the uninoculated (control) plots. Due to increased phosphatase and phytase enzyme activity of inoculated fungi, pearl millet saw improvements in grain yield, plant biomass, straw yield, and plant P uptake of 12–24%, 2–54%, 18–40%, and 3-12%, respectively. In pearl millet, 10-48%, 10-49%, and 6-47% improvement in acid phosphatase, alkaline phosphatase and phytase were recorded, respectively, at different growth periods compared to control. Among the four fungus examined, *Phoma species* was found to be the best P mobilizers, both under pearl millet and clusterbean. Irrespective of fungi and crops, P mobilisation from mineral sources was often higher than that from organic and phytin sources. Overall 40 to 85% more microbial than plant involvement was seen in the mobilisation of various P components. In general, 10.6-21.3% more mineral P and 11.5-19.2% more phytin P was hydrolyzed in clusterbean compared to pearl millet. Selected fungi are especially important for crop sustainability because they are effective under low phosphorus availability and when nutrients are bonded to organic matter and soil particles.

Keywords: Field assessment, Clusterbean, Pearl millet, Arid ecosystems. P mobilising fungi

INTRODUCTION

For the productivity of agroecosystems, phosphorus is a limiting nutrient (Jarvie *et al.*, 2019; Withers *et al.*, 2015; Filippelli, 2008). This is because of its limited solubility and high affinity for mineral surfaces, which result in low availability in soil. Plant size and growth are significantly hampered by phosphorus (P) supply issues or deficiencies. P is typically added to soil as a chemical P fertilizer to meet crop nutritional needs and produce adequate crop yields (Sharma *et al.*, 2013). However, the synthesis of chemical P fertilizer is a highly energy-intensive process. Overuse of chemical fertilizers can cause soil acidification and soil crust, which lower the amount of organic matter, humus, and beneficial organisms in the soil, stunt plant growth, alter the pH of the soil, increase pests, and may even contribute to the release of greenhouse gases. Crop growth is inhibited by soil acidity, which decreases the amount of phosphate that crops absorb and raises the concentration of harmful ions in the soil (Cooke, 1982), and it has long-term effects on the environment in the form of eutrophication of surface water (Turan *et al.*, 2006). The negative impacts of chemical fertilizers will start during the production of these chemicals, whose by-products include some dangerous chemicals or gases such as NH_4, CO_2, CH_4, etc. that will contribute to air pollution (Chandini *et al.*, 2019).

According to Schoumans *et al.* (2015), the majority of the P now utilised in chemical fertilizers comes from phosphate rocks, a limited resource that is unevenly distributed across the Earth's surface. It is anticipated that the price of P fertilizer will rise significantly on both an economic and environmental level because its extraction is becoming more difficult due to geopolitical instability (Liu *et al.*, 2022). Organic phosphorus (Po), which is derived from organic inputs and soil, is increasingly used as a supplement to mineral

P fertilizer in order to approach more sustainable sources for controlling phosphorus (P) nutrition in agro ecosystems (Amadou *et al.,* 2021). In order to increase plant photosynthetic efficiency, nutrient uptake, and biomass output, plant growth-promoting microorganisms have become an important component of sustainable agriculture practises (Mishra and Tailor 2023). Tian *et al.* (2021) reported that phosphate solubilizing microorganisms (PSMs) play a crucial role in the soil P cycle by mineralizing organic P, solubilizing inorganic P minerals, and storing significant amounts of P in biomass, which may in effect limit P bioavailability or cycling in soil-plant systems. PSMs are typically regarded as eco-friendly P fertilizers for raising agricultural output since they may mediate the basic soil P forms and orthophosphate levels. Soil microorganisms in particular have the capacity to liberate plant-accessible P from scarcely present forms of soil P through the solubilization and mineralization of inorganic and organic P, respectively (Barea and Richardson 2015; Whitelaw *et al.,* 1999). Microorganisms significantly increase nutrient uptake and crop sustainability (Hassan and Nawchoo 2022). Researchers (Alaylar *et al*., 2020; Wang *et al.,* 2020) have demonstrated that using phosphate solubilizing microorganisms (PSMs) is a proven method for managing phosphate rock mining and agricultural sustainability. It is also widely recognised as an eco-friendly P fertilizer for boosting agricultural productivity.

Biophos is a biofertilizer prepared by the fungus *Chaetomium globosum* to mobilize native phosphorus (Tarafdar and Gharu 2006). Five strains of filamentous fungi reported by Brazhnikova *et al.* (2022) had the highest level of phosphate-mobilizing ability and produced phytohormones to promote plant growth. Tarafdar (2019) reported a 16–25% increment in yield by inoculation of fungi such as *Aspergillus, Emericella, Gliocladium, Penicillium, Trichoderma,* and *Chaetomium*, which can mobilize phosphorus from agricultural soils by secreting phosphatases, phytases, and organic acids. A new white-rot fungus, *Ceriporia lacerata* HG2011, can mobilize P in soils for plant use, leading to improved crop agronomic performance (Sui *et al.,* 2022). It is well known that soil microorganisms may dissolve different types of insoluble P fractions thus; they qualify as bio inoculants and support soil sustainability (Rizwanuddin *et al.,* 2023). Less is known, however, about the capability of soil fungi to mediate P availability to plants from sources that would otherwise be scarce in field conditions (Richardson, 1994; Whitelaw *et al.,* 1999). The aim of this study was to assess the impact of phosphate solubilizing/mobilizing fungi on the clusterbean and pearl millet in natural environments and to compare the effectiveness of their releasing enzymes to those of the plant in mobilising/solubilising native phosphorus from phosphorus sources that are not easily accessible.

MATERIALS AND METHODS

Fungal isolation and identification. 32 fungi were isolated from 30 different Indian soils by utilising the dilution plate method of Tarafdar and Chhonkar (1979) and streptomycin sulphate (Allen, 1959) using Martin's Rose Bengal agar. On potato dextrose agar (PDA) medium, the pure cultures were maintained. The Agharkar Research Institute, Pune, India, identified the fungus. Based on their intra- and extracellular acid phosphatase, alkaline phosphatase, and phytase activities, four of the best fungi were selected for field trials.

Fungal enzyme activity and efficiency measurement. In 250-mL Erlenmeyer flasks of 100-mL Czapek-Dox broth, fungi were grown in order to measure the enzyme activity. The flasks were maintained at 30 °C for 21 days while 8-mm discs of fungal growth that were 4 days old (on PDA media) were placed in the medium. The contents of three flasks were filtered through Whatman No. 1 filter paper into another flask that had been chilled on ice. 100 mL of sterile, ice-cold, distilled water made up the entire final volume of each filtrate. The filtrate was used to evaluate the extracellular activity of phytase, alkaline phosphatase, and acid phosphatase. To measure intracellular activity, fungal mats from each replicate were pulverised in a mortar with quartz sand that had been acid-washed. By adding ice-cold, sterile, distilled water and centrifuging the mixture for 20 minutes at 12,000 rpm, a fine suspension was created. The supernatant, which was made to a specific volume, was carried out for the intracellular enzyme activity measurement.

The efficiency of the fungus was evaluated through the hydrolysis of phytin and Na-glycerophosphate. In a mortar, 1 g of fungal mat (in triplicate) was ground with acid-washed quartz sand and 30 mL of ice-cold, sterile, distilled water. The clear extract obtained after centrifugation was incubated for 24 h with 500 mg/L of phytin or Na-glycerophosphate at 30°C. The release of mineral P per minute per gm of fungal mat was calculated by the calorimetric method as given by Jackson (1967).

Seed inoculation. Clusterbean and pearl millet seeds were surface sterilised for two minutes with acidified 0.05% $HgCl_2$, then rinsed five to six times in sterilised deionized water. The seeds were treated with different fungi (100g kg^{-1} seed), air dried under a shed (avoid direct sun rays), and showed right away. The inoculation with different fungi was carried out in the slurry of carrier-based culture in sterilised (20%) jaggery (guar) solution. For the uninoculated treatment, the same quantity of sterilised culture (100g kg^{-1} seed) was used to inoculate the seed.

Field experiment- To examine the efficiency of four selected fungi, *Aspergillus candidus, Aspergillus ustus, Curvularia lunata* and *Phoma species*, a crop of pearl millet (*Pennisetum americanum* L.; cv.HHB67) and clusterbean (*Cyamopsis tetragonoloba* cv. RGC-936) was grown under *kharif* season at the Central Research Farm, ICAR-CAZRI, Jodhpur, during (July–September). The area is located at latitude of 26°18′ N and longitude of 73°01′E. During the cultivation period, 327 millimetres of rain and an average of 34.4 °C (maximum) to 25.5 °C (minimum) temperatures were recorded. The study included two treatments: with and

without inoculation, with a random block design using four replicates each. In the inoculated treatment, the seed was inoculated with selected fungi prior to sowing, while the seeds without fungal inoculation were employed in the control treatment. The test soil was a typical Camborthid (Table 1), and an 8 m x 8 m plot was used to sow each replicate. At 28, 35, 42, 49, and 56 days following planting, four plants from each replicate were carefully dug up from the ground with their roots. The crops were finally harvested after reaching maturity. During the growing phase, irrigation and fertilizer were not used.

Assessment and processing after sampling. After each sampling, the rhizosphere soil was tested for microbial population and enzyme activity. Martin's Rose Bengal agar medium was used to count the population of fungi (Allen, 1959). By using the usual approach of Tabatabai and Bremner (1969), acid and alkaline phosphatases were determined using acetate buffer (pH 5.4) and sodium tetraborate-NaOH buffer (pH 9.4), respectively. The enzyme substrate (4-nitrophenyl phosphate) mixture was incubated at 35 °C for 1 h, and the enzyme assay was expressed as enzyme units (EU). One unit is the amount of enzyme that hydrolyzes 1.0 µmol of p-nitrophenyl phosphate per minute at pH 5.4 (acid phosphatase) or 9.4 (alkaline phosphatase) at 35 °C. The amount of inorganic phosphate (Pi) released during the hydrolysis of sodium phytate (1 mM) in 100 M sodium acetate buffer (pH 4.5) was used to measure the activity of the phytase for 1 hour at 37 °C. By adding 0.5 mL of 10% trichloro-acetic acid (CCl_3COOH), the process was stopped. Centrifugation at 10,000 rpm for 10 min was used to remove proteins that had been precipitated by TCA, and the supernatant was then used to test for inorganic P that had been released (Ames, 1966). The quantity of the enzyme that released 1.0 mol inorganic P min^{-1} was considered to be one unit of phytase activity. The Tabatabai (1982) approach was used to quantify the dehydrogenase activity. 2,3,5-Triphenyl Tetrazolium Chloride was used to treat soil samples, and the triphenyl formazon it produced was detected at 485 nm. After removing the soil from the roots, root lengths were measured using Tennant's (1975) modified line-intersect method. At 60 °C, the plants were dried in oven, and the dry matter weight was recorded. Shoots and roots were ground to a fine powder. Using Kitson and Mellon's (1944) Vanadomolybadate method, plant P was determined after acid digestion of plant powder with 20 mL of concentrated HNO_3, $HClO_4$ and H_2SO_4 (ratio of 7:2:1). The wet digestion method of Walkley and Black, as explained by Jackson (1967), was used to determine the organic carbon in the soil. The standard methods were used to estimate the pH (1:2), EC (1:2), particle size distribution, and readily available P (Olsen's P) (Jackson, 1967). Mineral, organic and total P were estimated by Seeling and Jungk's (1996) method, while Mega's (1982) method was employed for estimating the phytin P in soil samples. The mineral, organic, or phytin P depleted at different stages of growth as a result of the inoculation of different fungi has been defined as the microbial contribution to P hydrolysis.

The relative growth rate for root was estimated after adopting the following equation:

$$\text{Relative growth rate for root (Kr)} = \frac{l_n\left(L_2 / L_1\right)}{t_2 - t_1}$$

The P-influx, was calculated using the formula after Römer and Schenk (1998) as follows:

$$\text{P- influx} = \frac{u_2 - u_1}{L_2 - L_1} \times \frac{l_n\left(\frac{L_2}{L_1}\right)}{t_2 - t_1}$$

Where u_2-u_1 (ΔU) is the change in P-uptake (mol $plant^{-1}$), L_2-L_1 (ΔL) is the change in total root length (cm $plant^{-1}$) at t_2 and t_1 respectively and t_2 –t_1 (Δt) is the change in time (second). The P-influx was expressed as (mol cm^{-1} root s^{-1}).

An analysis of variance was performed on the data, and vertical bars represent the standard errors of the differences between means (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

Four fungi, *Aspergillus candidus*, *Aspergillus ustus*, *Curvularia lunata*, *and Phoma species*, shown in Fig. 1, were chosen for an in-depth study in order to examine the native P mobilisation and P inflow to the clusterbean and pearl millet under field conditions. This was proven by its higher phytase and phosphatase activities compared to all other isolated species, as well as its ability to hydrolyze inaccessible P (results not shown). Table 1 shows the initial enzyme activity, microbial population, and phosphorus status that were observed in the experimental soil. A total of 1% of the total P was available, 29% was found in unavailable inorganic form, and 70% was present in organic form in which phytin P constitutes a large fraction (68%) of the organic P in soils. According to research, the majority of the phosphorus in the soil exists in the form of phytin and its derivatives (Mengel *et al*., 2001; Liu *et al*., 2022), and the activity of rhizosphere microorganisms (Saeed *et al.,* 2021) and plant P uptake mechanisms (Shen *et al*., 2011) regulates the amount of phosphorus that is available to plants.

Acid phosphatase activity: A significantly higher acid phosphatase activity throughout the crop growth period (4 to 8 weeks) was observed in the rhizosphere of clusterbean (Fig. 2) due to the inoculation of selected fungi. The maximum inoculation effect was observed in *Phoma species*. More activity was noticed after 7 weeks of plant growth; between 8 and 58% improvement in acid phosphatase secretion due to the inoculation of selected fungi was observed. In pearl millet, the highest acid phosphatase activity was noticed after 7 weeks of growth (Fig. 3). Inoculation with fungi significantly enhanced the acid phosphatase secretion from 4 to 8 weeks; the effect was greater between 4 and 6 weeks in all the fungi tested. In general, between 10 and 48% improvement in acid phosphatase secretion due to the

inoculation of different fungi was observed in the pearl millet crop throughout the growth period.

Fungi are particularly effective producers of both acid and alkaline phosphatases (Tarafdar, 1989). It has been hypothesised that higher fungal accumulation and more root exudation in the rhizosphere are caused by increased phosphatase activity (Fig. 2 and 3) and increased P mobilisation. Fungal activity may potentially have a qualitative and quantitative impact on the composition of root exudates due to the breakdown of exudates molecules and the release of microbial metabolites (Neumann and Roemheld 2000). Due to plant root exudates, the total microbial population may be higher, which influences more acid phosphatase activity as root exudates influence the growth and development of other organisms (Dhungana *et al*., 2022). Depending on the fungus, the P chemical nature changed the phosphatases release pattern (Della Mónica *et al*., 2018). Fungi can break the C-O-P ester link of organic P with the help of phosphatases and phytase which they release into the system. According to Kucey et al. (1989), microbial activity is a crucial component of the soil organic P cycle and affects the transformation. Phosphatase activity enables the mineralization of organic P to enhance P availability in plants and soil organisms as a mechanistic response to P deprivation (Janes-Bassett *et al.,* 2022). The phosphate group is liberated from its substrates by phosphatase enzymes, which are produced by bacteria, fungi, and plant roots. This process converts complicated and occasionally inaccessible forms of organic P into phosphate, which may be assimilated. Therefore, the availability of organic P substrate, P limitation in the soil, and P demand from plants and microorganisms are all factors that affect phosphatase production (Margalef *et al.,* 2017).

Alkaline phosphatase activity. Alkaline phosphatase activity between 4 and 8 weeks in the rhizosphere of clusterbean (Fig. 4) resulted in significantly higher activity throughout the period, which reached its maximum after 7 weeks. The increase in activity varies from 59 to 64% after fungal inoculation with clusterbean. All the organisms tested were found to have efficient development of alkaline phosphatase under the clusterbean rhizosphere. A similar trend in improvement of alkaline phosphatase activity was observed with pearl millet (Fig. 5), and the improvement varied from 43 to 48%. The highest activity in the pearl millet rhizosphere was observed after 7 weeks. *Phomaspecies*, among the four fungal species tested, was found to be more efficient to build up alkaline phosphatase activities in the soil. *Aspergillus ustus* was the second-most efficient fungus to build up alkaline phosphatase activity in the rhizosphere.

According to Spohn and Kuzyakov (2013), alkaline phosphatase is largely produced by microbes, whereas acid phosphatase originates primarily from plant roots and microorganisms in terrestrial ecosystems. According to Dhungana *et al.* (2022) plant root exudates influences the growth and development of microorganisms which increases the alkaline phosphatases in rhizosphere soil which is a subclass of phosphomonoesterase enzymes with a characteristic alkaline pH optimum that catalyses the breaking of ester (C-O-P) bonds. It has been used to examine the bioavailability of phosphate compounds in surface water (Suzumura *et al.,* 2012).

Phytase activity. Fig. 6 shows the increase in phytase activity with crop age in clusterbean. A significantly higher phytase activity was observed after 4 weeks due to the inoculation of efficient phosphatase and phytase-producing fungi. The inoculation effect was almost similar for all the fungi tested. A similar trend was observed when efficient fungi were inoculated in pearl millet (Fig. 7). However, the increase in phytase activity was higher under clusterbean than pearl millet, which was estimated to be 1 to 3% more than pearl millet at 8 weeks after inoculation.

However, both plants and microorganisms have an impact on the phytase activity in clusterbean and pearl millet (Fig. 4 and 5). The hydrolysis of organic P esters in the rhizosphere by rhizosphere microorganisms and enhanced phytase production by plant roots could help in the uptake of inorganic P. Fungi secrete phosphatases such as phytases and acidic/alkaline phosphatases to hydrolyze the organic form of phosphorus, or phytate, in order to increase the utilisation of their phytate-phosphorus (Singh & Satyanarayana, 2011; Liu *et al.,* 2022). According to Elhaissoufi *et al*. (2022), small amounts of inorganic phosphorus given to the rhizosphere may work as a stimulant for phytic acid mineralization, boosting plant phosphorus feeding. As competent, stable, and promising bio-inoculants (Rizwanuddin *et al.,* 2023), phytase from fungal sources has been found to be more effective than phytase from plant sources in similar amounts (Dhariwal and Tarafdar 2023).

Dehydrogenase activity: A significant increase in dehydrogenase activity in the clusterbean rhizosphere was observed, which was progressively expand with crop age up to 8 weeks after inoculation of efficient phosphatase and phytase-releasing strains of different fungi (Fig. 8). The more increase in activity was observed due to *Phomaspecies* (111%) closely followed by *Aspergillus candidus* (108%). The least increase in activity (103%) was observed with the inoculation of *Curvularia lunata* in clusterbean. *Phomaspecies* and *Aspergillus candidus* were found to be most effective for improving dehydrogenase activity in both clusterbean and pearl millet. The effect of inoculation on pearl millet (Fig. 9) showed significant improvement in dehydrogenase activity for 8 weeks under all the efficient fungi tested. In general, fungi were more effective with time and an increase in crop age. The increase in dehydrogenase activity under pearl millet was 106% with both *Aspergillus candidus* and *Curvularia lunata,* whereas an increase of 109% and 103%, respectively, was observed after inoculation of *Phomaspecies* and *Aspergillus ustus*.

The biological oxidation of soil organic material is significantly facilitated by dehydrogenase, which do not accumulate extracellularly in the soil (Zhang *et al*., 2010). It, therefore, acts as an indicator of the microbial

activity in soil (Wolinska & Stepniewsk 2012; Kaczyska *et al.,* 2015; Kaur and Kaur 2021). Other organisms in the rhizosphere are affected by the root exudates in terms of their growth and development (Upadhyay *et al.,* 2022; Dhungana *et al.,* 2022). One of the most effective, significant, and responsive indicators of soil fertility is the enzyme activity of dehydrogenase.

Exploitation of unavailable P compounds: The effect of seed inoculation with different fungi during the cultivation of clusterbean and pearl millet (Table 2) showed gradual depletion of different unavailable P fractions during the growth period. The maximum depletion of different forms of unavailable P (mineral, organic and phytin) was observed with the inoculation of *Phomaspecies*in both crops, while the minimum depletion was observed with *Aspergillus candidus* among the four different fungi tested. In clusterbean, the contribution by *Phomaspecies* was higher by 26% to exploit mineral-P, 55% more to exploit organic-P and 46% larger to exploit phytin-P as compared to *Aspergillus candidus*. Organic P hydrolyzed by *Aspergillus ustus* was 11.2% higher as compared to *Curvularia lunata*. However, *Curvularia lunata* hydrolyzed 14.7% more mineral P compared to *Aspergillus ustus*. The mobilization of P from mineral sources follows the order *Phomaspecies>Curvularia lunata>Aspergillus ustus>Aspergillus candidus*. But phytin P and organic P depletion in different fungi follow the order of *Phomaspecies>Aspergillus ustus>Curvularia lunata>Aspergillus candidus*. The hydrolysis of mineral P was higher than organic P and phytin P by all the fungi.

Total depletion of unavailable P fractions during the growth of pearl millet (Table 2) showed maximum P depletion by *Phomaspecies*. It hydrolyses 4.3% more mineral P, 3.4% more organic P, and 42.9% more phytin P as compared to *Curvularia lunata, which has* the next higher P mobilization. *Aspergillus ustus* hydrolyzed 3.6% more mineral P, 4.1% higher organic P, and 36.4% more phytin P as compared to *Aspergillus candidus*, which mobilizes the least unavailable phosphorus from the native soil sources. In general, the mineralization of unavailable P under clusterbean was higher than that under pearl millet, and 10.6-21.3% more mineral P and 11.5-19.2% more phytin P mobilized in clusterbean. *Phomaspecies* performed best to mobilize P among the four fungi tested, both under pearl millet and clusterbean. In general, P mobilization from mineral sources was more as compared to mobilization from organic and phytin P sources, irrespective of the crops.

Both crops benefited and improved from seed inoculation with various fungus species and results clearly depicted that *Phoma species* was most effective. According to Fiorentino *et al.* (2018), there is a species-dependent relationship between biostimulants' beneficial effects on nutrient uptake and crop development. In order to solubilise and mineralize complex organic phosphorous into a number of small molecules that are available to plants, they stimulate enzymes such as inorganic acids, organic acids, phosphatases, and phytases (Malgioglio *et al.,* 2022). Phosphatases, including acidic, alkaline, and phytase, hydrolyze the organic form of phosphorus (Tarafdar and Gharu 2006; Singh and Satayanarayan 2011; Tazisong *et al.,* 2015). According to Sui *et al*. (2022), the organic acid and phytase activities of the fungus (*Ceriporia lacerata*) solubilise the phosphates. It was demonstrated (Fatima et al. 2021) that phosphate can also be solubilized by *Aspergillus awamori* and *Penicillium digitatum*. Additionally, *Trichoderma sp*. and *T. viride* were found to help in the absorption of minerals and nutrients, particularly Fe, K, N, and P (Lin *et al*., 2021; Paul & Rakshit 2022).

Partition of plant and microbial contributions: Table 3 shows the partition of depletion of unavailable P for plants and *Aspergillus candidus*. A gradual increase in depletion of different forms of unavailable P with crop age after inoculation of *Aspergillus candidus* was observed (Table 3). With an increase in crop age, the plant contribution increases, whereas the microbial contribution decreases in the hydrolysis of all three unavailable P fractions (mineral P, organic P, and phytin P). In general, 40 to 79% of unavailable P hydrolysis in the rhizosphere of clusterbean was observed due to the inoculation of *Aspergillus candidus*. The plant contribution to mobilising unavailable P compounds varies between 21 and 60% of the total P hydrolyzed from particular unavailable P fractions. Phytin P was found to be the least hydrolysable organic P compound. The contribution of *Aspergillus candidus* to hydrolyze different unavailable P fractions under pearl millet showed (Table 3) a similar trend to that of clusterbean. The percent of total unavailable P hydrolysis was 2% higher in clusterbean than pearl millet, irrespective of different unavailable P fractions. The pearl millet plant seems to be more effective in the hydrolysis of phytin P as compared to the hydrolysis of the other two unavailable P fractions. In general, *Aspergillus candidus* contribution was higher (39 to 77%) than plant contribution (23 to 61%) in the hydrolysis of different unavailable P compounds.

The inoculation of *Aspergillus ustus* in clusterbean showed a similar trend as that of *Aspergillus candidus* (Table 4) to exploit different unavailable P fractions. In general, plant contributions varied between 18 to 55%, while microbial contributions ranged from 45 to 82%. The depletion of unavailable P increased with the increase in plant age of clusterbean and the rate of increase was higher up to a 6-week period. The percent plant contribution increased with crop age, while the percent microbial contribution showed a reverse trend. A similar trend was observed in pearl millet (Table 4). After 56 days of plant growth, among the total hydrolysis of phytin P, 42% of phytin-P was hydrolyzed by *Aspergillus ustus* and 58% by plants.

The partition of plant and microbial contributions to the hydrolysis of different unavailable P compounds with the inoculation of *Curvularia lunata* (Table 5) under clusterbean resulted in a higher depletion of unavailable P compounds with the increase in age of the plant. In general, plant contribution varies between 20 to 59%, while microbial contribution ranges from 41 to 80%.

The plant seems to be more efficient in the hydrolysis of mineral and organic P than phytin P. A similar trend was observed when pearl millet was inoculated with *Curvularia lunata* (Table 5), where plant contribution varies between 22 to 52% for hydrolysis of mineral P, 40 to 61% for hydrolysis of organic P, and 21 to 58% for hydrolysis of phytin P. The microbial contribution varies between 48 to 78% for the breakdown of mineral P, 39 to 59% for organic P breakdown, and 42 to 78% for phytin P hydrolysis. In general, in the early stage of plant growth the microbial contribution was more but in the later stages of growth the plant contribution to mobilize P was higher than microbial contribution.

A gradual increase in depletion of different forms of unavailable P with the inoculation of *Phomaspecies* under clusterbean (Table 6) with plant age was observed. With increase in crop age plant contribution was increases while the percent of microbial contribution was reduced drastically. Although the higher plant contribution to hydrolyze phytin P was observed only 56 days after cropping, plant contribution was a change between 15 to 52%, while microbial contribution ranges from 48 to 85%. *Phomaspecies*was found to be the most efficient fungus, especially in mobilising P from organic sources. The contribution of *Phomaspecies* to hydrolyze different unavailable P fractions under pearl millet (Table 6) showed a similar trend as in clusterbean. At initial stages, plant contribution was less compared to microbial contribution in all unavailable P fractions. But with increases in plant age, the contribution of plants increased to P hydrolysis and ranged from 16 to 64%. The microbial contribution ranges between 36 to 84% during the same growth period.

In the hydrolysis of different unavailable P fractions, the microbial contribution overall was significantly greater (40–85%) than the plant contribution (Tarafdar & Yadav 2011; Liu *et al.,* 2022). The transport of P between various soil P pools depends on microorganisms, which are crucial for the soil P cycle. The enzymes produced by PSM, phosphatases and phytase, aid in the breakage of the C-O-P link and the release of phosphorus from phytate-stored organic molecules in soil (Nannipieri *et al*., 2011; 2011; Liu *et al.,* 2022). Microorganisms may also produce organic acids such as malate, citrate, and oxalate, which may also help in the release of inorganic P (Jones, 1998; Zhu *et al.,* 2022). According to Richardson & Simpson (2011), organic acids are the primary solubilizers of the scarcely accessible phosphorus. By replacing P with a carboxylate anion, organic acids can mobilise phytate. They can also use ligand exchange to desorb phosphate anions from the soil. Tribasic citrate releases more P because it has more carboxyl groups and a pK_2 value that is closer to the soil pH range (Menezes-Blackburn *et al*., 2016). Organic matter that is bound to P by Fe/Al bridges is dissolved by organic acids, releasing phosphate (Gerke, 2010). The plant contribution increases with the growth period. In a lack of P, the root's secreted extracellular phytase is crucial for soil phytate hydrolysis. To catalyse the phytate hydrolysis, they either remain affixed to the root's cell walls or are released directly into the rhizosphere (Sun *et al*., 2022). Increased secretion of phosphatases and phytase by plant roots and rhizosphere microorganisms may help plants acquire inorganic P through the hydrolysis of organic P esters in the rhizosphere (Li *et al.,* 1997; Yadav and Tarafdar, 2007; Liu *et al.,* 2022; Rizwanuddin *et al.,* 2023). The study also revealed the potential for soil microorganisms to increase the availability of P from phytate both through phytase activity and perhaps by affecting the availability of phytate itself.

Plant biomass: A significant increase in dry matter was observed in clusterbean and pearl millet (Table 7) due to the inoculation of different fungi as compared to the control (without inoculation). The increase in dry matter of clusterbean varies between 10 and 51% after inoculation of *Aspergillus candidus*, 11 and 54% after inoculation of *Aspergillus ustus*, 10 and 52% after inoculation of *Curvularia lunata,* and 11 to 57% after inoculation of *Phomaspecies* compared to control soil. The increase in dry matter due to inoculation of different fungal species in pearl millet varies between 24-54% as compared to control soil (without inoculation), which was higher with *Phomaspecies.* In general, biomass production was 2.2 to 2.4 fold higher in pearl millet than clusterbean after 56 days of crop growth. The inoculation effect was greater with *Phoma species* than with the other fungi tested. The rate of increase in plant biomass in pearl millet crops was more up to 42 days, while in clusterbean it was higher up to 35 days. Thereafter, a gradual decline in the rate of biomass accumulation with plant age was observed under both clusterbean and pearl millet, although the total biomass increased until 56 days of crop age.

By enhancing nutrient availability, regulating phytohormones, and increasing systemic resistance, plant growth promoting microorganism directly boosts plant growth (Abhilash *et al*., 2016; Bhat *et al.,* 2019; Khan *et al*., 2020; Khoshru *et al.,* 2020). According to Adedayo and Babalola (2023), the development of roots, the solubilization of minerals from fossils, and the production of secondary metabolites all contribute to improving plant growth promotion under abiotic stress conditions. They can strengthen plant's defences and promote plant growth even more in stressful situations.

Root length. The root length of clusterbean and pearl millet at different crop growth periods after inoculation with tested fungi is presented in Table 8. In general, root length increased after inoculation. The increase in root length due to inoculation varies between 19 to 29%, 22 to 31%, 21 to 30%, and 21 to 32% in clusterbean due to the inoculation of *Aspergillus candidus*, *Aspergillus ustus*, *Curvularia lunata,* and *Phomaspecies*, respectively. The increase in root length between 4 to 8 weeks of crop age of pearl millet after inoculation with different fungi ranges from 18 to 24% for *Aspergillus candidus*, 19 to 26% for *Aspergillus ustus*, 19 to 25% for *Curvularia lunata* and 20 to 25% for *Phomaspecies*. The root length of clusterbean was 4 to 8 times less than pearl millet under different growth stages of crops. In general, inoculation with four

different fungi helped in additional root build-up, which was more due to *Phomaspecies* inoculation in clusterbean and pearl millet, followed by *Aspergillus ustus*. *Aspergillus candidus* seems to be least effective to improving root length after inoculation in both the crops.

According to López-Bucio *et al.* (2015), multilayer root-shoot communication is a key component of *Trichoderma's* stimulation process. *Trichoderma* applications have been shown to have a variety of direct and indirect effects on plants, including the release of substances with auxin activity, small peptides, and volatile organic compounds, which enhance root system architecture (total root length, density, and branching) and assimilate or mobilise macronutrients (P) and micronutrients (Fe, Mn, and Zn), thereby promoting plant growth and crop productivity (Contreras-Cornejo *et al*., 2009).

Effect on phosphorus uptake. The result presented (Table 9) clearly demonstrated that with the inoculation of efficient phosphatase and phytase-producing fungi, the total P content of the plant improved significantly after four weeks onward in both the crops studied. The increase in P uptake due to inoculation of different fungi in clusterbean varies between 2 to 9% for *Aspergillus candidus*, 5 to 10% for *Aspergillus ustus*, 4 to 9% for *Curvularia lunata*, and 3 to 10% for *Phomaspecies* as compared to un-inoculated control. The improvement of P uptake in pearl millet due to inoculation varies between 4 and 11% due to both *Curvularia lunata* and *Phomaspecies*, 3 to 10 due to *Aspergillus candidus*, and 4 to 12% due to *Aspergillus ustus*. In general, the P uptake by clusterbean was 49 to 52% higher than pearl millet after 56 days of crop growth. Through various solubilization and mineralization mechanisms, phosphate solubilizing microorganisms (PSM) are able to convert inorganic and organic soil P into a bio available form that facilitates uptake by plant roots (Khan *et al.*, 2009).

Relative root growth (Kr). The relative root growth (Kr) of clusterbean between 28 to 56 days was studied (Table 10) under field conditions. The growth rate was highest between 28 to 35 days, which is considered a critical growth period. Thereafter, the growth rate was reduced considerably, to 49 days. However, an increase in growth rate was observed further between 49 to 56 days. A considerable increase in Kr value was observed after inoculation with different phosphatases and phytase-producing fungi at any growth stage of clusterbean except between 42 to 49 days of crop growth while inoculated with *Aspergillus candidus* and *Aspergillus ustus*. In general, the increase in root growth over control due to inoculation, irrespective of the crop age, varied between 11 to 39 percent and was found as follows: 11.2% higher for *Aspergillus candidus*, 34.8% higher due to *Aspergillus ustus*, 34.3% more due to *Curvularia lunata*, and 39.1% higher due to inoculation with *Phomaspecies* A considerable improvement in Kr value was observed in the case of pearl millet (Table 10). The maximum growth rate of the root was observed between 28 to 35 days, which was three times higher than the growth rate between 35 and 42 days. The root growth rate of pearl millet, in general, reduced considerably after 42 days of plant growth and almost remained constant until 56 days. The Kr value of pearl millet was increased with the inoculation of efficient fungi (Table 10). With the inoculation, the Kr value increased from 4.5 to 10.6%, which was more with *Aspergillus ustus* during plant growth. As roots dig the soil, the release of organic molecules, especially organic acids, by the roots may change (Goss *et al.*, 1993). Hormones control the root architecture, which allows plant roots to respond to nutrition (Dong *et al*., 2018). During root development, hormones act as internal mediators between soil conditions and the development of the root architecture (López-Bucio *et al.*, 2003).

P-influx: Table 11 shows the P-influx of clusterbean varies between 4.91 and 16.98 (mol cm^{-1} s^{-1} × 10^{-14}) under different treatments. In general, P-inflow was higher at 28 to 35 days of the crop growth period and declined thereafter with crop age. The highest P-influx was observed between 6 and 7 weeks after seed inoculation with *Aspergillus candidus.* Although fungus inoculation does not always improve the P inflow to the plant per cm of root, due to the higher root length in inoculated treatments, the total P inflow to the plant was much higher than in uninoculated controls. The P-influx in pearl millet was highest after 5 to 6 weeks of crop growth, irrespective of the treatment (Table 11). P-influx in pearl millet varies between 0.29 and 1.16 (mol cm^{-1} s^{-1} × 10^{-14}) under different treatments. The higher P-inflow under inoculated treatment seems to be due to higher root length after inoculation.

In sustainable agriculture, Etesani *et al.* (2021) demonstrated that phosphate-solubilizing microorganisms improved phosphate availability and plant uptake. P efficiency was linked to plant uptake efficiency, which is calculated by the absorption rate per unit of root (influx) and the relative root growth rate (Kr) (Föchse *et al*., 1988). The current findings demonstrate that P-influx varied significantly during different stages of the growth of clusterbean and pearl millet (Table 11). During different stages of growth, efficient phosphatase and phytase producing organisms showed higher P-influx in inoculated than control. P concentration increased the response time of plant growth, and increasing P concentration increased the growth of root and shoot (Poudyal *et al.*, 2021). The morphology and development of the roots were associated with access to P. Their different uptake kinetics can be used to explain the variations in P-influx. Changes in the plant's root characteristics would be responsible for the effect of P-status on absorption efficiency. By mineralizing organic phosphorus, resolving inorganic phosphorus minerals, and storing significant amounts of phosphorus in biomass, phosphorus solubilizing microorganisms (PSMs), an important microflora that mediates accessible soil phosphorus, perform a critical function in soil (Tian *et al.*, 2021).

Grain and straw yield: The grain and straw yields of clusterbean and pearl millet (Table 12) showed consistently higher yields (both grain and straw) due to

the inoculation of efficient fungi. The grain yield of clusterbean was increased by 16 to 28% due to the inoculation of different fungi. The increase in grain yield of clusterbean was highest with *Phomaspecies* (28%), followed by *Aspergillus ustus* (22%), *Curvularia lunata* (20%), and *Aspergillus candidus* (16%) as compared to control (without inoculation). The grain yield of pearl millet was increased by 24% after inoculation with *Phomaspecies*, 19% by *Aspergillus ustus*, 17% by *Curvularia lunata,* and 12% by *Aspergillus candidus* as compared to control. A simultaneous improvement in straw yield was observed with the inoculation of different fungi. The increase in straw yield of clusterbean was found to be 38% with *Phomaspecies*, 26% with *Aspergillus ustus*, 20% with *Aspergillus candidus*, and 19% with *Curvularialunata*, respectively, as compared to control (uninoculated plants). The improvement in straw yield of pearl millet was 40% with the inoculation of *Phomaspecies*, 31% with the inoculation of *Aspergillus ustus*, 23% with the inoculation of *Curvularia lunata,* and 18% with the inoculation of *Aspergillus candidus*. In general, phosphatase and phytase-producing fungi were found to be most effective for higher crop production. *Phomaspecies* was found to be most efficient in enhancing the yield of pearl millet and clusterbean among the four selected fungi tested.

Inoculation with *Rhizobium species* and phosphate solubilizing microorganisms (PSM), *Pseudomonas striata*, or *Penicillium variabilis* greatly increased plant production and nutrient uptake (Zaidi *et al*., 2003). The rhizosphere flora of higher plants, in particular, has a substantial impact on the bioavailability of phosphorus in soils. Microbial generated carboxylic acids cause the release of phosphorus that is bonded to iron, aluminium, and calcium phosphate. Phosphatases enhances higher plant's utilisation of organic P compounds; thus, microbial mineralization of organic matter is essential to the cycling of nutrients in soils. Non-pathogenic fungi called plant growth promoting fungi (PGPF) can boost agricultural yields, reduce the use of agrochemicals, and support the production of crops (Motaher Hossain & Sultana, 2020). PGPF are biotic inducers that help agriculture and perform crucial tasks for its sustainability. By enhancing growth, germination, chlorophyll production, and abundance, they raise agricultural productivity. To help plants cope with stress, they produce phytohormones, induced resistance, and defence-related enzymes (Adedayo and Babalola 2023). By lowering the requirement for chemical inputs, the use of PGPF inoculants is a possible strategy to boost plant development and crop production in a more environmentally sustainable manner (Lopes *et al.,* 2021).

Table 1: Initial soil characteristics under field condition.

Parameter	Characteristics*
pH (soil:water)	7.8±0.05
EC (dSm^{-1})	0.23±0.01
Organic matter (%)	0.22±0.02
Sand (%)	84.1±0.1
Silt (%)	5.8±0.1
Clay (%)	8.0±0.05
Total P ($mg\ kg^{-1}$)	1262.8±13.4
Mineral P ($mg\ kg^{-1}$)	887.9.3±8.5
Organic P ($mg\ kg^{-1}$)	365.2±5.4
Olsen's P ($mg\ kg^{-1}$)	10.7±1.1
Water soluble Pi ($mg\ kg^{-1}$)	1.8±0.01
Phytin P ($mg\ kg^{-1}$)	254.3±7.6
Acid phosphatase activity ($EU \times 10^{-4}$)	0.06±0.01
Alkaline phosphatase activity ($EU \times 10^{-4}$)	0.09±0.01
Phytase activity ($EU \times 10^{-4}$)	1.58±0.18
Dehydrogenase activity ($p\ kat\ g^{-1}$)	1.09±0.20
Fungi ($\times 10^{-4}$)	15±1.2
Bacteria ($\times 10^{-4}$)	156±9.1
Actinomycetes ($\times 10^{-4}$)	110±9.7

Table 2: Depletion of different unavailable P fractions by clusterbean and pearl millet grown under field condition.

Fungi	Clusterbean			Pearl millet		
	Mineral P	Organic P	Phytin P	Mineral P	Organic P	Phytin P
Aspergillus candidus	275.5	230.4	151.5	249.1	239.4	128.1
Aspergillus ustus	289.8	269.5	208.5	258.2	249.2	174.8
Curvularia lunata	332.4	242.3	157.8	274.0	258.5	138.9
Phoma sp.	346.6	358.7	221.4	285.7	267.3	198.5
LSD (p = 0.05)	15.7	12.6	11.4	14.8	13.2	12.6

Table 3: Microbial and plantcontribution to hydrolyze different unavailable P fractions during the growth of seed inoculated by *Aspergillus candidus* under field condition.

Plat age (day)	Clusterbean						Pearl millet					
	Depletion of total unavailable P (mg kg^{-1})						Depletion of total unavailable P (mg kg^{-1})					
	Mineral P		Organic P		Phytin P		Mineral P		Organic P		Phytin P	
	PC*	MC**	PC	MC	PC	MC	PC*	MC**	PC	MC	PC	MC
28	3.18±0.05 (21.1)	11.9±1.51 (78.9)	11.76±0.09 (39.49)	18.01±1.01 (60.51)	3.58±0.08 (22.5)	12.32±1.04 (77.5)	2.96±0.21 (22.8)	10.03±0.52 (77.2)	12.26±0.88 (41.8)	17.07±1.40 (58.2)	3.37±0.61 (24.4)	10.43±1.09 (75.6)
35	10.54±0.09 (25.8)	30.33±3.10 (74.2)	16.86±1.04 (45.3)	20.35±1.31 (54.7)	8.99±1.30 (41.9)	12.47±1.06 (58.1)	11.61±0.74 (27.5)	30.60±2.01 (72.5)	18.58±1.15 (46.1)	21.72±1.81 (53.9)	8.42±0.92 (43.3)	10.48±1.05 (56.7)
42	20.70±1.21 (41.6)	29.07±3.22 (58.4)	24.27±1.09 (51.6)	22.77±1.21 (48.4)	14.68±1.36 (48.6)	15.53±1.21 (51.4)	25.04±1.20 (43.8)	32.12±2.12 (56.2)	25.90±1.22 (53.5)	22.51±1.92 (46.5)	13.21±0.01 (49.6)	13.42±1.21 (50.4)
49	28.87±2.12 (49.2)	29.80±4.11 (50.8)	29.10±1.51 (54.6)	24.19±2.41 (45.4)	19.78±1.21 (51.4)	18.70±1.15 (48.6)	31.30±1.40 (47.9)	34.04±2.41 (52.1)	31.96±1.40 (59.2)	22.02±1.43 (40.8)	17.05±1.08 (53.5)	14.82±1.10 (46.5)
56	58.23±4.31 (52.4)	52.89±3.87 (47.6)	36.73±2.71 (58.2)	26.38±1.21 (41.8)	27.42±1.22 (60.3)	18.05±1.26 (39.7)	38.29±2.09 (53.7)	33.02±2.30 (46.3)	43.52±2.09 (64.6)	23.85±1.80 (35.4)	22.84±1.59 (61.3)	14.42±1.20 (38.7)

* Plant contribution, ** Microbial contribution; Figure in parenthesis denotes the percent of total mineral/organic/phytin-P depleted

Table 4: Microbial and plantcontribution to hydrolyze different unavailable P fractions during the growth of seed inoculated by *Aspergillus ustus* under field condition.

Plat age (day)	Clusterbean						Pearl millet					
	Depletion of total unavailable P (mg kg^{-1})						Depletion of total unavailable P (mg kg^{-1})					
	Mineral P		Organic P		Phytin P		Mineral P		Organic P		Phytin P	
	PC*	MC**	PC	MC	PC	MC	PC*	MC**	PC	MC	PC	MC
28	3.61±0.72 (18.4)	15.98±1.92 (81.6)	12.52±1.01 (36.5)	21.77±1.78 (63.5)	8.98±1.31 (17.8)	18.38±1.81 (82.2)	2.83±0.56 (19.6)	11.62±0.99 (80.4)	4.63±0.31 (15.3)	25.61±1.71 (84.7)	3.92±0.52 (21.2)	14.58±1.05 (78.8)
35	11.51±0.95 (23.5)	37.46±2.31 (76.5)	17.03±1.52 (38.2)	27.55±1.92 (61.8)	10.61±0.98 (36.5)	18.46±1.31 (63.5)	10.35±0.84 (22.8)	32.05±1.30 (77.2)	18.06±1.92 (37.8)	29.72±1.31 (62.2)	9.59±0.80 (37.3)	16.12±1.04 (62.7)
42	21.68±1.05 (35.7)	39.05±3.11 (64.3)	25.24±1.83 (44.6)	31.34±2.52 (55.4))	17.04±1.11 (41.2)	24.32±2.60 (58.8)	19.78±1.04 (34.2)	38.06±1.49 (65.8)	21.33±1.31 (44.1)	27.04±1.51 (55.9)	15.79±0.95 (43.1)	20.84±1.07 (56.9)
49	29.64±1.51 (38.9)	46.56±3.12 (61.1)	30.02±1.51 (48.1)	32.39±3.11 (51.9)	24.0±2.06 (47.7)	26.31±1.80 (52.3)	26.64±1.92 (39.5)	40.80±1.82 (60.5)	26.97±1.42 (47.2)	30.17±1.40 (52.8)	20.21±1.03 (46.5)	23.26±1.12 (53.5)
56	37.49±1.80 (44.5)	46.75±3.40 (55.5)	35.26±2.70 (49.2)	36.41±3.11 (50.8)	33.26±4.11 (55.1)	27.11±1.99 (44.9)	32.49±2.14 (42.8)	43.42±2.72 (57.2)	32.02±1.39 (48.4)	33.60±1.31 (51.6)	29.09±1.31 (57.6)	21.41±1.11 (42.4)

* Plant contribution, ** Microbial contribution; Figure in parenthesis denotes the percent of total mineral/organic/phytin-P depleted

Table 5: Microbial and plant contribution to hydrolyze different unavailable P fractions during the growth of seed inoculated by *Curvularia lunata* under field condition.

Plant Age (days)	Clusterbean						Pearl millet					
	Depletion of total unavailable P (mg kg^{-1})						Depletion of total unavailable P (mg kg^{-1})					
	Mineral P		Organic P		Phytin P		Mineral P		Organic P		Phytin P	
	PC*	MC**	PC	MC	PC	MC	PC*	MC**	PC	MC	PC	MC
28	6.52±0.80 (20.5)	15.52±1.82 (79.5)	11.91±1.06 (40.0)	17.86±1.26 (60.0)	3.53±0.81 (20.7)	13.54±3.77 (79.3)	8.38±0.17 (21.8)	12.12±0.83 (78.2)	12.02±0.99 (40.5)	17.66±1.02 (59.5)	3.26±0.29 (21.5)	11.90±1.50 (78.5)
35	16.05±1.31 (25.2)	47.64±1.89 (74.8)	17.96±1.86 (44.7)	22.22±1.86 (55.3)	8.76±2.11 (40.1)	13.09±1.89 (59.9)	13.74±0.75 (26.9)	37.35±2.10 (73.1)	21.07±1.21 (45.8)	24.93±1.90 (54.2)	6.56±0.69 (32.8)	13.45±1.11 (67.2)
42	29.44±1.84 (40.6)	43.07±3.72 (59.4)	26.83±1.76 (52.7)	24.08±2.00 (47.3)	14.0±1.33 (46.3)	16.23±1.59 (53.7)	24.18±1.26 (39.8)	36.58±2.22 (60.2)	26.38±1.53 (50.8)	25.55±2.10 (49.2)	11.56±1.02 (40.1)	17.25±1.62 (59.9)
49	37.33±1.25 (46.4)	43.12±3.99 (53.6)	31.56±3.23 (55.8)	25.0±2.13 (44.2)	22.19±1.19 (56.5)	17.09±1.63 (43.5)	30.43±1.55 (47.7)	33.37±2.51 (52.3)	33.54±1.57 (56.5)	25.82±1.80 (43.5)	18.52±1.21 (54.3)	15.59±1.70 (45.7)
56	48.33±3.92 (51.6)	45.34±4.11 (48.4)	38.48±3.11 (59.3)	26.41±2.51 (40.7)	28.33±1.86 (57.4)	21.03±2.81 (42.6)	40.77±2.31 (52.4)	37.05±2.73 (47.6)	43.56±2.45 (60.9)	27.97±2.15 (39.1)	23.73±1.28 (58.2)	17.05±1.73 (41.8)

* Plant contribution, ** Microbial contribution; Figure in parenthesis denotes the percent of total mineral/organic/phytin-P depleted

Table 6: Microbial and plantcontribution to hydrolyze different unavailable P fractions during the growth of seed inoculated by *Phoma species* under field condition.

Plant Age (days)	Clusterbean						Pearl millet					
	Depletion of total unavailable P (mg kg^{-1})						Depletion of total unavailable P (mg kg^{-1})					
	Mineral P		Organic P		Phytin P		Mineral P		Organic P		Phytin P	
	PC*	MC**	PC	MC	PC	MC	PC*	MC**	PC	MC	PC	MC
28	3.62±0.52 (14.9)	20.65±1.81 (85.1)	12.08±1.06 (26.9)	32.81±1.82 (73.1)	4.30±4.16 (16.6)	21.59±1.09 (83.4)	2.54±0.17 (16.3)	13.06±1.20 (83.7)	8.66±0.96 (27.5)	22.82±1.52 (72.5)	4.12±0.29 (19.5)	16.99±0.09 (80.5)
35	14.24±1.40 (22.4)	49.34±3.82 (77.6)	16.98±1.31 (30.5)	38.69±1.83 (69.5)	10.61±0.81 (31.3)	23.30±1.81 (68.7)	11.59±0.75 (23.2)	38.36±2.51 (76.8)	15.81±1.11 (31.6)	24.23±1.30 (68.4)	7.38±0.78 (24.8)	22.22±1.00 (75.2)
42	26.23±1.82 (35.3)	40.05±3.66 (64.7)	29.11±1.50 (38.6)	46.31±3.93 (61.4)	16.71±1.31 (40.6)	24.45±2.52 (59.4)	22.74±1.26 (36.6)	39.39±2.70 (63.4)	20.98±1.30 (39.2)	32.54±1.71 (60.8)	15.52±1.19 (37.7)	25.64±1.12 (62.3)
49	33.77±1.96 (37.2)	57.00±4.76 (62.8)	33.39±1.61 (40.2)	49.66±4.11 (59.8)	26.37±1.52 (46.8)	29.97±1.96 (53.2)	26.81±1.58 (38.0)	43.75±7.01 (62.0)	32.99±1.52 (51.7)	30.82±1.82 (48.3)	20.20±1.81 (41.6)	28.35±1.80 (58.4)
56	41.79±2.89 (41.1)	59.89±3.99 (58.9)	44.45±2.76 (44.6)	55.21±4.63 (55.4)	33.14±1.08 (51.7)	30.96±1.82 (48.3)	37.76±2.11 (43.2)	49.65±3.50 (56.8)	50.41±2.15 (64.3)	27.99±2.10 (35.7)	30.48±2.54 (52.5)	27.57±1.91 (47.5)

* Plant contribution, ** Microbial contribution; Figure in parenthesis denotes the percent of total mineral/organic/phytin-P depleted

Table 7: Gradual increase in Plant biomass under field condition due to inoculation of different fungi (± indicate the standard errors of mean).

Plant Age (days)	Clusterbean Plant biomass (g plant^{-1})					Pearl millet Plant biomass (g plant^{-1})				
	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* species	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.
28	1.81±0.16	2.73±0.25	2.80±0.21	2.76±0.26	2.84±0.28	5.35±0.58	8.11±0.78	8.22±0.74	8.18±0.79	8.25±0.62
35	2.52±0.24	3.35±0.30	3.43±0.35	3.37±0.41	3.48±0.31	7.51±0.69	10.58±0.97	10.69±0.98	10.60±0.99	10.56±0.98
42	3.14±0.26	3.65±0.35	3.70±0.36	3.68±0.38	3.72±0.34	10.16±0.99	13.82±1.30	14.11±1.19	14.0±1.35	14.20±1.01
49	3.36±0.31	3.86±0.39	3.87±0.39	3.87±0.41	3.90±0.39	13.18±1.06	16.55±1.64	16.85±1.76	16.69±1.25	16.72±1.43
56	3.67±0.32	4.04±0.39	4.10±0.38	4.06±0.39	4.10±0.42	15.45±1.45	19.25±1.72	19.38±1.84	19.29±1.37	19.41±1.69

Table 8: Gradual increase in root length of clusterbean under field condition due to inoculation of different fungi (± indicate the standard errors of mean).

Plant Age (days)	Clusterbean Root length(cm)					Pearl millet Root length(cm)				
	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.
28	446±30	530±40	544±47	540±48	542±42	2011±135	2380±180	2417±190	2419±196	2434±198
35	482±35	595±44	608±52	598±48	604±52	3241±147	3831±202	3868±212	3863±347	3879±214
42	505±42	638±49	660±58	640±57	648±50	3718±274	4441±307	4489±305	4446±387	4571±330
49	525±47	656±62	676±61	670±58	682±60	4065±318	4970±355	5035±308	4961±414	5098±352
56	552±48	713±68	725±67	719±64	729±62	4460±327	5530±450	5631±420	5564±451	5597±405

Table 9: Gradual increase in P-uptake of clusterbean under field condition due to inoculation of different fungi (± indicate the standard errors of mean.

Plant Age (days)	Clusterbean Total P- uptake (µ mol plant^{-1})					Pearl millet Total P- uptake (µ mol plant^{-1})				
	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.
28	141.35±7.76	154.53±8.67	155.35±9.30	154.65±10.10	155.49±11.52	118.27±5.89	130.16±6.78	132.19±7.49	130.92±8.14	131.85±7.12
35	182.79±9.15	195.85±10.14	196.93±12.14	196.43±12.13	197.95±13.40	131.37±6.22	139.43±7.65	140.76±8.56	139.80±9.25	141.37±8.77
42	212.76±11.31	226.94±12.14	226.16±13.19	226.80±13.16	226.53±15.10	154.30±6.64	164.87±8.03	166.80±9.30	165.16±10.37	167.15±9.14
49	247.52±14.33	262.28±15.20	262.85±14.06	262.80±16.52	262.95±15.73	162.47±7.95	173.88±10.04	175.92±11.41	174.32±10.11	175.68±11.21
56	276.31±15.20	282.61±16.81	290.41±15.25	287.58±17.70	284.89±16.39	182.74±8.95	188.31±10.24	190.60±11.42	189.77±11.31	190.81±12.11

Table 10: Relative growth rate for root (Kr) of plant under field condition due to inoculation of different fungi (± indicate the standard errors of mean).

Crop age (days)	Clusterbean Relative root growth (cm $s^{-1} \times 10^{-8}$)					Pearl millet Relative root growth (cm $s^{-1} \times 10^{-8}$)				
	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.
28-35	12.83±0.69	19.13±0.82	18.39±0.79	16.87±0.55	17.91±0.62	12.83±0.69	19.13±0.82	18.39±0.79	16.87±0.55	17.91±0.62
35-42	7.71±0.54	11.54±0.59	13.57±0.63	11.22±0.49	11.63±0.48	7.71±0.54	11.54±0.59	13.57±0.63	11.22±0.49	11.63±0.48
42-49	6.42±0.48	4.60±0.74	3.96±0.37	7.57±0.39	8.45±0.27	6.42±0.48	4.60±0.74	3.96±0.37	7.57±0.39	8.45±0.27
49-56	8.29±0.50	13.78±0.94	11.57±0.73	11.67±0.59	11.02±0.38	8.29±0.50	13.78±0.94	11.57±0.73	11.67±0.59	11.02±0.38

Table 11: P-influx of plants under field condition due to inoculation of different fungi (± indicate the standard errors of mean).

Crop age (days	Clusterbean P - influx (mol cm^{-1} $s^{-1} \times 10^{-14}$)					Pearl millet P - influx (mol cm^{-1} $s^{-1} \times 10^{-14}$)				
	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.	Control	*Aspergillus candidus*	*Aspergillus ustus*	*Curvularia lunata*	*Phoma* sp.
28-35	14.77±0.77	12.16±0.63	11.95±0.18	12.15±0.31	12.26±0.43	0.84±0.09	0.50±0.03	0.46±0.03	0.47±0.03	0.51±0.04
35-42	10.05±0.75	8.34±0.52	7.63±0.31	7.98±0.45	7.55±0.61	1.16±0.14	1.02±0.10	1.03±0.10	1.01±0.09	1.01±0.10
42-49	11.16±0.88	16.98±0.94	9.08±0.84	9.21±0.89	8.64±0.79	0.35±0.02	0.32±0.02	0.32±0.03	0.32±0.02	0.29±0.01
49-56	8.84±0.79	4.91±0.11	6.51±0.54	5.90±0.43	6.75±0.53	0.79±0.06	0.45±0.03	0.60±0.04	0.49±0.01	0.47±0.03

Table 12: Straw and grain yield in clusterbean and pearl millet (kg ha^{-1}) after harvesting the crop.

Fungi	Clusterbean		Pearl millet	
	Dry matter	Grain yield	Dry matter	Grain yield
Control	22.0	5.0	19.4	8.8
Aspergillus candidus	26.5	5.8	22.9	9.9
Aspergillus ustus	27.8	6.1	25.5	10.5
Curvularia lunata	25.7	6.0	23.8	10.3
Phoma sp.	30.3	6.4	27.1	10.9
LSD (p = 0.05)	1.7	0.6	1.0	0.8

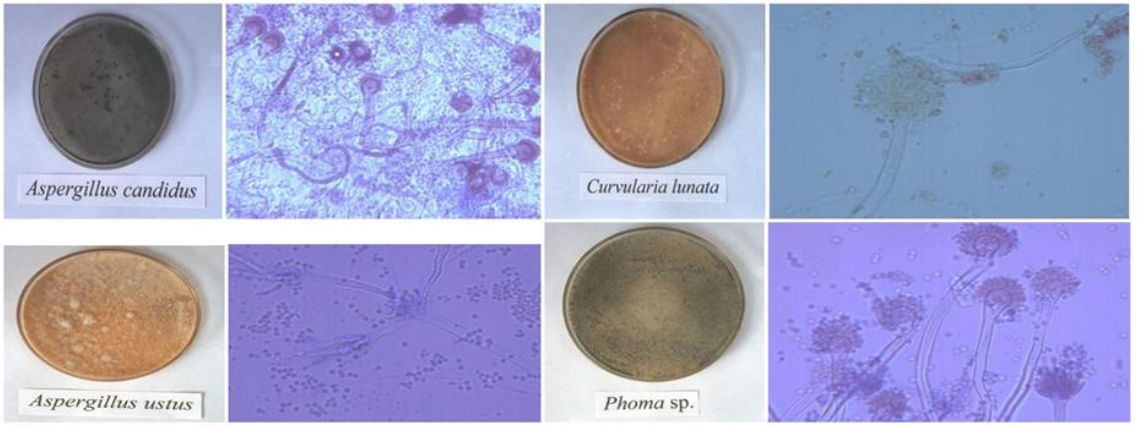

Fig. 1. Identified efficient phosphatase and phytase producing fungi and their spore (×312.5)

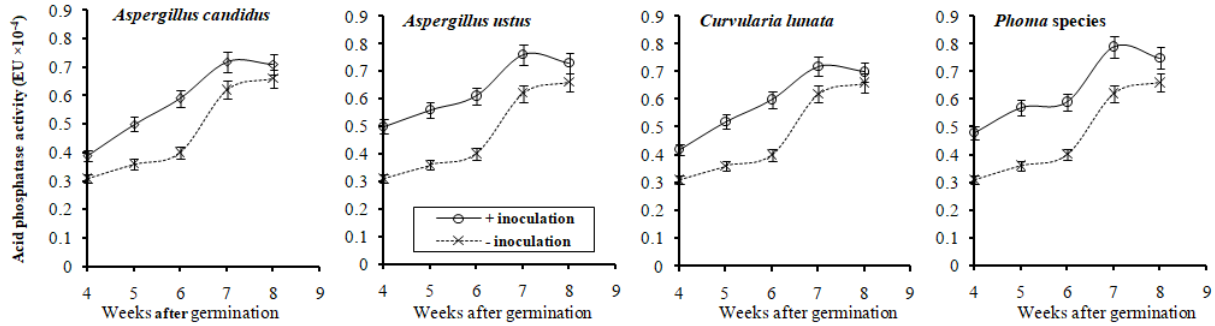

Fig. 2. Acid phosphatase activity of clusterbean after inoculation of fungi under field condition. I represent standard error of mean.

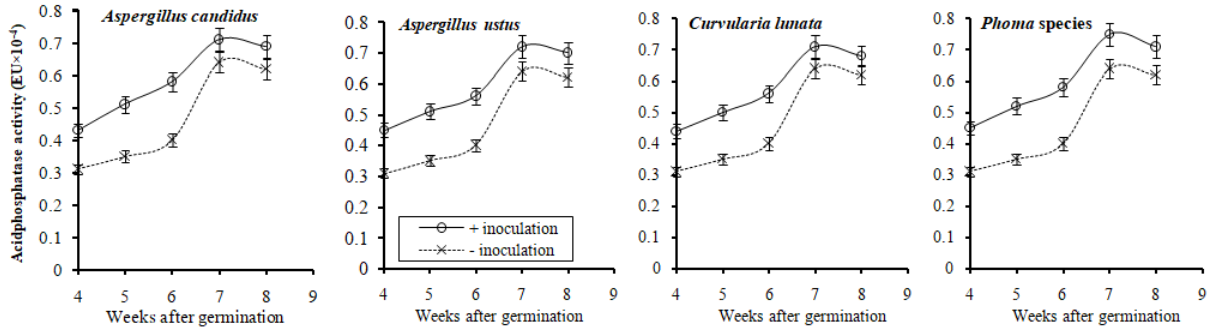

Fig. 3. Acid phosphatase activity of pearl millet after inoculation of fungi under field condition. I represent standard error of mean.

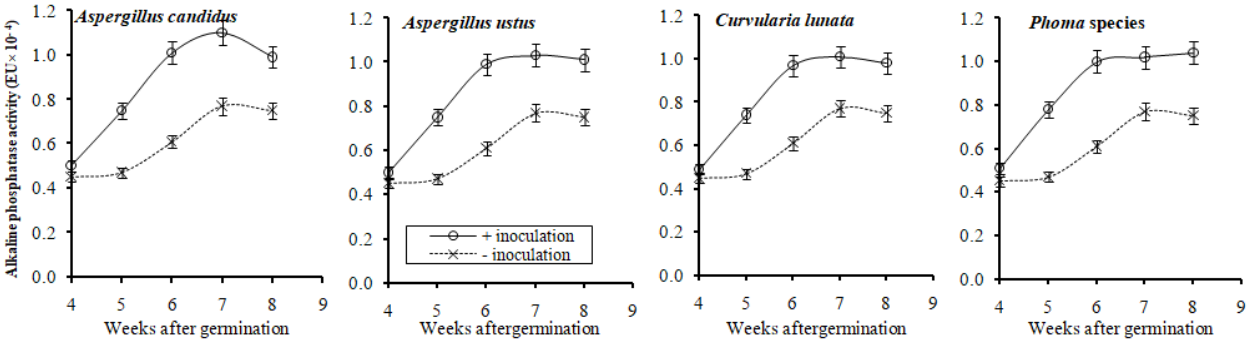

Fig. 4. Alkaline phosphatase activity of clusterbean after inoculation of fungi under field condition. I represent standard error of mean.

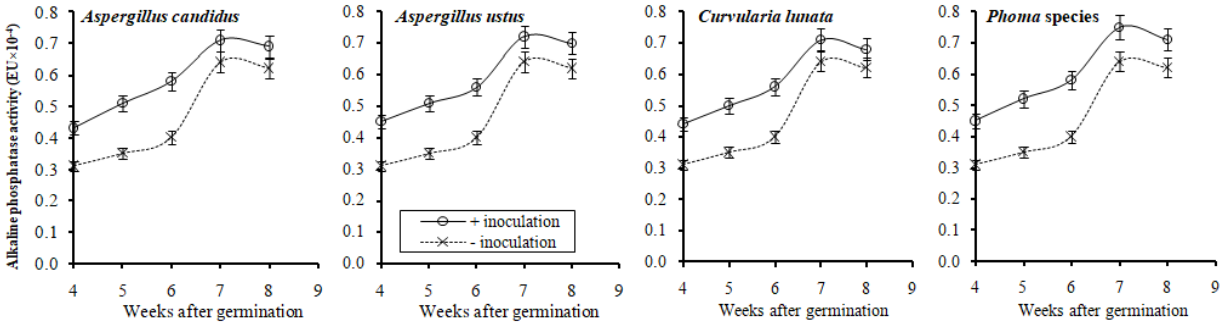

Fig. 5. Alkaline phosphatase activity of pearl millet after inoculation of fungi under field condition. I represent standard error of mean.

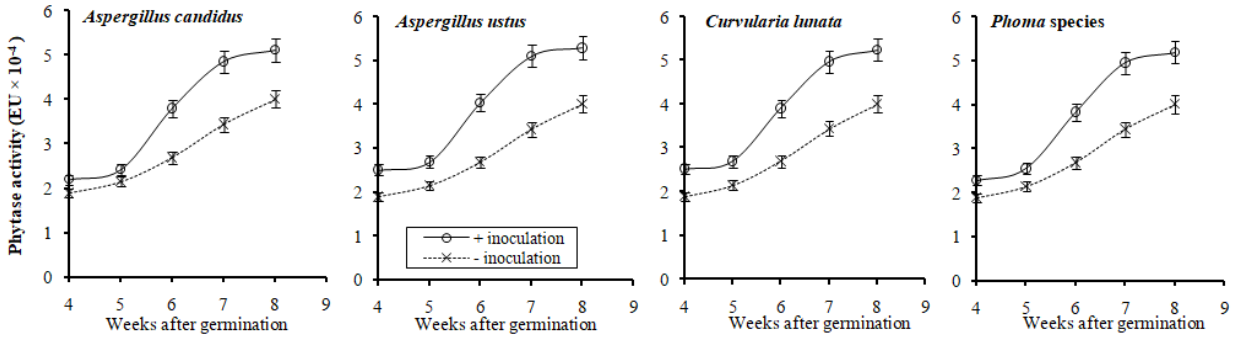

Fig. 6. Phytase activity of clusterbean after inoculation of fungi under field condition. I represent standard errors of mean.

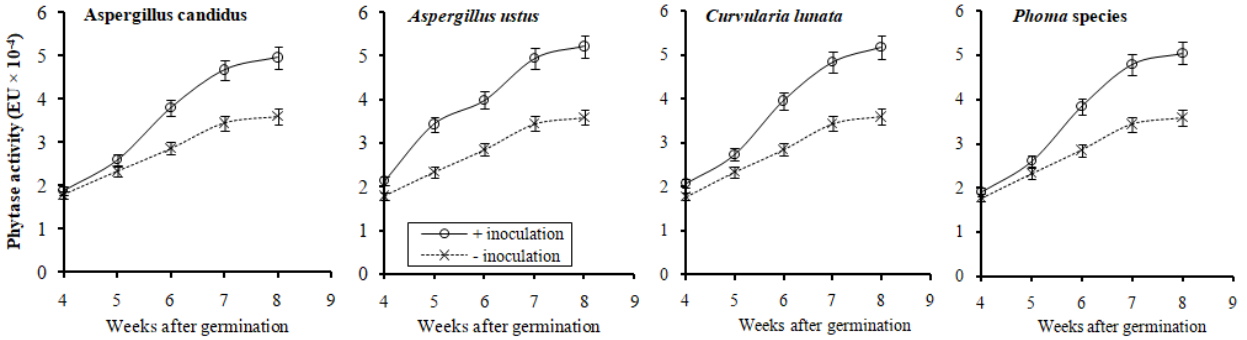

Fig. 7. Phytase activity of pearl millet after inoculation of fungi under field condition. I represent standard errors of mean.

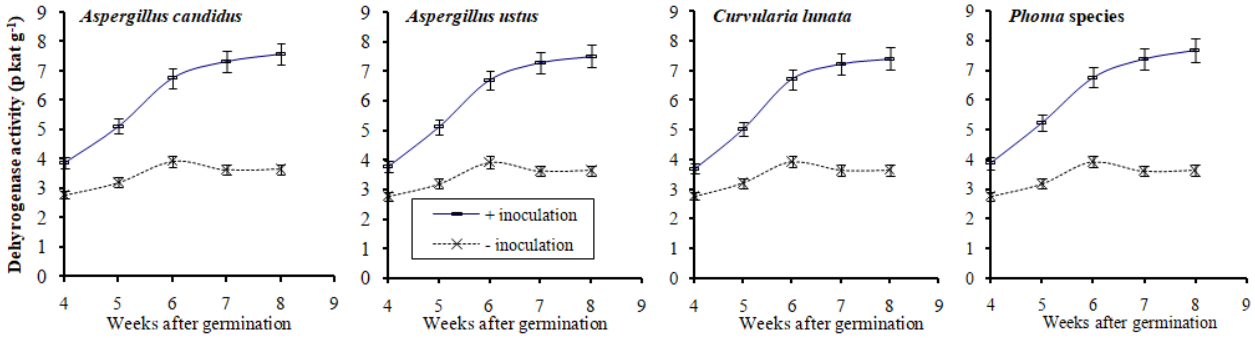

Fig. 8. Dehydrogenase activity of clusterbean after inoculation of fungi under field condition. I represent standard errors of mean.

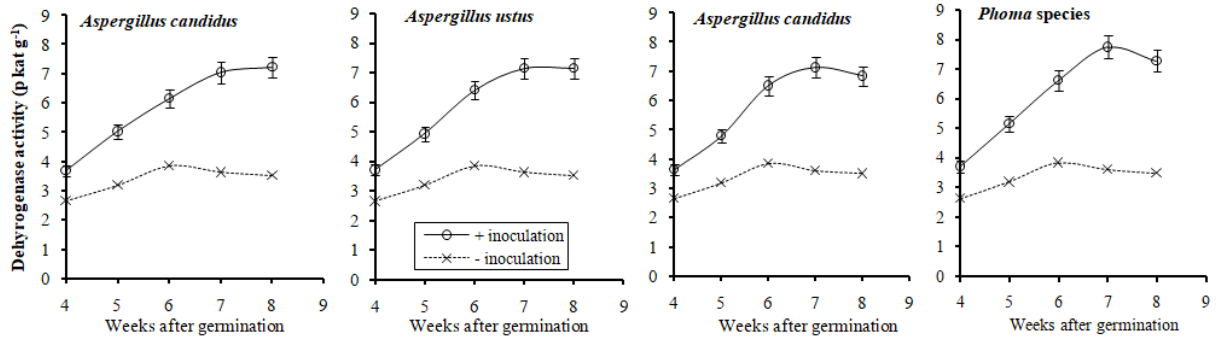

Fig. 9. Dehydrogenase activity of pearl millet after inoculation of fungi under field condition. I represent standard errors of mean.

CONCLUSIONS

The present result clearly demonstrated the importance of efficient phosphatases and phytase producing fungi for growth, biomass, yield and P nutrition of two most important arid crops namely pearl millet (cereal) and clusterbean (legume) under harsh arid environment. The results also pointed out how the P inflow as well as plant and microbial contribution to acquire P changes in different growth stages under field condition. The contribution from different P fractions for plant nourishment was also distinctly demonstrated. The results also emphasises the importance of plant growth promoting fungi for global food production.

FUTURE SCOPE

The phosphatase and phytase producing fungi can be use as seed inoculants to exploit P from the native soil. It can also try to apply by fertigation or in hydroponic operations.

Acknowledgment. The financial support received from ICAR and UGC during the course of investigation are acknowledged by the authors.
Conflict of Interest. None.

REFERENCES

Abhilash, P. C., Dubey, R. K., Tripathi, V., Gupta, V. K. and Singh, H. B. (2016). Plant growth-promoting microorganisms for environmental sustainability. *Trends Biotechn., 34*, 847–850.

Adedayo, A. A. and Babalola, O. O. (2023). Fungi That Promote Plant Growth in the Rhizosphere Boost Crop Growth. *J. Fungi, 9* (2), 239.

Alaylar, B., Egamberdieva, D., Gulluce, M., Karadayi, M. and Arora, N. K. (2020). Integration of molecular tools in microbial phosphate solubilization research in agriculture perspective. *World J. Microbiol. Biotechnol., 36*, 93.

Allen, O. N. (1959). Experiments in Soil Bacteriology, third ed. Burgess Publishing Co., p. 117.

Amadou, I., Houben, D. and Faucon, M. P. (2021) Unravelling the Role of Rhizosphere Microbiome and RootTraits in Organic Phosphorus Mobilization for Sustainable Phosphorus Fertilization. A Review. *Agronomy* 2021, *11*, 2267.

Ames, B. N. (1966). Assay of inorganic phosphate, total phosphate and phosphatases. *Method Enzymol., 8*, 115–118.

Barea, J. M. and Richardson, A. (2015). Phosphate mobilisation by soil microorganisms. In: Lugtenberg, B. (Eds.) Principles of plant- microbe interactions. *Switzerland, Springer International Publishing, 24*, 225-234.

Bhat, M. A., Rasool, R., and Ramzan, S. (2019). Plant growth promoting rhizobacteria (PGPR) for sustainable and eco-friendly agriculture. *Acta Sci. Agric., 3*, 23–25.

Brazhnikova, Y. V., Shaposhnikov, A. I., Sazanova, A. L., Belimov, A. A., Mukasheva, T. D. and Ignatova, L. V. (2022). Phosphate Mobilization by Culturable Fungi and Their Capacity to Increase Soil P Availability and Promote Barley Growth. *Current microbiology*, *79*(8), 240.

Chandini, R.K., Kumar, R. and Om, P. (2019). The Impact of Chemical Fertilizers on our Environment and Ecosystem. In Research Trends in Environmental Sciences, 2nd ed.; AkiNik Publications, New Delhi, India, pp. 71–86.

Contreras-Cornejo, H. A., Macias-Rodriguez, L., Cortes-Penagos, C. and López-Bucio, J. (2009). *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. *Plant Physiol., 149*, 1579–1592.

Cooke, G. W. (1982). Fertilizing for maximum yield. Third Edition English Language Book society/Collins, 457.

Della Mónica, I. F., Godoy, M. S., Godeas, A. M. and Scervino, J. M. (2018). Fungal extracellular phosphatases: their role in P cycling under different pH and P sources availability. *Journal of applied microbiology*, *124*(1), 155–165.

Dhariwal, A. G. and Tarafdar, J. C. (2023). A comparison of phytase efficiency originated from plant and fungal sources. *GSC Biol. Pharm. Sci., 23* (01), 117-126.

Dhungana, I., Michael, B. K. and Nguyen, N. H. (2022). Root exudate composition from different plant species influences the growth of rhizosphere bacteria. *Rhizosphere*, *25*, 100645.

Dong, J., Jones, R. H. and Mou, P. (2018). Relationships between Nutrient Heterogeneity, Root Growth, and Hormones: Evidence for Interspecific Variation. *Plants (Basel, Switzerland)*, *7*(1), 15.

Elhaissoufi, W., Ghoulam, C., Barakat, A., Zeroual, Y. and Bargaz, A. (2022). Phosphate bacterial solubilization: a key rhizosphere driving force enabling higher P use efficiency and crop productivity. *J. Adv. Res., 38*, 13–28.

Etesami, H., Ryong, J. B. and Glick, B. R. (2021). Contribution of Arbuscular Mycorrhizal Fungi, Phosphate–Solubilizing Bacteria, and Silicon to P Uptake by Plant. *Front. Plant Sci.,12*

Fatima, F., Ahmad, M. M., Verma, S. R. and Pathak, N. (2021). Relevance of phosphate solubilizing microbes in sustainable crop production: A review. *Int. J. Environ. Sci. Technol., 19*, 9283–9296.

Filippelli, G. M. (2008). The Global Phosphorus Cycle: Past, Present, and Future. *Elements*, *4*(2), 89–95.

Fiorentino, N., Ventorino, V., Woo, S.L., Pepe, O., De Rosa, A., Gioia, L., Romano, I., Lombardi, N., Napolitano, M., Colla, G. and Rouphael, Y. (2018) Trichoderma-Based Biostimulants Modulate Rhizosphere Microbial Populations and Improve N Uptake Efficiency, Yield, and Nutritional Quality of Leafy Vegetables. *Front. Plant Sci*., *9*, 743.

Föhse, D., Claassen, N. and Jungk, A. (1988). Phosphorus efficiency of plants I. External and internal P requirement and P uptake efficiency of different plant species. *Plant and Soil*., *110*, 101-109.

Gerke, J. (2010). Humic (organic matter)-Al (Fe)-phosphate complexes: an underestimated phosphate form in soils and source of plant-available phosphate. *Soil Sci*., *175*, 417–425.

Goss, M. J., Miller, M. H., Bailey, L. D. and Grant, C. A. (1993). Root growth and distribution in relation to nutrient availability and uptake, *Eur. J. Agron., 2*(2) 57-67.

Hassan, A. and Nawchoo, I. A. (2022). Rhizosphere Improvement role of biotechnology and microbioengineering. In Parry, A. N., Shameem, E.F., Abd-Allah and Mir, M. Y. (Eds.), Microbiome: Improving Crop Quality and Productivity. pp 268-287.

Jackson, M. L. (1967). Soil Chemical Analysis. Prentice-Hall of India, Delhi, 498 p.

Janes-Bassett, V., Martin, S.A.B, Gordon, B., Jess, D., Philip, M. H., Malika, M. M. and Gavin, S. (2022). A meta-analysis of phosphatase activity in agricultural settings

in response to phosphorus deficiency. *Soil Biology and Biochemistry, 165*, 108537

Jarvie, H. P., Flaten, D., Sharpley, A. N., Kleinman, P. J., Healy, M. G. and King, S. M. (2019). Future Phosphorus: Advancing New 2D Phosphorus Allotropes and Growing a Sustainable Bioeconomy. *J. Environ. Qual., 48*, 1145–1155.

Jones, D. L. (1998). Organic acids in the rhizosphere—A critical review. *Plant Soil, 205*, 25–44.

Kaczyńska, G., Borowik, A. and Wyszkowska, J. (2015). Soil Dehydrogenases as an Indicator of Contamination of the Environment with Petroleum Products. *Water Air Soil Pollut.*, *226*(11), 372.

Kaur, J. and Kaur, G. (2021). Dehydrogenase Activity as a Biological Indicator of Soil Health. *Chem. Sci. Rev. Lett.*, *10* (39), 326-329.

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.

Khan, N., Bano, A., Ali, S. and Babar, M.d. A. (2020). Crosstalk amongst phytohormones from planta and PGPR under biotic and abiotic stresses. *Plant Growth Regul., 90*, 189–203.

Khoshru, B., Mitra, D., Khoshmanzar, E., Myo, E. M., Uniyal, N., Mahakur, B., Mohapatra, P. K. D., Panneerselvam, P., Boutaj, H., Alizadeh, M., Cely, M. V. T., Senapti, A. and Rani, A. (2020). Current scenario and future prospects of plant growth-promoting rhizobacteria: an economic valuable resource for the agriculture revival under stressful conditions. *J. Plant Nutr., 43*, 3062–3092.

Kitson, R. E. and Mellon, M. G. (1944). Colorimetric determination of phosphorus molybdovanadophosphoric acid. *Ind. Eng. Chem. Anal. Ed.*,*16*, 379–383.

Kucey, R.M.N., Janzen, H. H. and Leggett, M. E. (1989). Microbially mediated increases in plant-available phosphorus. *Adv. Agron*., *42*, 199–228.

Li, M. G., Osaki, M., Honma, M. and Tadano, I. (1997). Purification and characterization of phytase induced in tomato roots under phosphorus deficient conditions. *Soil Sci. Plant Nutr*., *43*, 179– 190.

Lin, S., Gunupuru, L. R., Ofoe, R., Saleh, R., Asiedu, S.K., Thomas, R. H. and Abbey, L. (2021). Mineralization and nutrient release pattern of vermicast-sawdust mixed media with or without addition of *Trichoderma viride*. *PLoS ONE, 16*, e0254188.

Liu, X., Han, R., Cao, Y. and Turner, B. L. (2022). Enhancing Phytate Availability in Soils and Phytate-P Acquisition by Plants: A Review. *Environ. Sci. Technol., 56*, 9196–9219.

Lopes, M. J. S., Dias-Filho, M. B. and Gurgel, E. S. C. (2021) Successful Plant Growth-Promoting Microbes: Inoculation Methods and Abiotic Factors. *Front. Sustain. Food Syst*., *5*, 606454.

López-Bucio, J., Cruz-Ramírez, A. and Herrera-Estrella, L. (2003). The role of nutrient availability in regulating root architecture. *Curr. Opin. Plant Biol., 6*, 280–287.

López-Bucio, J., Pelagio-Flores, R. and Herrera-Estrella, A. (2015). Trichoderma as biostimulant: exploiting the multilevel properties of a plant beneficial fungus. *Scientia Horticulturae,196*, 109-123.

Malgioglio, G., Rizzo, G.F., Nigro, S., Lefebvre Du Prey, V., Herforth-Rahmé, J., Catara, V. and Branca, F. (2022). Plant-Microbe Interaction in Sustainable Agriculture: The Factors That May Influence the Efficacy of PGPM Application. *Sustainability, 14*, 2253.

Margalef, O., Sardans, J., Fernández-Martínez, M., Molowny-Horas, R., Janssens, I.A., Ciais, P., Goll, D., Richter, A., Obersteiner, M., Asensio, D. and Peñuelas, J. (2017). Global patterns of phosphatase activity in natural soils. *Sci. Rep., 7*, 1337.

Mega, J.A. (1982). Phytate: its chemistry, occurrence, food interactions, nutritional significance, and method of analysis. *J. Agril. Food Chem.*,*30*, 1–9.

Menezes-Blackburn, D., Inostroza, N. G., Gianfreda, L., Greiner, R., Mora, M. L. and Jorquera, M. A. (2016). Phytase-producing bacillus sp. inoculation increases phosphorus availability in cattle manure. *J. Soil Sci. Plant Nutr., 16*, 200–210.

Mengel, K., Kirkby, E.A., Kosegarten, H. and Appel, T. (2001). Phosphorus. In: Mengel, K., Kirkby, E. A., Kosegarten, H., Appel, T. (Eds.), Principles of Plant Nutrition. Springer, Dordrecht. p 453-479.

Mishra, A. and Tailor, S. (2023). Plant-microbe interactions in photosynthesis, nutrient acquisition, and plant growth In: Swapnil, P., Meena, M., Harish, Marwal, A., Vijayalakshmi, S. and Zehra, A. (Eds.), Plant-Microbe Interaction - Recent Advances in Molecular and Biochemical Approaches, Academic Press, p. 421-434.

Motaher Hossain, M. and Sultana, F. (2020). Application and Mechanisms of Plant Growth Promoting Fungi (PGPF) for Phytostimulation. In: Kumar Das, S. (Ed.), Organic Agriculture. Intech Open.

Nannipieri, P., Giagnoni, L., Landi, L. and Renella, G. (2011). Role of phosphatase enzymes in soil. In Phosphorus in Action. Soil Biology; Springer: *Heidelberg, 2011*; pp 215−243.

Neumann, G. and Romheld, V. (2000). The release of root exudates as affected by the plant's physiological status. In Pinton, R., Varanini, Z. and Nannipieri, P. (Eds.), The rhizosphere, biochemistry and organic substances at the soil-plant interface, Marcel Dekker Inc, pp 41–93.

Paul, S. and Rakshit, A. (2022). Evaluating Lignification, Antioxidative Defense, and Physiochemical Changes in Soybean through Bio-Priming under Graded Soil Fertilization. *J. Soil Sci. Plant Nutr*., *22*, 2295–2306.

Poudyal, S., Owen, J.S. Jr., Sharkey, T.D., Fernandez, R.T. and Cregg, B. (2021). Phosphorus requirement for biomass accumulation is higher compared to photosynthetic biochemistry for three ornamental shrubs. *Sci. Hortic., 275*, 109719.

Richardson, A.E. (1994). Soil microorganisms and phosphorus availability. In: Pankhurst, C.E., Doulse, B.M., Gupta, V.V.S.R., Grace, P.R. (Eds.), Soil Biota Management in Sustainable Farming System. CSIRO, Australia, pp. 50–62.

Richardson, A. E. and Simpson, R. J. (2011). Soil Microorganisms Mediating Phosphorus Availability. *Plant Physiol.*, *156*, 989–996.

Rizwanuddin, S., Kumar, V., Singh, P., Naik, B., Mishra, S., Chauhan, M., Saris, P. E. J., Verma, A. and Kumar, V. (2023). Insight into phytase-producing microorganisms for phytate solubilization and soil sustainability. *Frontiers in microbiology*, *14*, 1127249.

Römer, W. and Schenk, H. (1998). Influence of genotype on phosphate uptake and utilization efficiencies in spring barley. *Eur. J. Agron*., 8, 215-224.

Saeed, Q., Xiukang, W., Haider, F.U., Kučerik, J., Mumtaz, M.Z., Holatko, J., Naseem, M., Kintl, A., Ejaz, M., Naveed, M., Brtnicky, M. and Mustafa, A. (2021). Rhizosphere Bacteria in Plant Growth Promotion, Biocontrol, and Bioremediation of Contaminated

Sites: A Comprehensive Review of Effects and Mechanisms. *Int. J. Mol. Sci*., *22*(19), 10529.

Schoumans, O. F., Bouraoui, F., Kabbe, C., Oenema, O. and Van Dijk, K. C. (2015). Phosphorus Management in Europe in a Changing World. *AMBIO*, *44*, 180–192.

Seeling, B. and Jungk, A. (1996). Utilization of organic phosphorus in calcium chloride extract of soil by barley plants and hydrolysis by acid and alkaline phosphatase. *Plant Soil*, *178*, 179–184.

Sharma, S. B., Sayyed, R. Z., Trivedi, M. H. and Thivakaran, A. G. (2013). Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springer Plus*, *2*, 587.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W., & Zhang, F. (2011). Phosphorus dynamics: from soil to plant. *Plant physiology*, *156*(3), 997–1005.

Singh, B., and Satyanarayana, T. (2011). Microbial phytases in phosphorus acquisition and plant growth promotion. *Physiol. Mol. Biol. Plants*, *17*(2), 93–103.

Sokal, R. R. and Roholf, F. J. (1981). Biometry- The Principles and Practice of Statistics in Biological Research. W.H. Freeman and Co, New York.

Spohn, M. and Kuzyakov, Y. (2013). Distribution of microbial- and root-derived phosphatase activities in the rhizosphere depending on P availability and C allocation – Coupling soil zymography with 14C imaging. *Soil Biol. Biochem*., *67*, 106-113.

Sui, Z., Yin, J., Huang, J. and Yuan, L. (2022). Phosphorus mobilization and improvement of crop agronomic performances by a new white-rot fungus *Ceriporia lacerata* HG2011. *Journal of the science of food and agriculture*, *102*(4), 1640–1650.

Sun, R., Yi, Z., Fu, Y. and Liu, H. (2022). Dynamic changes in rhizosphere fungi in different developmental stages of wheat in a confined and isolated environment. *Appl. Microbiol. Biotechnol*., *106*, 441–453.

Suzumura, M., Hashihama, F., Yamada, N., and Kinouchi, S. (2012). Dissolved phosphorus pools and alkaline phosphatase activity in the euphotic zone of the Western north pacific ocean. *Frontiers in microbiology*, *3*, 99.

Tabatabai, M. A. (1982). Soil enzymes. In: page, A.L. (Eds.), Methods of Soil Analysis Part-2, Chemical and Microbiological Properties. American Society of Agronomy, Madison, pp. 903–947.

Tabatabai, M. A. and Bremner, J. M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. *Soil Biol. Biochem.,1*, 301–307.

Tarafdar, J. C. (1989). Use of electro focussing technique for characterizing the phosphatases in the soil and root exudates. *J. Ind. Soc. Soil. Sci*., *37*, 393–395.

Tarafdar, J.C. (2019). Fungal Inoculants for Native Phosphorus Mobilization. In: Giri, B., Prasad, R., Wu, QS., Varma, A. (eds) Biofertilizers for Sustainable Agriculture and Environment . Soil Biology, vol 55. Springer, Cham.

Tarafdar, J. C. and Yadav, R. S. (2011). Hydrolysis of P fractions by phosphatase and phytase producing fungi. *Agrochimica*, *55*(4), 1–13.

Tarafdar, J. C. and Chhonkar, P. K. (1979). Phosphatase production by microorganisms isolated from diverse types of soils. *Zbl. Bakt.,134*, 119–122.

Tarafdar, J.C. and Gharu, A. (2006). Mobilization of organic and poorly soluble phosphates by Chaetomium globosum. *Appl. Soil Ecol*., *32*, 273–283.

Tazisong, I., Zachary, S. and Zhongqi, H. (2015). Phosphatase Hydrolysis of Organic Phosphorus Compounds. *Adv. Enzym. Res*., *3* (2), 39-51.

Tennant, D. (1975). A test of a modified line intersects method of estimating root length. *J. Ecol., 63*, 995-1001.

Tian, J., Ge, F., Zhang, D., Deng, S. and Liu, X. (2021). Roles of Phosphate Solubilizing Microorganisms from Managing Soil Phosphorus Deficiency to Mediating Biogeochemical P Cycle. *Biology*, *10* (2),158.

Turan, M., Ataoğlu, N. and ¸Sahın, F. (2006). Evaluation of the Capacity of Phosphate Solubilizing Bacteria and Fungi on Different Forms of Phosphorus in Liquid Culture. *J. Sustain. Agr., 28*, 99–108.

Upadhyay, S. K., Srivastava, A. K., Rajput, V. D., Chauhan, P. K., Bhojiya, A. A., Jain, D., Chaubey, G., Dwivedi, P., Sharma, B., and Minkina, T. (2022). Root Exudates: Mechanistic Insight of Plant Growth Promoting Rhizobacteria for Sustainable Crop Production. *Frontiers in microbiology*, *13*, 916488.

Wang, Q., Xiao, C.Q., Feng, B. and Chi, R. (2020). Phosphate rock solubilization and the potential for lead immobilization by a phosphate solubilizing bacterium (*Pseudomonas* sp.). *J. Environ. Sci. Health. A, 55* (4), 411–420.

Whitelaw, M.A., Harden, T. J. and Helyar, K. (1999). Phosphate solubilization in solution culture by the soil fungus Penicillium radicum. *Soil Biol. Biochem*., 31, 655–665.

Withers, P. J., van Dijk, K. C., Neset, T. S., Nesme, T., Oenema, O., Rubæk, G. H., Schoumans, O. F., Smit, B. and Pellerin, S. (2015). Stewardship to tackle global phosphorus inefficiency: The case of Europe. *Ambio, 44 (2)*, 193–206.

Wolinska, A. and Stepniewsk, Z. (2012). Dehydrogenase Activity in the Soil Environment. In Canuto, R. A. (Eds.), Dehydrogenases . pp. 183-210.

Yadav, B. K. and Tarafdar, J. C. (2007). Ability of Emericella rugulosa to mobilize unavailable P compounds during Pearl millet *(Pennisetum glaucum (*L.) R. Br.) crop under arid condition. *Ind. J. Microbiol*., *47*, 57–63.

Zaidi, A., Khan, M. S. and Amil, M. (2003). Interactive effect of rhizotrophic microorganisms on yield and nutrient uptake of chickpea (*Cicerarietinum* L.). *Eur. J. Agron*., *19* (1), 15-21.

Zhang, N., He, X., Gao, Y., Li, Y., Wang, H., Ma, D., Zhang, R. and Yang, S. (2010). Pedogenic Carbonate and Soil Dehydrogenase Activity In Response To Soil Organic Matter in Artemisia ordosica Community. *Pedosphere, 20*, 229-235.

Zhu, H., Bing, H. and Wu, Y. (2022). Citric Acid Promotes the Mobilization of Phosphorus under the Lower Concentration of Low Molecular Weight Organic Acids in Acidic Forest Soil. *Adsorp. Sci. Technol*., 2022, 5071907.

How to cite this article: Amita Gharu Dhariwal, Indira Rathore and J.C. Tarafdar (2023). Field Evaluation of Phosphatase and Phytase Producing Fungi under Clusterbean and Pearl Millet in arid Ecosystems. *Biological Forum – An International Journal, 15*(5): 1466-1481.