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Assessing Fungal Inoculants for Native P Mobilization

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ABSTRACT: Four phosphate solubilizing/mobilizing fungi (*Aspergillus candidus, Aspergillus ustus, Curvularia lunata* and *Phoma* species) were isolated and tested for their population growth and efficiency towards phosphorus solubilization and mobilization from the native soil under both sterilised and non-sterile soil conditions after taking wheat as a test crop. The study is absolutely necessary in the present situation to know the exact contribution of plants and inoculated microorganisms for the mobilization of P from the native sources. The plant available phosphorus (P) in the experimental soil was less than 1% and among the 29% of organic phosphorus (Po), 70% was present as phytin form in the soil. The fungal population increased with time and a maximum of 15 to 16 times (under sterilized condition) and 12 to 13 times (under non-sterilized soil) was observed within four-week period. In sterilised soil, mobilization of plant unavailable P was higher, primarily because of an increase in the population of inoculated fungi. A positive influence on acid phosphatase and phytase activity was observed under wheat after inoculation, while alkaline phosphatase activity was not significantly affected in the test plants. In general, one third of the total plant-unavailable P was hydrolyzed by plants, whereas the contribution of inoculated fungi was observed at two-thirds. Plant contribution gradually increased with the plant growth period; maximum mineral and organic P hydrolysis generate by the plant sources was seen between 7 and 21 days. In general, more phytin P was hydrolyzed after 28 days of plant growth. The organic P was exhausted more than mineral P as well as contribution from microbial sources for mobilization of different P fractions was much more (52 to 87% of min-P, 53 to 87% of org-P and 50 to 80% phytin-P) than plant contribution (13 to 48%, 13 to 43% and 20 to 50% for min-P, org-P and phytin-P, respectively). The inoculation effect was more in an increase in plant biomass, root length, and plant P concentration. Among the four tested fungi, *Phoma species* was more efficient in disintegrate org-P as phytin, while *Aspergillus ustus* was establish to be the most efficient in min-P hydrolysis and enhance P nutrition for wheat plant. Our result clearly demonstrated the exact contribution from the efficient microorganisms for P mobilization from the different native P sources.

Keywords: Arid soil, Fungal contribution, Plant contribution, Native soil phosphorus, Wheat.

INTRODUCTION

Plants take up monobasic ($H_2PO_4^-$) and dibasic phosphate (HPO_4^{2-}) from the soil, In many agricultural soils, phosphorus (P) is insufficient, because 95–99% of total soil phosphorus is not available for plants as it is present in precipitated, immobilized, and insoluble forms (Gouda *et al.,* 2018). In general, phosphate deficiency by plants during their growth is compensated by applying chemical or phosphate fertilizers (Rodrıguez and Fraga 1999). Long-term application of P fertilizers has led to eutrophication of surface water (Turan *et al*., 2006) and accumulation of total soil P, the majority of which is poorly soluble, because most of the phosphate in soluble forms are supplied to soils as fertilizer is "fixed" in soil and rendered less available to plants (Whitelaw, 1999). Therefore, there is a need for novel approaches that decrease the amount of fertilizer applied while maintaining appropriate crop yields are necessary during the development of sustainable agricultural systems. Future promise may lie in the use of biological resources to make use of soil nutrients (Jeffries and Barea 1994). The soil P mainly occurs in insoluble inorganic or organic forms, neither of which is readily available to plants. According to Tarafdar *et al.* (2002), phosphorus (P) mineralization rate is affected by microbial activity, free phosphatase and phytase enzyme activity and the concentration of P (McGill and Cole 1981) and pH (Rawal *et al*., 2022) in soil solution. Phosphorus bioavailability or cycling in soil-plant systems is fundamentally regulated by

microorganisms (Tian *et al.,* 2021) because they take parts a key role in a number of processes that control P availability and transformations. Soil microorganisms have the potential to liberate plant-accessible P by the way of solubilization and mineralization of inorganic and organic P, respectively, from the sparsely available forms of soil P (Barea and Richardson 2015; Whitelaw *et al.,* 1999). The uptake of nutrients and crop sustainability are greatly improved by microorganisms (Hassan and Nawchoo 2022). According to Tarafdar and Marschner (1995), microorganisms in soil may play a crucial role in phytate and glycerophosphate accessibility to plant roots. They demonstrated that in the co-inoculated plants with the mycorrhizal fungus *Glomus mosseae* and the fungus *Aspergillus fumigatus*, which is known to have phytase activity, the P nutrition of wheat grown in phytate-supplied soil was increased (Wyss *et al.,* 1999).

To avoid phosphorus deficiency, it is possible for phosphate-solubilizing microorganisms (PSM) to play a leading role in contribute phosphate to plants in a more environmentally friendly and viable manner (Vaghasia *et al.,* 2017). Yadav and Tarafdar (2003) find that dissimilar organic P compounds could be hydrolyzed differently by different fungal isolates which indicate that efficient phosphatase and phytase-releasing organisms can take advantage of the significant amounts of less reachable soil phosphorus. It has been proved beyond doubt (Alaylar *et al.,* 2020; Wang *et al*., 2020) that the application of phosphate solubilizing microorganisms is an accepted solution for successful phosphate rock mining management and agricultural sustainability and is widely accepted as eco-friendly P fertilizers for enhancing agricultural productivity. The focus of this study was to evaluate the effects of phosphate solubilizing/mobilizing fungi on the growth of wheat plants under green house conditions and examine the efficiency of their enzymes, compared to the plant releasing enzymes, in deliver available phosphorus from unavailable P sources for plant nutrition.

MATERIAL AND METHODS

Fungal isolation, identification and selection. Dilution plate technique (Tarafdar and Chhonkar 1979) was used for isolating the fungi amongst 30 different types of soil collected from different areas of India on streptomycin sulphate-containing Martin's Rose Bengal agar (Allen, 1959). Twenty-five organisms that produce phosphatase and phytase were isolated. The pure cultures of isolated fungi were maintained on a potato dextrose agar (PDA) medium. The most effective fungi were chosen based on their intra- and extracellular acid phosphatase, alkaline phosphatase and phytase activity. Accordingly the best fungi was selected and identified by Agharkar Research Institute, Pune, India, as *Aspergillus candidus*, *Aspergillus ustus*, *Curvularia lunata* and *Phoma species.*

Fungal enzyme activity and efficiency. Fungi were cultured in 250 mL Erlenmeyer flasks containing 100 mL Czapek-Dox broth to measure enzyme activity. The flasks were incubated at 30 °C for 21 days, while the medium was incubated with 8 mm discs of fungal growth that were 4 days old (on PDA media). The material in three flasks was filtered through Whatman No. 1 filter paper into another flask that had been refrigerated on ice. Each filtrate's final volume was made up of 100 mL of sterilised, ice-cold distilled water. The extracellular activity of different enzymes (phytase, alkaline phosphatase and acid phosphatase) was measured in the filtrate. Acid-washed quartz sand was used to grind the fungal mats at each sampling interval, to determine intracellular activity. A fine suspension was prepared by adding ice-cold sterilised distilled water and being centrifuged for 20 minutes at 12,000 rpm. The intracellular enzymes were found in a clear supernatant that was make up to a specified volume.

The efficiency of enzymes produced by fungi was assessed using Na-glycerophosphate and phytin as an organic P compounds. 1 g of fungal mat (in triplicate) was ground in a mortar with 30 mL of ice-cold sterilized distilled water and acid-washed quartz sand. The extract followed centrifugation, as mentioned previously. The clear supernatant was incubated with 500 mg/L phytin and incubated at 30°C for 24 hours. According to Jackson (1967), the release of min-P was calculated calorimetrically and expressed as micrograms of P released per minute per gram of fungal mat.

Pot experiment. We have used 25-cm-high and 10-cm-wide pots for the study. The textural classes of soil used was alalysed loamy sand which was dug out of seven distinct soil pits at the Central Research Farm, ICAR-CAZRI, in Jodhpur to a depth of about 30 cm. The collected soil was ground and sieved through a 20-mesh (0.9 mm) sieve. Four different treatments were used in the experiment: sterilized soil with plants, sterilized soil without plants, non-sterilized soil with plants, and non-sterilized soil without plants. On three alternate days, soil was autoclaved for two hours at 105°C and 1.05 kg cm^{-2} pressure to sterilise it. Each pot acquires300 g of sterilised or unsterilized soil. Table 1 presents the initial physico-chemical characteristics of soil. The entire soil in each pot was thoroughly mixed before planting and added with the 20 mL of fungal culture. In any of the treatments, no fertiliser was applied. Four pots were harvested per week from each of the sixteen pots that had been used for each treatment. Each pot contained 20 wheat (*Triticum aestivum* L.) seeds (cv. Raj 3077), of which 15 plants were continued after germination for the study. On every alternate day, water was added to the soil at 50% water holding capacity (WHC), and after 7, 14, 21, and 28 days, the sampling was done.

Biochemical evaluation and Processing after sampling. The acid and alkaline phosphatases from rhizosphere soil were estimated after adopting the standard procedure of Tabatabai and Bremner (1969) using acetate buffer (pH 5.4) and sodium tetraborate–NaOH buffer (pH 9.4), respectively. The mixture (enzyme and 4-nitrophenyl phosphate) was incubated at 35 °C for 1 h and the analysis was expressed as enzyme

units (EU). One unit represent the amount of enzyme that hydrolyses 1.0 µmol of p-nitrophenyl phosphate per minute at pH 5.4 (acid phosphatase) or 9.4 (alkaline phosphatase) at 35°C. Phytase activity was evaluate by measuring the amount of inorganic phosphate (Pi) released by hydrolysis of sodium phytate (1 mM) in 100 M sodium acetate buffer (pH 4.5) and incubating at 37°C for 1 h. The reaction was terminated by the addition of 0.5 mL 10% trichloro acetic acid (CCl_3COOH). Proteins precipitated by TCA were removed by centrifugation at 10,000 rpm for 10 min, and the supernatant was analyzed for liberated inorganic P (Ames, 1966). One unit of phytase activity was describing as the amount of enzyme that liberated 1.0 µmol Pi min^{-1}. Tabatabai (1982) method was used to estimate dehydrogenase activity, which is a measure of total microbial activity. In this method samples were incubated with 2,3,5-triphenyl tetrazolium chloride, and the triphenyl formazon produced was determined at 485 nm.

Immediately after each sampling, roots were thoroughly cleaned with tap water and then in distilled water to remove any remaining soil. Modified line-intersect method of Tennant (1975) was used to measure root lengths after separating the roots from the soil. The plants were dried in oven at 60 °C and the dry matter weight was recorded. After that it was ground to a fine powder for analysis. The plant P content was evaluated using Kitson and Mellon's (1944) Vanadomolybadate method which was done after acid digestion of plant powder with 20 mL concentrated HNO_3, $HClO_4$ and H_2SO_4 (ratio of 7:2:1). Martin's Rose Bengal agar medium was used to count the fungal population presents (Allen, 1959). The organic carbon content in the soil was estimated by the wet digestion method of Walkley and Black, as described by Jackson (1967). According to Seeling and Jungk's (1996) description, estimations of mineral, total, and organic P were established. The phytin P was determined by extracting the phytate with 15% CCl_3-COOH (trichloro-acetic acid) as described by Mega (1982). The pH (1:2), EC (1:2), particle size distribution, and available P (Olsen's P) were determined using the standard methods (Jackson, 1967). The microbial contribution for hydrolysis of P was defined as the mineral, organic, or phytin P depleted from the pots as a result of the inoculation of fungus in pots without plants while the plant contribution referred to as the P depletion after introducing plants to the inoculated pots. The statistical analysis was conducted after analysis of variance on the data, and vertical bars represent the standard errors of the differences between means (Sokal and Rohlf 1981).

RESULTS AND DISCUSSION

To study the native P mobilization and P inflow to the wheat plants, four efficient and isolated organisms (*Aspergillus candidus*, *Aspergillus ustus*, *Curvularia lunata* and *Phoma* species)were pick out for detailed study under green house conditions. This was determined by its better phosphatase and phytase activity among all other isolated species, as well as its capacity to hydrolyze unavailable P (results not shown). They were all a rapidly growing organism that grew 6 to 7 g of biomass in approximately 14 days in liquid medium. Table 1 shows the initial phosphorus status, enzyme activity and microbial population observed in the experimental soil. Approximately 1% of total P in the studied soil was available for plant uptake, while 99% of total P exists in an unavailable form: 70% in mineral (Pi) and 29% in organic (Po). Most of the organic P in the soil was found as phytin, which made up 68% of the total Po (Dalal, 1978; Mengel *et al.,* 2001).

Population build-up. The gradual population build-up of different fungi under various conditions is presented in Fig. 1. The population build–up increased with time, and a maximum 15–16 fold increase in fungal population from the initial value was observed within a four-week period under sterilized soil conditions. But due to competition with the native population already present in the soil, non sterilized soil registered only a 12–13-fold increase in population within the same period. *Aspergillus ustus* showed maximum population build-up in all soil conditions after 4 weeks, which were 16 times more in sterilized soil conditions and 13 times higher in non-sterilized soil situations. A gradual increase in population from 1.3 to 2.1 times after the first week of inoculation, 2.3 to -4.0 times at the second week of inoculation, 5.1 to 8.8 times throughout the third week of inoculation, and 7.4 to 16.0 times in the course of fourth week of inoculation. It was found to fewer increases in fungal population where wheat had been grown as compared to control soil (without plants). The population build-up under control (barren soil) was 9.3 to 10.1 times higher in sterilized conditions and 7.4 to 8.7 times higher in unsterilized soil conditions after four weeks of inoculation. In general, a 27–39% decrease in fungal population was noticed under unsterilized soil conditions after four weeks of plant growth as compared to the fungal population build up under sterilized soil conditions within the same period. The growth of population of microorganisms was more under sterilised conditions. Sterilisation of soil increases electrical conductivity and water-soluble C, which further increases the microbial population (Mahmood *et al*., 2014). Plant introduction reduces the increase rate in microbial population build-up (Fig. 1) as root exudates may influence the growth and development of other organisms (Dhungana *et al.,* 2023), which may give stiff competition to microorganisms (Tarafdar and Gharu 2006). It was noticed that the activity of microorganisms consequence in quantitative and qualitative alterations in the composition of the root exudates mainly due to the degradation of the exudates and the release of microbial metabolites. Miller *et al.* (1989) found that the composition of plant root exudates and rhizosphere microflora varies with plant types (Miller *et al.,* 1989). The changes in structure of microbial community in the rhizosphere may occur due to the rhizodeposition or extent of C loss through roots (De Leij *et al.,* 1993). Organic compounds having low molecular weight in

root exudates also influence the structure and function of soil microbial communities in the rhizosphere (Shi *et al.,* 2011). Our result clearly depicted that the population of the microorganisms built gradually (Fig. 1), this is mainly due to the exudation of higher amounts of diverse C compounds by roots, as it was found responsible for 'booster' of soil microbial activity (Hinsinger, 2001). A similar observation was also noticed by Bowen and Roviera (1999), they concluded that root exudates being a major source of energy for microbial growth (Bowen and Rovira 1999; Dakora and Phillips 2000). Soil is considered a mixture of water, air, minerals, organic matter, and living organisms, root exudates present at the interface between root and soil will have an initial effect on the physical and chemical properties of the soil as well as the microorganisms and plants that exist in the soil (Brevik *et al*., 2015; Giri *et al.*, 2018). It was found that plant species, growth stage, environmental factors, and microorganisms are primary factors that affect the composition and amount of root exudates (Ma *et al*., 2022) and their production mechanisms.

Enzyme activity. The increase in activity of acid phosphatase in our experimental soil with or without inoculation of wheat plants under sterilized and non-sterilized soil conditions is presented in Fig. 2. The result indicates more acid phosphatase activity under sterilized soil conditions where fungi are introduced. The gradual elevated activity was noticed with crop age, up to four weeks. The escalation in acid phosphatase activity varied between 20 to 30 times in sterilized soil and 4.5 to 6.9 times in non-sterilized soil due to the inoculation of four selected efficient phosphatase and phytase producing fungi during the growth period of four weeks. The more release of acid phosphatase activity was observed with *Phoma* Species followed by *Aspergillus ustus*. The presence of plants into the soil influences the acid phosphatase activity. This was between 19 and 59% more under unsterilized soil conditions as well as between 13 and 23% more under sterilized soil conditions as compared to the soil without plants.

In sterilized soil the increase in alkaline phosphatase activity was significantly more (54–82%) due to the inoculation of different fungi in comparison to the non-sterilized soil condition (Fig. 3). More (82% higher) alkaline phosphatase activity was observed with *Phoma* sp. while a 54% improvement in alkaline phosphatase activity under sterilized soil was perceive in *Aspergillus ustus*. The introduction of wheat plant on the increase in alkaline phosphatase activity was not always significant under sterilized soil conditions. However, the effect was more visible at later stages of plant growth. In general, 34 to 81% more alkaline phosphatase activity was visualized under sterilized soil compared to non-sterilized soil with wheat plantations. The significant differences in activity (21 to 45%) under non-sterilized soil conditions were noticed after the second week of plant growth.

The introduction of wheat plant in experimental soil resulted significant improvement in phytase which was also true with the inoculation of wheat seed with selected fungi. The more effect was under sterilized soil conditions than in non-sterilized conditions (Fig. 4). The swell in phytase activity was 10.5 to 11.7 times higher with fungal inoculation under sterilized soil conditions, whereas only 8.1 to 9.1 time more under non-sterilized soil after four weeks of inoculation. The wheat rhizosphere help in increase in phytase build up further; which were 17.4% in seed inoculated with *Aspergillus candidus*, 14.4% with *Aspergillus ustus*, 14.5% with *Curvularia lunata* and 14.8% with *Phoma* species, under controlled soil condition. When soil conditions are shifted to non-sterilized, wheat plants helps to increase phytase activity from 5.1 *(Aspergillus ustus)* to 16.9% *(Aspergillus candidus)* as compared to unplanted soil.

With the introduction of wheat plant and inoculation with different fungi to wheat seed, the dehydrogenase activity was increased, which was maximum after 4 weeks of plant growth. The plant introduction also resulted more dehydrogenase activity which varies between 6 to 20% due to *Aspergillus candidus* inoculation, 18 to 38% with *Aspergillus ustus*, 4 to 22% with *Curvularia lunata* and 5 to 16% with *Phoma* sp. inoculation in sterilized soil condition whereas 10 to 23%, 20 to 40%, 19 to 49% and 11 to 21% in *Aspergillus candidus*, *Aspergillus ustus*, *Curvularia lunata* and *Phoma* species, respectively, under non-sterilized soil condition, respectively (Fig. 5).

It was reported that both plants and microorganisms are released acid phosphatase (Tarafdar, 1989). The more the biomass accumulation, the more likely to be that phytase and phosphatase will be released. Therefore, in unsterilized soil, due to plant root exudates, the total microbial population may be higher, which influences more acid phosphatase activity (Fig. 2). Plant induced acidification in the rhizosphere created conditions conducive to the dissolution of the sparingly soluble soil P. Plant introduction does not significantly increase alkaline phosphatase activity (Fig. 3), as microorganisms may only contribute alkaline phosphatase in soil (Tarafdar, 1989). Both plants and fungi contributed phytase activity. Enhanced secretion of phytase (Liu *et al.,* 2022) by plant roots and rhizosphere microorganisms (Tarafdar and Marschner 1994; Singh and Satyanarayana 2011) may contribute to inorganic P acquisition by hydrolysis of organic esters in the rhizosphere. It was reported that (Naves *et al.,* 2012) the activity of phytases depends on pH and temperature as well as phytases produced by microorganisms are more thermo-stable than plant phytases (Azeem *et al*., 2015). Although, it was found that the variation in their optimum temperature and pH level varies with microorganisms and their species. The fungal phytases have a pH value ranging from 4.5 to 6.5, like *Aspergillus fumigatus*, where 80% of activity takes place (Wyss *et al.,* 1999). It was reported that the fungal phytases mostly to the acidic ones, having a pH optimum range of 4.5 to 6.0 (Konietzny and Greiner 2002). Beissner (1997) observed that oxalic acid in the root exudates can, to some extent, contribute to phytase

mobilization in soils. It was general believe that the dehydrogenase activity is an indicator of total microbial activity (Quilchano and Marañon 2002; Gu *et al*., 2009; Salazar *et al.,* 2011). Due to higher microbial activity, higher dehydrogenase activity was observed in unsterilized than sterilized soils (Fig. 5). The decrease in dehydrogenase activity reflects a considerable decrease in microbial biomass in the rhizosphere, as root exudates influence the growth and development of other organisms (Dhungana *et al.,* 2023). Both dehydrogenase and alkaline phosphatase activities were associated with the total microbial activity of the soil (Skujins, 1973; Tarafdar and Claassen 1988). This may partly be attributed to better-specific interactions between plant roots and microbes in the rhizosphere (Attiwill and Adams 1993). The important factors to control the soil phosphatase activity may be the P concentration in soil solution (McGill and Cole 1981), the humus content (Tarafdar *et al.,* 1989), the presence of organic P substrates (Tarafdar and Claassen 1988; Helal, 1990).

Hydrolysis of different native plant unavailable P compounds. In general, contribution from fungi was much higher than offering from plants in the hydrolysation of different unavailable P compounds tested (Figs. 6–8). The total mineral P depletion under sterilized soil conditions varied between 52 to 86.5% due to different fungal inoculations between 7 to 28 days of plant age, while only 13.5 to 48% exploitation of unavailable mineral P due to wheat plants within the same period was observed (Fig. 6). The maximum unavailable mineral P depletion under sterilized soil conditions was observed after four weeks of inoculation of *Aspergillus ustus* (86.5%), followed by *Aspergillus candidus* (83%), *Phoma* species (82%) and *Curvlaria lunata* (81%). Under non-sterilized soil conditions, fungal contribution varies between 64 to 82.5%, while plant contribution ranges only from 17.5 to 36%. The maximum mineral P deletion under non-sterilized soil condition was observed in *Aspergillus ustus* inoculation (82%) at four weeks of plant growth, which is followed by *Phoma* species (79%) and least in *Curvularia lunata*. In general, mineral P depletion under both soil conditions increases with time, and *Aspergillus ustus* is most efficient for mobilising plant-unavailable mineral P.

Out of total organic P mobilization, the microbial contribution was found to be 55 to 87% under sterilized condition and 53 to 83% under non-sterilized soil condition, while plant contribution ranges from 13 to 45% under sterilized condition and 17 to 47% under non-sterilized soil condition (Fig. 7). The more hydrolysis of organic P due to fungal inoculation observed in *Aspergillus candidus* at sterilized soil condition (87%) (Fig. 7). The maximum hydrolysis due to plant growth was observed in *Curvularia lunata* at sterilized soil condition (45%) (Fig.7). The more microbial organic P depletion under non-sterilized soil condition observed in *Aspergillus candidus* (83%) at four week after inoculation, followed by *Phoma* species (81%) and least (79%) was observed with *Curvularia lunata*. Maximum organic P depletion due to plant growth under non-sterilized condition was observed in *Curvularia lunata* (47%) followed by *Aspergillus ustus* (44%) after 7 days of plant growth. However, the *Aspergillus candidus* was efficient under sterilized soil condition while the *Aspergillus ustus* was effective both under sterilized as well as normal soil condition. In general, the microbial contribution towards the organic P hydrolysis in both soil conditions increases with plant growth (7 to 28 days), while the plant contribution towards the organic P hydrolysis decreases with plant growth.

The microbial role to hydrolysis of phytin P was 52 to 80% and 50 to 72% under sterilized and non-sterilized soil condition respectively, while plant contribution was only 20 to 48% under sterilized soil and 28 to 50% under non-sterilized soil condition (Fig. 8). More hydrolysis of phytin P due to fungal inoculation under sterilized condition was observed after seed inoculation with *Aspergillus candidus*. However, under non-sterilized condition more hydrolysis of phytin-P was recorded after seed inoculation with *Phoma* species. In general, out of the total plant-unavailable P hydrolysis in native soil, one-third was contributed by plants and two-thirds was contributed by inoculated fungi. With the increase in plant growth period, a gradual increase in plant contribution was noticed. Maximum hydrolysis of mineral and organic P due to the plant was observed between 7 to 21 days, while more phytin P was hydrolyzed after 28 days of plant growth. The consistently higher hydrolysis, after introduction of phosphatase and phytase-producing fungi, was observed in organic P, followed by mineral P and phytin P.

For disintegration of different unavailable P fractions, the microbial contribution was much higher than the plant contribution (Fig 6-8). The phosphatases and phytase released by microorganisms may cleavage of C-O-P ester bond; moreover, the microorganisms can produce organic acids such as malate, citrate and oxalate, which may possibly help in the greater release of inorganic P from organic sources (Jones, 1998; Rashid *et al*., 2004). Santi *et al.* (2000) reported that *Aspergillus niger* BCC F194 was able to solubilise different types of hardly soluble phosphates, i.e., CRP (Cileungsi rock phosphate), MRP (madura rock phosphate), $Ca_3(PO_4)_2$ (calcium phosphate) and $AlPO_4$ (aluminum phosphate) by producing oxalic acid (3.75 mM), citric acid (2.0 mM), and gluconic acid (0.9 mM). The oxalic and citric acids were two major acids produced by *Aspergillus flavus*, *Aspergillus niger* and *Penicillium canescens* (Rashid *et al*., 2004) and that *Aspergillus flavus* produced a greater amount of acids as compared to two other fungal species. Shen *et al*. (2001) identified that elephant grass ([*Pennisetom purpureum*] Cv Napier) exudes pentane dioic acid under P deficient condition which has high P mobilizing activity, and mobilize $FePO_4$ and $AlPO_4$. Moreover, the contribution of soil microorganisms to solubilize various forms of precipitated P is well documented (Kucey *et al.,* 1989; Richardson, 1994;

Whitelaw *et al.,* 1999; Deubel and Merbach 2005; Tian *et al*., 2021). However, the potential of soil fungi to mediate the availability of P to plants from otherwise poorly available sources such as phytin is uncertain. The importance of soil microorganisms for increasing the available P from phytate and glycerophosphate to plant roots has similarly been suggested by Tarafdar and Marschner (1995). Tarafdar *et al*. (2003) identified seven efficient phosphatase-producing fungi (PPF) represented the genera *Aspergillus*, *Pseudeurotium* and *Trichoderma* and reported that hydrolyzing efficiency from mono-phosphate was four times higher than hexa-phosphate. They also propose that *Trichoderma harzianum* was found to be the most efficient organic P mobilizers compared to other fungi tested. Tian *et al.* (2021) point out the potential of soil microorganisms for increasing the bioavailability of P. But the extent to which such microorganisms release P from phytate in soil for subsequent uptake by plant roots still to be determined (Richardson *et al*., 2001).

More secretion of phosphatases and phytase (Li *et al.,* 1997; Yadav and Tarafdar 2001) by plant roots and rhizosphere microorganisms (Tarafdar and Marschner 1994) may give to inorganic P acquisition by hydrolysis of organic P esters in the rhizosphere. Richardson *et al.* (2001) spotlighted the potential of soil microorganisms for increasing the availability of P from phytate through the delivery of phytase activity and presumably by affecting the availability of phytase itself. However, they mentioned that the extent to which such microorganisms release P from phytate in soil for subsequent uptake by plant roots remains to be determined. Our result clearly demonstrated the efficiency of fungi in releasing P from phytate (Phytin-P) and other unavailable P sources. Fungal phytase (Dhariwal and Tarafdar 2023) and acid phosphatase was more efficient than plant phytase and acid phosphatase as it released three times more inorganic P from phytin and two times more from lecithin (Tarafdar *et al.,* 1992).

Plant biomass and root development. Plant biomass of wheat both in sterilized and non-sterilized soil due to inoculation of different phosphatase and phytase producing fungi was presented (Table 2). The result demonstrated significantly more plant biomass with crop age, which was higher in sterilized soil than in nonsterilized soil. The plant biomass accumulation was 6.6 to 13.2% higher in sterilized soil than nonsterilized soil due to the inoculation of different fungi during the plant growth period (7 to 28 days). In general, inoculation with *Phoma* species resulted in more plant biomass under sterilized and non-sterilized soil condition in comparison to the other fungal species tested. *Aspergillus candidus* inoculation resulted in the least biomass accumulation under both soil conditions.

Higher root length due to the inoculation of different fungi with crop age was noticed (Table 3). The increase in root length was significantly higher in sterilized soil than in non-sterilized soil. A positive effect of soil sterilisation on root biomass was also reported by Mahmood *et al*. (2014). Seedlings grown in sterilised soils produced higher root length, surface area, volume, number of tips, and rhizo-sheath soil (RS) mass as compared to those grown in unsterilized soil. The increase in root length varied between 5 to 41% in sterilized soil whereas it was 4 to 39% in non-sterilized soil after inoculation with different fungi. In general, *Aspergillus candidus* inoculation helps improve in root length by 4 to 24%. *Aspergillus ustus* could improve root length by 5 to 27%. The *Curvularia lunata* inoculation helps to increase in root length by 7 to 34%, while *Phoma* species improves the root length of wheat by 9 to 41%. Although a higher root development in terms of root length was observed under sterilized soil conditions as compared to non-sterilized conditions, the differences during the plant growth period were not always significant (Table 3).

P content in plant. Improvement in phosphorus (P) content due to the inoculation of different efficient fungi (Table 4) was presented. The build up of P was significantly higher in sterilized soil than non-sterilized soil due to the inoculation of efficient fungi until 3 weeks of plant growth; thereafter, the increase was marginal and not significant. *Phoma* species could help wheat plants accumulate more phosphorus, both under sterilized and non-sterilized soil conditions. The P acquisition by wheat plants due to different fungal species followed the order: *Phoma* species>*Curvularia lunata* ≈ *Aspergillus ustus*>*Aspergillus candidus*.

Relative growth rate for root (Kr) and P-inflow. Relative growth rate of wheat root (Kr) under sterilized soil condition was higher than that of non-sterilized soil at the initial stages (7 to 14 days) of plant growth. But a reverse trend, in general, was observed after 14 days of plant growth (Table 5). Within the inoculation of efficient fungi, about 12 to 49% improvement in Kr value was observed under sterilized soil conditions, while 20 to 27% improvement was noticed under nonsterilized soil conditions. In general the relative growth rate of the root was found to be higher when *Phoma* species was inoculated as compared to the other species tested.

P-inflow to wheat both in sterilized and non-sterilized soil condition due to inoculation with efficient phosphatase and phytase producing fungi demonstrated higher P-influx in non-sterilized soil than sterilized soil in all the treatments during different crop growth stages (Table 6). The P-inflow was highest between 7 to 14 days of plant growth and significantly declined thereafter with crop age under all treatments. In general, P inflow was more due to the inoculation of *Phoma* species as compared to other species tested. However, in most cases, inoculation by phosphatase and phytase releasing organisms helps in enhancement of P inflow.

The P efficiency which was related to the uptake efficiency of the plant, is determined by the absorption rate per unit of root (influx) (Fohse *et al*., 1988) as well as the relative growth rate of the root (Kr). The present results show that P-influx differed greatly at different growth periods (Table 6) of wheat. Efficient phosphatase and phytase producing organisms showed higher P-influx in non sterilized soil than sterilized soil

during different growth stages (Table 6). Plant growth response to P concentration increased, and increasing P concentration increased roots and shoot growth (Poudyal *et al.,* 2021). The ability to access P was related to root morphology and development. The differences in P-influx can be explained by differences in their uptake kinetics. The influence of P-status on uptake efficiency would be attributed to changes in the root parameters of the plant. Phosphate solubilizing microorganisms (PSMs), a significant micro flora that mediates accessible soil phosphorus, serve a crucial function in soil by mineralizing organic phosphorus, resolving inorganic phosphorus minerals, and storing significant amounts of phosphorus in biomass (Tian *et al.,* 2021).

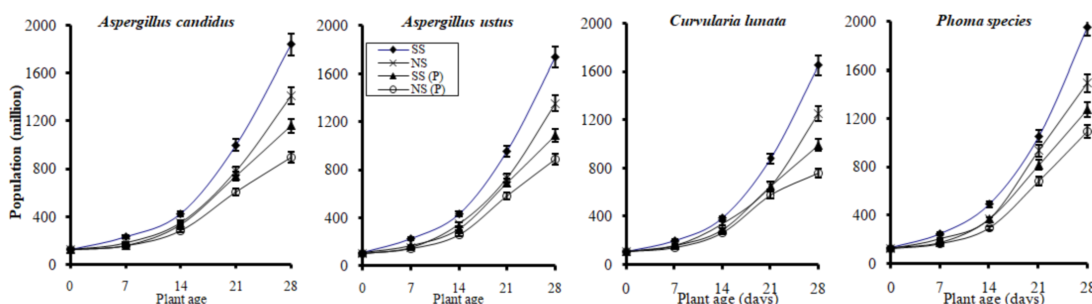

Fig. 1. Competitive ability of selected fungi to population builds up under wheat rhizosphere. Bar represent standard errors of mean. SS: Sterilized soil; NS: Non-sterilized soil; SS(P): Sterilized soil with plant; NS(P): Non-sterilized soil with plant.

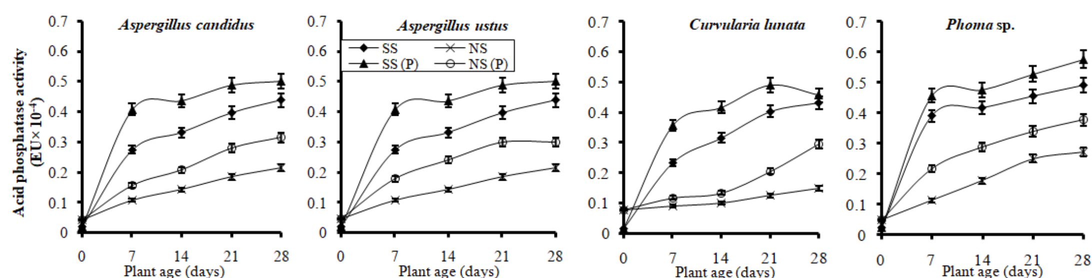

Fig. 2. Build up of acid phosphatase in the wheat rhizosphere after inoculation of four different fungi. Bar represent standard errors of mean. SS: Sterilized soil; NS: Non-sterilized soil; SS(P): Sterilized soil with plant; NS(P): Non-sterilized soil with plant.

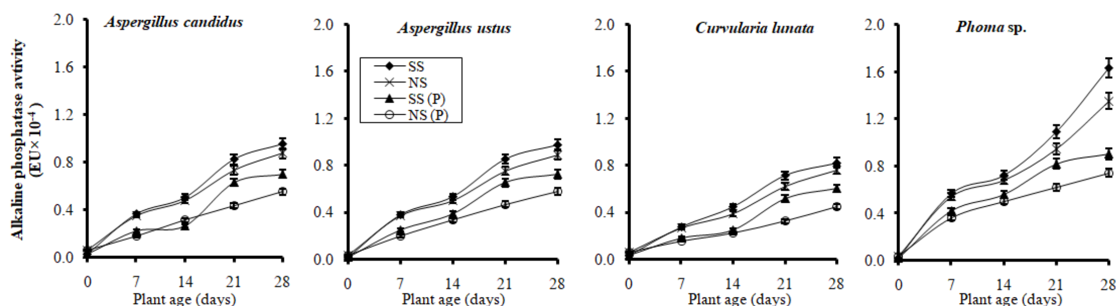

Fig. 3. Alkaline phosphatase activity after inoculation of four different fungi. Bar represent standard errors of mean. SS: Sterilized soil; NS: Non-sterilized soil; SS(P): Sterilized soil with plant; NS(P): Non-sterilized soil with plant.

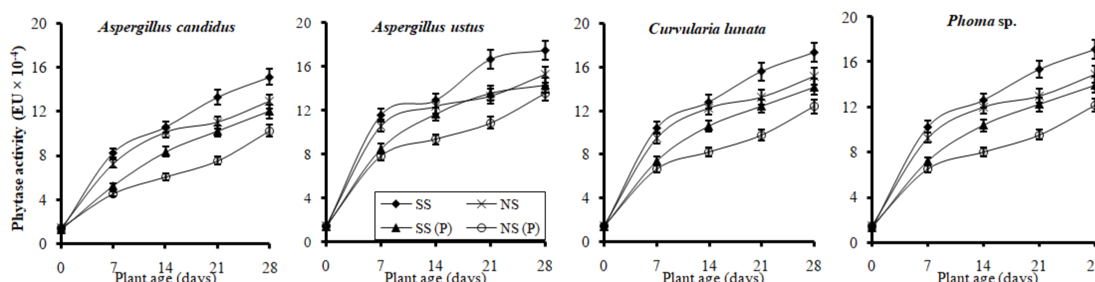

Fig. 4. Phytase activity after inoculation of four different fungi. Bar represent standard errors of mean. SS: Sterilized soil; NS: Non-sterilized soil; SS(P): Sterilized soil with plant; NS(P): Non-sterilized soil with plant.

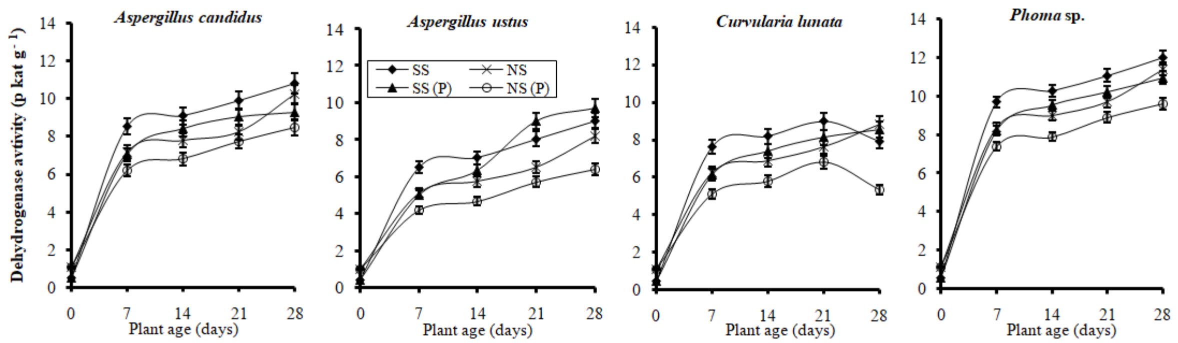

Fig. 5. Dehydrogenase activity of wheat rhizosphere after inoculation of four different fungi. Bar represent standard errors of mean. SS: Sterilized soil; NS: Non-sterilized soil; SS(P): Sterilized soil with plant; NS(P): Non-sterilized soil with plant.

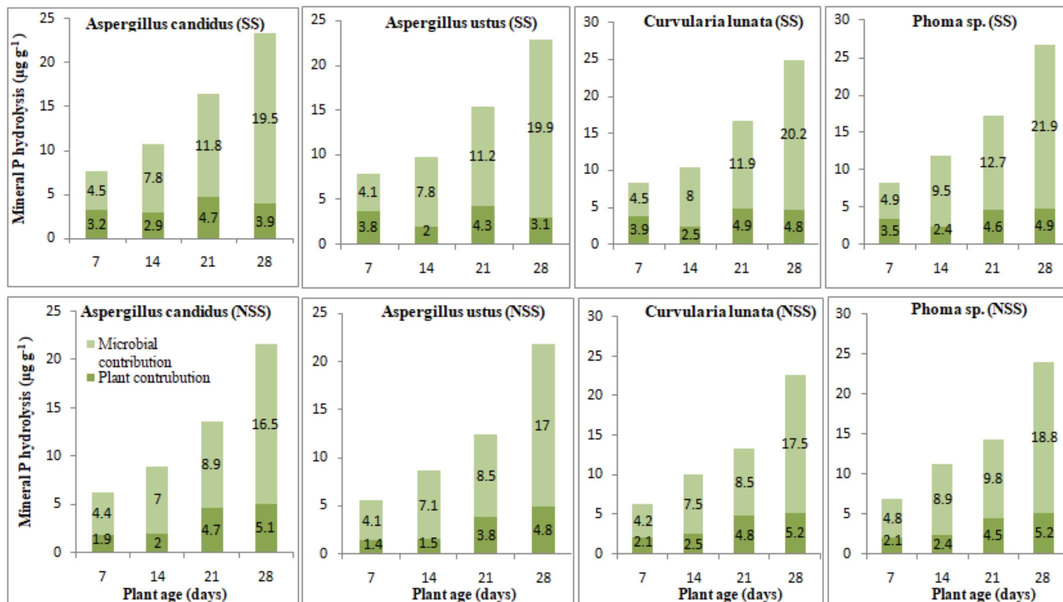

Fig. 6. Partition of wheat plant and fungal contribution in native mineral P hydrolysis under different soil conditions with plant age. SS: Sterilized soil; NSS: Non-sterilized soil.

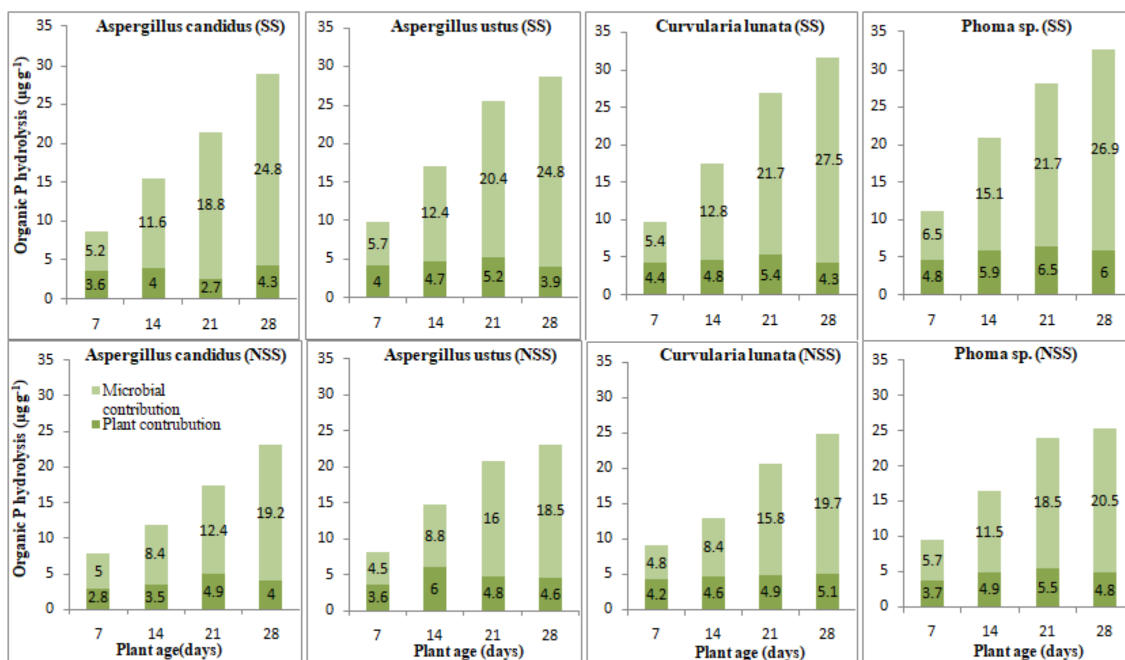

Fig. 7. Partition of wheat plant and fungal contribution in native organic Phydrolysis under different soil conditions with plant age. SS: Sterilized soil; NSS: Non-sterilized soil.

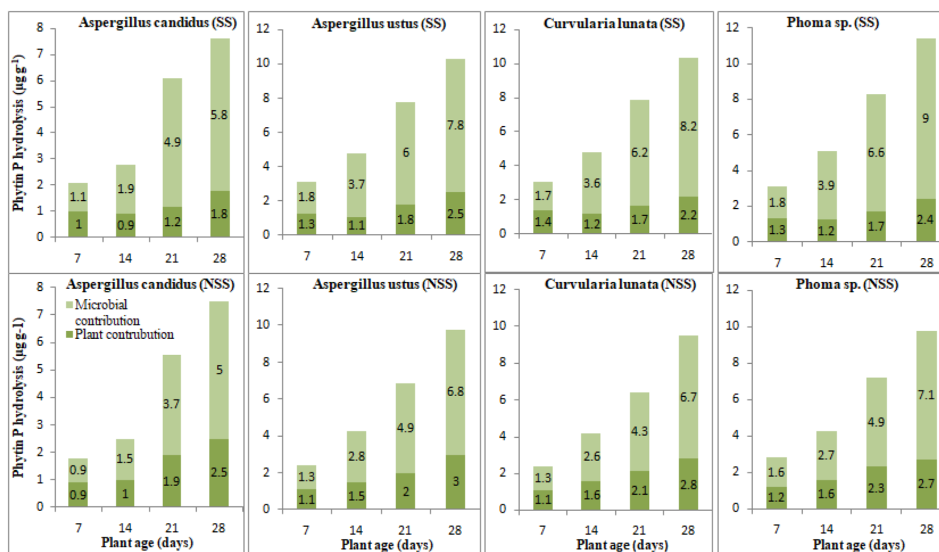

Fig. 8. Partition of wheat plant and fungal contribution in native Phytin P hydrolysis under different soil conditions with plant age. SS: Sterilized soil; NSS: Non-sterilized soil.

Table 1: Initial characteristics of soil used in experiment.

Parameter	Characteristics*
pH (soil : water, 1:25)	7.5±0.06
EC (dSm^{-1})	0.22±0.01
Organic matter (%)	0.24±0.01
Sand (%)	85.5±0.01
Silt (%)	5.8±0.01
Clay (%)	8.1±0.06
Total P (mg kg-1)	1269±15.5
Mineral P (mg kg-1)	889.6±9.4
Organic P (mg kg-1)	369.2±6.1
Olsen's P (mg kg-1)	10.9±1.5
Water soluble Pi (mg kg-1)	1.9±0.02
Phytin P (mg kg-1)	257.8±8.4
Acid phosphatase activity (EU × 10-4)	0.04±0.01
Alkaline phosphatase activity (EU × 10-4)	0.07±0.01
Phytase activity (EU × 10-4)	1.40±0.16
Dehydrogenase activity (p kat g-1)	1.09±0.21
Fungi (× 10-4)	15±1.5
Bacteria (× 10-4)	156±10.5
Actinomycetes (× 10-4)	110±10.2
* Mean value, ± indicate the standard errors of mean.	

Table 2: Gradual increase in plant biomass of wheat under sterilized and non-sterilized soil condition due to inoculation of four efficient selected fungi (± indicate standard error of mean).

Fungi	Plant biomass (mg $plant^{-1}$)							
	Sterilized soil				Non-sterilized soil			
	Crop age (days)				Crop age (days)			
	7	14	21	28	7	14	21	28
Control	204±12	301±15	352±18	484±20	198±10	282±14	324±18	439±20
Aspergillus candidus	245±17	360±21	470±24	600±48	220±12	321±18	430±21	530±25
Aspergillus ustus	263±16	379±25	480±28	613±49	241±20	340±19	440±22	545±31
Curvularialunata	268±14	392±26	493±32	630±51	240±21	345±22	448±29	552±34
Phoma sp.	276±18	393±28	495±40	643±55	259±22	351±25	452±31	578±39

Table 3: Gradual increase in root length of wheat under sterilized and non-sterilized soil condition due to inoculation of four efficient selected fungi (± indicate standard error of mean).

Fungi	Root length (cm $plant^{-1}$)							
	Sterilized soil				Non-sterilized soil			
	Crop age (days)				Crop age (days)			
	7	14	21	28	7	14	21	28
Control	250±14	272±16	290±19	321±22	241±13	258±17	271±18	314±20
Aspergillus candidus	262±17	292±18	342±25	398±24	250±19	274±15	314±20	379±27
Aspergillus ustus	275±19	312±22	365±26	409±33	253±21	280±19	344±25	398±29
Curvularialunata	279±21	325±25	380±30	431±30	258±20	302±21	358±24	413±31
Phoma sp.	284±21	339±25	392±32	454±35	262±21	310±25	367±26	438±32

Table 4: Plant P content of wheat with time under sterilized and non-sterilized soil condition due to inoculation of four efficient fungal species (± indicate standard error of mean).

Fungi	P content (μ mol plant^{-1})							
	Sterilized soil				Non-sterilized soil			
	Crop age (days)				Crop age (days)			
	7	14	21	28	7	14	21	28
Control	89±2.7	119±10.4	136±10.7	139±10.5	114±9.7	137±9.5	156±11.2	160±12.5
Aspergillus candidus	171±11.5	198±11.5	214±12.3	218±12.9	119±8.5	149±14.3	168±13.5	175±10.3
Aspergillus ustus	167±12.2	197±12.9	215±14.3	220±13.5	122±10.0	157±11.5	179±14.3	185±12.9
Curvularialunata	169±14.9	200±15.6	217±13.9	222±15.4	120±10.3	156±14.0	178±15.0	184±14.3
Phoma sp.	174±15.7	206±16.0	226±15.7	231±15.8	125±12.5	164±15.8	189±16.0	197±15.1

Table 5: Relative growth rate (Kr) for root of wheat under sterilized and non-sterilized soil due to inoculation of four efficient fungi (± indicate standard error of mean).

Fungi	Relative root growth (cm s^{-1} × 10^{-8})					
	Sterilized soil			Non-sterilized soil		
	Crop age (days)			Crop age (days)		
	7-14	14-21	21-28	7-14	14-21	21-28
Control	13.94±1.0	10.59±1.5	16.79±1.8	11.27±1.1	8.13±0.8	24.35±2.1
Aspergillus candidus	17.92±1.7	26.13±2.1	25.07±1.9	15.16±1.6	22.53±1.7	31.11±2.9
Aspergillus ustus	20.87±1.8	25.94±2.9	18.82±2.1	16.77±1.9	34.04±2.2	24.11±2.7
Curvularialunata	25.23±1.9	25.85±2.7	20.82±2.8	26.04±1.8	28.13±1.9	23.63±2.6
Phoma sp.	29.27±1.6	24.02±2.2	24.28±2.4	27.81±2.0	27.91±2.0	29.24±2.8

Table 6: P-influx of wheat under sterilized and non-sterilized soil due to inoculation of four efficient fungi (± indicate standard error of mean)

Fungi	P influx (mole cm s^{-1} ^{1}× 10^{-14})					
	Sterilized soil			Non-sterilized soil		
	Crop age (days)			Crop age (days)		
	7-14	14-21	21-28	7-14	14-21	21-28
Control	15.31±2.12	10.01±0.92	1.62±0.02	19.89±1.51	11.88±1.31	2.26±0.26
Aspergillus candidus	16.13±3.14	8.36±0.75	1.79±0.01	20.51±1.72	10.70±1.08	3.35±0.29
Aspergillus ustus	16.91±4.13	8.81±0.87	2.13±0.03	21.73±1.82	11.70±1.10	2.68±0.21
Curvularialunata	17.00±6.30	7.99±0.69	2.04±0.04	21.30±2.10	11.05±1.15	2.58±0.20
Phoma sp.	17.03±6.90	9.06±0.98	1.96±0.03	22.60±1.95	12.24±1.08	3.29±0.31

CONCLUSIONS

The present result clearly demonstrated the role of phosphatase solubilizing/mobilizing microorganisms for utilizing of different unavailable native P sources for plant nutrition. Our results also clearly partitioned the gradual plant and microbial contribution towards the exploitation of P from different native P sources. It was also clear from our result that *Aspergillus ustus* can be used in future for mineral P exploitation from the soil dominant in mineral P and *Phoma species* may be recommended for organic P exploitation from the soil dominant in organic P for plant nutrition.

FUTURE SCOPE

There is enormous scope to find out some more fungi and their role in exploitation of native P. *Aspergillus ustus* and *Phoma species* can be used for large scale production by the industry as future inoculums.

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Conflict of Interest. None.

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