INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
22–0071/2022/27–4–284–288
DOI: 10.17957/IJAB/15.1927
http://www.fspublishers.org

Full Length Article

Enhancing Phosphorus Bioavailability in Maize through Phosphorus Solubilizing Fungi

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Received 11 February 2022; Accepted 09 April 2022; Published 30 April 2022

Abstract

This study was to explore the beneficial interactive effects of fungal inoculum alone or in conjunction with phosphatic fertilizer on maize (*Zea mays* L.). We conducted a greenhouse pot experiment in soil which included four treatments such as control, *Penecillium oxalicum*, *Aspergiilus niger*, *P. oxalicum* + *A. niger* at three different levels of phosphatic fertilizers (0, 100, and 200 mg kg^{-1}). The plant height, fresh weight of root/shoot biomass and dry weight of root/shoot biomass and soil chemical properties were recorded. We evaluated the growth parameters of maize and phosphorus (P) uptake in maize were highest in treatment $I_3 \times F_2$ (*Penecillium*+*Aspergillus* + 200 mg P kg^{-1}) followed by $I_2 \times F_2$ (*Aspergillus* + 200 mg P kg^{-1}) over rest of the treatments. The beneficial effect was decreased where alone fertilizer dose was added as compared to those where it was added with the co-inoculation of phosphorus solubilizing fungi (PSF). It was observed that soil pH, total organic carbon and available P significantly changed because of various treatments. Based on these evidences, it could be inferred that increasing the maize growth by fungal culture improves soil P fertility, which is favorable to succeeding crops. Further research is needed to understand the chemistry of co-inoculation of fungal inoculum with phosphatic fertilizer.

Keywords: Phosphorus; Bioavailability; Fungi; Phosphatic fertilizers; Maize

Introduction

Phosphorus (P) is the second most crucial macronutrient after nitrogen, and due to its low bio availability in soil, limits plant growth and development (Anand *et al.* 2016). Many physiological processes, such as photosynthesis, respiration, and cell division, depend on plants *via* P availability. It also aids in the formation of a more profound and more abundant root system (Farooq *et al.* 2009). Phosphate anions, such as primary orthophosphate ($H_2PO_4^-$) or secondary orthophosphate (HPO_4^{2-}), are used by plants to obtain phosphate anions, on the other hand, they are highly reactive and could be immobilized through precipitation with cations such as Ca^{2+}, Mg^{2+}, $Fe^{3+,}$ and Al^{3+}, depending on the specific properties of the soil. Phosphate is exceedingly insoluble and inaccessible to plants in these forms, which consequences a minimal amount of P available to plants (Sharma *et al.* 2011).

Despite the widespread deficiency of soil P (Pradhan and Sukla 2006; Richardson and Simpson 2011), plants are usually unable to obtain it in adequate quantities, even in fertile soils (Talboys *et al.* 2016). Athar (2005) and Fatima *et al.* (2006) reported that 90% of the soils have P deficient in Pakistan. However, there is a need to overcome this problem for getting high yield in crop production. Chemical P fertilizers are often used to minimize the P shortage issues and increase plant yield; however, phosphatic fertilizers are costly. The bulk of P applied in phosphatic fertilizer is transformed to insoluble forms (Chen *et al.* 2006). As a result, only a small portion of the fertilizer supply reaches the plant roots. Excessive use of phosphatic fertilizers has resulted in a significant buildup of P in most agricultural soils. Phosphorus recovery from phosphatic fertilizer, on the other hand, is often just 10 to 30% (Schröder *et al.* 2011). To achieve sustainable agriculture, it is vital to limit the use of chemical fertilizers while providing the necessary P by other methods. In this context, studies on the efficacy of biological fertilizers containing the needed quantity of P for diverse crops are particularly important.

Despite the widespread deficiency of soil P (Pradhan

To cite this paper: Khan M, A Latif, M Arsalan, M Aziz, R Ullah, M Bilal, W Asghar, R Latif, M Ehsan, MT Mehmood (2022). Enhancing phosphorus bioavailability in maize through phosphorus solubilizing fungi. *Intl J Agric Biol* 27:2843–288

and Sukla 2006; Richardson and Simpson 2011), plants are usually unable to obtain it in adequate quantities even in fertile soils (Talboys *et al*. 2016). In Pakistani soils, almost 90% of the soils are P deficient (Athar 2005; Fatima *et al*. 2006). Chemical P fertilizers are often used to address P shortage issues and increase plant yield, however phosphatic fertilizers are costly. The bulk of P applied in the form of phosphatic fertilizer is transformed to insoluble forms (Chen *et al.* 2006). On the other hand, biological fertilizers containing the needed quantity of P for diverse crops seems to be a cheap and environmental friendly option.

Phosphorus is essential to growth, and microorganisms are fierce competitors for the nutrient. In addition to compensating for the higher cost of fertilizer production, they also activate the fertilizer applied to the soil (Pradhan and Sukla 2006). Phosphorous solubilizing micro-organisms (PSMs) include a variety of fungi, bacteria, *actinomycetes*, and yeast due to their capacity to solubilize sparingly soluble P (Wani *et al.* 2007). PSMs are involved in numerous mechanisms for phosphate solubilization in the growth environment, including acid generation that lowers pH, exchange reactions, and ion chelation (Khan *et al*. 2009).

Fungi are a major component in the solubilization of insoluble P by generating by releasing organic acids such as lactic, citric, 2-ketogluconic, glycolic malic, malonic, oxalic, succinic and tartaric acids, all of which have chelating properties (Krishnaraj *et al*. 2014), and also by decreasing pH (Seshadri *et al*. 2004). According to Pradhan and Sukla (2006), *Aspergillus* and *Penecillium* strains exhibit varying amounts of phosphate solubilization activity in liquid broth culture in the presence of different carbon and nitrogen sources. Dual inoculation of these strains also has a significant effect on increasing yield of soybeans plant (Soe *et al*. 2012). However, there is not much literature available on the inoculation of phosphorus solubilizing fungi (PSF) and phosphatic fertilizer on maize crop. This study was conducted with the objective to compare P absorption by maize and to evaluate the impact of PSF on soil and plant growth parameters.

Materials and Methods

Soil collection and preparation

Soil was taken from the field surface (0–15 cm) at experimental area of PMAS-Arid Agriculture University Rawalpindi. A composite soil sample was drawn from the whole soil lot and analyzed for soil texture, soil moisture content, pH, ECe, bulk density, calcium carbonate, total organic carbon (TOC), exchangeable K and available phosphorous.

Fungal strains selection, preparation and application of PSF inoculants

Two strains of fungi (*Aspergillus* and *Penicillium*) were selected based on of their high P solubilizing efficiency and obtained from the Department of Soil Science, PMAS-Arid Agriculture University, Rawalpindi. These strains were cultured in 250 mL broth by using reciprocal shaker at 200 rpm for 4–5 days. Before seed sowing, ten milliliters of each fungal strain (3.93×10^5 CFU/mL) was inoculated with irrigation water (500 mL) to each pot (6 kg) of respective treatment kept under greenhouse. Maize variety "NARC-2704" was used in this pot experiment. Seven seeds of maize were sown at 2 cm depth of earthen pots. The distance between two plants was maintained by thinning and keeping five seedlings per pot after ten days of sowing.

Experimental layout and treatments

It was a two factors factorial experiment arranged in the completely randomized design (CRD) with three replications. Treatments were inoculum strains such as I_0 = Control, I_1 = *P. oxalicum*, I_2 = *A. niger* and I_3 = *P. oxalicum* + *A. niger*, and phosphorus fertilizer levels such as F_0= Control, F_1 = Fertilizer @ 100 mg P kg^{-1} soil and F_2 = Fertilizer @ 200 mg P kg^{-1} soil.

Basic dose of N and K fertilizers were applied at 120 and 50 mg kg^{-1}, respectively. The sources for N, P, and K were used as urea, single super phosphate, and murate of potash, respectively. To control insects and pests of maize carbofuran 7G was applied at 5 g/pot. Pots were irrigated with tap water according to the need of crop. At flowering stage, the data were recorded on crop growth parameters. The plant samples were collected and analyzed for total phosphorus.

Data recording

Data on plant growth parameters such as plant height, dry weight of shoot/root, and dried weight of shoot/root and root length were collected. From each pot plant height was measured in centimeters with the help of meter rod and then averaged. From each pot fresh weight of shoot and root was determined in grams with the help of weighing balance. Shoot and root sample were placed in oven at 65°C for 48 h and dry matter yield was recorded.

Plant analysis for phosphorus

Total phosphorus was determined from plant samples through dry ashing. For this, plant sample were washed, air dried and dried in oven for 24 h at 60°C. Samples were ground and then again dried at 60°C to obtain constant weight prior to sample preparation. The concentration of phosphorus was determined using this curve through spectrophotometer reading at 440 nm (Chapman and Pratt 1961).

$$\mathrm{P}\ (\%) = \frac{\text{ppm P (from calibration curve)} \times R \times 100}{\text{Wt} \times 10000}$$

Where, R = Ratio between total volume of the aliquot and the aliquot volume, W_t = Weight of dry plant sample (g).

Soil characterization

Soil was analyzed for the following characteristics: Soil moisture content, particle size distribution, soil pH, electrical conductivity, available phosphorus, TOC, calcium carbonate (Table 1).

Statistical analysis

This experiment was conducted using a completely randomized design (CRD). The acquired data was statistically analyzed using the analysis of variance (ANOVA) technique and the mean value was compared using the least significant difference (LSD) test at a probability level of 5%.

Results

Plant height

The results of interactive effect of inoculation and phosphorus fertilizer showed that $I_3 \times F_2$ (*Penecillium*+*Aspergillus* + 200 mg P kg^{-1}) recorded the highest plant height (137.67 cm) followed by $I_2 \times F_2$, and $I_1 \times F_2$. The $I_0 \times F_0$ recorded the minimum value of plant height (106.67 cm) (Table 2).

Dry weight of shoot and root

The results of interactive effect of inoculation and phosphorus fertilizer showed that $I_3 \times F_2$ (*Penecillium* + *Aspergillus* + 200 mg P kg^{-1}) recorded the highest dry weight of shoot and root (7.67 g and 0.56 g, respectively), followed by $I_2 \times F_2$, and $I_1 \times F_2$. The $I_0 \times F_0$ recorded the minimum value of dry weight of shoot and root (3.03 g and 0.13 g, respectively) (Table 2).

Root length

The results of interactive effect of inoculation and phosphorus fertilizer showed that $I_3 \times F_2$ (*Penecillium* + *Aspergillus* + 200 mg P kg^{-1}) recorded the highest root length (30.67 cm), followed by $I_2 \times F_2$, and $I_1 \times F_2$. The $I_0 \times F_0$ recorded the minimum value of root length (7.67 cm) (Table 3).

Total phosphorus of plant

The results of interactive effect of inoculation and phosphorus fertilizer showed that $I_3 \times F_2$ (*Penecillium* + *Aspergillus* + 200 mg P kg^{-1}) with a value of 0.28% proved best in P uptake followed by $I_2 \times F_2$, and $I_1 \times F_2$. The minimum value of P uptake (0.2%) was observed where $I_0 \times F_0$ recorded were treated. The results of individual effect of inoculation showed that I_3 recorded the highest P uptake (0.25%), followed by I_2 and I_1. The minimum P uptake was observed in I_0 (0.21%) (Table 3).

Table 1: Physico-chemical properties of soil before the start of experiment

Characteristics	Values
Clay	15%
Sand	71%
Silt	14%
Texture	Sandy loam
EC_e	0.314 dS m^{-1}
Soil pH	7.61
Soil Moisture	14.6%
Bulk Density	1.40 Mg m^{-3}
Available P	7.63 μg g^{-1}
Exchangeable K	129 μg g^{-1}
Calcium carbonate	0.139 g kg^{-1}
Total Organic Carbon	0.50%

EC_e= Electrical conductivity extract, P = Phosphorous, K= Potassium

Table 2: Interactive effect of phosphatic fertilizer and P solubilizing fungal inoculum on plant height (cm), shoot and root dry weight (g) of maize

Parameter of maize plant	Inoculum (I)	Fertilizer levels		
		F_0	F_1	F_2
Plant height	I_0	106.67 f	102.67 g	132.00 b
	I_1	94.67 h	114.67 de	133.00 b
	I_2	112.33 e	115.33 d	133.33 b
	I_3	116.33 cd	118.67 c	137.67 a
Shoot dry weight				
	I_0	3.03 j	3.67 i	6.30 d
	I_1	3.83 h	4.83 g	6.57 c
	I_2	4.83 g	5.53 f	7.20 b
	I_3	6.00 e	6.23 d	7.67 a
Root dry weight				
	I_0	0.13 d	0.20 cd	0.43 ab
	I_1	0.30 bc	0.33 bc	0.36 bc
	I_2	0.30 bc	0.36 bc	0.46 ab
	I_3	0.40 ab	0.43 ab	0.56 a

Table 3: Interactive effect of phosphatic fertilizer and P solubilizing fungal inoculum on root length (cm) Phosphorous contents (%) of maize plants of maize

Parameter of maize plant	Inoculum (I)	Fertilizer levels		
		F_0	F_1	F_2
Root length	I_0	7.67 i	11.67 h	20.00 d
	I_1	13.33 g	15.00 f	25.67 c
	I_2	13.33 g	16.67 e	27.67 b
	I_3	19.33 d	19.00 d	30.67 a
Phosphorous contents (%)	I_0	0.2 d	0.23 d	0.23 d
	I_1	0.21 f	0.21 f	0.25 c
	I_2	0. 22 e	0.22 e	0.26 b
	I_3	0.23 d	0.24 cd	0.28 a

Change in soil pH, TOC and available P after crop harvesting

The results of interactive effect of inoculation and phosphorus fertilizer showed that $I_3 \times F_2$ recorded the maximum change % in soil pH, TOC and available P (19.71, 30.0 and 38.40%), followed by $I_2 \times F_2$, and $I_1 \times F_2$. The $I_0 \times F_0$ recorded the minimum change % in pH, TOC and available P (1.5, 4.0 and 1.70%) Table 4.

Table 4: Effect of different fungal inoculum and phosphorous rates on soil pH, TOC and available P

Treatments	pH	Change after harvest (%)	TOC (%)	Change after harvest (%)	Available P	Change after harvest (%)
$I_o \times F_o$ (Control)	7.53	1.05	0.52	4	7.5	1.70
$I_1 \times F_o$ (*Penecillium spp.*)	6.91	9.19	0.55	10	7.8	2.22
$I_2 \times F_o$ (*Aspergillus spp.*)	6.82	10.38	0.55	10	7.9	3.53
$I_3 \times F_o$ (*Penecillium + Aspergills)*	6.54	14.06	0.6	20	8.2	7.47
$I_o \times F_1$(100 mg P kg^{-1})	7.21	5.25	0.54	8	8.0	4.84
$I_1 \times F_1$ (*Penecillium* + 100 mg P kg^{-1})	6.72	11.69	0.55	10	8.4	10.09
$I_2 \times F_1$ (*Aspergillus* + 100 mg P kg^{-1})	6.63	12.87	0.56	12	8.7	14.02
$I_3 \times F_1$ (*Penecillium* + *Aspergillus* + 100 mg P kg^{-1})	6.42	15.24	0.63	26	9.0	17.95
$I_o \times F_2$ (200 mg P kg^{-1})	7.12	6,43	0.54	8	9.2	20.57
$I_1 \times F_2$ (*Penecillium* + 200 mg P kg^{-1})	6.71	11.82	0.56	12	9.5	24.50
$I_2 \times F_2$ (*Aspergillus* + 200 mg P kg^{-1})	6.51	14.45	0.57	14	10.23	34.07
$I_3 \times F_2$ (*Penecillium* + *Aspergillus* + 200 mg P kg^{-1})	6.11	19.71	0.65	30	10.56	38.40

Discussion

In this study it was observed that the interaction PSF with P fertilizer (*Penecillium* + *Aspergillus* + 200 mg P kg^{-1}) had a profound effect on plant height, fresh weight of shoot/root, and dried weight of shoot/root and root length as compared to control. With the application of PSF, it can increase the soil P-available so that plant growth increase, this is because the application of PSF can produce organic acids that can chelate Al and Fe so that P is available for plants (Silitonga *et al*. 2019). The PSF strain with inorganic phosphatic fertilizer greatly influenced the plant height, which could be due to increased bioavailability of nutrients (K, N and P). These findings are consistent with Wu *et al*. (2005), who reported that combination inoculation of PSF and inorganic phosphatic fertilizer resulted in a significant increase in plant height of maize.

Similarly, the interactive effect of phosphatic fertilizer and PSF inoculum had significant effect on shoot and root dry weight. This is because plants are able to absorb P nutrients, with the increasing of P-available causing P content to also increase (Silitonga *et al*. 2019). These results were in agreement with the finding of Wakelin *et al*. (2007), who tested the combination of PSF inoculum (*P. radicum* and *P. bilaiae*) with phosphate fertilizer, and found a 15% increase in shoot growth and shoot dry matter of lentil crop. Similarly, Richa *et al*. (2007) found that when phosphatic fertilizer was combined with PSF inoculum, root dry weight was considerably higher than when fertilizer or fungus were applied alone.

The root length of maize crops at various levels of phosphatic fertilizer with various fungal inoculum combinations revealed the fact that interactive effect of both fertilizer and inoculum had the highest root length. With the application of phosphate solubilizing fungi, it can increase the soil P-available so that plant growth increase, this is because the application of phosphate solubilizing fungi can produce organic acids that can chelate Al and Fe so that P is available for plants (Silitonga *et al*. 2019). These results are in line with that of Wu *et al*. (2005), who stated that growth of maize plant increased by combined inoculation of PSF and chemical fertilizer include increase in plant height, plant weight and root length. The results regarding effect of individual fungal strains, rate of fertilizer application and combination of both factors on total P of maize crop are consistent with those of Wahid and Mehana (2000), who concluded that mixed inoculation of rock phosphate and super phosphate with PSF (*Penecillium*) increases the production of Faba bean (*Vicia faba* L.) and the P uptake. Wahid and Mehana were of the view that these strains secrete organic acid in soil that increases the plant uptake of P from a water soluble P.

The data after the maize crop has been harvested revealed that the interaction PSF with P fertilizer (*Penecillium* + *Aspergillus* + 200 mg P kg^{-1}) significantly altered the soil pH, TOC and available P as compared to control. The decrease in soil pH could be due to release of organic acids by PSF (He *et al.* 2002). These results are in accordance with the finding of Iman and Azouni (2008), who suggested that co inoculation of PSF with phosphate fertilizer cause decrease in pH as compared to single inoculation of these strains and fertilizer. Similarly, Caravac *et al*. (2004) found more available P, more water soluble carbon, and a lower soil pH when compared to the control soil upon the addition of phosphatic fertilizer and PSF.

Conclusion

The results of the study revealed that growth parameters of maize and P uptake were improved where PSF (*Aspergillus and Penecillium*) co-inoculated with phosphatic fertilizer at 200 mg kg^{-1}. The combined impacts of amendments were greater than the effects of individual amendments and control. Solubilized P increased soil fertility and as a result yield of maize was increased. Thus, it is concluded that the combined use of PSF and phosphatic fertilizer may increase the P uptake and yield of crops.

Acknowledgements

Not applicable to this article

Author Contributions

Madeeha Khan planned and performed the experiments**,** Abdul Latif assisted in research theme and writing, Muhammad Arsalan technical guidance**,** Marjan Aziza data analysis, Rehmat Ullah and Muhamad Bilal technical guidance**,** Waleed Asghar data analysis**,** Rizwan Latif, Muhammad Ehsan and Muhammad Tariq Mehmood data analysis.

Conflicts of Interest

Authors declare no conflicts of interests among institutions

Conflict of interest

The authors declare that they have no competing interests.

Data Availability

The datasets generated during the study are all included in the manuscript

Ethics Approval

Not applicable to this article

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