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Effect of phytase and phosphorus levels on soil phosphatase activity and nutrient availability in an inceptisol under fodder maize

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Abstract
The pot culture experiment was conducted to assess the 'Effect of phytase and phosphorus levels on soil phosphatase activity and nutrient availability in an Inceptisol under fodder maize' cultivation during *kharif* season, 2016-17 at Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri. The treatment consisted four levels of phytase (1200, 2400, 3600 and 4800 IU) and phosphorus (0, 25, 50 and 75 kg P_2O_5 ha^{-1}) with absolute control. Enzyme activity and nutrient availability in soil was assessed at 30 days after sowing and at harvest of fodder maize. It was observed that, application of phytase @ 4800 IU and 0 kg P_2O_5 ha^{-1} recorded significantly higher acid (14.74 and 15.83 µg PNP g^{-1} soil hr^{-1}) and alkaline phosphatase activity (27.81 and 29.09 µg PNP g^{-1} soil hr^{-1}, respectively) at 30 days after sowing then declined at harvest of fodder maize. The application of phytase @ 4800 IU and 75 kg P_2O_5 ha^{-1} recorded significantly higher available nitrogen (103.08 and 106.40 mg kg^{-1}), phosphorus (11.35 and 11.67 mg kg^{-1}) and potassium (185.63 and 186.50 mg kg^{-1}, respectively) at 30 days after sowing then declined at harvest. In general, the application of phytase @ 4800 IU and 00 kg P_2O_5 ha^{-1} significantly increased enzyme activity at 30 days after sowing then declined at harvest as well as significantly increased the available nutrient status at 30 days after sowing then declined at harvest by the application of phytase @ 4800 IU and 75 kg P_2O_5 ha^{-1}. This study suggests that, application of phytase and phosphorus increasing the enzyme activity as well as nutrient availability in soil 30 days after sowing and at harvest as compared to the initial status.

Keywords: Phytase, phosphorus levels, acid and alkaline phosphatase activity, available nutrient status

Introduction

Phosphorus is the second most essential major plant nutrient after nitrogen. It is least mobile and is unavailable to plants in most soil conditions. The P content in average soil is about 0.05% (w/w) but only 0.1% of total P is available to plants because of its low solubility and its fixation in soil (Scheffer and Schachtschabel, 1992) [15]. There is no atmospheric source of P that could be made available to plants (Ezawa *et al.*, 2002) [3]. Phosphorus is added in soil through application of chemical fertilizers but major part of it gets fixed in the soil, and only 15-20% is utilized by the crops (Gaur, 1985). In acidic, calcareous or normal soils, P reacts with reactive metals such as Al^{3+}, Fe^{3+} and Ca^{2+}, there by becoming unavailable for plant uptake (Gyaneshwar *et al.*, 2002) [7]. The transformation of phosphorus in soil is dependent upon a large number of factors *viz.*, soil pH, other nutrients, organic matter, types of clay, soil temperature, aeration, moisture, crop species and time of applications. Soil P exists both in organic and inorganic form, the organic P compounds can be classified into i) inositol phosphate plant origin comprising up to 60% of soil organic P, ii) the nucleic acids, and iii) phospholipids. In soils 20-85% of the total P is in organic form but plants can only utilize this organic P after its mineralization. The importance of soil organic P as a source of plant available P depends on its rate of solubilization and release of inorganic P.

Phytases (myo-inositol hexakisphosphate phosphohydrolases) are enzymes that catalyze mineralization – conversion of organic P from phytate to inorganic P, which can be readily taken up by the plants (Yadav and Tarafdar, 2007) [26]. Phosphatase and phytase which catalyzes hydrolytic cleavage of the C-O-P ester bond of organic P present in soil and release plant available inorganic forms (Yadav and Tarafdar, 2004) [25]. George *et al.*, (2007) [5] demonstrated three discrete categories of phytase on the basis of protein sequencing and the succession of dephosphorylation of phosphate moieties (myo-inositol-hexaphosphate

Phosphohydrolases, i.e. EC 3.1.3.8, EC 3.1.3.26 and EC 3.1.3.72). P occurs at three and six positions, respectively. Their names were derived from three and six phosphate bonds of myo-IP6. Usually 3-phytases are found in microorganisms and filamentous fungi while 6-phytases are in plant tissues. The enzyme 3-phytase (EC 3.1.3.8) obtained from *Aspergillus sp.* and 6-phytase (EC 3.1.3.26) from wheat.

Maize (*Zea mays* L.) is a member of family Gramineae, and its rank third position after wheat and rice throughout the world. The crop has a wide range of uses as forage to feed animals. About 60 per cent of the global harvest of maize is fed to livestock (Dowsell *et al.*, 1996) [2]. It is a miracle crop. Maize (*Zea mays*) is an ideal forage crop grown throughout the country; it is quick growing high yielding and supplies palatable and nutritious forage which can be fed at any stage of growth without any risk to animals. Maize is an exhaustive crop having higher potential than other cereals and absorbs large quantity of nutrients from the soil during different growth stages. The information about enzymatic mineralization of P along with other nutrients in black soil for fodder maize through phytase enzyme in presence of phosphorus fertilizers is not available in literature. Hence, a pot culture experiments was carried out to assess the effect of phytase and phosphorus levels on soil phosphatase activity and nutrient availability in an Inceptisol under maize cultivation.

Material and Methods

The pot culture experiment was conducted at Department of Soil Science and Agricultural Chemistry, Mahatma phule Krishi Vidyapeeth, Rahuri to study 'Effect of phytase and phosphorus levels on soil phosphatase activity and available nutrient status in an Inceptisol under fodder maize' during *kharif* 2016-17. The experiment was laid out in factorial completely randomized design with two replications and seventeen treatments, consisting combination of phytase @ 1200, 2400, 3600 and 4800 IU and phosphorus @ 0, 25, 50 and 75 kg P_2O_5 ha^{-1} levels with absolute control. The required quantity of surface soil of 0-15 cm depth was collected from Post Graduate Institute Research Farm and the quantity of FYM required for the experiment was procured from Department of Animal Husbandry and Dairy Science, Mahatma phule Krishi Vidyapeeth, Rahuri. The phytase isolated from *Aspergillus Niger* was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune, (MS) India. The enzyme unit was expressed as IU which is the amount of enzyme that liberates 1 µmol of inorganic orthophosphate from phytate per minute at pH 5.5 and 37 ^{0}C (Zyla *et al.* 1995) [27].

The thirty four earthen pots filled with 10 kg (2 mm sieved) black soil were used for experiments. At the time of filling the pots, treatment wise required quantity of FYM and fertilizer dose was thoroughly mixed with soil. Before sowing, treatment wise recommended dose of nitrogen and potassium was applied through urea and muriate of potash. Application of phosphorus (*viz.*, 0, 25, 50 and 75 kg P_2O_5 ha^{-1}, respectively) through single superphosphate was given to crop as per treatments. Thereafter, pots were irrigated. Dibbling of three maize seed equidistantly in the pot were carried out on the same day at field capacity. The maize seeds were sown on 23rd August, 2016. Thirty two pots each with P_2O_5 and phytase as per levels and one pot of absolute control. At field capacity, moisture of soil was maintained by irrigating the pots at an interval of four to six days starting from 2 to 3 days after sowing till the tasseling stage by using moisture meter. The maize plants after flowering were harvested for green fodder yield and nutrient uptake studies. The periodical enzyme activities and availability of nutrients in soil were analyzed by using standard methods at 30 days after sowing and at harvest of fodder maize. The acid and alkaline phosphatase activity in soil was assessed by using method advocated by Tabatabai and Bremner (1969) [18]. The soil $KMnO_4$-N, Olsen-P and N *N* NH_4OAc-K was analyzed by methods given by Subbiah and Asija (1956); Watanable and Olsen (1965) and Jackson (1973), respectively. The soil used in this study was higher in pH (8.36), medium in organic carbon (6.7 g kg^{-1}), low in available N (84.70 mg kg^{-1}), medium in available P (6.84 mg kg^{-1}), and very high in available K (162.00 mg kg^{-1}). Statistical analysis was done by following standard procedures by Panse and Sukhatme (1985) [11].

Result and Discussion

Effect on Soil phosphatase activities

The soil phosphatase activity was significantly influenced by the application of phytase and phosphorus levels at 30 days after sowing and at harvest are presented in table 1.

Acid phosphatase and alkaline phosphatase in soil was significantly increased by phytase and phosphorus levels imposed to fodder maize at 30 days after sowing and declined at harvest. Application of phytase @ 4800 IU recorded significantly higher acid phosphatase (14.74 and 10.72 µg PNP g^{-1} soil hr^{-1}) and alkaline phosphatase (27.81 and 23.05 µg PNP g^{-1} soil hr^{-1}) at 30 days after sowing and at harvest, respectively. The acid phosphatase (15.83 and 11.84 µg PNP g^{-1} soil hr^{-1}) and alkaline phosphatase (29.09 and 24.38 µg PNP g^{-1} soil hr^{-1}) was significantly higher by the application of 0 kg P_2O_5 ha^{-1} at 30 days after sowing and at harvest, respectively. The combined application of phytase and phosphorus showed non-significant to acid and alkaline phosphatase activity, but soil enzyme activity was significantly increased in treated pots as compared to control.

Higher soil acid phosphatase activity in FYM amended pots might be ascribed to enhanced microbial population and activity. Further these secret acid phosphatases which have positive effect on native P solubilization (Yadav and Verma, 2012). Dotaniya *et al.* (2014) [1] concluded that maximum acid phosphatase activities were observed at 75 DAT (days after transplanting) in rice (0.206 mg PNP g^{-1} soil h^{-1}), 90 DAS in sorghum (0.194 mg PNP g^{-1}soil h^{-1}) and pearl millet (0.201 mg PNP g^{-1}soilh^{-1}) 50 DAS in soybean (0.127 mg PNP g^{-1} soil h^{-1}). But alkaline phosphatase activities were maximum at 75 DAS in all the crops; i.e. rice, sorghum, pearl millet, soybean, respectively. Further, they concluded that, enzyme activities are associated with higher availability and uptake of phosphorus.

Higher alkaline phosphatase activity in alkaline calcareous soil was reported by Kramer and Green, (2000) [9] as against acid phosphatase which is higher in acidic soils. Similar results were also reported by Wang *et al.* (2011) [23] that activity of acid phosphatase is predominant in acid soils whereas, alkaline phosphatase is in alkaline soil. Further, Tarafdar and Chhonkar, (1978) [19] reported that, soil fungi are effective producers of alkaline phosphatase. Higher alkaline phosphatase activity and significantly high fungal population under legumes than cereals reported for arid soils in India by Tarafdar *et al.* (1989) [21]. Manna *et al.* (2007) [10] reported that, the activity of alkaline phosphatase was significantly increased (12-67%) by the application of FYM (4-16 Mg ha^{-1}) compared to control.

Table 1: Effect of phytase and phosphorus levels on enzyme activity in soil at 30 DAS and at harvest of fodder maize

Treatment	Acid phosphatase (μg PNP g^{-1} soil hr^{-1})		Alkaline phosphatase	
	30 DAS	At harvest	30 DAS	At harvest
Phytase levels (P)				
P_1 (1200 IU)	13.69	9.35	25.61	22.08
P_2 (2400 IU)	13.84	9.66	26.17	22.19
P_3 (3600 IU)	14.34	10.33	27.08	22.31
P_4 (4800 IU)	14.74	10.72	27.81	23.05
S.Em. ±	0.15	0.07	0.058	0.14
C.D at 5 (%)	0.43	0.20	0.16	0.42
P_2O_5 levels (F)				
F_1 (00 kg ha^{-1})	15.83	11.84	29.09	24.38
F_2 (25 kg ha^{-1})	14.40	10.35	27.14	22.83
F_3 (50 kg ha^{-1})	13.55	9.28	26.18	21.77
F_4 (75 kg ha^{-1})	12.82	8.59	24.26	20.66
S.Em. ±	0.15	0.07	0.058	0.14
C.D at 5 (%)	0.43	0.20	0.16	0.42
In.(P x F)				
P_1F_1	15.11	11.08	28.20	24.26
P_1F_2	13.69	9.62	25.83	23.01
P_1F_3	13.47	8.56	25.26	21.16
P_1F_4	12.49	8.13	23.14	19.92
P_2F_1	15.67	11.40	28.97	24.73
P_2F_2	14.04	10.10	26.26	21.99
P_2F_3	13.21	9.00	25.92	21.46
P_2F_4	12.44	8.17	23.54	20.57
P_3F_1	16.08	12.16	29.32	23.78
P_3F_2	14.67	10.84	27.78	22.56
P_3F_3	13.57	9.50	26.44	22.00
P_3F_4	13.05	8.82	24.78	20.92
P_4F_1	16.48	12.71	29.86	24.76
P_4F_2	15.23	10.84	28.70	23.75
P_4F_3	13.98	10.08	27.10	22.46
P_4F_4	13.30	9.25	25.60	21.24
S.Em. ±	0.30	0.14	0.12	0.30
C.D at 5 (%)	NS	NS	0.33	NS
Tr. Vs Control				
Treated	14.15	10.01	26.67	22.41
Control	7.76	7.59	16.86	14.48
S.Em. ±	0.31	0.14	0.11	0.30
C.D at 5 (%)	0.89	0.42	0.34	0.87
Initial	6.72		11.93	

*DAS- Days after sowing

Effect on nutrient availability

The soil available nitrogen, phosphorus and potassium was significantly influenced by the application of phytase and phosphorus levels at 30 days after sowing and at harvest are presented in table 2.

Available nitrogen

The application of phytase @ 4800 IU recorded higher soil available N (103.08 and 87.15 mg kg^{-1}) and application of 75 kg P_2O_5 ha^{-1} recorded the higher soil available nitrogen (106.40 and 88.90 mg kg^{-1}) at 30 days after sowing and at harvest, respectively. The combined application of phytase and phosphorus recorded non-significant for the available nitrogen. However, the soil available nitrogen was numerically increased upto 30 days after sowing and decreased at harvest in all the levels of phytase enzyme. This might be associated with to release of organically bound phosphorus by phytase enzyme, simultaneously the carbon also released from organic matter in which phosphorus was bound. These released organic carbon act as source of energy to microorganisms responsible for nitrogen release and fixing the atmospheric nitrogen in soil.

The increase in nitrogen availability in soil can be attributed to release of ammonium ions bound to organic phosphate degradation by phytase. Similar results were reported by Gujar *et al.* (2013) [6]. The decreases in soil available nitrogen might be because of uptake of nitrogen by crops for growth and development. Reduction in soil available nitrogen may be due to microbial immobilization. Similar results were also reported by Suresh and Suryaprabha (2005) [17].

Available phosphorus

Result revealed that use of phytase and phosphorus levels increased soil available phosphorus at 30 days after sowing and at harvest over initial. The application of phytase @ 4800 IU recorded higher soil available P (11.35 and 9.27 mg kg^{-1}) and application of 75 kg P_2O_5 ha^{-1} recorded the higher soil available nitrogen (11.67 and 9.82 mg kg^{-1}) at 30 days after sowing and at harvest, respectively. The combine application of phytase and phosphorus showed non-significant for available phosphorus at 30 days after sowing, but significant at harvest by the application of phytase @ 4800 IU and 75 kg P_2O_5 ha^{-1} (10.27 mg kg^{-1}). The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the soil available phosphorus also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to increase the phosphorus in soil. Higher availability of phosphorus in soil with individual phytase, phosphorus levels and their combine application to pots might be due to plants and micro-organisms increases exudation of phosphorus hydrolyzing enzymes. These enzymes breakdown organic phosphorus thus, making more phosphorus available. Further, phytase catalyzes the organic phosphorus present in soil in the form of inositol hexaphosphate (major form of organic phosphorus in soil). Application of phytase along with phosphorus levels might have played key role in enhancing microbial population and their activity there by exudation of microbial phytase causing higher availability of soil phosphorus.

Table 2: Effect of phytase and phosphorus levels on available nitrogen, phosphorus and potassium in soil at 30 DAS and at harvest of fodder maize

Treatment	Available N (mg kg^{-1})		Available P (mg kg^{-1})		Available K (mg kg^{-1})	
	30 DAS	At harvest	30 DAS	At harvest	30 DAS	At harvest
Phytase levels (P)						
P_1 (1200 IU)	97.65	82.78	10.16	8.52	177.38	168.63
P_2 (2400 IU)	99.05	84.70	10.58	8.92	181.38	172.63
P_3 (3600 IU)	101.33	86.10	10.79	8.95	183.88	174.63
P_4 (4800 IU)	103.08	87.15	11.35	9.27	185.63	176.00
SEm±	0.44	0.47	0.069	0.025	0.35	0.32
C.D at 5 (%)	1.29	1.35	0.19	0.07	1.03	0.92

P_2O_5 levels (F)						
F_1 (00 kg ha^{-1})	93.45	81.38	9.03	7.60	176.00	167.25
F_2 (25 kg ha^{-1})	96.25	82.95	10.82	8.87	181.56	172.81
F_3 (50 kg ha^{-1})	105.0	87.50	11.36	9.36	184.19	174.50
F_4 (75 kg ha^{-1})	106.40	88.90	11.67	9.82	186.50	177.31
SEm±	0.44	0.47	0.069	0.025	0.35	0.32
C.D at 5 (%)	1.29	1.35	0.19	0.07	1.03	0.92
In.(P x F)						
P_1F_1	90.30	79.10	8.43	7.26	174.50	165.75
P_1F_2	93.10	79.80	10.15	8.49	177.00	168.25
P_1F_3	102.90	85.40	10.82	8.92	178.00	169.25
P_1F_4	104.30	86.80	11.25	9.41	180.00	171.25
P_2F_1	91.70	81.20	8.92	7.50	175.25	166.50
P_2F_2	94.50	82.60	10.64	8.79	180.75	172.00
P_2F_3	104.30	86.80	11.19	9.47	183.50	174.75
P_2F_4	105.70	88.20	11.56	9.90	186.00	177.25
P_3F_1	94.50	82.60	9.10	7.75	177.00	168.25
P_3F_2	98.00	84.00	10.95	8.98	183.25	174.50
P_3F_3	105.70	88.20	11.38	9.35	186.50	175.75
P_3F_4	107.10	89.60	11.75	9.72	188.75	180.00
P_4F_1	97.30	82.60	9.66	7.87	177.25	168.50
P_4F_2	99.40	85.40	11.56	9.23	185.25	176.50
P_4F_3	107.10	89.60	12.05	9.72	188.75	178.25
P_4F_4	108.50	91.00	12.12	10.27	191.25	180.75
SEm±	0.90	0.94	0.13	0.05	0.72	0.64
C.D at 5 (%)	NS	NS	NS	0.14	2.07	1.86
Tr. Vs Control						
Treated	100.28	85.18	10.72	8.91	182.06	172.97
Control	78.40	72.80	4.37	3.44	151.75	143.00
SEm±	0.92	0.97	0.14	0.052	0.74	0.66
C.D at 5 (%)	2.66	2.79	0.41	0.15	2.13	1.91
Initial	84.70		6.84		162.00	

*DAS- Days after sowing

Vibha *et al.* (2014) [22] reported significant improvement in soil P availability under mung bean cultivation in sandy loam soil with inoculation of *Aspergillus niger* + *Penicillium citrinum* and *Aspergillus niger 2* + *Aspergillus niger* 3. Higher soil available phosphorus due to inoculation of *Bacillus* isolates observed by Ramesh *et al.* (2011) [12]. The increase in phosphatase and phytase activity with inoculation of *Bacillus* isolates might be due to P mobilization and acquisition by plants. This is in consonance with the study revealing that inoculation of *Aspergillus* strains significantly improved P uptake by plants and extracted P status in soil. Similar results were reported by Tarafdar and Rao (1996) [20]. Richardson and Simpson (2011) [13] concluded that microorganisms are the key driver in regulating mineralization of phytate in soil and their presence within the rhizosphere may compensate for a plants inability to otherwise acquire P directly from phytate.

Available potassium

Phytase application @ 4800 IU recorded significantly higher soil available K (185.63 and 176.00 mg kg^{-1}) at 30 days after sowing and at harvest as compared to other levels of phytase. The application of 75 kg P_2O_5 ha^{-1} recorded the higher soil available potassium (186.50 and 177.31 mg kg^{-1}) at 30 days after sowing and at harvest, respectively. The higher availability of potassium by the combined application of phytase @ 4800 IU and 75 kg P_2O_5 ha^{-1} (191.25 and 180.75 mg kg^{-1}) at 30 days after sowing and at harvest. The soil available potassium was found reduced at harvest but increased over control. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of potassium in soil. Similar results were reported by Santhy *et al.* (1998) [14]. Vibha *et al.* (2014) [22] reported significant improvement in soil in K availability under mung bean cultivation in sandy loam soil with inoculation of *Aspergillus niger* 2 + *Aspergillus niger* 3.

Conclusion

In an Inceptisol, the availability of P is strongly related with activity of enzymes and soil chemical processes. Combined application of phytase @ 4800 IU and 75 kg P_2O_5 ha^{-1} significantly improved soil phosphatase activity resulted in greater availability of soil P and other nutrients.

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