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Effect of Phytase Enzyme and Phosphatic Fertilizers on Available Phosphorus in an Alfisol

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Abstract: An experiment involving sixteen treatments with four levels of phytase (0, 240, 480, 720 IU) and four levels of phosphorus (0, 25, 50, 100 kg ha^{-1}) was carried out during *kharif* 2017 to assess the availability of phosphorus at 0, 15, 30, 45, 60 and 90 days (DAI) after incubation. There was an increase in soil available phosphorus up to 45th days of incubation either with the application of phytase or P and it reduced after 60 days of incubation. Significantly higher (12.98 kg ha^{-1}) available phosphorus was observed with the application of phytase @ 720 IU (16.14 kg ha^{-1}) and phosphatic fertilizer @ 100 kg ha^{-1}within 24 hours of incubation over initial available P (5.20 kg ha^{-1}). Application of 480 IU phytase resulted in significantly higher available P (51.98 kg ha^{-1}) which was at par with 720 IU (50.45 kg ha^{-1}). However, combined application of either 480 or 720 IU phytase along with either 50 or 100 kg P ha^{-1} through single super phosphate (SSP) had in statistically at par results for available phosphorus. Higher cumulative available phosphorus was recorded with the application of phytase @ 720 IU along with 100 kg P ha^{-1} through SSP (247.23 kg ha^{-1}) throughout the incubation period. Further phosphorus release potential indicated that application of phytase @ 720 IU along with 100 kg P ha^{-1} through SSP mobilized 0.65 kg ha^{-1} day^{-1} phosphorus.

Key words: *Phytase, phosphatic fertilizer, available phosphorus, P release potential*

Introduction

The availability of P in soil is influenced by pH of the soil. In case of soils with low pH, applied soluble phosphorus get converted into insoluble complexes of Fe and Al phosphates (Yadav and Verma 2012). However, in case of soils with slightly acidic pH, soluble phosphorus gets converted into insoluble complexes of Fe and Al hydroxides. The availability of soil phosphorous (potential or applied) is largely governed by micro flora and soil enzymes. Acid alkaline phosphatase and phytase are the soil enzymes mainly involved in the mineralization of fixed phosphorous. The acid phosphatase and alkaline phosphatase are predominant in acid and alkaline soil respectively (Yadav and Tarafdar 2003). Phytin is an organic form of phosphorus present in plant, soil and organic matter which is hydrolyzed by phytase enzyme. In soil, phosphorus exists both in organic and inorganic form, the organic phosphorus compounds can be classified into i) the inositol phosphate, primarily of plant origin up to 60 % of soil organic phosphorus, ii) the nucleic acids and iii) phospholipids (Aseri *et al*. 2009). In soils, 20-85 % of the total phosphorus remains in organic form, but

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plants can only utilize the organic phosphorus after its mineralization. Phytases are synthesized by many microorganisms, including various bacteria, fungi, micromycetes and other microbes which help to increase soil phosphorus availability for plant nutrition (Aseri *et al*. 2009). The importance of soil organic phosphorus as a source of plant available phosphorus depends on its rate of solubilisation and release of inorganic phosphorus. Phosphatase and phytase catalyzes hydrolytic cleavage of the C-O-P ester bond of organic phosphorus present in the soil and release plant available inorganic form (HPO_4^{-2} and $H_2PO_4^-$) (Yadav and Tarafdar 2003).

Till date scanty information is available on the use of different levels of phytase and inorganic phosphorus in an Alfisols of Konkan region and hence, the present investigation was undertaken with objectives to assess the effect of phytase and phosphatic fertilizer levels on periodical phosphorus availability and its release potential.

Materials and Methods

An incubation (0, 15, 30, 45 and 90 days) study (pot culture) was conducted at Division of Soil Science and Agricultural Chemistry, College of Agriculture, Pune during *kharif* 2017 to assess the effect of phytase enzyme and phosphatic fertilizers on available phosphorus in a surface soil (0-15 cm). The experiment having sixteen treatments with four levels of phytase enzyme (@ 0, 240, 480 and 720 IU) and four levels of phosphatic fertilizers (@ 0, 25, 50 and 100 kg ha^{-1}) was replicated thrice in Factorial Completely Randomized Design. The initial physio-chemical and biochemical properties of soil were analyzed using standard methods.

The phytase enzyme isolated from *Aspergillus niger* by National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune, India was procured for present study. The enzyme unit was expressed as IU, which is the amount of enzyme that liberated 1 µmol of inorganic orthophosphate from phytin per minute at pH 5.5 at $37^{\circ}C$. The plastic pots (288) with an inner diameter of 12 cm were filled with 200 g soil (2.0 mm). Treatment-wise quantity of phytase enzyme and phosphatic fertilizers (after dilution) were mixed in soil. In order to apply phytase @ 240, 480 and 720 IU, the procured phytase @ 2.6 g was dissolved in 1 liter water to have 1 lakh IU stock solution. From this stock solution, working standards @ 115.2, 230.4 and 345.6 ml were pipetted and volume was made to 480 ml and applied @ 10 ml to 48 pots to have 240, 280, 720 IU phytase enzyme respectively, and for control only de-ionised water was applied. In order to provide 25, 50 and 100 kg ha^{-1} P through phosphatic fertilizer 669.5, 1339.2 and 2678.4 mg of SSP were weighed, dissolved and volume made to 480 ml and applied @ 10 ml pot^{-1} respectively to 48 pots. Treatment-wise phytase enzyme and P (through SSP) was thoroughly mixed in soil. At each incubation period (0, 15, 30, 45, 60 and 90 days) 48 pots were used for assessing the availability of P. The soil available phosphorus was assessed periodically by Bray's and Kurtz (1945) method.

The phosphorus release potential (kg ha^{-1} day^{-1}) was calculated and presented (Table 6) for each incubation period using following mathematical expression by considering respective incubation days.

$$\text{Phosphorus release potential (kg ha}^{-1}\text{ day}^{-1}) = \frac{\text{Soil available P at X day - Soil Available P at 0 day}}{X}$$

Where, X = 15, 30, 45, 60 and 90

Results and Discussion

Availability of phosphorus was significantly influenced by application of phytase and phosphatic fertilizer at all the incubation periods. The combined application of higher level of phytase and inorganic P resulted in higher soil available phosphorus. Results revealed that significantly higher available phosphorus was noticed with the application of phytase @ 720 IU (16.14 kg ha^{-1}) and phosphatic fertilizer @ 100 kg ha^{-1}

within 24 hours of incubation over initial value of 5.20 kg ha^{-1} (Table 1). However, combined application of phytase @ 720 IU + 100 kg ha^{-1} P recorded significantly higher soil available P (17.10 kg ha^{-1}) than other interactions at zero day of incubation. There was increase in available phosphorus at 15th and 30th days of incubation with phytase @ 720 IU while it was higher at 45th day of incubation in the pots receiving 480 IU phytase. Application of 100 kg P ha^{-1} recorded higher phosphorus available at 15th and 30th days of incubation than other treatments but at 45th DAI, application of 50 kg P ha^{-1} recorded higher phosphorus availability (Table 2).

The individual effects of phytase and phosphatic fertilizer levels on periodical mean soil available phosphorus is presented in table 3 and 4. It was noticed that there was consistent increase in soil available phosphorus due to application of increasing levels of phytase or phosphatic fertilizer from 0 to 45th day of incubation but at 60th and 90th DAI, slight decrease in phosphorus availability was noticed for 480 IU phytase as well as 50 kg ha^{-1} P and it was statistically at par with the application of phytase @ 720 IU and 100 kg P ha^{-1}. The application of higher levels of phytase @ 480 or 720 IU was more effective in releasing the phosphorus during early incubation periods (15 day and 30 day) than the application of higher levels of P @ 50 kg ha^{-1} and 100 kg ha^{-1}. The reduction in soil available phosphorus at 45th, 60th and 90th DAI was more pronounced with 720 IU phytase. The availability of soil-P was higher at initial incubation periods 0, 15 days and 30 DAI with the application of phytase @ 720 IU than 480 IU at 0, 15 and 30 DAI.

There was an increase in available phosphorus up to 45th DAI in all the treatments of phytase and P. Application of phytase @ 480 IU with 100 kg ha^{-1} P had highest available phosphorus at 45th DAI followed by phytase @ 240 IU with 50 kg ha^{-1} P. The cumulative available phosphorus was maximum (247.23 kg ha^{-1}) with the application of phytase @ 720 IU with 100 kg ha^{-1} P (Table 5).

The P release potential was found to be maximum (0.65 kg ha^{-1} day^{-1}) with the application of phytase @ 720 IU + 100 kg ha^{-1} P followed by application of phytase @ 480 IU with 50 kg ha^{-1} P. Lowest (0.36 kg ha^{-1}) available phosphorus potential per day was recorded with application of phytase @ 0 IU + 25 kg ha^{-1} P.

In acid soils, precipitation of phosphorus as Fe phosphate ($FePO_4.2H_2O$) and Al phosphate ($AlPO_4.2H_2O$) or by adsorption to Fe and Al oxides (labile inorganic P) govern the availability of phosphorus. Phosphate solubilizing microorganisms play an important role in releasing plant available inorganic phosphate into soil from unavailable inorganic P (Ca-P, Fe-P, Al-P) through secretion of organic acids (Subba Rao 1995). Prasad and Ram (1986) found higher available P in the rhizophere soil of greengram inoculated with *Rhizobium* than the uninoculated soil. Gujar *et al.* (2013) also reported that microbial released phytase had high efficiency to mineralize P in soils. The organic acids released by phosphorus solubilizing organisms chelates the cations like Al and Fe or lower the pH and compete with phosphate for adsorption sites in soil (Whitelaw 1999). This may be the reason for higher phosphorus in phytase treated pots. The decrease in available phosphorus could be attributed to increase in fungal biomass resulting in enhanced uptake of soluble phosphate for their growth (Coutinho *et al.* 2012).

Conclusions

The application of phytase either @ 480 or 720 IU along with phosphatic fertilizers @ 50 or 100 kg ha^{-1} were found to be beneficial for availability of phosphorus in an Alfisol up to 45th DAI. Similar levels of phytase and phosphatic fertilizer were found superior for cumulative available P with per day release potential.

Table 1. Physico-chemical properties of initial soil

Parameter	Values
pH (1:2.5)	5.5
EC (1:2.5) dS m^{-1}	0.09
Organic carbon (%)	1.38
Calcium carbonate (%)	1.50
Available N (kg ha^{-1})	486
Available P (kg ha^{-1})	5.20
Available K (kg ha^{-1})	235
Fe (mg kg^{-1})	8.46
Mn (mg kg^{-1})	44.20
Zn (mg kg^{-1})	0.40
Cu (mg kg^{-1})	2.42

Table 2. Effect of phytase and phosphatic fertilizer on availability of phosphorus

a) At 0 days of incubation

Phosphorus (kg ha^{-1}) / Phytase (IU)	P (kg ha^{-1})				
	0	25	50	100	Mean
0	4.99	5.97	7.56	8.98	6.87
240	9.55	10.83	11.26	11.40	10.76
480	12.25	12.26	13.11	14.39	13.00
720	14.86	15.96	16.67	17.10	16.14
Mean	10.40	11.25	12.15	12.98	
	P		**S**		**P x S**
S.E. ±	0.152		0.152		0.304
CD at 5%	0.450		0.450		0.900

Initial soil available P: 5.20 kg ha^{-1}

b) At 15th days of incubation

Phosphorus (kg ha^{-1}) / Phytase (IU)	kg ha^{-1}				
	0	25	50	100	Mean
0	4.96	12.11	12.53	16.96	11.64
240	23.80	24.79	24.79	25.70	25.34
480	27.08	30.07	34.20	35.34	34.16
720	37.05	38.76	39.05	41.47	39.08
Mean	23.97	27.46	28.58	30.21	
	P		**S**		**P x S**
S.E. ±	0.586		0.586		1.446
CD at 5%	1.748		1.748		3.496

c) At 30th days of incubation

Phosphorus (kg ha^{-1}) / Phytase (IU)	kg ha^{-1}				
	0	25	50	100	Mean
0	4.81	44.18	49.02	51.86	37.47
240	43.70	51.01	53.15	47.31	48.79
480	49.59	51.58	52.45	54.29	51.98
720	48.69	49.59	51.73	52.87	50.45
Mean	36.70	48.84	51.59	51.58	
	P		S		P x S
S.E. ±	0.849		0.849		1.698
CD at 5%	2.777		2.777		5.554

d) At 45th days of incubation

Phosphorus (kg ha^{-1}) / Phytase (IU)	kg ha^{-1}				
	0	25	50	100	Mean
0	4.81	44.18	49.02	51.86	37.47
240	43.70	51.01	53.15	47.31	48.79
480	49.59	51.58	52.45	54.29	51.98
720	48.69	49.59	51.73	52.87	50.45
Mean	36.70	48.84	51.59	51.58	
	P		S		P x S
S.E. ±	0.849		0.849		1.698
CD at 5%	2.777		2.777		5.554

e) At 60th days of incubation

Phosphorus (kg ha^{-1}) / Phytase (IU)	kg ha^{-1}				
	0	25	50	100	Mean
0	4.84	38.05	41.33	46.03	32.56
240	38.47	46.03	50.65	38.62	43.44
480	44.60	49.16	51.30	52.72	49.44
720	41.90	44.46	47.45	48.73	45.64
Mean	32.45	44.42	47.68	46.52	
	P		S		P x S
S.E. ±	0.76		0.76		1.53
CD at 5%	2.30		2.30		4.59

f) At 90th days of incubation

Phosphorus (kg ha^{-1}) / Phytase (IU)	kg ha^{-1}				
	0	25	50	100	Mean
0	4.90	39.04	42.04	45.60	32.89
240	38.47	46.03	50.45	41.18	44.03
480	46.45	47.31	48.31	50.16	48.06
720	43.89	44.32	46.65	46.88	45.43
Mean	33.43	44.17	46.86	45.95	
	P		S		P x S
S.E. ±	0.064		0.064		0.128
CD at 5%	0.192		0.192		0.385

Table 3. Effect of phytase on soil available P throughout incubation period

Phytase enzyme (IU)	Available P kg ha^{-1} Incubation days						Mean
	0	15	30	45	60	90	
0	6.87	9.19	11.64	37.47	32.56	32.89	21.77
240	10.76	18.42	25.34	48.79	43.44	44.03	31.80
480	13.00	25.90	34.16	51.98	49.44	48.06	37.09
720	16.14	33.17	39.08	50.45	45.64	45.43	38.32
Mean	11.69	21.67	27.56	47.17	42.77	42.60	

Table 4. Effect of phosphatic fertilizer on soil available throughout incubation period

P_2O_5 levels (kg ha^{-1})	Available P kg ha^{-1} Incubation days						Mean
	0	15	30	45	60	90	
0	10.40	18.10	23.97	36.70	32.45	33.43	25.84
25	11.25	20.84	27.46	48.84	44.42	44.17	32.83
50	12.15	22.83	28.58	51.59	47.68	46.86	34.95
100	12.98	24.90	30.21	51.58	46.52	45.95	35.36
Mean	11.70	21.67	27.56	47.18	42.77	42.60	

Table 5. Effect of phytase and phosphatic fertilizer levels on cumulative available phosphorus

Treatments	Incubation days						Cumulative soil available P (kg ha^{-1})
	0	15	30	45	60	90	
P_0S_0	4.99	4.84	4.96	4.81	4.84	4.9	29.34
P_0S_1	5.97	8.69	12.11	44.18	38.05	39.04	148.04
P_0S_2	7.56	10.12	12.53	49.02	41.33	42.04	162.60
P_0S_3	8.98	13.11	16.96	51.86	46.03	45.60	182.54
P_1S_0	9.55	15.25	23.80	43.70	38.47	38.47	169.24
P_1S_1	10.83	17.24	24.79	51.01	46.03	46.03	195.93
P_1S_2	11.26	19.09	24.79	53.15	50.65	50.45	209.39
P_1S_3	11.40	22.09	25.70	47.31	38.62	41.18	186.30
P_2S_0	12.25	23.94	27.08	49.59	44.60	46.45	203.91
P_2S_1	12.26	26.36	30.07	51.58	49.16	47.31	216.74
P_2S_2	13.11	27.21	34.20	52.45	51.30	48.31	226.58
P_2S_3	14.39	26.08	35.34	54.29	52.72	50.16	232.98
P_3S_0	14.86	28.35	37.07	48.69	41.90	43.89	214.76
P_3S_1	15.96	31.07	38.76	49.59	44.46	44.32	224.16
P_3S_2	16.67	34.91	39.05	51.73	47.45	46.65	236.46
P_3S_3	17.1	38.33	41.47	52.87	48.73	48.73	247.23
S.E$\pm$	0.304	1.377	1.446	1.698	1.530	0.128	
C.D at 5 %	0.900	4.197	3.496	5.554	4.590	0.385	

Table 6. Effect of phytase and phosphatic fertilizer levels on phosphorus release potential

Treatment combinations	Per day potential (kg ha^{-1}) Incubation days					Mean
	15	30	45	60	90	
P_0S_0	-0.01	0.00	0.00	0.00	0.00	0.00
P_0S_1	0.18	0.20	0.85	0.53	0.37	0.36
P_0S_2	0.17	0.17	0.92	0.56	0.38	0.37
P_0S_3	0.28	0.27	0.95	0.62	0.41	0.42
P_1S_0	0.38	0.48	0.76	0.48	0.32	0.40
P_1S_1	0.43	0.47	0.89	0.59	0.39	0.46
P_1S_2	0.52	0.45	0.93	0.66	0.44	0.50
P_1S_3	0.71	0.48	0.80	0.45	0.33	0.46
P_2S_0	0.78	0.49	0.83	0.54	0.38	0.50
P_2S_1	0.94	0.59	0.87	0.62	0.39	0.57
P_2S_2	0.94	0.70	0.87	0.64	0.39	0.59
P_2S_3	0.78	0.70	0.89	0.64	0.40	0.57
P_3S_0	0.90	0.74	0.75	0.45	0.32	0.53
P_3S_1	1.01	0.76	0.75	0.48	0.32	0.55
P_3S_2	1.22	0.75	0.78	0.00	0.00	0.46
P_3S_3	1.42	0.81	0.79	0.53	0.37	0.65
Mean	0.67	0.50	0.79	0.49	0.33	

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