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Effect of Phytase and Farm Yard Manure on Growth, Nutrient Uptake and Yield of Soybean in Non-calcareous Soil

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Abstract: A pot culture experiment was conducted during *Kharif* -2014 at Division of Soil Science and Agricultural Chemistry, College of Agriculture, Pune to assess the effect of phytase and FYM levels on growth, nutrient uptake and yield of soybean in non-calcareous soil. The treatment consisted of four levels of each phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5, 5, 7.5 t ha^{-1}) replicated thrice in factorial randomized block design. Growth attributes (*viz.,* plant height, root length, root shoot ratio, fresh and dry weight of roots, number and weight of root nodules), plant dry matter accumulation were studied at 50% flowering of soybean. Further, grain and straw yield along with nutrient uptake were also studied at harvest of soybean. Application of phytase @ 3600 IU or FYM @ 7.5 t ha^{-1} or their combine application reported significantly higher plant height, root length, root shoot ratio, fresh and dry weight of roots, number and weight of root nodules than rest of the treatment at 50 % flowering of soybean. Significantly higher mean straw yield was recorded with the application of phytase @ 3600 IU (12.86 and 14.31 g pot^{-1}) at 50 % flowering and at harvest. Significantly higher grain and straw yield was recorded with the application of phytase @ 3600 IU (17.34 and 14.31 g pot^{-1}) which was statistically at par with phytase @ 2400 IU (17.28 and 13.74 g pot^{-1}). Combine application of phytase @ 3600 IU along with FYM @ 7.5 t ha^{-1} recorded significantly higher nitrogen (643.93 and 801.95 mg pot^{-1}), phosphorus (82.89 and 128.08 mg pot^{-1}) and potassium (354.75 and 114.72 mg pot^{-1}) uptake was recorded with the application of phytase @ 3600 IU at 50 % flowering and at harvest of soybean respectively.

Keywords: *Phytase, FYM, soybean growth, yield, nutrient uptake and non-calcareous soil*

Introduction

In soils 20-85 per cent of the total phosphorus is in organic form but plants can only utilize this organic phosphorus after its mineralization. The amount of organic phosphorus in soil is strongly correlated with total organic carbon and depth of soil. The importance of soil organic phosphorus as a source of plant available phosphorus depends on its rate of solubilization and release of inorganic phosphorus. Several types of phosphatases like phytases are able to increase the rate of dephosphorylation (hydrolysis of organic phosphorus). The availability of soil phosphorous (potential or applied) is largely governed by microflora and soil enzymes. Soil enzymes like acid and alkaline phosphatase and phytase are mainly involved in mineralization of fixed phosphorous. Phytate is an organic form of phosphorus present in plant, soil and organic matter which is hydrolyzed by phytase enzyme. Phytase enzyme catalyzes the hydrolysis of organic form

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of phosphorus into plant available P. Legumes secrete more phosphatase enzymes than cereals; this may be due to higher requirement of phosphorus by legumes especially soybean.

A large proportion of phosphorus applied to soil as a fertilizers rapidly becomes unavailable to plants due to its fixation by adsorption and precipitation. The soybean growing soils in Maharashtra are either of alkaline in nature with high clay content applied fertilizer phosphorus is not available to plant roots and get converted into unavailable form. The object of this experiment is to assess the efficiency of phytase enzyme in presence of FYM for soybean. Therefore, the phytase enzyme isolated from *Aspergillus niger* by National Chemical Laboratory, Pune was used along with FYM for soybean to assess its effect on growth, nutrient uptake and yield.

Materials and Methods

A pot culture experiment was conducted at the Division of Soil science and Agricultural Chemistry, College of Agriculture, Pune, during *Kharif* 2014. The experiment consisted of sixteen treatment combinations involving four levels of phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5, 5 and 7.5 t ha^{-1}) replicated thrice in Factorial Completely Randomized design. IU (International unit) is the amount of enzyme that liberates 1µmol of inorganic orthophosphate from phytin per minute at pH 5.5 and 37° C. (Zyla *et al*. 1995).

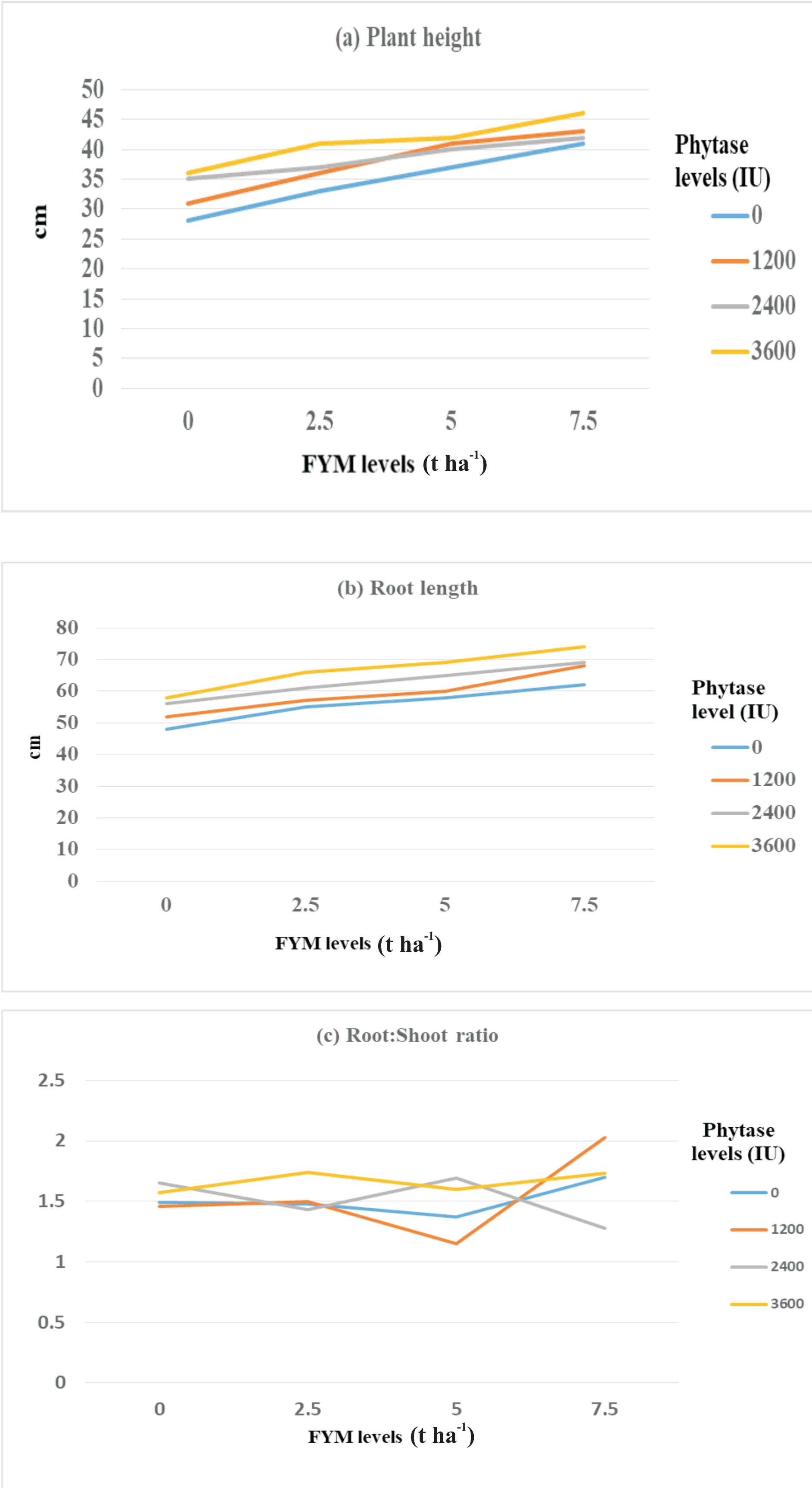
The soil $KMnO_4$-N, Olsen-P and N *N* NH_4OAc-K was analyzed by methods given by Subbia and Asija (1956); Olsen *et al*. (1954) and Hanway and Heidel (1952), respectively. The soil $CaCO_3$ content was assessed by rapid titration method prescribed by Jackson (1973). The soil used in this study was higher in pH (8.0), medium in O.C. (0.7 %), low in free calcium carbonate content (4.5%), low in available N (245 kg ha^{-1}), medium in available P (16.04 kg ha^{-1}) and K (310 kg ha^{-1}). The black non-calcareous soil was collected from Agronomy farm, College of Agriculture, Pune while required FYM was procured from Division of Animal Husbandry and Diary Science, College of Agriculture, Pune. However, the phytase isolated from *Aspergillus niger* was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune, India. The FYM used in the experiment was analyzed by using triacid digestion method. The FYM contains 11.7% organic carbon, total N (0.56%), total P (0.37%) and total K (0.52%) with 21.0 C:N ratio and 31.78 C:P ratio.

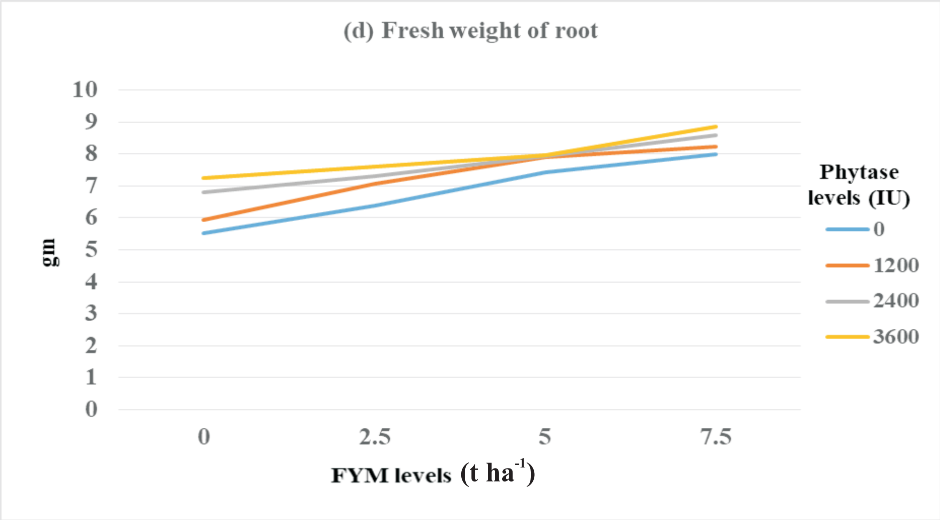
Pots were filled with 10 kg 2 mm sieved soil and treatment-wise quantity of phytase and FYM were thoroughly mixed and soybean seeds were sown. Another set of sixteen pots were maintained separately with similar treatments. These pots were broken at 50% flowering of soybean for recording observations on root length, root:shoot ratio, fresh and dry weight of roots, number and active weight of root nodules. The processed straw and grain samples were digested in sulphuric–salicylic acid and H_2O_2 system (Novozamsky *et al.* 1983). Total phosphorus was determined by vanodomolybdophosphoric acid yellow colour method (Tondon 1993) while total potassium was determined flam-photometrically (Jackson 1973). Dry matter and nutrient uptake at 50% flowering and at harvest of soybean was also estimated and reported. The grain and straw yield of soybean was reported at harvest.

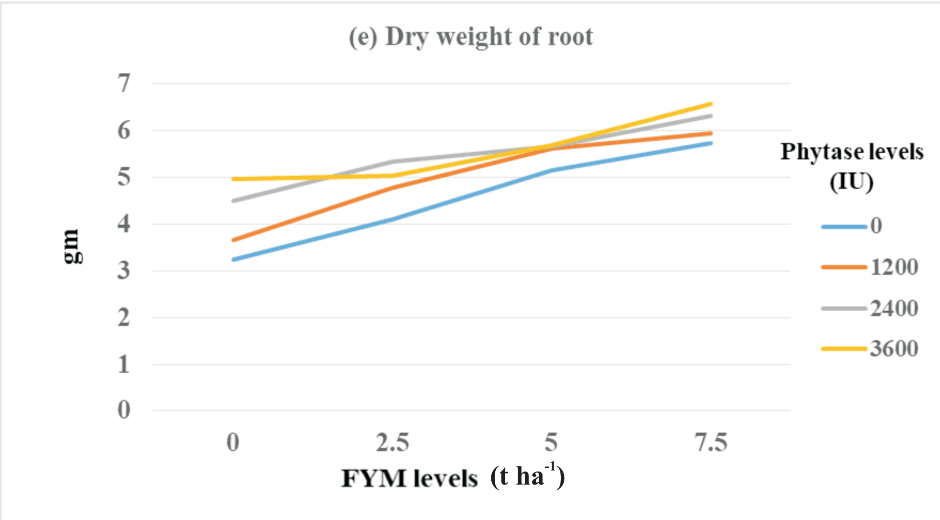
Results and Discussion

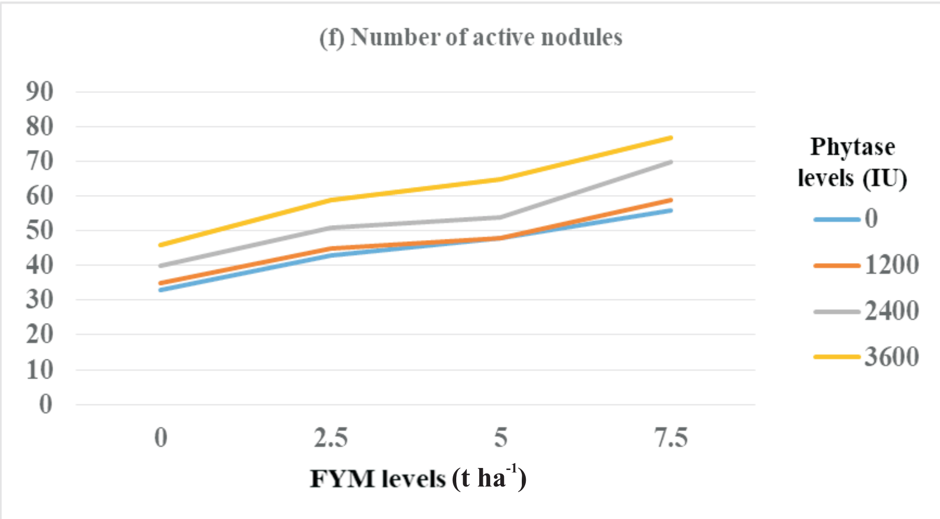
Growth attributes

Growth attributes *viz.,* plant height, root length, root-shoot ratio, fresh and dry weight of roots, number and weight of active nodules at 50% flowering of soybean were significantly influenced by the application of phytase enzyme and FYM levels (Fig. 1a to 1g). Significantly higher mean plant height (41 cm) of soybean was reported with the application of 3600 IU phytase followed by 2400 and 1200 IU (38 cm). The application of FYM @7.5 t ha^{-1} had significantly higher plant height (43 cm). The Interaction effect between 3600 IU phytase with 7.5 t ha^{-1} also had significantly higher plant height (46 cm). Significantly higher root length at 50% flowering was recorded with the application of 3600 IU phytase (67 cm) whereas FYM @7.5 t ha^{-1} had 68 cm. The combine application of


(a) Plant height
cm
50
45
40
35
30
25
20
15
10
5
0
0
2.5
5
7.5
FYM levels (t ha-1)
Phytase levels (IU)
0
1200
2400
3600
(b) Root length
cm
80
70
60
50
40
30
20
10
0
0
2.5
5
7.5
FYM levels (t ha-1)
Phytase level (IU)
0
1200
2400
3600
(c) Root:Shoot ratio
2.5
2
1.5
1
0.5
0
0
2.5
5
7.5
FYM levels (t ha-1)
Phytase levels (IU)
0
1200
2400
3600


(d) Fresh weight of root
10
9
8
7
6
5
4
3
2
1
0
gm
0
2.5
5
7.5
FYM levels (t ha-1)
Phytase levels (IU)
0
1200
2400
3600


(e) Dry weight of root
7
6
5
4
3
2
1
0
gm
0
2.5
5
7.5
FYM levels (t ha-1)
Phytase levels (IU)
0
1200
2400
3600


(f) Number of active nodules
90
80
70
60
50
40
30
20
10
0
0
2.5
5
7.5
FYM levels (t ha-1)
Phytase levels (IU)
0
1200
2400
3600

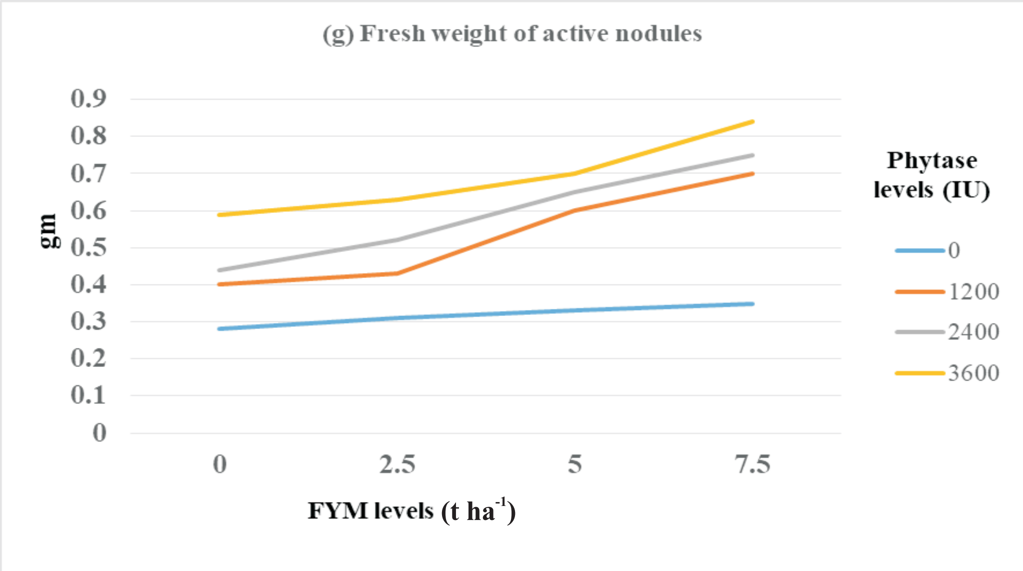


Fig.1 (a to g) : Effect of phytase and farm yard manure on growth attributes of soybean at 50% flowering in non-calcareous soil

phytase and FYM were found non-significant for root length but numerically higher root length (74 cm) recorded with the application of 3600 IU phytase along with 7.5 t ha^{-1} FYM. Significantly higher root-shoot ratio and dry weight of roots at 50% flowering of soybean were recorded with 3600 IU phytase or 7.5 t ha^{-1} FYM.

Significantly higher number of active nodules were observed with phytase @ 3600 IU or FYM 7.5 t ha^{-1} and their conjoint application produced higher number of nodules. Application of either phytase @ 3600 IU or FYM @ 7.5 t ha^{-1} had significantly higher fresh weight of active root nodules and interaction of these two also observed higher fresh weight of nodules (0.69 and 0.66 gm). Numerically higher weight of active root nodules (0.84 g) was recorded with 3600 IU phytase + 7.5 t ha^{-1} FYM. The increase in plant height, root length, root-shoot ratio, root weight and nodule number might be ascribed to solubilisation of inorganic phosphorus by action of organic and inorganic acid secreted by microorganism and ultimately reduction in pH of soil which cause higher P solubility (Yadav and Verma 2012).

Gujar *et al.* (2013) studied effect of phytase from *Aspergillus niger* on plant growth and mineral assimilation in wheat (*Triticum aestivum* Linn.*)* on non-calcareous soil and concluded that phytase isolated from *Aspergillus niger* promoted plant growth (upto 200 %) and root length. Vibha *et al. (*2014) reported significantly higher root length of mung bean with inoculation of *Aspergillus niger* strains No. 2. Tarafdar and Rao (2001) reported increased root nodulation of cluster bean with the inoculation of AMF + *Rhizobium* and FYM.

Grain yield and dry matter production

The dry matter accumulation at 50% flowering ranged between 8.52 and 15.08 g pot^{-1} (Table 1). An increase in mean dry matter was noticed with the increase in levels of FYM or phytase. Significantly higher mean dry matter was recorded with phytase @ 3600 IU (12.86 g pot^{-1}) followed by 2400 IU (12.83 g pot^{-1}). Application of FYM @ 7.5 t ha^{-1} had a significantly higher mean soybean dry matter (14.08 g pot^{-1}) followed by FYM @ 5 t ha^{-1} (12.86 g pot^{-1}).

Significantly higher grain (17.34 and 17.77 g pot^{-1}) and straw yield (14.31 and 14.17 g pot^{-1}) was recorded with the application of 3600 IU phytase and 7.5 t ha^{-1} FYM respectively (Table 2a and b). Combine application of 3600 IU phytase along with 7.5 t ha^{-1} recorded numerically higher grain yield of soybean (18.68 g pot^{-1}). However, similar treatment reported significantly higher straw yield of soybean (14.84 g pot^{-1}). Tomar (1998) showed that application of phosphate solubilizing bacteria along with FYM @ 5 t ha^{-1} beneficial for higher grain and straw yield. The increased dry matter and grain yield by the addition of FYM might be due to extensive root, tapping a large volume of soil for water and nutrients. Muhammad Iqbal *et al*. (2013) also reported simultaneous exudation of organic acids by maize roots with higher yield due to inoculation of phosphate solubilizing bacteria.

Table 1. Dry matter production of soybean at 50% flowering as influenced by the application of phytase enzyme and FYM in non-calcareous soil

Phytase (P) \ FYM (F)	Dry matter (g pot^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	8.52	10.18	12.17	13.61	11.12
1200 IU	9.49	11.58	12.84	13.19	11.77
2400 IU	11.63	12.20	13.08	14.43	12.83
3600 IU	11.11	11.93	13.34	15.08	12.86
Mean	10.19	11.47	12.86	14.08	
	P	F	P × F		
S.E. ±	0.327	0.327	0.654		
CD at 5%	0.946	0.946	NS		

Table 2 (a and b) . Grain and straw yield of soybean as influenced by the application of phytase enzyme and FYM in non-calcareous soil

a. Grain yield

Phytase (P) \ FYM (F)	Grain (g pot^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	14.33	15.32	16.44	15.55	15.41
1200 IU	15.37	16.41	16.81	18.21	16.70
2400 IU	15.91	17.02	17.55	18.65	17.28
3600 IU	15.89	16.91	17.89	18.68	17.34
Mean	15.37	16.41	17.17	17.77	
	P	F	P × F		
S.E. ±	0.17	0.17	0.341		
CD at 5%	0.493	0.493	NS		

b. Straw yield

FYM (F) / Phytase (P)	Straw (g pot^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	12.37	12.42	13.44	13.68	12.98
1200 IU	12.58	12.45	12.32	13.51	12.71
2400 IU	13.25	13.39	13.65	14.67	13.74
3600 IU	13.81	14.30	14.30	14.84	14.31
Mean	13.00	13.14	13.43	14.17	
	P	F	P × F		
S.E. ±	0.059	0.059	0.118		
CD at 5%	0.17	0.17	0.341		

Nutrient uptake

The uptake of N, P and K at 50% flowering of soybean ranged between 238.03-643.93 mg pot^{-1}, 10.26-29.64 mg pot^{-1} and 29.15-84.60 mg pot^{-1} respectively (Table 3a, b and c). Application of 3600 IU phytase and FYM @ 7.5 t ha^{-1} recorded significantly higher nitrogen (495.53 and 593.44 mg pot^{-1}), phosphorus (66.43 and 82.89 mg pot^{-1}) and potassium (285.12 and 354.75 mg pot^{-1}) uptake by soybean at 50% flowering respectively.

Significantly higher nitrogen (755.37 mg pot^{-1}), phosphorus (111.23 mg pot^{-1}) and potassium (96.41 and 354.75 mg pot^{-1}) uptake were recorded with the application of 3600 IU phytase and 7.5 t ha^{-1} FYM at harvest of soybean (Table 4a, b and c). Combine application of phytase @ 3600 IU along with FYM @ 7.5 t ha^{-1} recorded significantly higher nitrogen (643.93 and 801.95 mg pot^{-1}), phosphorus (82.89 and 128.08 mg pot^{-1}) and potassium (354.75 and 114.72 mg pot^{-1}) uptake was recorded with the application of phytase @ 3600 IU at 50 % flowering and at harvest of soybean respectively.

Tarafdar and Rao (1996) also reported higher nitrogen, phosphorus, and potassium and calcium uptake by cluster bean, chickpea and wheat due to inoculation

Table 3 (a, b and c). Effect of phytase and FYM levels on nitrogen, phosphorus and potassium uptake of soybean at 50 percent flowering

a) Nitrogen uptake

FYM (F) / Phytase (P)	mg pot^{-1}				
	0 t ha^{-1}	**2.5 t ha^{-1}**	**5 t ha^{-1}**	**7.5 t ha^{-1}**	**Mean**
0 IU	238.03	340.13	416.27	566.47	390.23
1200 IU	264.80	405.50	477.07	578.40	431.44
2400 IU	365.23	445.37	560.87	584.97	489.11
3600 IU	354.73	507.37	476.10	643.93	495.53
Mean	305.70	424.59	482.58	593.44	
	P	F	P × F		
SE ±	11.086	11.086	22.171		
CD at 5%	32.079	32.079	64.158		

b) Phosphorus uptake

FYM (F) / Phytase (P)	mg pot^{-1}				
	0 t ha^{-1}	**2.5 t ha^{-1}**	**5 t ha^{-1}**	**7.5 t ha^{-1}**	**Mean**
0 IU	29.10	42.67	60.13	66.61	49.63
1200 IU	36.35	47.81	59.18	69.81	53.29
2400 IU	52.07	60.05	62.07	72.27	61.62
3600 IU	53.64	55.22	73.98	82.89	66.43
Mean	42.79	51.44	63.84	72.90	
	P		F		P × F
SE $\pm$	1.811		1.811		3.623
CD at 5%	5.242		5.242		10.483

c) Potassium uptake

FYM (F) / Phytase (P)	mg pot^{-1}				
	0 t ha^{-1}	**2.5 t ha^{-1}**	**5 t ha^{-1}**	**7.5 t ha^{-1}**	**Mean**
0 IU	135.85	228.40	282.84	303.85	237.74
1200 IU	173.11	221.71	258.55	298.64	238.00
2400 IU	194.29	222.67	280.50	330.51	256.99
3600 IU	242.21	250.97	292.56	354.75	285.12
Mean	186.37	230.94	278.61	321.94	
	P		F		P × F
SE $\pm$	7.183		7.183		14.366
CD at 5%	20.786		20.786		NS

Table 4 (a, b and c). Effect of phytase and FYM levels on nitrogen, phosphorus and potassium uptake of soybean (grain) at harvest

FYM (F) / Phytase (P)	mg pot^{-1}				
	0 t ha^{-1}	**2.5 t ha^{-1}**	**5 t ha^{-1}**	**7.5 t ha^{-1}**	**Mean**
0 IU	534.08	587.77	602.18	689.09	603.28
1200 IU	570.20	655.96	659.04	697.74	645.74
2400 IU	676.87	712.55	716.34	780.81	721.64
3600 IU	711.26	750.77	757.50	801.95	755.37
Mean	623.10	676.76	694.88	731.29	
	P		F		P × F
SE $\pm$	4.029		4.029		8.058
CD at 5%	11.659		11.659		23.318

b) Phosphorus uptake

FYM (F) / Phytase (P)	mg pot^{-1}				
	0 t ha^{-1}	**2.5 t ha^{-1}**	**5 t ha^{-1}**	**7.5 t ha^{-1}**	**Mean**
0 IU	50.42	78.31	84.94	97.20	77.72
1200 IU	68.36	88.03	94.83	99.84	87.77
2400 IU	83.67	88.80	95.78	115.18	95.86
3600 IU	97.17	97.72	121.96	128.08	111.23
Mean	74.91	88.21	99.38	110.08	
	P	F	P × F		
SE ±	3.958	3.958	7.915		
CD at 5%	11.452	11.452	22.905		

c) Potassium uptake

FYM (F) / Phytase (P)	mg pot^{-1}				
	0 t ha^{-1}	**2.5 t ha^{-1}**	**5 t ha^{-1}**	**7.5 t ha^{-1}**	**Mean**
0 IU	50.69	52.58	70.32	101.63	68.81
1200 IU	62.87	65.16	79.21	94.54	75.45
2400 IU	69.08	82.98	95.95	111.00	89.75
3600 IU	74.66	92.87	103.39	114.72	96.41
Mean	64.33	73.40	87.22	105.47	
	P	F	P × F		
SE ±	0.674	0.674	1.347		
CD at 5%	1.949	1.949	3.898		

with phosphatase producing fungi. Gujar *et al.* (2013) opined that phytase promoted phosphorus mineral assimilation much more efficiently than chemical fertilizers.

Conclusion

Application of phytase @ 3600 IU, FYM @7.5 t ha^{-1} and their combine application was found superior for growth attributes of soybean at 50% flowering. However individual application or combine application of higher level of either phystae or FYM was found to be better for grain and straw yield and nutrient uptake of soybean at 50% flowering or at harvest.

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