EFFECT OF PHYTASE AND FYM LEVELS ON SOIL ENZYME ACTIVITIES, YIELD AND NUTRIENT UPTAKE BY SOYBEAN ON NON CALCAREOUS SOIL

By

Mr. Suradkar Rahul Kamlesh
(Reg. No. 013/228)

A thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI – 413722, DIST. AHMEDNAGAR,
MAHARASHTRA STATE (INDIA)

In partial fulfilment of the requirements for the degree
Of

MASTER OF SCIENCE (AGRICULTURE)
In

SOIL SCIENCE AND AGRICULTURAL CHEMISTRY

DIVISION OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY,
COLLEGE OF AGRICULTURE, PUNE – 411 005
MAHARASHTRA STATE (INDIA)

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Approved by

Dr. A. B. Jadhav
(Chairman and Research Guide)

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DIVISION OF SOIL SCIENCE AND AGRICULTURAL CHEMISTRY,
COLLEGE OF AGRICULTURE, PUNE – 411 005
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2015

CANDIDATE'S DECLARATION

I, hereby declare that thesis entitled **" EFFECT OF PHYTASE AND FYM LEVELS ON SOIL ENZYME ACTIVITIES, YIELD AND NUTRIENT UPTAKE BY SOYBEAN ON NON CALCAREOUS SOIL**" or part of there has not been submitted by me or any other person to any other University or Institute for a Degree or a Diploma.

Place : Pune

Date : / /15 **(SURADKAR RAHUL KAMLESH)**

Dr. A. B. Jadhav,
M. Sc. (Agri.), Ph. D.
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CERTIFICATE

This is to certify that the thesis entitled, **"EFFECT OF PHYTASE AND FYM LEVELS ON SOIL ENZYME ACTIVITIES, YIELD AND NUTRIENT UPTAKE BY SOYBEAN ON NON CALCAREOUS SOIL" ,** submitted to the Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**, embodies the results of a *bonafide* research carried out by **Mr. SURADKAR RAHUL KAMLESH** under my guidance and supervision and that no part of the thesis has been submitted for any other Degree or Diploma.

The assistance and help rendered during the course of this investigation have been duly acknowledged.

Place : Pune
Date : / /15

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Chairman and Research Guide

Dr. J. V. Patil,
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Associate Dean,
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CERTIFICATE

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Place : Pune (**J. V. Patil**)
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Place: *Pune*

Date: */ / 2015* ***[Suradkar Rahul Kamlersh]***

CONTENTS

ABSTRACT

" EFFECT OF PHYTASE AND FYM LEVELS ON SOIL ENZYME ACTIVITIES, YIELD AND NUTRIENT UPTAKE BY SOYBEAN ON NON CALCAREOUS SOIL"

by
Mr. RAHUL KAMLESH SURADKAR

A candidate for the
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2015

Research Guide : Dr. A. B. Jadhav
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The effect of phytase and FYM levels on soil enzyme activities, yield and nutrient uptake by soybean on non calcareous soil was studied by conducting a pot culture experiment at the Division of Soil Science and Agricultural Chemistry, College of Agriculture, Pune, during *Kharif* 2014. The experiment consisted of sixteen different treatment combinations. The four levels of phytase (0, 1200, 2400, 3600 IU) and FYM (0, 2.5, 5, 7.5 t ha^{-1}) replicated thrice in Factorial Completely Randomised Design. Required quantity of phytase in liquid form was procured from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune. The phytase enzyme used in the experimentation was extracted from *Aspergillus niger* NCIM 563.

The data revealed that application of phytase either @ 3600 IU or 2400 IU along with 7.5t ha^{-1} FYM were found statistically at par for higher acid phosphatase, alkaline phosphatase and dehydrogenase activity in soil at 50 percent flowering and at harvest of soybean.

It could be concluded from the data that application of phytase either @ 1200 IU, 2400 IU or 3600 IU along with 7.5 t ha^{-1} or 5 t ha^{-1} FYM recorded significantly higher soil organic carbon at 50 percent flowering and at harvest of soybean.

Considerable decrease (13.20 %) in calcium carbonate content in soil was recorded from 4.24 per cent (0 IU phytase) to 3.68 per cent (3600 IU). However, decreasing magnitude to the tune of 10.37 per cent was recorded with the application of 7.5 t ha^{-1} FYM (3.80 %) than 0 t ha^{-1} (4.24 %) at 50 % flowering of soybean. Similar trend in the reduction of calcium carbonate content in soil was recorded at harvest of soybean.

It could be concluded from the data that availability of nitrogen, phosphorus and potassium in soil were found significant and higher with the application of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM which was found statistically at par with 2400 IU with 7.5 t ha^{-1} at 50 percent flowering and at harvest.

Application of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM recorded significantly higher soil DTPA extractable iron, manganese and copper at 50 percent flowering of soybean. However in case of DTPA extractable zinc application of phytase either @ 3600 IU or 2400 IU along with 7.5 t ha^{-1} FYM recorded statistically at par results with each other. Application of phytase either @ 2400 IU or 3600 IU along with 5 t ha^{-1} or 7.5 t ha^{-1} FYM recorded at par results for DTPA extractable zinc and manganese at harvest of soybean.

Application phytase @ 3600 IU or 2400 IU along with 7.5 t ha^{-1} FYM recorded statistically at par soil bacterial, fungal and actinomycetes population.

Rahul Kamlesh Suradkar **1 to 152**

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1. INTRODUCTION

Phosphorus is critically involved in energy production, transfer and storage of ATP for every biological process in nature. In plant, phosphorus is essential for photosynthesis, transpiration, respiration, energy, metabolism, synthesis of nucleic acids and membranes, nitrogen fixation, gene transfer, reproduction, root growth, flowering and root setting. Phosphorus is a major plant nutrient next to nitrogen in augmenting plant metabolic activity ultimately reflecting on the crop yield. It plays a key role in various physiological processes concerning root system development, nodulation and nitrogen fixation (Siva Sankar *et al*., 1984). It plays an important role in physiological function such as conservation and transfer of energy in metabolic reactions of all living cells of the plants (Havlin *et al*. 2007).

Phosphorus is least mobile and is unavailable to plants in most of soil conditions. The phosphorus content in average soil is about 0.05 % (W/W) but only 0.1% of the total phosphorus is available to plants because of its low solubility and its fixation in soil (Scheffer and Schachtschabel, 1992). Phosphorus is added in soil through application of chemical fertilizers but major part of it gets fixed in the soil, while 15-20 % is utilized by the crop (Wani, 1980 and Gaur, 1985). Major limitation of adequate phosphorus supply to plant is phosphorus fixation. In neutral to alkaline calcareous soil, phosphorus retention is dominated by precipitation reaction of calcium and in acid soil it is fixed with iron and aluminium (Lindsay *et al*., 1989) or on the surface of clay mineral (Devau *et al*., 2010).

Soil phosphorus exists both in organic and inorganic form, the organic phosphorus compounds can be classified into i) the inositol phosphatase primarily of plant origin comprising up to 60% of soil organic phosphorus, ii) the nucleic acids and iii) phospholipids. In soils 20-85 per

cent of the total phosphorus is in organic form but plants can only utilize this organic phosphorus after it's 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 rate of nitrogen & carbon mineralization is positively correlated with that of organic phosphorus mineralization. The presence of carbon, nitrogen and phosphorus in the organic material like FYM with microbial activity, phosphatase and phytase enzyme activity proportionately regulate the mineralization of organic phosphorus, nitrogen & carbon. Phosphatase & phytase which catalyzes hydrolytic cleavage of the C-O-P ester bond of organic phosphorus present in soil and release plant available inorganic form (HPO_4^{2-} and $H_2PO_4^-$) (Yadav and Tarafdar, 2004). The C:P ratio of an organic manure like FYM determines the extent of which inorganic phosphorus is immobilized. Micro-organisms may produce both acid and alkaline phosphatase but plant can only secrete acid phosphatase. phosphorus mineralization is an enzymatic process which involves group of phosphstase enzyme catalyzes different soil biochemical reactions & release plant usable phosphorus (Tarafdar,1989).

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.The acid phosphatase is predominant in acid soil and alkaline phosphatase is predominant in alkaline soil (Juma and Tabatabai 1987). The acid and alkaline phosphatase and phytase have an important role in mineralization of phosphorous in association with microbial population. Phytate is an organic form of phosphorus present in plant, soil

and organic matter which is hydrolyzed by phytase enzyme. Phytase enzyme not only catalyzes the hydrolysis of organic form of phosphorus but also involved in improvement of other soil enzyme activities and microflora. Legumes secrete more phosphatase enzymes than cereals; this may be due to higher requirement of phosphorus by legumes especially soybean.

The soybean growing areas in Maharashtra are either of calcareous or black clayey soils. These soils are alkaline in nature (8-8.5 pH) with moderate to high calcium carbonate content. The content of free calcium carbonate and clay content dominated soils are the main constraints for phosphorous availability. Both these constrains are attributed to the phosphorus fixation either by calcium as calcium phosphate or adsorption of phosphate ions on surface of clay, there by resulting in lower recovery by crops & buildup of its status in soil. A large proportion of phosphorus applied to soil as fertilizer rapidly becomes unavailable to plants due to fixation of inorganic phosphorus by adsorption & precipitation whereas, organic phosphorus fractions gets immobilized in soil organic matter.

Soybean (*Glycine max* L. Merril) is one of the important pulse as well as oilseed crop of the *Leguminoseae* family. In India, area under soybean is 120.327 lakh hectares with a production of 122.345 lakh MT in *Kharif* 2013. Whereas in Maharashtra state it is (38.704 lakh hectares) with production of 46.459 lakh MT in *Kharif* 2013 (SOPA – The Soybean Processors Association of India).

Soybean is an excellent source of crude protein (43.2 %) and oil (19.5 %). Soybean protein is an important source of lysine (8.4%) and all other essential amino acids. It also contains carbohydrates (26%), minerals (45 %) and phospholipids (2%) (Halwankar *et al*., 1992). It is rich source of vitamin A, B and D. Soybean sprouted seed contains protein (38-42%), oil (20%), carbohydrate (20%), crude fibre (3.82%), 710 IU vitamin A, 300 IU

vitamin B. Further it is important source of mineral like calcium, sodium, magnesium, phosphorus, iron, potassium and sulphur (Kale, 1985). Soybean oil is enriched with vitamin A and lecithin and resembles butter ghee. Soy protein is superior to most of the plant protein by virtue of its high biological value (78.41), protein efficiency ratio (2.47 %) and its essential amino acids pattern resemble to those of cow milk. Soybean therefore has a potential to combat protein calorie malnutrition, in developing countries like India.

The information about enzymatic mineralization of phosphorous in calcareous soil for soybean through phytase enzyme in presence of FYM is not available. Hence a pot culture experiment was planned to assess the effect of phytase and FYM levels on soil enzyme activities, yield and nutrient uptake by soybean on calcareous soil with following objectives.

1. To study the effect of phytase and FYM levels on soil enzyme activities at 50 percent flowering and harvest of soybean.
2. To assess the effect of phytase and FYM levels on nutrient availability at 50 percent flowering and harvest of soybean.
3. To study the effect of phytase and FYM levels on yield and nutrient uptake by soybean.

2. REVIEW OF LITERATURE

2.1 Effect of phytase and FYM levels on soil enzyme activities at flowering and harvest of soybean

Dinkelaker and Marschner (1992) demonstrated acid phosphatase activity in the rhizosphere of soil-grown plants and they noticed that the production and secretion of acid phosphatases by some plant species are stimulated by deficient levels of soil phosphorus. Jha *et al.* (1992) studied the soil microbial population in relation to altitude and forest degradation. They noticed that at both the altitudes fungi and bacteria population were higher in less degraded forest than in the more degraded one. Further, they calculated a co-relation coefficient between fungi and bacteria population, organic carbon and moisture.

Kumar *et al*., (1992) conducted an experiment on the soil microbial population number and Enzyme activities in relation to altitude and forest degradation. Their study indicated that dehydrogenase activity display the close, positive correlations not only with organic matter but also fungal population abundance in four forest stands (two at low and two at higher attitudes).

Martens *et al*., (1992) conducted a field study to determine the activity and persistence of soil enzymes with the repeated addition of different organic residues at Department of Environment and Soil Sciences, California. They concluded that the enzyme activity in the amended soil was increased by an average of 2 to 4 fold by incorporation of organic amendments as compared to the non amended soils. Further they stated that the increased levels of enzyme activity in the organic amended soil may be a

reflection of the increased protective sites within the soil as a result of enhanced humus content.

Asmar and Gissel-Nielsen (1996) worked on the extracellular phosphomono and phosphodiesterase associated with and released by the roots of barley genotypes. They concluded that the activity of phytase and phosphatase in the rhizosphere originates mainly from roots when the plants are grown under P-stress.

Wodzinski and Ullah (1996) reported enzymes are proteins and they are succeptible to possible denaturation or destruction by digestive enzymes or anything that can change their structure. Enzymes typically have ideal conditions (temperatures, pH, etc.) where they function more readily. Further they reported plant phytase works better at 45 to 60ºC whereas microbial phytases work more readily at wider temperature ranges i.e. 35 to 63ºC.

Malewar *et al.* (1999) studied the CO_2 evolution and microbial population in soil as influenced by organic and NPK fertilizers under sorghum-wheat system at MAU, Parbhani during 1994-1996 on *Typic Haplusterts.* They were indicated higher population of microbes was observed with 50:50 inorganic + organic combinations. The conjoint application of organic manure along with fertilizers were able to enhance the microbial population rather fertilizer alone.

Yadav and Tarafdar (2003) compared relative efficiency of phosphatase and phytase for the hydrolysis of different organic P compounds at various enzyme concentrations. They concluded that phytase

has a slight edge over phosphatases in hydrolysis of different organic P compounds.

Charlile and Dickinson (2004) studied dehydrogenase enzyme as an indicator of microbial activity in growing media. Dehydrogenase activity was low in a peat-based growing medium, but higher in media prepared from composted materials, and was highest in a medium containing 50 per cent pine bark with 50 per cent chipboard waste. During storage, the activity of dehydrogenase declined in all media. Microbial activity as measured by CO_2 evolution from media did not always correspond to dehydrogenase activity, particularly in media containing composted spruce bark and paper.

A. Aziz Qureshi *et al*., (2005) conducted pot experiment in slightly alkaline *Typic Haplustepts* soil at Indian Agricultural Research Institute, New Delhi. They studied the direct and residual effect of phosphate rocks in presence of phosphate solubilizers and FYM on the available P, organic carbon and viable counts of phosphate solubilizers in soil after soybean, mustard and wheat crops and concluded that combine application of phosphate solubilizers in presence of FYM improved organic carbon status and proliferation of phosphate solubilizers. Amongst the phosphate solubilizers, *Aspergillus awamori* showed significant effect in improving available P. Lung *et al*., (2006) conducted an experiment on tobacco (*N. tabacum*) crop showing assimilation of phytate-phosphorus by the extracellular phytase activity affected by the availability of soluble phytate. They concluded that P starvation of tobacco seedlings triggered a substantial increase in the specific phytase activity in root exudates.

George *et al* (2005) studied the behaviour of plant derived extracellular and microbial origin phytase upon addition to three different soils (spodosols, oxisols, alfisols). They reported notable increase in pH of rhizospheral soil to the tune of 0.5 and 0.7 units by exposing above soils to the roots of phytase exuding fungal phytase.

Urease, acid phosphatase, and dehydrogenase activities in soil as influenced by integrated nitrogen management treatment imposed for banana on Inseptisol at MPKV, Rahuri was studied by Jadhav *et al.*,(2008).They concluded that higher activities of urease and dehydrogenase was reported at 120DAP of banana while acid phosphatase activity was higher upto 165 DAP. Further they reported positive correlation among urease, acid phosphatase, and dehydrogenase, microbial population and organic carbon.

Tarafdar (2008) studied mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He observed that phytase has a slight edge over phosphatases in hydrolysis of different organic P compounds. Secretion of phytase was much higher than acid phosphatase secreted by plants. He also concluded that inoculation with *Aspergillus* strains significantly increase the phosphorus concentration, total phosphorus uptake as well as soil available phosphorus status in rhizosphere of chickpea and wheat than in bulk soil.

Aseri *et al*., (2009) studied hydrolysis of organic phosphate forms by phosphatases and phytase producing fungi of arid and semi arid soils of India at Amity University, Rajasthan and Central Arid Zone Research Institute, Jodhpur during March 2004. They reported that several

types of phosphatases, such as phytases, are able to increase the rate of the dephosphorylation (hydrolysis) of organic P. Phosphatases in the rhizosphere may arise from plant roots or soil microbes. The addition of phytase increased the P content of maize seedlings when supplied with phytate by plant which concluded that the utilization of phytate by plant was limited by low rates of hydrolysis. They concluded that *Trichoderma spp.* was found to be most efficient for hydrolysis of organic P by secreting phosphatases and phytase enzymes.

Kaya *et al.,* (2009) grown different chickpea genotypes on clay loam soil at Suleyman Demirel University research and experimental farm, Isparta-Turkey. They determined zinc, phosphorus, phytic acid, protein concentrations and phytase activity. They concluded that phytases are enzymes that degrade phytate and permit higher availability of Zn and other nutrients such as P and Fe. Further they stated that N and Zn fertilization showed different effect on phytase activity for different varieties.

Kouas *et al*., (2009) conducted an experiment intended to measure the nodulated roots oxygen consumption and phytase activity in 2 lines of common bean (*Phaseolus vulgaris*) at two levels of P supply at Montpellier, France during 2009. They reported that the induction of higher phytase activity under P deficiency as observed in bean roots and nodules may contribute the P utilization from phytate in the plant and contribute to improve its adaptation to low P soils.

Yadav *et al*., (2009) reported enhanced secretion of phytase in phosphorus deficient soil as compared to phosphorus sufficient soil under *Cenchrus ciliaris*. Solubilization of phosphorus was also more in soil with

lower native unavailable phosphorus. Extracellular phytase activity of buffel grass genotypes grown on nutrient solution under P deficient (5 µg P L^{-1}) and P sufficient (250 mg P L^{-1}) sterile conditions were compared by Yadav and Tarafdar (2009). They concluded that activity of root associated phytase of seven buffel grass genotypes was significantly more than that of root released phytase. Buffel grass genotype CAZRI 116 has reported 35 % more efficient in solubilization of native phosphorus.

Yadav *et al*., (2009) studied the mobilization of phosphorus in presence of organic matter inoculated with *chaetomium globosum* with cluster bean under arid ecosystem and concluded increase in acid phosphatase (15%), alkaline phosphatase (12%) and phytase (71%) as against without inoculated organic matter. Nakhro and Dkhar (2010) reported that the application of organic manures increased the organic carbon content of the soil and thereby increasing the microbial counts and microbial biomass carbon.

A pot culture experiment was conducted by Rai and Yadav (2011) during *rabi*-2004-05 and 2005-06 at BHU, Varanasi on the influence of inorganic and organic nutrient sources on soil enzyme activities and reported that the activity of dehydrogenase (DHA) and alkaline phosphatase were more with absolute treatments of organic sources of nutrients. In case of incubation study, highest and significant urease (300 µg urea hydrolyzed g^{-1} h^{-1}) activity was recorded at 30 DAI in the treatment receiving full doses of RDF + 85 kg CW ha^{-1} followed by treatments of RDF +2 t FYM and RDF +1 t FYM, which were significantly superior over absolute control. Similarly, maximum build up of dehydrogenase (74.5 µg TPF g^{-1} day^{-1}) and

alkaline phosphatase (90.7 µg PNP produced g^{-1} h^{-1}) was found in the soils treated with FYM and with pressmud at 30 DAI and 120 DAI, respectively.

Ramesh *et al*., (2011) studied phytase, phosphatase activity and P-nutrition of soybean as influenced by inoculation of *Bacillus* sp. at Directorate of Soybean Research (ICAR), Indore. They reported that phytic acid-P as a percentage of total P content in soybean seeds decreased with the inoculation of *Bacillus* isolates as compared to un-inoculated control. Phytate constitutes upto 50% of total organic P in soil. More than 75% of the total P in soybean exists in phytic acid or its complex anion form, phytate salt.

Adnane Bargaz *et al.*, (2012) conducted field trials in semi arid zone of Haouz area at the region of Marrakesh, Morocco using six common bean genotypes in late April and harvested in late June during two successive years (2009-2010) to study-low soil phosphorus availability increases acid phosphatases activities and affects phosphorus partitioning in nodule, seeds and rhizosphere of *Phaseolus vulgaris*. They reported significant increase in acid phosphatase and phytase activity in rhizospheral soil of *Phaseolus vulgaris* under low soil phosphorus situations as against soil with sufficient phosphorus.

Rai &Yadav (2011) concluded that application of 100% N through FYM recorded highest dehydrogenase activity where as 100% N through press mud application recorded higher alkaline phosphatase activity while conducting pot culture experiment during *rabi*-2004-05 and 2005-06 at BHU, Varanasi.

Ushari *et.al.*,(2013) studied the enzymatic activity by isolating phytase producing bacteria (*Bacillus* sps NBtRS6) from the rhizosphere soil of NBt cotton field in Andhra Pradesh and they observed that phosphatase is an enzyme that release inorganic phosphate from organic moiety and complex inorganic materials. It is known to play an essential role in phosphorus cycle. Further they stated that phosphatases play akey role in phosphorus cycle by solubilizing organic and inorganic phosphates into available forms that support growth of crop plants.

Dotaniya *et al* (2014) studied the rhizosphere effect of *kharif* crops on soil enzymes in typic Haplustert (Vertisols) at IISS, Bhopal during 2011. They concluded maximum acid phosphatase activities were observed at 75 DAT (days after transplanting) in rice (0.206 mg PNP g^{-1} h^{-1}), 90 DAS in sorghum (0.194 mg PNP g^{-1} h^{-1}) and pearl millet (0.201 mg PNP g^{-1} h^{-1}) 502 DAS in soybean (0.127 mg PNP g^{-1} 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.

2.2 Effect of phytase and FYM levels on nutrient availability at flowering and harvest of soybean.

Jain *et al.* (1995) reported that the application of FYM and sugar pressmud had significantly enhanced the plant height, number of leaves per plant, number of branches per plant over control in soybean when grown in medium black soil in Madhya Pradesh. Tomar (1998) showed that the application of phosphate solubilizing bacteria (10 g/kg seed) combined with farm yard manure (5 tonnes ha^{-1}) gave the maximum grain and straw yields than the control. Reddy *et al.* (1999) reported that the P uptake by

soybean and wheat increased with increasing rates of manure and fertilizer P and was relatively larger in soybean than in wheat. The per cent P recovery by the crops from fertilizer P decreased with increasing fertilizer P rate, while it was improved in the presence of manure.

Sharma *et al.,* (2000) observed that DTPA-extractable micronutrient like Zn, Fe, Mn and Cu were found to be enhanced significantly due to application of organic material as compared to chemical fertilizers. Richardson *et.al* (2001) studied the utilization of soil organic phosphorus by higher plants and they concluded that soil organisms play a key role in soil P dynamics and subsequent availability of phosphate to plants.

Sihag *et.al.,* (2005) conducted a manurial experiment on rice-wheat cropping system in *Typic Haplustepts* type soil during *kharif*- 1997 at Rice Research Station, Kaul to study the effect of integrated use of inorganic fertilizers and organic materials on the distribution of different forms of N and P in soil . They concluded significant increase in the saloid-P, Al-P and Ca-P forms with the application of inorganic fertilizers along with organic materials. Further, they were reported highest amount of all the forms of P was obtained with FYM followed by green manuring and pressmud treatment.

Phosphorus availability in soils with low organic matter is very low. The organic carbon in the soil increase P availability by formation of organophosphate complexes, anion replacement of H_2PO_4 on adsorption sites, coating of humus on Fe/Al oxides which reduces adsorption and increases the quantity of organic P mineralized to inorganic P. The complex

anions formed by addition of organic matter such as citrate, oxalate, tartarate and malate are the most effective in replacing H_2PO_4 from the adsorption sites (Havlin *et al*., 2007).

Tarafdar, (2008) conducted an experiment on mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He concluded that inoculation with *Aspergillus* strains significantly increased phosphorus concentration, total phosphorus uptake, available phosphorus (25-62%) for chickpea and wheat. Seed inoculation with *Aspergillus fumigates* and soil inoculation with *Glomus mosseae* increased shoot concentrations of phosphorus and lesser extent potassium and manganese. El-Azouni., (2008) reported that the dual inoculation of phosphate solubilising fungi (*Aspergillus niger* and *Penicillium digitarium*) significantly increased the organic carbon levels of the treated soil when compared to untreated soil.

Pattanayak *et al.,* (2009) carried out a study work in relation to the availability of phosphorus at Orissa university of Agriculture and Technology, Bhubaneswar. They concluded that the rate of P mineralization depends on microbial activity and on the activity of free phosphatases. Solubilization of inorganic phosphorus in soil is mostly mediated by microbial activity (*Pseudomonas strita, Bacillus polymixa, bacillus megatherium, penicillium digitatum, Aspergillus awamori*) due to secretion of organic acids which prevents fixation of phosphate ion with chelating effect. Further they reported that the availability and forms of P in soil at one point of time depend upon the native and/or added organic matter content from external sources. Further they stated that the released organic acids

during the process of decomposition of organic matter also solubilize native P leading to increased P availability.

Yankaraddy *et al*., (2009) conducted a field experiment with treatments consisted of organic sources *viz.* coffee pulp compost, rice hull ash and FYM combined with 50-100 per cent recommended dose of fertilizers. The results revealed that application of FYM recorded highest grain yield, straw yield, and nutrient content and nutrient uptake. It was followed by application of coffee pulp compost. After harvest of the crop, available nitrogen, phosphorus and potassium in the treated soil were maximum. Zubair *et al.*, (2012) reported that an improvement in the soil organic carbon (OC) and nitrogen contents ranging from 0.10 to 1.40 % and 0.05 to 0.55 % respectively were achieved in a salt affected soil through amendment of soil with green and farm yard manure.

Gujar *et.al.*,(2013) studied effect of phytase from *Aspergillus niger* on plant growth & mineral assimilation in wheat (*Triticum aestivum Linn.)* on calcareous soil and its potential for use as a soil amendment at NCL, Pune during 2012 and concluded that phytase isolated from *Aspergillus niger* showed improvement in phosphorus and nutrient availability. Nitrogen availability in soil significantly increased by 12%, whereas potassium availability decreased by about 3% and increase in phosphorus content of soil by about 1.18 fold due to phytase application. They further reported that the phytase was able to degrade about 30 % of the phytic acid in the soil. Increase in nitrogen availability can be attributed to release of ammonium ions bound to phytate following degradation by phytase. Phytate being a chelating agent filches the metal ions essential to soil fertility and plant growth. Phytase releases phosphorus from phytate,

leading to loss of ability of phytase to bind or chelate minerals and consequently amend the availability of nutrients (nitrogen, calcium, magnesium, zinc and iron) in soil.

K. Ushari *et.al.*,(2013) studied the enzymatic activity and role of microbial biomass in plant nutrition by isolating phytase producing bacteria (*Bacillus* sps NBtRS6) from the rhizosphere soil of NBt cotton field in Andhra Pradesh. They concluded that microorganisms are integral to the soil P cycle and as such play an important role in mediating the availability of P to plants. Further they stated that organic phosphate can be solubilized by bacterial organic acids and free phosphates may liberated by hydrolysis. Vibha *et al*., (2014) studied the effect of phosphorus solubilizing fungi on major and minor nutrient availability under mung bean for pot experiment in sandy loam soil at Rajendra Agricultural University, Pusa during 2011-12. Availability of nitrogen, potassium and phosphorus significantly improved for inoculated soil than for uninoculated control.

2.3 Effect of phytase and FYM levels on yield and nutrient uptake by soybean

Tarafdar and Rao, (2001) conducted a field experiment under rain-fed conditions in loamy sand soil at Central Arid Zone Research Institute, Jodhpur, Rajasthan from mid-July to end of October in 1995 and 1996. They studied the response of clusterbean to Glomus mosseae and Rhizobium in an arid soil fertilized with nitrogen, phosphorus and Farmyard manure and reported that concentrations of iron, copper and zinc were enhanced. Further, root nodulation, dry matter production and seed yield of clusterbean were increased with inoculation of AMF + Rhizobium and FYM. Application of FYM had additive effect on dry matter production and

seed yield. This could be attributed to a more extensive root system, tapping a large volume of soil for water and nutrients.

Tarafdar, (2008) conducted an experiment on mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He concluded that inoculation with *Aspergillus* strains significantly increased phosphorus concentration, total phosphorus uptake, available phosphorus (25-62%) for chickpea and wheat. Seed inoculation with *Aspergillus fumigates* and soil inoculation with *Glomus mosseae* increased shoot and root dry weight, root length and shoot concentrations of phosphorus and lesser extent potassium and manganese. Yadav and Tarafdar (2010) conducted an experiment and reported that grain yield of cluster bean was increased by 26% of straw yield by 42% plant phosphorus content by 12% due to inoculation of *Emericella rugulosa* (phosphorus solubilising fungi).

Gujar *et.al.*,(2013) studied effect of phytase from *Aspergillus niger* on plant growth & mineral assimilation in wheat (*Triticum aestivum Linn.)* on calcareous soil and its potential for use as a soil amendment at NCL, Pune during 2012 and concluded that phytase isolated from *Aspergillus niger* promoted plant growth (up to 200%). The length of shoot, root and shoot:root ratio was higher in pots supplemented with phytase than uninoculated control. Phosphorus uptake (approximately 74%) and assimilation of micronutrients (calcium, magnesium, zinc and iron) were enhanced due to application of phytase.

Muhammad Iqbal *et al.,* (2013) conducted pot culture experiment using five bacterial strains at different levels of manure and

studied impact of phosphate solubilizing bacteria on growth and yield of maize at Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. They concluded from the experimentation that phosphate solublizing bacteria enhance the growth (plant height, root length, dry weight of shoot and root) through simultaneous exudation of organic acids (by decreasing pH) and/or through releasing phosphatases and ACC-deaminase.

Vibha *et al*., (2014) studied impact of phosphorus solubilisation of fungi on yield of mung bean on sandy loam soil and concluded significantly higher root length, dry weight and seed yield with inoculation of *Aspergillus niger* strains, *Penicilium citrinum* strains and phosphate solubilising bacteria over uninoculated control.

3. MATERIALS AND METHODS

The present investigations entitled “Effect of phytase and FYM levels on soil enzyme activities, yield and nutrient uptake by soybean on non calcareous soil” carried out by conducting pot culture experiment at Division of Soil Science and Agricultural Chemistry during *Kharif* 2014. The details of the material used and methods adopted are depicted in this chapter.

3.1 Material

3.1.1. Soil

The required quantity of surface non calcareous soil of 0-15 cm depth was collected from plot number A-3 from Agronomy farm, College of Agriculture, Pune. The soil was grouped under inceptisol order which comprises of montmorillonite clay. The soil was medium black with 50 cm depth. The important physical, chemical, biological and biochemical properties of soil were determined by using standard methods (Table I)

3.1.2 FYM

The quantity of farm yard manure required for experiment was procured from the Division of Animal Husbandry and Dairy science, College of Agriculture, Pune. The FYM were analyzed for nutrient content by using standard methods and data were reported in table.

3.1.3 Soybean seed

Soybean seed of variety JS-335 was obtained from the Central Seed Cell, Mahatma Phule Krishi Vidhyapeeth, Rahuri, for the experiment.

3.1.4 Phytase enzyme

In order to conduct the pot culture experiment required quantity of phytase enzyme isolated from *Aspergillus niger* was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune, India. The phytase extraction methodology was given by Gujar *et al*., for determining the effect of phytase from *Aspergillus niger* on plant growth and mineral assimilation in wheat and its potential for use as a soil amendment.

3.1.5 Pots

Earthen pots of 10 kg capacity were used for experiment. Due care was taken to ensure enough aeration and no water stagnation with holes in pots.

3.1.6 Insecticide

Cypermethrin 25 % EC @ 2ml in 4L of water was sprayed twice at 30 and 45 DAS as a preventive measure for leaf eating caterpillar.

3.1.7 Fertilizer

Required quantity of recommended dose of nitrogen through urea was applied in this experiment.

3.2 Methods

3.2.1 Analysis

The soil, FYM and plant samples were analyzed by using the following standard analytical methods:

Table I. Methods adopted for soil, FYM and plant analysis

Sr. No.	Parameter	Methods used	Reference
A.	**Soil Chemical analysis**		
1.	pH (1:2.5)	Potentiometric	Jackson (1973)
2.	EC (1:2.5)	Conductometric	Jackson (1973)
3.	Organic carbon	Wet oxidation	Nelson and Sommer (1982)
4.	Calcium carbonate	Rapid titration method	Jackson (1973)
5.	Available N	Alkaline permanganate	Subbia & Asija (1956)
6.	Available P	0.5 *M* $NaHCO_3$ (pH 8.5)	Olsen *et.al* (1954)
7.	Available K	Neutral *N* NH_4OAC	Knudsen *et.al* (1982)
8.	DTPA extractable micro nutrients	Atomic absorption spectro photometer	Lindsay and Norwell (1978)
B.	**Soil Biological analysis**		
1.	Total Microbial population	Serial dilution plate technique	Pramer & Schmidt (1964)
C.	**Soil enzyme analysis**		
1.	Phytase	Modified Ammonium molybdate colorimetric method	Heinonen, J.K & Lahati,R.J (1981)
2.	Acid phosphatase	Colorimetric	Tabatabai & Bremner (1969)
3.	Alkaline phosphatase	Colorimetric	Tabatabai & Bremner (1969)
4.	Dehydrogenase	Colorimetric	Casida *et.al* (1968)
D.	**FYM**		
1.	Organic matter	Dry combustion method	Gorsuch (1970)
2.	Total nitrogen	Macro-Kjeldahl	Piper (1966)

Table I. Contd….

3.	Total phosphorus	Vanadomolybdate yellow color method in Nitric acid system	Jackson (1973)
4.	Total Potassium	Flame photometric	Chapman & Pratt (1961)
5.	Micro nutrients (Fe,Mn,Zn,Cu)	Atomic Absorption Spectro photometer	Zorowski & Burau (1977)
E.	**Plant Analysis**		
1.	Total nitrogen	Micro-kjeldahl	Piper (1966)
2.	Total phosphorus	Vanadomolybdate yellow colour method in nitric acid System	Jackson (1973)
3.	Total potassium	Flame photometery	Chapman and Pratt, (1961)
4.	Micro nutrients (Fe,Mn,Zn,Cu)	Atomic Absorption Spectro photometric	Zorowski & Burau (1977)
5.	Phytate	Colorimetric	Latta & Eskin (1980)

3.2.2 Preparation of pots

Earthen pots were washed with water and used for experiment. Sixty four pots each with 10 kg (2mm sieved) calcareous soil were filled. Before filling the pots treatment wise required quantity of FYM was thoroughly mixed with soil. Thereafter, pots were irrigated. Sowing of soybean equidistantly in the pots was carried out on the same day. After sowing treatment wise recommended dose of nitrogen were applied through urea.

3.2.3 Phytase application

In order to supply treatment wise liquid solution of phytase @ 1200, 2400 and 3600 IU, required quantity of liquid culture @ 24 ml, 48 ml and 96 ml were applied uniformly over the soil after sowing of soybean (plate-1).

Table II. Initial soil analysis:

Sr. No.	Parameters	Value
A.	**Biochemical properties**	
1.	Phytase	Traces
2.	Acid phosphatase (μM 'P' g^{-1} soil hr^{-1})	13.37
3.	Alkaline phosphatase (μM 'P' g^{-1} soil hr^{-1})	21.73
4.	Dehydrogenase (μg TPF g^{-1} soil hr^{-1})	11.06
B.	**Biological properties**	
1.	Bacteria population (x 10^5 CFU g^{-1} soil)	18.76
2.	Fungal population (x 10^4 CFU g^{-1} soil)	9.23
3.	Actinomycetes (x 10^5 CFU g^{-1} soil)	16.02
C.	**Chemical properties**	
1.	pH (1:2.5)	8.0
2.	EC (1:2.5) (dSm^{-1})	0.29
3.	Organic carbon (%)	0.70
4.	$CaCO_3$ (%)	4.5
5.	Available N (kg ha^{-1})	245
6.	Available P (kg ha^{-1})	16.04
7.	Available K (kg ha^{-1})	310
8.	DTPA Fe (mg kg^{-1})	2.4
9.	DTPA Mn (mg kg^{-1})	2.23
10.	DTPA Zn (mg kg^{-1})	1.23
11.	DTPA Cu (mg kg^{-1})	4.55

3.2.4 Characteristics of FYM

The FYM used in the pot culture study was analyzed for different chemical properties and the data are presented in Table 3.

Table III. FYM analysis

Sr. No.	Chemical properties	Value
1.	Organic carbon (%)	11.76
2.	Total N (%)	0.56
3.	Total P (%)	0.37
4.	Total K (%)	0.52
5.	Fe (mg kg^{-1})	1627.47
6.	Mn (mg kg^{-1})	327.85
7.	Zn (mg kg^{-1})	123.87
8.	Cu (mg kg^{-1})	43.57
9.	C:N ratio	21
10.	C:P ratio	31.78

3.2.5 Experimental details

The pot culture experiment was carried out with four phytase and four FYM levels in Factorial Complete Randomized Design (FCRD) design (Plate 2: Experimental view). There were total sixteen treatment combinations with three replications (Fig. 2 plan and lay out of treatment combinations). Details of treatments are depicted in Table IV.

Table: IV Details of treatments:

a) Details of treatment:

Sr. No.	Levels of phytase (IU)	Levels of FYM (t ha^{-1})
1.	0	0
2.	1200	2.5
3.	2400	5.0
4.	3600	7.5

b) Quantity of phytase and FYM

Sr. No.	phytase (ml)	FYM (g pot^{-1})
1.	0	0
2.	24	11
3.	48	22
4.	96	33

- Phytase levels @ 120 IU kg^{-1} soil were formulated on the basis of lab scale study conducted by NCIM, NCL, Pune.
- IU is the amount of enzyme that liberates 1 µmol of inorganic orthophosphate from phytin per minute at pH 5.5 and temperature 37ºC.

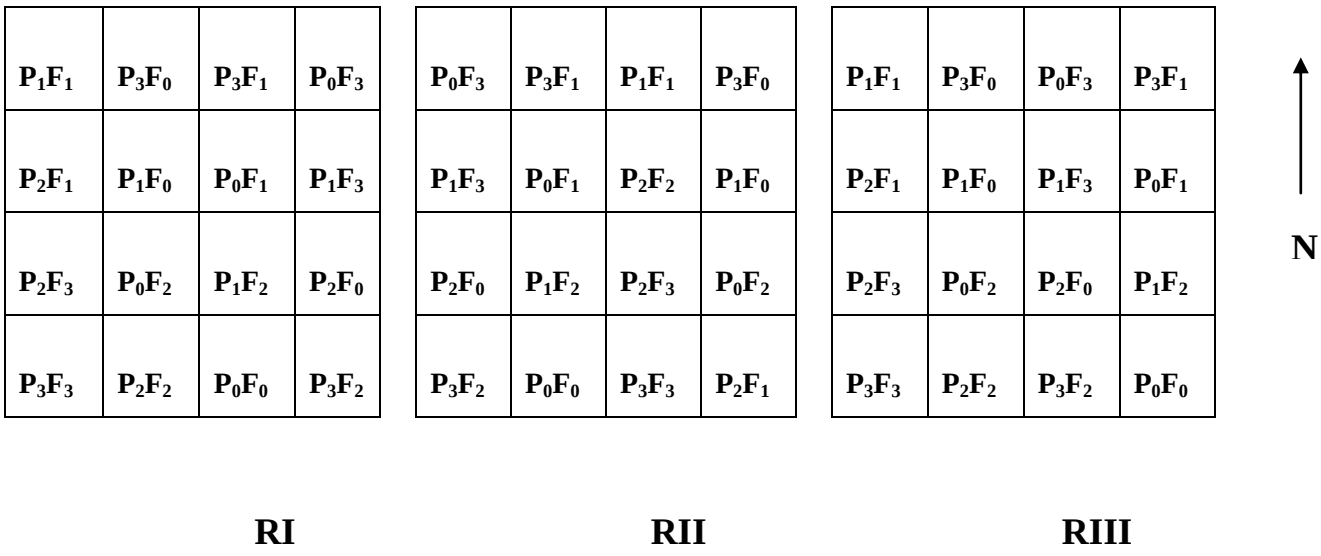


Fig 1: Plan of layout

Table: V Treatment combinations:

P_0F_0	Phytase 0 IU and FYM 0 tha^{-1}	P_2F_0	Phytase 2400 IU and FYM 0tha^{-1}
P_0F_1	Phytase 0 IU and FYM 2.5tha^{-1}	P_2F_1	Phytase 2400IU and FYM 2.5tha^{-1}
P_0F_2	Phytase 0 IU and FYM 5tha^{-1}	P_2F_2	Phytase 2400 IU and FYM 5tha^{-1}
P_0F_3	Phytase 0 IU and FYM 7.5tha^{-1}	P_2F_3	Phytase 2400 IU and FYM 7.5tha^{-1}
P_1F_0	Phytase 1200 IU and FYM 0tha^{-1}	P_3F_0	Phytase 3600 IU and FYM 0tha^{-1}
P_1F_1	Phytase 1200 IU and FYM 2.5tha^{-1}	P_3F_1	Phytase 3600IU and FYM 2.5tha^{-1}
P_1F_2	Phytase 1200 IU and FYM 5tha^{-1}	P_3F_2	Phytase 3600 IU and FYM 5tha^{-1}
P_1F_3	Phytase 1200 IU and FYM 7.5tha^{-1}	P_3F_3	Phytase 3600 IU and FYM 7.5tha^{-1}

3.2.6 Soil sampling

Soil samples were analyzed for soil enzymes and nutrient availability at initial, 50% flowering and harvest by adopting standard methods of analysis. Separate sixteen pots were maintained upto 50% flowering and thereafter they were broken and rhizospheral soil sample collected (plate-3 a)

3.2.7 Soil microbial count

The soil samples were collected at initial, 50% flowering and at harvest (rhizospheral soil) and assessed for total microbial count (fungi, bacteria and actinomycetes)

3.2.8 Methodology of soil enzyme assay

The rhizosphere soil samples were used for assessing the enzyme activities (phytase, acid and alkaline phosphatase and dehydrogenase) at initial, 50% flowering and at harvest of soybean.

3.2.8.1 Phytase enzyme

Microorganism, culture media and enzyme production

Aspergillus niger strain number- NCIM 563, used in the present study for extraction of phytase was obtained from National Collection of Industrial Microorganism (NCIM), National Chemical Laboratory, Pune, India. The stock culture was maintained on potato dextrose agar (PDA) slant and stored at 4 ºC. Spores for inoculation were obtained by culturing the strain at 30 ºC on a PDA slant for 7 days, followed by washing with 10ml sterile containing 0.1ml L^{-1} Tween 80. *Aspergillus niger* was cultivated in production medium at room temperature for 15 days. After fermentation, mycelia were separated by filtration, followed by centrifugation at 10,000 × g for 30 min, and the clear supernatant was collected. Solid ammonium sulfate (95 % saturation) was added to the supernatant with constant stirring. The precipitate was collected by centrifugation at 15000 × g for 20 min and dissolved in the smallest possible volume of glycine-HCl buffer (100 mmol L^{-1}, pH 2.5). Salt was removed by passing through a Sephadex G-25 column and active fractions were concentrated through a YM-30 membrane (Millipore). The enzyme (specific activity approximately 625 IU mg^{-1}) was thus obtained.

Phytase determination

Determination of phytase activity is based on the colorimetrical quantification (Molybdate-Blue method) at 700 nm of free phosphorus released by the hydrolysis of phytate using ammonium molybdate as color reagent.

Reagents:

1. Water- Distilled water
2. Buffer solution (0.1 mol/L)- Dissolve 5.742 g sodium acetic acid, 0.5 g Titron X-100 and 0.5 g bovine serum albumin in 900 ml water, adjust to pH 5.0 with acetic acid (100%) and dilute to 1L with water.
3. Substrate solution-Dissolve 577.4 mg sodium phytate ($C_6H_6O_{24}P_6Na_{12}$) from rice and 574.2 mg sodium acetic acid in 90ml water, adjust the pH to 5.0 with acetic acid (100%) and dilute to 100ml with water. Prepare this solution fresh daily.
4. Reaction stop solution- Trichloroacetic acid (5%)
5. Ammonium heptamolybdate stock solution (Solution A)- Dissolve 7.5 g ammonium heptamolybdate ($N_6H_{24}Mo_7O_{24}.4H_2O$) in 400ml distilled water. This solution may be kept at 4 ºC shielded from light for 1 month.
6. Ferrous sulfate stock solution (Solution B)- Ferrous sufate (2.7%). This solution may be kept at 4 ºC and shielded from light for 1month.
7. Color mix- Mix 100ml solution A and 25 ml solution B. Prepare this solution fresh daily.
8. Potassium dihydrogen phosphate stock solution-Prepare potassium dihydrogen phosphate to constant weight at 60 ºC before dissolving it to a final concentration of 4.0 mmol/L using buffer solution. Prepare this solution fresh daily.

Preparation of sample:

Dilute the weighted sample in duplicate (sample and blank) with buffer solution to a phytase activity within 0.03-0.08 IU/ml.

Assay:

In this procedure, interval of adding reagents to every tube should be completely coincident after the substrate is added to the reaction mixture.

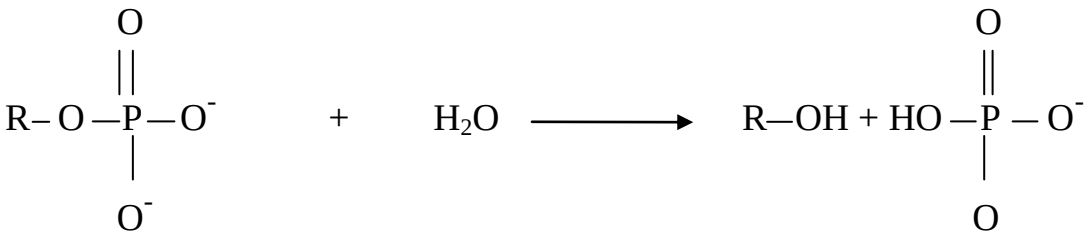
Procedure	Sample, Standards	Sample blank	Standards blank
Sample solution (ml)	0.2	0.2	__
Buffer solution (ml)	__	__	0.2
5 min at 37ºC	√	√	√
Substrate solution (ml)	0.8	0.8 (the second step)	0.8(the second step)
Mixing	√	√	√
30min at 37ºC	√	__	__
Stop solution (ml)	1.0	1.0 (the first step)	1.0 (the first step)
Color reagent (ml)	1.0	1.0	1.0
Mixing	√	√	√
Total volume (ml)	1.0	3.0	3.0

Centrifuge all the tubes for 10 min at 4,000 rpm before standing for 10 min at room temperature. Measure the absorbance of sample and its blank at 700 nm with the spectrophotometer after zeroing the instrument with standards blank. Determine the enzyme activity by reading the corrected absorbance difference for the sample and calculating the released phosphorus. Enzyme activity is expressed in activity units (FYU). 1 FYU is

the enzyme that liberates 1µmol inorganic orthophosphate per minute under test conditions (pH 5.0; temperature 37 ºC; and substrate concentration, sodium phytate [$C_6H_6O_{24}P_6Na_{12}$] at 0.005 mol/L).

3.2.8.2 Acid and alkaline phosphatase

The assay method for phosphomonoesterases (acid and alkaline phosphatase) was given by Tabatabai and Bremner, 1969; Eivazi and Tabatabai, 1977. The general equation of the reaction catalyzed by acid and alkaline phosphatase is:



Reagents:

1. Toluene, Fisher certified reagent
2. Modified universal buffer (MUB) stock solution: Dissolve 12.1 g of tris (hydroxymethyl) aminomethane (THAM), 11.6 g of maleic acid, 14.0 g of citric acid, and 6.3 g of boric acid in 488 ml of 1*N* sodium hydroxide (NaOH) and dilute the solution to 1 liter with water. Store it in a refrigerator.
3. Modified universal buffer (MUB), pH 6.5 and11: Place 200 ml of MUB stock solution in a 500–ml beaker containing a magnetic stirring bar, and place the beaker on a magnetic stirrer. Titrate the solution to pH 6.5 with 0.1 *N* hydrochloric acid, and adjust the volume to 1 liter

with water. Titrate another 200 ml of the MUB stock solution to pH 11 by using 0.1 *N* NaOH, and adjust the volume to 1 liter with water.

4. *p*-Nitrophenyl phosphate solution, 0.025 *M*: Dissolve 0.420 g of disodium *p*-nitrophenyl phosphate tetra hydrate in about 40 ml of modified universal buffer pH 6.5 or pH 11 and dilute the solution to 50 with MUB of the same pH. Store the solution in a refrigerator.
5. Calcium chloride ($CaCl_2$) 0.5 *M*: Dissolve 73.5 g of $CaCl_2.H_2O$ in about 700 ml of water, and dilute the volume to 1 liter with water.
6. Sodium hydroxide, 0.5 *M*: Dissolve 20 g of NaOH in about 700 ml of water, and dilute the volume to 1 liter with water.
7. Standard *p*-nitrophenol solution: Dissolve 1.0 g of *p*-nitrophenol in about 70 ml of water and dilute the solution to 1 liter with water. Store the solution in a refrigerator.

Procedure:

Place 1g of soil (< 2mm) in a 50 ml Erlenmeyer flask.

⇩

Add 0.2 ml of toluene

⇩

4ml of MUB (pH-6.5 for acid phosphatase & 11 for alkaline phosphatase)

1 ml of *p*-nitrophenyl phosphate solution made in the same buffer

⇩

Swirl the flask, stopper it and place it in incubator at 37ºC

⇩

After one hour remove stopper, add 1ml 0.5 M $CaCl_2$ and 4ml 0.5 *M* NaOH

⇩

Swirl the flask for few seconds and filter the soil suspension through Whatman no. 2 filter paper

⇩

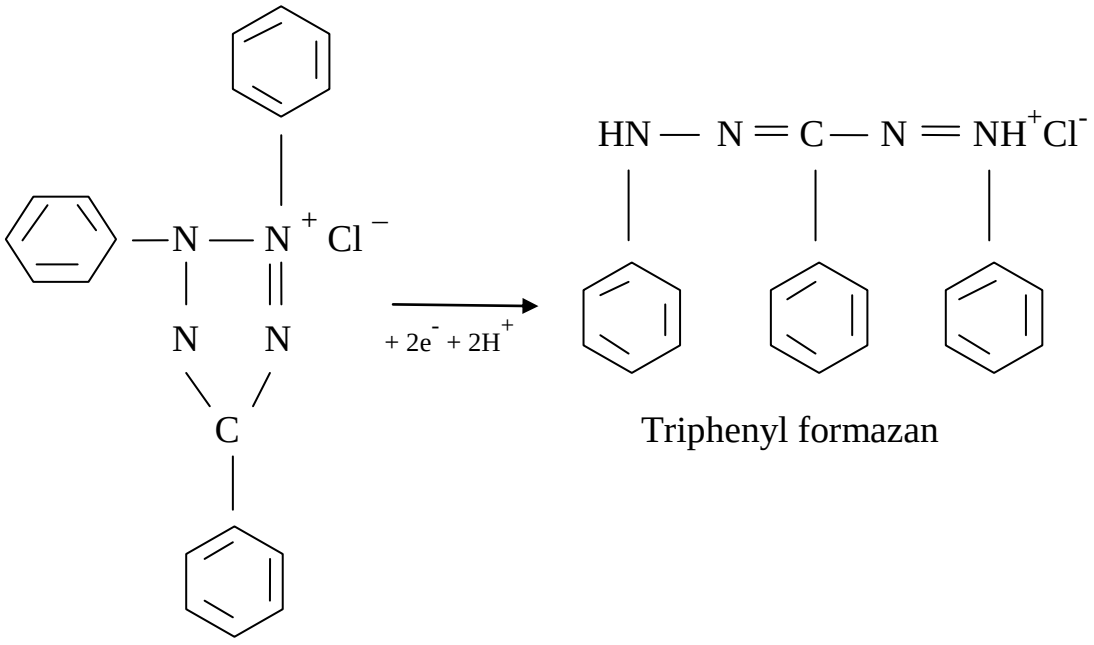
Measure the yellow color intensity of the filtrate colorimeter

⇩

Calculate *p*-nitrophenol content of filtrate by reference to a calibration graph

3.2.8.3 Dehydrogenase

The assay method for dehydrogenase was given Casida, 1968. The method is based on extraction with methanol and colorimetric determination of the TPF produced from the reduction of TTC in soils.



Triphenyl formazan

2,3,5- Triphenyltetrazolium chloride

Reagents:

1. Calcium carbonate
2. 2,3,5-Triphenyltetrazolium chloride (TTC), 3%: Dissolve 3g of TTC in about 80ml of water and adjust the volume to 100 ml with water
3. Methanol
4. Triphenyl formazon (TPF) standard solution: Dissolve 100 mg of TPF in about 80ml of methanol, and adjust the volume to 100ml with methanol.

Procedure:

Mix thoroughly 20g air dried soil (< 2mm) and 0.2g $CaCO_3$ and place 6g of this mixture in each three test tubes

⇩

Add 1ml of 3% aqueous solution of TTC and 2.5 ml of distilled water (small amount of free liquid appears at top).

⇩

Mix the content, stopper tube and incubate at 37ºC

⇩

After 24 hours, add 10ml methanol, stopper and shake for one minute

⇩

Filter the suspension through glass funnel plugged with absorbent cotton into 100ml volumetric flask.

⇩

Wash tube with methanol (10ml portion) until reddish color disappears from cotton

⇩

Dilute the filtrate to 100ml volume with methanol

⇩

Measure intensity of color by spectrophotometer at wavelength of 484 nm and methanol as blank

⇩

Calculate amount of TPF produced by reference to a calibration graph

3.2.9 Plant morphological characteristics:

In order to carry out biometric observations of soybean at 50% flowering, sixteen pots along with soybean plants were maintained. These pots were broken down carefully without disturbing the soil-root biomass (plate-3 a). After breaking the pots, rhizospheral soil sample were collected from the soil-root biomass and used for analysis of chemical, biological and biochemical properties. The same mass of soil along with plant was slowly dipped in water in order to discard all the soil attached to the roots (plate-3 b). The fresh weight of shoot and root were taken after wiping with filter paper and drying in shade thereafter biometric observations were recorded accordingly.

The following observations were taken at 50% flowering:

3.2.9.1 Plant height and root length (cm)

The plant height (shoot length) and root length were taken with the help of thread and scale. Measurement was taken from ground level to the top for plant height. In case of root, plants were uprooted and roots detached which were subsequently measured.

3.2.9.2 Root shoot ratio (cm)

The values obtained after measuring the plant height and root length were used to formulate the root-shoot ratio.

3.2.9.3 Fresh and dry weight of shoot and root (g)

Fresh weight of shoot was recorded after detaching the root from the plant. The same plant was air dried and then oven dried at 70 ºC temperature till constant weight and used for dry weight.

3.2.9.4 Dry matter and plant analysis

After biometric observations, same plants were used for dry matter observation and used for nutrient analysis.

3.2.9.5 Yield and nutrient uptake at 50% flowering and at harvest

The dry matter yield of soybean was taken at 50% flowering for straw and at harvest for grain and straw by estimating their dry weight. Uptakes of major and micro nutrients were determined by estimating the dry weight and nutrient concentration of soybean grain. The uptake of each nutrient was calculated separately for grain and straw.

4. RESULT AND DISCUSSION

4.1 Effect of phytase and FYM levels on biochemical properties in non calcareous soil under soybean at 50% flowering and at harvest

4.1.1 Phytase activity:

Phytase activity in non calcareous soil as influenced by various levels of phytase and FYM imposed to soybean was assessed at 50 percent flowering and at harvest of soybean was found significant and presented in Table 1 a and b; Fig. 1a and b.

a) At 50 percent flowering: Application of phytase @ 0, 1200, 2400 and 3600 IU recorded increasing trend for soil phytase activity as 0.04, 0.09, 0.12 and 0.16 IU respectively at 50 per cent flowering. While application of FYM @ 0, 2.5, 5 and 7.5 t ha^{-1} also recorded increasing trend for soil phytase activity i.e. 0.04, 0.09, 0.10 and 0.16 IU. Application of phytase @ 3600 IU (0.23 IU) recorded significantly higher soil phytase activities followed by use of phytase @ 2400 IU (0.18 IU).

Application of FYM @ 7.5 t ha^{-1} (0.22 IU) recorded significantly higher soil phytase activity followed by application of FYM @ 5 t ha^{-1} (0.18 IU). Lower mean phytase activity was recorded in no phytase and no FYM (0.10 IU) amended pots.

Interaction effect of combine application of phytase and FYM was found significant on soil phytase activity of non calcareous soil. Phytase application @ 3600 IU (0.29 IU) along with 7.5 t ha^{-1} FYM recorded significantly higher and statistically at par soil phytase activity, followed by phytase @ 3600 IU + FYM @ 5 t ha^{-1} (0.26 IU) and phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (0.25 IU).

Table 1: Effect of phytase and FYM levels on phytase activity in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	Phytase activity (IU)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.04	0.09	0.12	0.16	0.10
1200 IU	0.09	0.11	0.14	0.19	0.13
2400 IU	0.10	0.17	0.21	0.25	0.18
3600 IU	0.16	0.22	0.26	0.29	0.23
Mean	0.10	0.15	0.18	0.22	
	P		F		P × F
S.E. ±	0.002		0.002		0.004
CD at 5%	0.005		0.005		0.011

b) At harvest of soybean

FYM (F) / Phytase (P)	Phytase activity (IU)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.07	0.12	0.17	0.22	0.14
1200 IU	0.11	0.14	0.20	0.24	0.17
2400 IU	0.17	0.20	0.24	0.27	0.22
3600 IU	0.21	0.25	0.30	0.35	0.28
Mean	0.14	0.18	0.23	0.27	
	P		F		P × F
S.E. ±	0.002		0.002		0.005
CD at 5%	0.007		0.007		0.013

Initial phytase activity: 0 IU

In general soil phytase activity was higher with the combine application of phytase and FYM rather than only phytase.

b) At harvest of soybean: The observed activity of phytase in non calcareous soil as influenced by phytase and FYM levels imposed to soybean at harvest are presented in Table 1 b; Fig. b.

Consistent increasing trend in phytase activity was noticed at 50 percent flowering and at harvest over initial activity (0 IU). The results revealed that the application of phytase @ 3600 IU (0.28 IU) recorded significantly higher soil phytase activity followed by 2400 IU (0.22 IU), and 1200 IU (0.17 IU). While lower phytase activity was noticed with the application of phytase @ 0 IU (0.14 IU).

In case of FYM application, FYM @ 7.5 t ha^{-1} recorded significantly higher mean phytase activity (0.27 IU) than rest of the treatments followed by 5 t ha^{-1} (0.23 IU). Lowest mean phytase activity was recorded with the application of 0 t ha^{-1} FYM (0.14 IU)

Conjoint application of FYM and phytase were significantly influenced phytase activity in soil. Combine application of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM (0.35 IU) recorded significantly higher phytase activity followed by phytase @ 3600 IU + FYM @ 5 t ha^{-1} (0.30 IU) and 2400 IU + 7.5 t ha^{-1} FYM (0.27 (IU) . Lower phytase activity was recorded in no phytase and no FYM (0.07 IU).

Yadav *et al.*, (2009) reported 15 % increase in acid phosphatase, 12% increase in alkaline phosphatase, 71% increase in soil phytase activity in soil due to inoculation of *Chaetomium globosum* with organic matter compared

to application of only organic matter by conducting an experiment on phosphorus mobilization and yield of cluster bean under arid ecosystem.

Ushari *et.al.*,(2013) studied the enzymatic activity by isolating phytase producing bacteria (*Bacillus* sps NBtRS6) from the rhizosphere soil of NBt cotton field in Andhra Pradesh and they observed that phosphatase is an enzyme that release inorganic phosphate from organic moiety and complex inorganic materials. It is known to play an essential role in phosphorus cycle. Further they stated that phosphatases play akey role in phosphorus cycle by solubilizing organic and inorganic phosphates into available forms that support growth of crop plants. Similar results were also found by Adnane Bargaz, they reported significant increase in acid phosphatase and phytase activity in rhizospheral soil of *Phaseolus vulgaris* under low soil phosphorus situations as against soil with sufficient phosphorus. Lung *et al*., (2006) conducted an experiment on tobacco (*N. tabacum*) crop showing assimilation of phytate-phosphorus by the extracellular phytase activity affected by the availability of soluble phytate. They concluded that P starvation of tobacco seedlings triggered a substantial increase in the specific phytase activity in root exudates. Aseri *et al*., (2009) studied hydrolysis of organic phosphate forms by phosphatases and phytase producing fungi of arid and semi arid soils of India at Amity University, Rajasthan and Central Arid Zone Research Institute, Jodhpur during March 2004. They reported that several types of phosphatases, such as phytases, are able to increase the rate of the dephosphorylation (hydrolysis) of organic P. Phosphatases in the rhizosphere may arise from plant roots or soil microbes. The addition of phytase increased the P content of maize seedlings when supplied with phytate by plant which concluded that the utilization of phytate by plant was limited by low rates of hydrolysis. They concluded that

Trichoderma spp. was found to be most efficient for hydrolysis of organic P by secreting phosphatases and phytase enzymes.

Further, Wodzinski and Ullah (1996) reported superior stability and activity of microbial phytase at wide temperature range i.e. 35 to 63ºC than plant phytase i.e. 45 to 60ºC.

4.1.2 Acid phosphatase activity:

The acid phosphatase activity was assessed at 50% flowering and at harvest of soybean as influenced by graded levels of FYM and phytase are presented in Table 2 a and b; Fig. 2 a and b.

The consistent increase in soil acid phosphatase activity of non calcareous soil was noticed in all individual phytase and FYM levels and their combinations over initial (13.37 µM 'P' g^{-1} soil hr^{-1}). The soil acid phosphatase activity at both the growth stages (at 50% flowering and at harvest) was significantly influenced by levels of phytase (0, 1200, 2400, 3600 IU) and FYM (0, 2.5, 5, 7.5 t ha^{-1}) imposed to soybean.

a) At 50% flowering of soybean: Mean acid phosphatase activity was influenced significantly by different phytase levels. Singificantly higher soil acid phosphatase activity was recorded with the application of phytase @ 3600 IU (17.00 µM 'P' g^{-1} soil hr^{-1}) which was at par @ 2400 IU (16.67 µM 'P' g^{-1} soil hr^{-1}). However no phytase application recorded significantly lower acid phosphatase activity (13.83 µM 'P' g^{-1} soil hr^{-1}) than rest of the treatments.

Application of FYM @ 7.5 t ha^{-1} recorded significantly higher mean soil acid phosphatase activity (18.46 µM 'P' g^{-1} soil hr^{-1}) followed by 5 t ha^{-1} (17.29 µM 'P' g^{-1} soil hr^{-1}) FYM. However application of 0 and 2.5 t ha^{-1}

FYM recorded lower mean acid phosphatase activity i.e. 12.97 and 14.11 µM 'P' g^{-1} soil hr^{-1} respectively.

Soil acid phosphatase activity was significantly influenced by interaction between phytase and FYM levels. Application of 3600 IU phytase along with FYM @ 7.5 t ha^{-1} recorded significantly higher soil acid phosphatase activity (21.53 µM 'P' g^{-1} soil hr^{-1}). Application of phytase @ 3600 IU along with 5 t ha^{-1} FYM (20.70 µM 'P' g^{-1} soil hr^{-1}) recorded at par results for activity of soil acid phosphatase. However it is observed that only FYM application may be @ 2.5, 5 or 7.5 t ha^{-1} without phytase recorded higher magnitude of increase in acid phosphatase activity than combine application of phytase and FYM.

b) At harvest of soybean: Consistent increasing trend in relation to acid phosphatase activity was observed over initial, at 50% flowering and at harvest in individual phytase, FYM applied treatment and their interaction. Phytase application @ 3600 IU (20.68 µM 'P' g^{-1} soil hr^{-1}) and @ 2400 IU (19.36 µM 'P' g^{-1} soil hr^{-1}) recorded significantly higher and at par acid phosphatase activity in soil. While no phytase application recorded lower acid phosphatase activity (17.63 µM 'P' g^{-1} soil hr^{-1}).

FYM application @ 7.5 t ha^{-1} (20.40 µM 'P' g^{-1} soil hr^{-1}) recorded significantly higher activity of soil acid phosphatase. However the application of FYM @ 5 t ha^{-1} (20.30 µM 'P' g^{-1} soil hr^{-1}) was found at par over rest of the treatments. Lower soil acid phosphatase activity was noticed in no FYM amended pots.

Significant interaction effect of graded levels of phytase and FYM was observed on soil acid phosphatase activity. Conjoint application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (23.22 µM 'P' g^{-1} soil hr^{-1}) recorded

Table 2: Effect of phytase and FYM levels on acid phosphatase activity in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / phytase (P)	µM 'P' g^{-1} soil hr^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	11.36	12.42	14.63	16.92	13.83
1200 IU	12.99	15.04	14.69	17.38	15.03
2400 IU	12.54	16.98	19.14	17.99	16.67
3600 IU	14.98	12.01	20.70	21.53	17.00
Mean	12.97	14.11	17.29	18.46	
	P		F		P × F
S.E. ±	0.213		0.213		0.426
CD at 5%	0.616		0.616		1.232

b) At harvest of soybean

FYM (F) / Phytase (P)	µM 'P' g^{-1} soil hr^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	15.93	16.22	18.15	20.22	17.63
1200 IU	18.14	19.14	18.99	19.36	18.91
2400 IU	17.85	18.29	22.53	18.78	19.36
3600 IU	19.49	18.49	21.51	23.22	20.68
Mean	17.85	18.04	20.30	20.40	
	P		F		P × F
S.E. ±	0.335		0.335		0.67
CD at 5%	0.969		0.969		1.939

Initial acid phosphatase activity: 13.37 µM 'P' g^{-1} soil hr^{-1}

significantly higher soil acid phosphatase activity. However with application of phytase either @ 2400 IU (22.53 µM 'P' g^{-1} soil hr^{-1}) or 3600 IU (21.51 µM 'P' g^{-1} soil hr^{-1}) along with 5 t ha^{-1} recorded statistically at par results. Lowest acid phosphatase activity was noticed with absolute control (no phytase, no FYM).

The acid phosphatase activity was higher in FYM amended pots than phytase was might be due to more growth and number of fine roots of a small diameter and thereby causing exudation of intracellular acid phosphatase. Similar results were also reported by Adanane bargaz (2012).

Higher soil acid phosphatase activity in FYM amended pots might be ascribed to enhanced microbial population and activity. Further these secret acid phosphatase which have positive effect on native 'P' solubilization. (Yadav and Verma,2012).

Dotaniya *et al* (2014) studied the rhizosphere effect of *kharif* crops on soil enzymes in typic Haplustert (Vertisols) at IISS, Bhopal during 2011. They concluded maximum acid phosphatase activities were observed at 75 DAT (days after transplanting) in rice (0.206 mg PNP g^{-1} h^{-1}), 90 DAS in sorghum (0.194 mg PNP g^{-1} h^{-1}) and pearl millet (0.201 mg PNP g^{-1} h^{-1}) 502 DAS in soybean (0.127 mg PNP g^{-1} 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.

Isolation of gene with higher efficiency of acid phosphatase activity and introduced in soybean was carried out by Wang *et al* (2009) and concluded that enhanced acid phosphatase activity and it's secretion by hairy roots in transgenic lines of soybean over control.

Kumar *et al*., (1992) conducted an experiment on the soil microbial population number and enzyme activities in relation to altitude and forest degradation. Their study indicated that dehydrogenase activity display the close, positive correlations not only with organic matter but also fungal population.

Martens *et al*., (1992) conducted a field study to determine the activity and persistence of soil enzymes with the repeated addition of different organic residues at Department of Environment and Soil Sciences, California. They concluded that the enzyme activity in the amended soil was increased by an average of 2 to 4 fold by incorporation of organic amendments as compared to the non amended soils. Further they stated that the increased levels of enzyme activity in the organic amended soil may be a reflection of the increased protective sites within the soil as a result of enhanced humus content. Aseri *et al*., (2009) studied hydrolysis of organic phosphate forms by phosphatases and phytase producing fungi of arid and semi arid soils of India at Amity University, Rajasthan and Central Arid Zone Research Institute, Jodhpur during March 2004. They reported that several types of phosphatases, such as phytases, are able to increase the rate of the dephosphorylation (hydrolysis) of organic P. Phosphatases in the rhizosphere may arise from plant roots or soil microbes. The addition of phytase increased the P content of maize seedlings when supplied with phytate by plant which concluded that the utilization of phytate by plant was limited by low rates of hydrolysis. They concluded that *Trichoderma spp.* was found to be most efficient for hydrolysis of organic P by secreting phosphatases and phytase enzymes.

4.1.3 Alkaline phosphatase activity:

Alkaline phosphatase activity in non calcareous soil as influenced by various levels of phytase and FYM imposed to soybean was assessed at 50 percent flowering and at harvest of soybean was found significant and presented in Table 3 a and b; Fig. 3 a and b.

a) At 50% flowering of soybean: The data reported a significant increase in soil alkaline phosphatase activity with the application of alone phytase or FYM and various levels of combined treatments (phytase + FYM).

The application of phytase @ 3600 IU (30.96 µM 'P' g^{-1} soil hr^{-1}) recorded significantly higher soil alkaline phosphatase activity. While the use of 2400 IU (30.12 µM 'P' g^{-1} soil hr^{-1}) registered statistically at par values for soil alkaline phosphatase activity. Lower soil alkaline phosphatase activity was noticed with no use of phytase (27.92 µM 'P' g^{-1} soil hr^{-1}).

Data indicated that application of FYM @ 7.5 t ha^{-1} (32.33 µM 'P' g^{-1} soil hr^{-1}) noticed significantly higher soil alkaline phosphatase activity followed by 5 t ha^{-1} (30.89 µM 'P' g^{-1} soil hr^{-1}). Lower soil alkaline phosphatase activity was noticed with no use of FYM (25.95 µM 'P' g^{-1} soil hr^{-1}).

Significant interaction effect was reported from the present experimental data with the combined application of phytase and FYM. Conjoint application of phytase @ 3600 IU along with FYM @ 7.5 t ha^{-1} (34.36 µM 'P' g^{-1} soil hr^{-1}) recorded significantly higher soil alkaline phosphatase activity which was found statistically at par with the use of phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (33.09 µM 'P' g^{-1} soil hr^{-1}).

Table 3: Effect of phytase and FYM levels on alkaline phosphatase activity in non calcareous soil .

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	µM 'P' g^{-1} soil hr^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	23.89	27.5	29.68	30.6	27.92
1200 IU	25.85	28.65	30.28	31.28	29.02
2400 IU	26.92	29.09	31.36	33.09	30.12
3600 IU	27.14	30.12	32.22	34.36	30.96
Mean	25.95	28.84	30.89	32.33	
	P		F		P × F
S.E. ±	0.336		0.336		0.671
CD at 5%	0.971		0.971		1.943

b) At harvest of soybean

FYM (F) / Phytase (P)	µM 'P' g^{-1} soil hr^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	25.36	28	30.21	32.47	29.01
1200 IU	26.36	28.69	31.32	33.21	29.90
2400 IU	28.62	29.32	32.36	35.69	31.50
3600 IU	29.32	30.32	33.45	36.45	32.39
Mean	27.42	29.08	31.84	34.46	
	P		F		P × F
S.E. ±	0.433		0.433		0.865
CD at 5%	1.252		1.252		2.504

Initial alkaline phosphatase activity = 21.73 µM 'P' g^{-1} soil hr^{-1}

b) At harvest of soybean: The perusal of data revealed that significantly higher soil alkaline phosphatase activity was recorded with the application of phytase @ 3600 IU (32.39 µM 'P' g^{-1} soil hr^{-1}) which was statistically at par with the use of phytase @ 2400 IU (31.50 µM 'P' g^{-1} soil hr^{-1}).

With respect to the use of FYM significantly higher soil alkaline phosphatase activity was noticed with the application of FYM @ 7.5 t ha^{-1} (34.46 µM 'P' g^{-1} soil hr^{-1}) followed by FYM @ 5 t ha^{-1} (31.84 µM 'P' g^{-1} soil hr^{-1}) and 2.5 t ha^{-1} (29.08 µM 'P' g^{-1} soil hr^{-1}). Lower soil alkaline phosphatase activity was observed with no application of FYM (27.42 µM 'P' g^{-1} soil hr^{-1}).

Conjoint application of phytase and FYM in different treatment combinations reported a significant increase in soil alkaline phosphatase activity. The use of phytase @ 3600 IU along with FYM @ 7.5 t ha^{-1} (36.45 µM 'P' g^{-1} soil hr^{-1}) reported significantly higher soil alkaline phosphatase activity which was statistically at par with the application of phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (35.69 µM 'P' g^{-1} soil hr^{-1}) over control and rest of the treatments.

Yadav *et al.*, (2009) reported 15 % increase in acid phosphatase, 12% increase in alkaline phosphatase, 71% increase in soil phytase activity in soil due to inoculation of *Chaetomium globosum* with organic matter compared to application of only organic matter by conducting an experiment on phosphorus mobilization and yield of cluster bean under arid ecosystem. Higher alkaline phosphatase activity in alkaline calcareous soil was reported by Kramer and Green, (2000) as against acid phosphatase which is higher in acidic soils. Similar results were also reported by Wang *et al.*, (2011) that activity of acid phosphatase is predominant in acid soils whereas alkaline phosphatase is in alkaline soil. Further, as per Tarafdar and Chhonkar, (1978) 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). The activity of acid and alkaline phosphatase was found to correlate with organic matter in various studies (Guan, 1989; Jordan and Kremer, 1994; Aon and Colaneri, 2001). Release of organic anions and production of alkaline phosphatase enzyme hydrolyses the soil organic phosphorus and split phosphorus from organic residues (Tarafdar and Claassen, 1988). Nannipieri *et al*., (1983) studied the microbial biomass in relation to the soil enzyme activities. They reported that soil enzyme activities may be increased by the incorporation of organic materials in the soil. Kumar *et al*., (1992) conducted an experiment on the soil microbial population number and enzyme activities in relation to altitude and forest degradation. Their study indicated that dehydrogenase activity display the close, positive correlations not only with organic matter but also fungal population.

Martens *et al*., (1992) conducted a field study to determine the activity and persistence of soil enzymes with the repeated addition of different organic residues at Department of Environment and Soil Sciences, California. They concluded that the enzyme activity in the amended soil was increased by an average of 2 to 4 fold by incorporation of organic amendments as compared to the non amended soils. Further they stated that the increased levels of enzyme activity in the organic amended soil may be a reflection of the increased protective sites within the soil as a result of enhanced humus content.

Manna *et al.,* (2007) 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. Solubilization rate of TCP under soybean was significantly increased by increasing levels of FYM application. The rate

of organic –P mineralization was slower than inorganic -P solubilizing rate, and increased significantly with increasing FYM application.

4.1.4 Dehydrogenase activity:

Dehydrogenase activity in non calcareous soil as influenced by various levels of phytase and FYM imposed to soybean was assessed at 50 percent flowering and at harvest of soybean was found significant. The result regarding to the dehydrogenase activity is noted in Table 4 a and b; Fig. 4 a and b.

a**) At 50% flowering of soybean:** Application of phytase @ 0, 1200, 2400 and 3600 IU recorded increasing trend for soil dehydrogenase activity as 13.38, 14.01, 14.35 and 15.38 µg TPF g^{-1} soil hr^{-1} respectively at 50 per cent flowering. While application of FYM @ 0, 2.5, 5 and 7.5 t ha^{-1} also recorded increasing trend for soil dehydrogenase activity i.e. 13.38, 13.84, 14.42 and 15.36 µg TPF g^{-1} soil hr^{-1}.The magnitude of increase in soil dehydrogenase activity was higher with the application of FYM than phytase. Application of phytase @ 3600 IU (16.63 µg TPF g^{-1} soil hr^{-1}) and 2400 IU (16.56 µg TPF g^{-1} soil hr^{-1}) recorded significantly higher soil dehydrogenase activity which was statistically at par with each other.

With respect to use of FYM data noticed that application of 7.5 t ha^{-1} (16.90 µg TPF g^{-1} soil hr^{-1}) recorded significantly higher soil dehydrogenase activity followed by 5 t ha^{-1} (16.04 µg TPF g^{-1} soil hr^{-1}) and 2.5 t ha^{-1} (15.20 µg TPF g^{-1} soil hr^{-1}). Lower mean dehydrogenase activity was recorded in no phytase (15.47 µg TPF g^{-1} soil hr^{-1}) and no FYM (14.28 µg TPF g^{-1} soil hr^{-1}) amended pots.

Interaction effect of combine application of phytase and FYM was found significant on soil dehydrogenase activity of non calcareous soil. Phytase

application either @ 3600 IU (18.53 µg TPF g^{-1} soil hr^{-1}) or 2400 IU (18.01 µg TPF g^{-1} soil hr^{-1}) along with 7.5 t ha^{-1} FYM recorded significantly higher and statistically at par soil dehydrogenase activity. In general soil dehydrogenase activity was higher with the combine application of phytase and FYM rather than only phytase.

b) At harvest of soybean: The observed activity of dehydrogenase in non calcareous soil as influenced by phytase and FYM levels imposed to soybean are presented in Table 4 b and Fig. 4 b. Consistent increasing trend in dehydrogenase activity was noticed at 50 percent flowering and at harvest over initial activity (11.06 µg TPF g^{-1} soil hr^{-1}). Similar trend of higher magnitude of increase was reported with FYM amended pots than phytase. The results indicated that highest FYM levels (7.5 t ha^{-1}) without phytase (16.34 µg TPF g^{-1} soil hr^{-1}) recorded higher dehydrogenase activity than that of highest phytase level (3600 IU) without FYM (15.60 µg TPF g^{-1} soil hr^{-1}).

The results revealed that the application of phytase @ 3600 IU (16.80 µg TPF g^{-1} soil hr^{-1}), 2400 IU (16.69 µg TPF g^{-1} soil hr^{-1}), and 1200 IU (16.00 µg TPF g^{-1} soil hr^{-1}) recorded significantly higher and statistically at par soil dehydrogenase activity.

In case of FYM application @ 7.5 t ha^{-1} recorded significantly higher mean dehydrogenase activity (18.67 µg TPF g^{-1} soil hr^{-1}) than rest of the treatments followed by 5 t ha^{-1} (16.60 µg TPF g^{-1} soil hr^{-1}).

Conjoint application of FYM and phytase were significantly influenced dehydrogenase activity in soil. Combine application of phytase either @ 3600 IU (19.64 µg TPF g^{-1} soil hr^{-1}) along with 7.5 t ha^{-1} FYM recorded significantly higher soil dehydrogenase activity.

Table 4: Effect of phytase and FYM levels on dehydrogenase activity in non calcareous soil

a) At 50 % flowering of soybean

Phytase (P) \ FYM (F)	µg TPF g^{-1} soil hr^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	13.38	13.84	14.42	15.36	14.25
1200 IU	14.01	14.64	15.58	15.69	14.98
2400 IU	14.35	16.48	17.40	18.01	16.56
3600 IU	15.38	15.84	16.76	18.53	16.63
Mean	14.28	15.20	16.04	16.90	
	P		F		P × F
S.E. ±	0.255		0.255		0.509
CD at 5%	0.737		0.737		1.474

b) At harvest of soybean

Phytase (P) \ FYM (F)	µg TPF g^{-1} soil hr^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	14.10	15.61	13.21	16.34	15.31
1200 IU	15.58	14.12	15.53	18.73	16.00
2400 IU	15.04	14.65	19.00	18.00	16.69
3600 IU	15.60	13.38	18.55	19.64	16.80
Mean	15.08	14.44	16.60	18.67	
	P		F		P × F
S.E. ±	0.398		0.398		0.796
CD at 5%	1.152		1.152		2.303

Initial dehydrogenase activity: 11.06 µg TPF g^{-1} soil hr^{-1}

However application of phytase either @ 2400 IU (19.00 µg TPF g^{-1} soil hr^{-1}) or 3600 IU (18.55 µg TPF g^{-1} soil hr^{-1}) along with 5 t ha^{-1} FYM recorded statistically at par dehydrogenase activity. Lower dehydrogenase activity was recorded in no phytase along with 5 t ha^{-1} FYM (13.21 µg TPF g^{-1} soil hr^{-1}) and 3600 IU phytase with 2.5 t ha^{-1} FYM (13.38 µg TPF g^{-1} soil hr^{-1}).

Tarafdar and Rao, 1996 studied dehydrogenase and phosphatase activity as influenced by inoculation with *Aspergillus* in rhizosphere and non rhizosphere soils of cluster bean, chickpea and wheat. They concluded higher dehydrogenase, acid phosphatase and alkaline phosphatase activities in rhizospheral soil than non rhizospheral soil.

Rai and Yadav (2011) studied influence of inorganic and organic nutrient sources on enzyme activities by conducting an incubation study on sandy loam soil during 2004-05 and pot culture experiment during rabi season of 2004-05 and 2005-06. They reported that maximum build up of dehydrogenase (74.5 µg TPF produced g^{-1} soil d^{-1}) was found in soil treated with FYM and press mud at 30 DAI and 120 DAI.

Kumar *et al*., (1992) conducted an experiment on the soil microbial population number and enzyme activities in relation to altitude and forest degradation. Their study indicated that dehydrogenase activity display the close, positive correlations not only with organic matter but also fungal population.

Martens *et al*., (1992) conducted a field study to determine the activity and persistence of soil enzymes with the repeated addition of different organic residues at Department of Environment and Soil Sciences, California. They concluded that the enzyme activity in the amended soil was increased by an average of 2 to 4 fold by incorporation of organic amendments as compared

to the non amended soils. Further they stated that the increased levels of enzyme activity in the organic amended soil may be a reflection of the increased protective sites within the soil as a result of enhanced humus content. Asmar and Gissel-Nielsen (1996) worked on the extracellular phosphomono and phosphodiesterase associated with and released by the roots of barley genotypes. They concluded that the activity of phytase and phosphatase in the rhizosphere originates mainly from roots when the plants are grown under P-stress. Similar results were also reported by Charlile and Dickinson (2004).

4.2 Effect of phytase and FYM levels on biological properties in non calcareous soil under soybean at 50% flowering and at harvest

4.2.1 Bacterial population

Effect of phytase and FYM levels on soil bacterial population under soybean cultivation was assessed at 50 percent flowering and at harvest of soybean and presented in Table 5 a and b and graphically depicted in Fig. 5 a and b.

The incorporation of graded levels of phytase and FYM were significantly influenced soil bacterial population at both the growth stages of soybean.

Soil bacterial population as influenced by phytase and FYM levels were ranged between 28 and 52 x 10^5 CFU g^{-1} soil at 50 percent flowering and 25 to 41 x 10^5 CFU g^{-1} soil at harvest of soybean.

a) At 50% flowering of soybean: Application of phytase @ 3600 IU recorded maximum soil bacterial population (45 x 10^5 CFU g^{-1} soil) than rest of the treatments, followed by phytase @ 2400 (42 x 10^5 CFU g^{-1} soil) and 1200 IU (40 x 10^5 CFU g^{-1} soil). Mean lower soil bacterial population was observed

with 0 IU phytase (37 x 10^5 CFU g^{-1} soil). In respect to the use of FYM data indicated consistent increase in bacterial population of soil with the application of 0 t ha^{-1} (28 x 10^5 CFU g^{-1} soil) 2.5 t ha^{-1} (37 x 10^5 CFU g^{-1} soil), 5 t ha^{-1} (40 x 10^5 CFU g^{-1} soil) and 7.5 t ha^{-1} (43 x 10^5 CFU g^{-1} soil) FYM. Significantly higher soil bacterial population was noticed with the application of 7.5 t ha^{-1} FYM (48 x 10^5 CFU g^{-1} soil) followed by 5 t ha^{-1} FYM (44 x 10^5 CFU g^{-1} soil).

Use of phytase either @ 3600 IU (52 x 10^5 CFU g^{-1} soil) or 2400 IU (50 x 10^5 CFU g^{-1} soil) along with 7.5 t ha^{-1} FYM recorded significantly higher soil bacterial population over initial and rest of the treatments.

b) At harvest of soybean: Application of phytase @ 3600 IU recorded significantly higher (36 x 10^5 CFU g^{-1} soil) soil bacterial population over rest of the treatments. However application of 2400 IU (35 x 10^5 CFU g^{-1} soil) phytase observed statistically at par in respect of bacterial population. While lower soil bacterial population were recorded with 0 IU phytase (31 x 10^5 CFU g^{-1} soil)

Results revealed that FYM application @ 7.5 t ha^{-1} (39 x 10^5 CFU g^{-1} soil) recorded significantly higher soil bacterial population followed by 5 t ha^{-1} FYM (37 x 10^5 CFU g^{-1} soil). Application of 0 t ha^{-1} FYM recorded (27 x 10^5 CFU g^{-1} soil) lower soil bacterial population.

Significant interaction effect of phytase and FYM levels on soil bacterial population was observed. Combine application of phytase @ 3600 IU + 7.5 t ha^{-1} FYM registered significantly higher (41 x 10^5 CFU g^{-1} soil) soil bacterial population which was statistically at par with 2400 IU + 7.5 t ha^{-1} (40 x 10^5 CFU g^{-1} soil). Considerable increase in soil bacterial population was observed with 0 IU phytase + 0 tha^{-1} FYM (control) (25 x 10^5 CFU g^{-1} soil) at harvest over initial microbial count (18 x 10^5 CFU g^{-1} soil).

Table 5: Effect of phytase and FYM levels on bacterial population in non calcareous soil

a) At 50 % flowering of soybean

Phytase (P) \ FYM (F)	x 10^5 CFU g^{-1} soil				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	28	37	40	43	37
1200 IU	31	39	42	47	40
2400 IU	30	41	45	50	42
3600 IU	36	43	48	52	45
Mean	31	40	44	48	
	P	F	P × F		
S.E. ±	0.413	0.413	0.827		
CD at 5%	1.196	1.196	2.920		

b) At harvest of soybean

Phytase (P) \ FYM (F)	x 10^5 CFU g^{-1} soil				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	25	30	32	36	31
1200 IU	26	31	36	38	33
2400 IU	28	33	37	40	35
3600 IU	29	36	38	41	36
Mean	27	33	36	39	
	P	F	P × F		
S.E. ±	0.322	0.322	0.644		
CD at 5%	0.931	0.931	1.863		

Initial bacterial population = 18 x 10^5 CFU g^{-1} soil

In general FYM in pots reported consistent and higher increase in soil bacterial population than phytase. In other words the magnitude of increase in soil bacterial population was more pronounced with graded levels of FYM.

A. Aziz Qureshi *et al*., (2005) stated that in soybean rhizosphere the better proliferation of phosphate solubilizers may be due to relatively high amounts of organic carbon and also due to root exudates released in soybean rhizosphere as a source of energy. Similar findings were reported by Jha *et al* (1992) and Malewar *et al* (1999). Nakhro and Dkhar (2010) reported that the application of organic manures increased the organic carbon content of the soil and thereby increasing the microbial counts and microbial biomass carbon.

4.2.2 Fungal population

Fungal population in non calcareous soil as influenced by phytase and FYM application for soybean was assessed at 50 percent flowering and at harvest are depicted in Table no 6 a and b and graphically represented in Fig. 6 a and b.

a) At 50% flowering of soybean: Soil fungal population was significantly influenced by phytase and FYM levels and it was ranged between 9 to 23 x 10^4 CFU g^{-1} soils. Phytase application alone @ 0, 1200, 2400 and 3600 IU recorded more or less similar soil fungal population as 9, 10, 10 and 11 x 10^4 CFU g^{-1} soil respectively. Among phytase levels incorporation of 3600 IU phytase recorded significantly higher soil fungal population (17 x 10^4 CFU g^{-1} soil). However use of 2400 IU (16 x 10^4 CFU g^{-1} soil) phytase level recorded statistically at par population of fungi in soil. Lowest (13 x 10^4 CFU g^{-1} soil) fungal population was noticed with 0 IU phytase.

Incorporation of FYM levels only @ 0, 2.5, 5 and 7.5 t ha^{-1} noticed increasing trend as 9, 11, 14 and 17 x 10^4 CFU g^{-1} soil for soil fungal population.

In case of FYM levels application @ 7.5 t ha^{-1} (20 x 10^4 CFU g^{-1} soil) recorded significantly higher soil fungal population followed by FYM @ 5 t ha^{-1} (17 x 10^4 CFU g^{-1} soil) over 0 t ha^{-1} (10 x 10^4 CFU g^{-1} soil).

Conjunctive use of various levels of phytase and FYM were recorded significant interaction for fungal population in the soil at 50 percent flowering and at harvest of soybean. Combine application of phytase @ 3600 IU + 7.5 t ha^{-1} FYM (23 x 10^4 CFU g^{-1} soil) and 2400 IU + 7.5 t ha^{-1}(21 x 10^4 CFU g^{-1} soil) recorded significantly higher and statistically at par population of fungi in soil. Lowest fungal populations among the different levels were obtained with 0 IU phytase and 0 t ha^{-1} FYM (9 x 10^4 CFU g^{-1} soil).

b) At harvest of soybean: Soil fungal population was significantly influenced by phytase and FYM levels at harvest and it was ranged between 19 to 37 x 10^4 CFU g^{-1} soils. With the application of 3600 IU phytase recorded significantly higher soil fungal population (31 x 10^4 CFU g^{-1} soil) which was found statistically at par with the application of 2400 IU (29 x 10^4 CFU g^{-1} soil).

Addition of FYM at different levels also noted increasing trend in fungal population over control. Fungal population of soil with an initial value 9 x 10^4 CFU g^{-1} soil had increased significantly and attained a maximum value of 33 x 10^4 CFU g^{-1} soil in the treatment that has received 7.5 t ha^{-1} FYM. However treatments received 5, 2.5 and 0 t ha^{-1} FYM recorded 30, 27 and 22 x 10^4 CFU g^{-1} soil fungal population in soil respectively.

Significant interaction among various levels of phytase and FYM were observed for fungal population in soil. Application of either 3600 IU (37 x 10^4 CFU g^{-1} soil) or 2400 IU (34 x 10^4 CFU g^{-1} soil) phytase along with 7.5 t ha^{-1} FYM recorded significantly higher and statistically at par soil fungal population.

Table 6: Effect of phytase and FYM levels on fungal population in non calcareous soil

a) At 50 % flowering of soybean

Phytase (P) \ FYM (F)	x 10^4 CFU g^{-1} soil				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	9	11	14	17	13
1200 IU	10	12	16	18	14
2400 IU	10	14	17	21	16
3600 IU	11	15	19	23	17
Mean	10	13	17	20	
	P		F		P × F
S.E. ±	0.315		0.315		0.629
CD at 5%	0.91		0.91		1.821

b) At harvest of soybean

Phytase (P) \ FYM (F)	x 10^4 CFU g^{-1} soil				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	19	23	25	28	24
1200 IU	21	27	29	32	27
2400 IU	23	29	31	34	29
3600 IU	24	30	33	37	31
Mean	22	27	30	33	
	P		F		P × F
S.E. ±	0.437		0.437		0.874
CD at 5%	1.265		1.265		2.53

Initial fungal population: 9 x 10^4 CFU g^{-1} soil

A. Aziz Qureshi *et al*., (2005) stated that in soybean rhizosphere the better proliferation of phosphate solubilizers may be due to relatively high amounts of organic carbon and also due to root exudates released in soybean rhizosphere as a source of energy. Similar findings were reported by Mukherji *et al* (1991), Jha *et al* (1992) and Malewar *et al* (1999). Nakhro and Dkhar (2010) reported that the application of organic manures increased the organic carbon content of the soil and thereby increasing the microbial counts and microbial biomass carbon.

4.2.3 Actinomycetes population

Effect of phytase and FYM levels on soil actinomycetes population was assessed at 50 percent flowering and at harvest of soybean stated in Table 7 a, b and graphically presented in Fig 7 a, b.

The perusal of data indicated that increase in actinomycetes population was more pronounced in the application of FYM than phytase. The magnitude of increase to the tune of 55 % at 50 percent flowering and 104 % at harvesting stage over initial microbial count (16 x 10^5 CFU g^{-1} soil). Graded levels of FYM application were significantly affected actinomycetes population in soil, while levels of phytase did not affected. Actinomycetes population in soil was not affected significantly by phytase but increasing trend was observed at both the growth stages.

a) At 50% flowering of soybean: Data indicated phytase levels did not affect actinomycetes population statistically, however numerically more or less similar actinomycetes population were recorded with levels of phytase 0 IU: 14 x 10^5, 1200 IU: 15 x 10^5, 2400 IU: 17 x 10^5, 3600 IU: 18 x 10^5 CFU g^{-1} soil.

Incorporation of 7.5 t ha^{-1} FYM recorded significantly higher (29 x 10^5 CFU g^{-1} soil) mean actinomycetes population over rest of the treatments

followed by FYM @ 5 t ha^{-1} (26 x 10^5 CFU g^{-1} soil) and 2.5 t ha^{-1} (23 x 10^5 CFU g^{-1} soil).

Data indicated that soil actinomycetes population was significantly influenced by conjoint use of phytase and FYM. Significantly higher soil actinomycetes population was recorded with the application of phytase @ 3600 IU + 7.5 t ha^{-1} FYM (31 x 10^5 CFU g^{-1} soil). However application of phytase either @ 2400 IU (30 x 10^5 CFU g^{-1} soil) or 1200 IU (28 x 10^5 CFU g^{-1} soil) along with FYM @ 7.5 t ha^{-1} was found statistically at par with each other and over rest of the treatments. In general incorporation of higher levels of FYM either 5 t ha^{-1} or 7.5 t ha^{-1} observed higher actinomycetes population in soil.

b) At harvest of soybean: The effect on soil actinomycetes population was significant with the graded levels of FYM while phytase showed no significant effect. Although more or less similar values of soil actinomycetes population was observed between 22 x 10^5 CFU g^{-1} soil to 27 x 10^5 CFU g^{-1} soil with phytase application.

Application of FYM @ 7.5 t ha^{-1} (37 x 10^5 CFU g^{-1} soil) recorded significantly higher actinomycetes population, followed by FYM @ 5 t ha^{-1} (33 x 10^5 CFU g^{-1} soil). Whereas incorporation of 2.5 and 0 t ha^{-1} recorded 29 x 10^5

Table 7: Effect of phytase and FYM levels on actinomycetes population in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	x 10^5 CFU g^{-1} soil				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	14	22	24	27	22
1200 IU	15	23	26	28	23
2400 IU	17	24	27	30	25
3600 IU	18	24	28	31	25
Mean	16	23	26	29	
	P		F		P × F
S.E. ±	0.417		0.417		0.834
CD at 5%	NS		1.206		2.412

b) At harvest of soybean

FYM (F) / Phytase (P)	x 10^5 CFU g^{-1} soil				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	22	26	31	33	28
1200 IU	23	27	32	35	29
2400 IU	25	31	33	39	32
3600 IU	27	32	36	41	34
Mean	24	29	33	37	
	P		F		P × F
S.E. ±	0.396		0.396		0.793
CD at 5%	NS		1.147		2.294

Initial actinomycetes population = 16 x 10^5 CFU g^{-1} soil

CFU g^{-1} soil and 24 x 10^5 CFU g^{-1} soil actinomycetes population at harvest of soybean crop.

The actinomycetes population was significantly influenced by conjoint use of phytase and FYM. Significantly higher soil actinomycetes population was recorded with the application of phytase @ 3600 IU + 7.5 t ha^{-1} FYM (41 x 10^5 CFU g^{-1} soil) which was statistically at par with phytase @ 2400 IU along with 7.5 t ha^{-1} (39 x 10^5 CFU g^{-1} soil).

The microbial population of bacteria, fungi and actinomycetes were found lowest in without FYM application treatments. This may be due to the low amount of organic matter. A. Aziz Qureshi *et al*., (2005) stated that in soybean rhizosphere the better proliferation of phosphate solubilizers may be due to relatively high amounts of organic carbon and also due to root exudates released in soybean rhizosphere as a source of energy. Similar findings were reported by Mukherji *et al* (1991), Jha *et al* (1992) and Malewar *et al* (1999). Nakhro and Dkhar (2010) reported that the application of organic manures increased the organic carbon content of the soil and thereby increasing the microbial counts and microbial biomass carbon.

4.3 Effect of phytase and FYM levels on chemical properties in non calcareous soil under soybean at 50% flowering and at harvest of soybean

4.3.1 pH

Effect of phytase levels (0, 1200, 2400 and 3600 IU) and FYM levels (0, 2.5, 5 and 7.5 t ha^{-1}) on pH of non calcareous soil under soybean cultivation was assessed at 50 percent flowering and harvest of the crop. pH of soil was significantly influenced by various levels of phytase and FYM at both growth stages of soybean. Graded levels of FYM application had dominant

effect for soil pH reduction than phytase levels over initial which is presented in Table 8 a and b.

a) At 50% flowering of soybean: It is observed from the data that application of phytase and FYM levels recorded lower values of pH (by 0.5 units) over initial (8.0) at 50 percent flowering. Application of phytase @ 3600 IU recorded significantly higher soil pH value (7.57) followed by application of 2400 IU (7.52) and 1200 IU (7.47).

In case of FYM application @ 0 t ha^{-1} (7.50), 7.5 t ha^{-1} (7.50) and 2.5 t ha^{-1} (7.52) recorded significantly higher and at par results at 50 percent flowering. Application of phytase @ 3600 IU (7.59) along with 7.5 t ha^{-1} FYM recorded significantly higher and phytase @ 3600 IU either along with FYM @ 5 t ha^{-1} (7.58) or 2.5 t ha^{-1} (7.56) registered at par values of soil reaction.

b) At harvest of soybean: At harvest of soybean, soil pH was slightly increased with the application of either phytase or FYM. Soil pH at harvest stage of soybean was also significantly influenced by phytase and FYM levels. Application of phytase @ 2400 IU (7.62) recorded significantly higher results for soil pH which was statistically at par with phytase @ 3600 IU (7.60). However FYM application either @ 7.5 t ha^{-1} or 5 t ha^{-1} recorded similar values of soil pH (7.62). While application of 2.5 t ha^{-1} (7.55) and 0 t ha^{-1} (7.53) FYM were recorded more or less similar values for soil pH.

Interaction effect was significant for soil pH with the application of various levels of phytase and FYM. Phytase application @ 2400 IU (7.77) along with 7.5 t ha^{-1} FYM recorded significantly higher pH which was found to be at par either with the application of 3600 IU (7.66) or 1200 IU (7.65) phytase along with 5 t ha^{-1} FYM.

Table 8: Effect of phytase and FYM levels on soil pH of non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	Soil pH (1:2.5)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	7.45	7.48	7.46	7.45	7.46
1200 IU	7.52	7.50	7.42	7.43	7.47
2400 IU	7.5	7.52	7.51	7.53	7.52
3600 IU	7.54	7.56	7.58	7.59	**7.57**
Mean	7.50	7.52	7.49	7.50	
	P		F		P × F
S.E. ±	0.007		0.007		0.013
CD at 5%	0.019		0.019		0.038

b) At harvest of soybean

FYM (F) / Phytase (P)	Soil pH (1:2.5)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	7.60	7.55	7.57	7.63	7.50
1200 IU	7.47	7.52	7.65	7.55	7.55
2400 IU	7.56	7.63	7.62	7.77	7.62
3600 IU	7.47	7.49	7.66	7.61	7.60
Mean	7.53	7.55	7.62	7.62	
	P		F		P × F
S.E. ±	0.006		0.006		0.012
CD at 5%	0.017		0.017		0.034

Initial pH: 8.0

The reduction in soil pH over initial and during experimentation of soybean on calcareous soil may be attributed to release of different organic acids by plant roots i.e. citric acid, oxalic acid, malic acid (Zheng *et al*, 2005) and by microorganisms i.e. gluconic acid, 2-ketogluconic acid, citric acid,oxalic acid (Richardson, 2001). Further the reduction in soil pH may be attributed to release of organic acid (carbonic acid) and inorganic acid (sulphuric acid and nitric acid) during decomposition of organic matter. Similar results were reported by Gujar *et al*, 2013, that addition of phytase in soil led to increase in its fertility with obvious decrease in pH of soil by about 0.4 units (8.4 to 8.06). The decrease in magnitude of pH by application of phytase may be ascribed to that of the presence of phytate which is the most abundant form of organic P and constitute more than 50% of total organic P. The release of inorganic phosphate from phytate is mediated by phytase. The decrease in pH might also be due to the release of organic and inorganic acids secreted by Phosphorus soubilizing microbes, in which hydroxyl (OH^-) and carboxyl ($COOH^-$) groups of acid chelates cations (Ca^{2+} in calcareous soil) and decrease pH in basic soils. (Kpomblekou and Tabatabai, 1994). Further, Hinsinger, 2001 concluded that the pH of the rhizosphere is lowered through biotical production of proton or bicarbonate release (anion/ cation balance) and gaseous (O_2/ CO_2) exchanges. The increase in nutrient concentration, root and shoot length and root-shoot ratio 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 solubility (Yadav and Verma, 2012). The lowering in pH of the medium suggests the release of organic acids by the P-solubilizing microorganisms (Whitelaw 2000). Root exudation of high concentrations of organic acid anions as a result of P deficiency (Hoffland *et al*., 1989) lowers rhizosphere pH. However at harvest of soybean, slight enhancement in the pH was reported. These results are in accordance with

those reported by George *et al* (2005). They reported notable increase in pH of rhizospheral soil to the tune of 0.5 and 0.7 units by exposing above soils to the roots of phytase exuding fungal phytase.

4.3.2 Soil Electrical Conductivity

Electrical conductivity of non calcareous soil as influenced by different phytase and FYM levels imposed to soybean was assessed at 50 percent flowering and at harvest and presented in Table 9 a and b.

Data revealed that consistent increasing trend for electrical conductivity of soil was recorded by the application of either phytase or FYM at both the growth stages over initial (0.29 dSm^{-1}).

a) At 50% flowering of soybean: Application of phytase @ 2400 IU, 1200 IU, and 3600 IU recorded (0.36, 0.35 and 0.35 dSm^{-1}) electrical conductivity which was significantly superior and at par with each other over 0 IU phytase. In relation to FYM application significantly higher and statistically at par values of electrical conductivity were recorded with the application of 2.5 t ha^{-1} FYM (0.37 dSm^{-1}), 7.5 t ha^{-1} FYM (0.35 dSm^{-1}) and 5 t ha^{-1} (0.34 dSm^{-1}) FYM over 0 t ha^{-1} FYM (0.33 dSm^{-1}).

Combine application of phytase and FYM were affected electrical conductivity of soil significantly. However highest electrical conductivity of soil was recorded with the application of 2400 IU phytase along with 5 t ha^{-1} FYM (0.37 dSm^{-1}) which was found to be at par with the application of either 1200 IU (0.37 dSm^{-1}), 2400 IU (0.36 dSm^{-1}), 0 IU (0.36 dSm^{-1}) or 3600 IU (0.35 dSm^{-1}) phytase along with 2.5 t ha^{-1} FYM.

b) At harvest of soybean: Electrical conductivity of non calcareous soil as influenced by phytase and FYM levels was found to be significant at harvest of

Table 9: Effect of phytase and FYM levels on electrical conductivity in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	Electrical conductivity (dSm^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.27	0.36	0.29	0.35	0.32
1200 IU	0.34	0.37	0.35	0.35	0.35
2400 IU	0.37	0.36	0.37	0.35	0.36
3600 IU	0.33	0.35	0.36	0.35	0.35
Mean	0.33	0.37	0.34	0.35	
	P		F		P × F
S.E. ±	0.006		0.006		0.013
CD at 5%	0.018		0.018		0.036

b) At harvest of soybean

FYM (F) / Phytase (P)	Electrical conductivity (dSm^{-1})				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.33	0.36	0.36	0.37	0.36
1200 IU	0.35	0.37	0.37	0.38	0.37
2400 IU	0.37	0.39	0.44	0.36	0.39
3600 IU	0.38	0.46	0.38	0.39	0.40
Mean	0.36	0.40	0.39	0.38	
	P		F		P × F
S.E. ±	0.007		0.007		0.014
CD at 5%	0.021		0.021		0.041

Initial electrical conductivity: 0.29 dSm^{-1}

soybean. It is evident from the data that consistent increasing magnitude of electrical conductivity was observed to the tune of 1.3 times over initial (0.29 dSm^{-1}).

Phytase application @ 3600 IU, 2400 IU and 1200 IU (0.40, 0.39 and 0.37 dSm^{-1}) recorded significantly higher and at par electrical conductivity of soil respectively. FYM application @ 2.5 t ha^{-1} (0.40 dSm^{-1}) and 5 t ha^{-1} (0.39 dSm^{-1}) were recorded significantly higher and at par results for soil electrical conductivity.

Combine application of phytase @ 3600 IU along with 2.5 t ha^{-1} FYM (0.46 dSm^{-1}) reported significantly higher electrical conductivity of soil which was followed by 2400 IU phytase and 5 t ha FYM (0.44 dSm^{-1}).

The increase in electrical conductivity or total soluble salt content of soil was reported either with successive application of phytase or FYM which might be due to the release of organic acid during decomposition of FYM. However phytase might have enhanced the organic acid release by plant roots in association with microbes.

Further combine addition of phytase and FYM resulted higher electrical conductivity of soil which might be ascribed to organic matter decomposition, release of organic acids, enhanced microbial population and activity, higher secretion of intracellular phytase by soybean roots and microbial release of phytase.

4.3.3 Soil Organic carbon

Organic carbon content of soil as influenced by phytase and FYM levels applied to soybean was significantly influenced at both the growth stages

i.e. at 50 percent flowering and at harvest. The data regarding to organic carbon content is depicted in Table 10 a and b.

a) At 50 percent flowering of soybean: Data revealed that successive application of either phytase or FYM or their combinations recorded consistent increase in soil organic carbon content. However the magnitude of increase was to the tune of 1.5 times higher than the initial (0.70 %) at 50 percent flowering and 1.2 times at harvest. Increase in soil organic carbon was more pronounced with the application of phytase than FYM. Phytase application @ 3600 IU recorded significantly higher organic carbon (1.13 %) followed by phytase @ 2400 IU (1.06 %).

Significantly higher mean soil organic carbon content was observed with the application of 7.5 t ha^{-1} (1.20 %) followed by 5 t ha^{-1} (1.05 %) and 2.5 t ha^{-1} (1.00 %). Lower percent soil organic carbon was recorded in the control pot P_0F_0 (no phytase no FYM) (0.73 %).

Combine application of phytase and FYM and their interaction effect found significant at 50 percent flowering. Among the different treatment combinations application of phytase either @ 3600 IU (1.33 %) along with either 7.5 t ha^{-1} FYM (1.33 %) or 5 t ha^{-1} FYM (1.23 %) recorded statistically at par results for soil organic carbon content. However application of either 2400 IU (1.23 %) or 1200 IU (1.23 %) along with 7.5 t ha^{-1} FYM also recorded at par results for soil organic carbon.

b) At harvest of soybean: Organic carbon content in soil was significantly influenced by the successive levels of phytase and FYM. Similar trend with the application of phytase @ 3600 IU (1.23 %) recorded significantly higher soil organic carbon content at harvest. However 1200 IU (1.15 %) and 0 IU (0.92 %) phytase application recorded lower soil organic carbon in soil.

Table 10: Effect of phytase and FYM levels on organic carbon in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	per cent				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.73	0.79	0.83	1.01	0.84
1200 IU	0.75	0.97	1.03	1.23	1.00
2400 IU	0.79	1.1	1.1	1.23	1.06
3600 IU	0.83	1.13	1.23	1.33	1.13
Mean	0.78	1.00	1.05	1.20	
	P		F		P × F
S.E. ±	0.019		0.019		0.039
CD at 5%	0.056		0.056		0.112

b) At harvest of soybean

FYM (F) / Phytase (P)	per cent				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.79	0.83	0.93	1.13	0.92
1200 IU	0.83	1.13	1.29	1.33	1.15
2400 IU	1.00	1.03	1.20	1.20	1.11
3600 IU	1.13	1.17	1.27	1.33	1.23
Mean	0.94	1.04	1.17	1.25	
	P		F		P × F
S.E. ±	0.024		0.024		0.047
CD at 5%	0.068		0.068		0.136

Initial organic carbon = 0.70 per cent

Significantly higher mean soil organic carbon was recorded with the application of 7.5 t ha^{-1} FYM (1.25 %). FYM application @ 5 t ha^{-1} and 2.5 t ha^{-1} recorded 1.17 and 1.04 percent soil organic carbon respectively.

Application of phytase either @ 3600 IU (1.33 %) or 1200 IU (1.33 %) along with 7.5 t ha^{-1} FYM recorded significantly higher soil organic carbon content. However application of phytase either @ 3600 IU (1.27 %) or 2400 IU (1.20 %) or 1200 IU (1.29 %) along with 5 t ha^{-1} and phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (1.20 %) recorded statistically at par soil organic carbon content.

Consistent increase in soil organic carbon due to phytase and FYM application could be ascribed due to the addition of FYM helped in increasing organic carbon content. Combine application of FYM and phytase might have associated effect for enhancing microbial population and their activity thereby release of organic acids and higher secretion of phytase by plant roots.

The increase in organic carbon content in all treatment combinations might be due to organic matter addition through FYM and leaf fall of soybean. The increase in organic carbon content with addition of phytase might be due to mineralization of organic fraction of phosphorus (phytate). The potential role of soil microorganism for increasing availability of phosphorus from phytate through phytase is well defined and during this process of mobilization of organic phosphorus into inorganic phosphorus with association of root exudates, organic acids released by plants as well as during decomposition of organic matter present in soil. Similar results were reported by Aseri, 2009 and Tarafdar, 2008. El-Azouni., (2008) reported that the dual inoculation of phosphate solubilising fungi (*Aspergillus niger* and *Penicillium*

digitarium) significantly increased the organic carbon levels of the treated soil when compared to untreated soil. Increase in organic carbon content with the increase in FYM levels may be attributed to the added organic carbon biomass through FYM. (Havlin *et al*. 2007). A. Aziz Qureshi *et al*., (2005) concluded that combine application of phosphate solubilizers in presence of FYM improved organic carbon status and proliferation of phosphate solubilizers. Nakhro and Dkhar (2010) reported that the application of organic manures increased the organic carbon content of the soil. Zubair et al., (2012) reported that an improvement in the soil organic carbon (OC) and nitrogen contents ranging from 0.10 to 1.40 % and 0.05 to 0.55 % respectively were achieved in a salt affected soil through amendment of soil with green and farm yard manure.

4.3.4 Calcium carbonate

Perusal of data indicated a declining trend (4.5 to 3.66 %) in calcium carbonate content at 50 percent flowering and at harvest of soybean stated in Table 11 and graphically presented in Fig. 6 a and b.

The magnitude of decrease was more (i.e. 22 %) at 50 percent flowering than at harvest (i.e. 20 %). The magnitude of reduction of calcium carbonate content in soil was observed more or less similar with either application of phytase or FYM.

a) At 50 percent flowering of soybean Application of 3600 IU phytase recorded significantly lower (3.43 %) mean lower calcium carbonate content in soil than rest of the treatments. However incorporation of phytase @ 1200 IU or 2400 IU recorded 3.83 % and 3.63 % calcium carbonate content in soil respectively.

Table 11: Effect of phytase and FYM levels on calcium carbonate in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	per cent				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	4.24	4.00	4.05	3.80	4.02
1200 IU	4.12	3.86	3.74	3.60	3.83
2400 IU	3.94	3.75	3.50	3.32	3.63
3600 IU	3.68	3.50	3.35	3.20	3.43
Mean	4.00	3.78	3.66	3.48	
	P		F		P × F
S.E. ±	0.026		0.026		0.053
CD at 5%	0.076		0.076		0.152

b) At harvest of soybean

FYM (F) / Phytase (P)	per cent				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	4.20	3.70	3.60	3.50	3.75
1200 IU	4.10	3.20	3.52	3.60	3.61
2400 IU	3.87	3.70	3.37	3.42	3.59
3600 IU	3.60	3.54	3.60	3.37	3.53
Mean	3.94	3.54	3.52	3.47	
	P		F		P × F
S.E. ±	0.028		0.028		0.056
CD at 5%	NS		0.081		0.162

Initial calcium carbonate: 4.5 per cent

Data indicated efficiency of phytase for reduction of calcium carbonate was lower with successive levels. However application of 3600 IU phytase recorded lowest calcium carbonate content (3.43 %).

The perusal of data indicated a declining trend from its initial value (4.5 %) for calcium carbonate content with the application of successive levels of FYM. However significantly lower calcium carbonate content was recorded with the application of 0 t ha^{-1} FYM (4.0 %). Application of FYM @ 7.5 t ha^{-1} recorded lowest (3.48 %) calcium carbonate content in soil followed by 5 t ha^{-1} (3.66 %) and 2.5 t ha^{-1} (3.78 %).

Data showed significant interaction effect of phytase and FYM levels on calcium carbonate content of soil. It could be assessed from the data, application of higher levels of phytase (3600 IU) and FYM (7.5 t ha^{-1}) were found desirable for the reduction of calcium carbonate content (3.20 %) than successive lower levels (0 IU and 0 t ha^{-1}) (4.24 %).

Application of successive levels of only phytase were more pronounced for the reduction of calcium carbonate content than FYM.

b) At harvest of soybean Incorporation of various levels of phytase did not affect calcium carbonate content of soil significantly. But numerically higher calcium carbonate content was recorded with 0 IU phytase (3.75 %) followed by 1200 IU (3.61 %), 2400 IU (3.59 %) and 3600 IU (3.53 %).

Data indicated that significantly higher and statistically at par calcium carbonate content was recorded with the incorporation of 2.5 t ha^{-1}(3.54 %) and 5 t ha^{-1} (3.52 %). Whereas lower calcium carbonate content observed with 7.5 t ha^{-1} FYM (3.47 %).

Conjunctive use of phytase and FYM levels had significantly affected calcium carbonate content in the soil. Application of 3600 IU phytase with 7.5 t ha^{-1} recorded lower content of calcium carbonate (3.37 %).

Similar trend of results for reduction of $CaCO_3$ content to the tune of 37 per cent were observed by Gujar *et al*, 2013 due to application of phytase. They concluded that phytate is a primary storage form of organic phosphorus in plant (60-90%) (Vats *et al*, 2006).

The decrease in $CaCO_3$ content may be attributed to dissolution of $CaCO_3$ content with organic acids released during decomposition of organic matter added to soil (Zheng *et al*, 2005). Markovacki *et al*. (2008) reported that higher microbial population in soil decrease $CaCO_3$ content due to secretion of organic acid and respiration of CO_2 leading to formation of carbonic acid. The reduction in $CaCO_3$ might also be due to phytate acting as a chelating agent and having the ability to hydrolyze the phosphorus from calcium salts and clay as inorganic PO_4.

4.3.5 Available nitrogen

Soil available nitrogen at 50 percent flowering and at harvest was assessed t-o evaluate the effect of phytase and FYM levels imposed to soybean. Table 12 a and b.

Soil available nitrogen content was significantly influenced by phytase and FYM levels. Data revealed that the application of graded levels of phytase and FYM recorded consistent increase in soil available nitrogen. Further the magnitude of increase was more pronounced with higher levels of phytase and FYM. The increasing trend of soil available nitrogen was more dominated by FYM application than that of phytase application.

Table 12: Effect of phytase and FYM levels on available nitrogen in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	kgha^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	163.56	169.75	179.90	183.80	174.25
1200 IU	175.58	182.69	225.83	243.21	206.83
2400 IU	184.51	199.54	244.59	273.40	225.51
3600 IU	204.36	221.49	248.36	289.32	240.88
Mean	182.00	193.37	224.70	247.43	
	P		F		P × F
S.E. ±	1.029		1.029		2.057
CD at 5%	2.977		2.977		5.954

b) At harvest of soybean

FYM (F) / Phytase (P)	kgha^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	155.71	161.52	166.03	173.63	164.22
1200 IU	160.75	163.02	190.71	183.94	174.61
2400 IU	167.03	135.50	188.17	200.11	172.70
3600 IU	154.51	179.59	192.00	213.64	184.93
Mean	159.50	159.91	184.22	192.83	
	P		F		P × F
S.E. ±	4.271		4.271		8.541
CD at 5%	12.359		12.359		24.717

Initial available nitrogen = 245 kgha^{-1}

a) At 50 percent flowering of soybean: Application of phytase @ 3600 IU recorded significantly higher mean soil available nitrogen (240.88 kg ha^{-1}) followed by 2400 IU (225.51 kg ha^{-1}) and 1200 IU (206.83 kg ha^{-1}). Lower mean soil available nitrogen was reported with no phytase application (174.25 kg ha^{-1}).

Data revealed that FYM application @ 7.5 t ha^{-1} recorded significantly higher soil available nitrogen (247.43 kg ha^{-1}) followed by 5 t ha^{-1} (224.70 kg ha^{-1}) while lower soil nitrogen availability was recorded in the pots where no phytase and no FYM was given.

Significant interaction effect was observed on soil available nitrogen by the conjoint use of phytase and FYM in different treatment combinations. A combine application of phytase @3600 IU along with 7.5 t ha^{-1} FYM recorded significantly higher soil available nitrogen (289.32 kg ha^{-1}) than rest of the treatments.

b) At harvest of soybean: Application of phytase @ 3600 IU (184.93 kg ha^{-1}) recorded significantly higher soil available nitrogen which was statistically at par with phytase @ 1200 IU (174.61 kg ha^{-1}) and 2400 IU (172.70 kg ha^{-1}).

Results revealed that application of FYM @ 7.5 t ha^{-1} (192.83 kg ha^{-1}) recorded significantly higher soil available nitrogen followed by 5 t ha^{-1} FYM (184.22 kg ha^{-1}) at harvest.

It was observed from the data that application of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM recorded significantly higher soil available nitrogen (213.64 kg ha^{-1}) which was at par with 2400 IU phytase (200.11 kg ha^{-1}) with same level of FYM. Statistically at par soil available nitrogen content was also recorded with the application of phytase either 3600 IU (192.00 kg ha^{-1}) or 1200 IU (190.71 kg ha^{-1}) along with 5 t ha^{-1} FYM.

The magnitude of reduction in soil available nitrogen was more pronounced with no FYM level and lower levels of FYM application at both stages i.e. 50% flowering and harvest. However, reduction in soil available nitrogen at 50% flowering and harvest in all treatment combinations might be due to the crop uptake, leaching losses and evaporative losses. The available nitrogen content of soil in all treatment combinations was considerably reduced during 50% flowering (48 - 52 DAS). Reduction in soil available nitrogen may be due to microbial immobilization, denitrification losses and leaching losses. Similar results were also reported regarding FYM application on lower soil available nitrogen (Suresh and Suryaprabha, 2005). Similar results were recorded by Yankaraddy *et al* (2009) they reported that application of FYM recorded highest grain yield, straw yield, and nutrient content and nutrient uptake. It was followed by application of coffee pulp compost.

4.3.6 Available phosphorus

Soil available phosphorus as influenced by graded levels of phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5, 5, and 7.5 t ha^{-1}) imposed to soybean in non calcareous soil were assessed at 50 percent flowering and at harvest depicted in Table 13 a and b graphically presented in and Fig 7 a and b.

The results of this pot culture experiment indicated that use of phytase and FYM levels increased soil available phosphorus at 50 percent flowering by 6 kg ha^{-1} over initial (16.4 kg ha^{-1}). While it was found to be more or less similar at harvest of soybean. Use of phytase levels alone in absence of FYM (P_0: 0 IU, P_1: 1200 IU, P_2: 2400 IU and P_3: 3600 IU) recorded consistent increase (16.44, 18.44, 18.59 and 20.51 kg ha^{-1}) in soil available phosphorus. However similar trend with only FYM levels (F_0: 0, F_1: 2.5, F_2: 5 and P_3: 7.5 t

ha^{-1}) recorded slightly higher increase (16.44, 18.50, 20.15 and 23.02 kg ha^{-1}) in soil available phosphorus.

a) At 50 percent flowering: Application of phytase @ 3600 IU recorded significantly higher (22.68 kg ha^{-1}) soil available phosphorus than rest of the treatment followed by use of phytase @ 2400 IU (21.12 kg ha^{-1}) and 1200 IU (20.29 kg ha^{-1}). Use of phytase levels indicated slightly higher phosphorus than FYM levels.

Perusal of data indicated significantly higher soil available phosphorus by using 5 t ha^{-1} FYM (22.22 kg ha^{-1}) which was found statistically at par with 2.5 t ha^{-1} FYM (21.63 kg ha^{-1}). Further application of 7.5 t ha^{-1} FYM recorded 21.17 kg ha^{-1} soil available phosphorus.

Interaction effect among phytase and FYM levels were studied and found significant. Use of phytase @ 3600 IU along with 2.5 t ha^{-1} FYM recorded significantly higher soil available phosphorus (24.83 kg ha^{-1}) followed by phytase @ 2400 IU + 5 t ha^{-1} FYM ((24.26 kg ha^{-1}) which was found to be at par with phytase 3600 IU + 7.5 t ha^{-1} FYM (23.90 kg ha^{-1}) and phytase 2400 IU + 2.5 t ha^{-1} FYM (23.55 kg ha^{-1}). More availability of phosphorus in soil was observed with combine application of higher levels of phytase (2400 and 3600 IU) and FYM (2.5, 5 t ha^{-1}).

b) At harvest of soybean: Individual use of phytase, FYM and their combine application indicated significant variation in soil available phosphorus. Results presented in Table 13 b indicated that application of phytase @ 2400 IU recorded significantly higher soil available phosphorus (17.63 kg ha^{-1}), followed by application of phytase @ 1200 IU (16.13 kg ha^{-1}) and 3600 IU (15.63 kg ha^{-1}). Significantly lower available phosphorus in soil was recorded in control (no phytase and no FYM) (14.57 kg ha^{-1}).

Table 13: Effect of phytase and FYM levels on available phosphorus in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	kgha^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	16.44	18.50	20.15	23.02	19.53
1200 IU	18.44	19.64	22.98	19.69	20.29
2400 IU	18.59	23.55	24.26	18.07	21.12
3600 IU	20.51	24.83	21.49	23.90	22.68
Mean	18.60	21.63	22.22	21.17	
	P		F		P × F
S.E. ±	0.313		0.313		0.626
CD at 5%	0.906		0.906		1.813

b) At harvest of soybean

FYM (F) / Phytase (P)	kgha^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	11.99	14.42	15.96	19.92	14.57
1200 IU	13.29	14.65	17.82	18.77	16.13
2400 IU	13.63	15.64	19.30	21.95	17.63
3600 IU	13.74	15.20	14.53	18.55	15.63
Mean	13.16	14.98	16.90	19.80	
	P		F		P × F
S.E. ±	0.178		0.178		0.356
CD at 5%	0.515		0.515		1.03

Initial available phosphorus = 16.4 kgha^{-1}

FYM application @ 7.5 t ha^{-1} recorded significantly higher mean soil available phosphorus (19.80 kg ha^{-1}) followed by 5 t ha^{-1} FYM (16.90 kg ha^{-1}). Lower soil available phosphorus was reported with the application of 2.5 t ha^{-1} FYM (14.98 kg ha^{-1}) and control (no FYM) (13.16 kg ha^{-1}).

Conjunctive use of phytase and FYM levels were indicated significant interaction. However conjoint use of phytase @ 2400 IU + 7.5 t ha^{-1} FYM recorded significantly higher soil available phosphorus (21.95 kg ha^{-1}). Further combine use of phytase @ 2400 IU + 5 t ha^{-1} FYM recorded 19.30 kg ha^{-1} and phytase application either 1200 IU (18.77 kg ha^{-1}) or 3600 IU (18.55 kg ha^{-1}) along with 7.5 t ha^{-1} FYM soil available phosphorus. Available phosphorus status of soil at harvest of soybean was considerably more in the treatments comprising higher levels of phytase and FYM than lower levels.

Higher availability of phosphorus in soil with individual phytase, FYM and their combine Applied 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 catalyses the organic phosphorus present in soil in the form of inositol hexaphosphate (major form of organic phosphorus in soil). Application of phytase along with FYM might have played key role in enhancing microbial population and their activity thereby exudation of microbial phytase causing higher availability of soil phosphorus. Similar trends of results reported by Richardson *et al* (2001).

Higher build up of soil available phosphorus at harvest of soybean might be due to acid phosphates and phytase secreted by micro-organisms like fungi and bacteria. Kapoor *et al.* (1989) concluded fungal isolates exhibit greater phosphorus solubilizing ability than bacteria in both liquid and sand

culture. In the similar line phytase used the experiment was isolated from *Aspergillus niger* fungus species.

Most of the phosphate solubilizing micro-organisms solubilize Ca-P complexes. These (i.e. PSMs) could be effective in calcareous soil in which Ca-P complexes are more. Inorganic phosphorus soluilized by the action of organic acids (eg. acetate, lactate, oxalate, tartarate, succinate, gluconate, keto-gluconate, glyconatem) and inorganic acids secreted by PSMs. (Yadav and Verma,2012). Sihag *et.al.,* (2005) conducted a manurial experiment on rice-wheat cropping system in *Typic Haplustepts* type soil during *kharif*- 1997 at Rice Research Station, Kaul to study the effect of integrated use of inorganic fertilizers and organic materials on the distribution of different forms of N and P in soil . They concluded significant increase in the saloid-P, Al-P and Ca-P forms with the application of inorganic fertilizers along with organic materials. Further, they were reported highest amount of all the forms of P was obtained with FYM followed by green manuring and pressmud treatment.

Phosphorus availability in soils with low organic matter is very low. The organic carbon in the soil increase P availability by formation of organophosphate complexes, anion replacement of H_2PO_4 on adsorption sites, coating of humus on Fe/Al oxides which reduces adsorption and increases the quantity of organic P mineralized to inorganic P. The complex anions formed by addition of organic matter such as citrate, oxalate, tartarate and malate are the most effective in replacing H_2PO_4 from the adsorption sites (Havlin *et al*., 2007).

Pattanayak *et al.,* (2009) carried out a study work in relation to the availability of phosphorus at Orissa university of Agriculture and Technology, Bhubaneswar. They concluded that the rate of P mineralization depends on

microbial activity and on the activity of free phosphatases. Solubilization of inorganic phosphorus in soil is mostly mediated by microbial activity (*Pseudomonas strita, Bacillus polymixa, bacillus megatherium, penicillium digitatum, Aspergillus awamori*) due to secretion of organic acids which prevents fixation of phosphate ion with chelating effect. Further they reported that the availability and forms of P in soil at one point of time depend upon the native and/or added organic matter content from external sources. Further they stated that the released organic acids during the process of decomposition of organic matter also solubilize native P leading to increased P availability.

Soil microorganisms play a key role in soil phosphorus dynamics and subsequently availability of phosphate to plants (Richardson, 2001). Release of organic anions and production of siderophores and acid phosphatase by plant roots and microbes (Yadaf and Tarafdar, 2001) or alkaline phosphatase (Tarafdar and Claassen, 1988) enzymes hydrolyze the soil organic phosphorus and split phosphorus from organic residues. The largest portion of extracellular phosphatase is derived from the microbial population (Dodor and Tabatabai, 2003). Mixed cultures of PSMs (*Bacillus, Streptomyces* and *Pseudomonas* etc) are most effective in mineralizing organic phosphate (Molla, *et al*., 1984). Exploitation of microbes to increase the availability of phosphorus in soil therefore is an attractive suggestion for developing a more sustainable agriculture. Vibha *et al*., (2014), reported significant improvement in soil phosphorus availability under mung bean cultivation in sandy loam soil with inoculation of *Aspergillus niger + Penicillium citrinum* and *Aspergillus niger 2 + Aspergillus niger* 3. Vibha *et al.,* (2014), reported significant improvement in availability of soil phosphorus under mung bean on sandy loam soil with inoculation of bacterial and fungal bioagents. Ramesh *et al.*, (2011), observed higher soil available phosphorus due to inoculation of Bacillus isolates. The

increase in phosphatase and phytase activity with inoculation of *Bacillus* isolates might be due to phosphorus mobilization and acquisition by plants. This is in consonance with the study revealing that inoculation of *Aspergillus* strains significantly improved phosphorus uptake by plants and extractable phosphorus status in soil. Similar results were reported by Tarafdar and Rao, (1996). P availability is frequently greater in manured soils and with the addition of humic substances in lime-rich soil. The organic acids produced by microbes can either directly dissolve the mineral P as a result of anion exchange of phosphate by acid anion or can chelate Fe, Al and Ca ions associated with P (Omar 1998). Richardson and Simpson (2011) concluded that microorganisms are a key driver in regulating the mineralization of phytate in soil and their presence within the rhizosphere may compensate for a plants inability to otherwise acquire phosphorus directly from phytate. Root exudation of high concentrations of organic acid anions as a result of P deficiency (Hoffland *et al*., 1989) lowers rhizosphere pH, making P (Haynes, 1990; Jones and Darrah, 1994) more available in calcareous soils. Similar results were recorded by Yankaraddy *et al* (2009) they reported that application of FYM recorded highest grain yield, straw yield, and nutrient content and nutrient uptake. It was followed by application of coffee pulp compost. Ushari *et.al.*,(2013) studied the enzymatic activity by isolating phytase producing bacteria (*Bacillus* sps NBtRS6) from the rhizosphere soil of NBt cotton field in Andhra Pradesh and they observed that phosphatase is an enzyme that release inorganic phosphate from organic moiety and complex inorganic materials. It is known to play an essential role in phosphorus cycle. Further they stated that phosphatases play a key role in phosphorus cycle by solubilizing organic and inorganic phosphates into available forms that support growth of crop plants. Similar results were also reported by Dotaniya *et al* (2014).

Kaya *et al.,* (2009) grown different chickpea genotypes on clay loam soil at Suleyman Demirel University research and experimental farm, Isparta-Turkey. They determined zinc, phosphorus, phytic acid, protein concentrations and phytase activity. They concluded that phytases are enzymes that degrade phytate and permit higher availability of Zn and other nutrients such as P and Fe.

4.3.7 Available potassium

Data presented in the Table 14 a and b indicated that soil available potassium as influenced by phytase and FYM levels imposed to soybean were found to be significant at 50 percent flowering and at harvest.

The decreasing trend was noticed by the phytase, FYM and their combined application to soybean over initial (310 kg ha^{-1}).

a) At 50 percent flowering: Phytase application @ 3600 IU recorded significantly higher soil available potassium (291.55 kg ha^{-1}) followed by 2400 IU (287.27 kg ha^{-1}) and 1200 IU (284.28 kg ha^{-1}). Lower availability of potassium in soil was indicated by 0 IU phytase (281.41 kg ha-^{1}). Consistent increasing trend was obtained with the successive levels of phytase (0 IU: 278.05, 1200 IU: 280.13, 2400 IU: 280.49 and 3600 IU: 286.97 kg ha^{-1}).

Significantly higher soil available potassium was recorded in the pots receiving FYM @ 7.5 t ha^{-1} (291.90 kg ha^{-1}). Lower soil available potassium was recorded in control (281.41 kg ha^{-1}) where FYM was not applied.

Table 14: Effect of phytase and FYM levels on available potassium in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	kgha^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	278.05	277.31	282.95	286.97	281.32
1200 IU	280.13	282.54	284.61	289.82	284.28
2400 IU	280.49	285.88	287.73	294.96	287.27
3600 IU	286.97	290.75	292.61	295.85	291.55
Mean	281.41	284.12	286.98	291.90	
	P		F		P x F
S.E. $\pm$	0.551		0.551		1.102
CD at 5%	1.594		1.594		3.188

b) At harvest of soybean

FYM (F) / Phytase (P)	kgha^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	246.41	249.32	253.92	256.61	251.57
1200 IU	248.62	251.28	258.49	263.08	255.37
2400 IU	250.31	252.7	257.63	267.02	256.92
3600 IU	252.75	256.59	271.86	270.65	262.96
Mean	249.52	252.47	260.17	264.64	
	P		F		P × F
S.E. $\pm$	0.588		0.588		1.176
CD at 5%	1.701		1.701		3.402

Initial available potassium: 310 kgha^{-1}

Significant interaction effect was observed with various levels of phytase, FYM and their combine application. Conjoint application of phytase @ 3600 IU + 7.5 t ha^{-1} FYM (295.85 kg ha^{-1}) recorded significant interaction effect on soil available potassium which was found statistically at par with 2400 IU phytase along with 5 t ha^{-1} FYM application (294.96 kg ha^{-1}).

b) At harvest of soybean: Availability of potassium in soil was also significantly affected by different levels of phytase and FYM at harvest of soybean. It is revealed from the data that 3600 IU phytase application recorded significantly higher soil available potassium (262.96 kg ha-^{1}) followed by phytase application @ 2400 IU (256.92 kg ha-^{1}). Data indicated decreasing trend in soil available potassium at harvest of soybean than 50 percent flowering of soybean over initial.

As regard with the FYM application data stated significant effects on soil available potassium. The pot supplied with 7.5 t ha^{-1} FYM recorded significantly higher soil available potassium (264.64 kg ha-^{1}).

The pot culture study indicated significant interaction for soil available potassium by phytase and FYM levels. Combine application of 3600 IU phytase + 7.5 t ha^{-1} FYM recorded significantly higher (271.86 t ha^{-1}) and statistically at par with same level of phytase along with 5 t ha^{-1} FYM (270.65 kgha^{-1}). Similar results were recorded by Yankaraddy *et al* (2009) they reported that application of FYM recorded highest grain yield, straw yield, and nutrient content and nutrient uptake. It was followed by application of coffee pulp compost.

Vibha *et al.,* 2014, reported significant improvement in soil in potassium availability under mung bean cultivation in sandy loam soil with inoculation of *Aspergillus niger* + *Penicillium citrinum* and *Aspergillus niger* 2

+ *Aspergillus niger* 3. Yankaraddy et al., (2009) conducted a field experiment with treatments consisted of organic sources viz. coffee pulp compost, rice hull ash and FYM combined with 50-100 per cent recommended dose of fertilizers. The results revealed that application of FYM recorded highest grain yield, straw yield, and nutrient content and nutrient uptake.

4.3.8 DTPA iron

Data pertaining to soil DTPA Fe as influenced by phytase and FYM levels applied ton soybean on non calcareous soil were found to be significantly influenced at 50 percent flowering and at harvest presented in Table 15 a and b.

The initial DTPA Fe content of soil was deficient and low (2.4 mg kg^{-1}) than soil critical limit (4.6 mg kg^{-1}).

a) At 50 percent flowering: Data indicated decreasing trend for soil DTPA Fe but the magnitude of decrease was higher at harvest than that of 50 percent of soybean over initial (2.4 mg kg^{-1}). Data show increasing trend in soil available Fe (DTPA Fe) with successive levels of phytase and FYM (0 IU: 2.11, 1200 IU: 2.26, 2400: 2.30 and 3600 IU: 2.34 mg kg^{-1}) and (0 t ha^{-1}: 2.11, 2.5 t ha^{-1}: 2.19, 5 t ha^{-1}: 2.32 and 7.5 t ha^{-1}: 2.38 mg kg^{-1}).

Phytase application @ 3600 IU recorded significantly higher mean DTPA Fe (2.57 mg kg^{-1}) than rest of the treatments, followed by use of phytase 1200 IU (2.39 mg kg^{-1}), or 2400 IU (2.44 mg kg^{-1}).

With respect to FYM application data stated that pots received 7.5 t ha^{-1} recorded significantly higher DTPA Fe (2.63 mg kg^{-1}) followed by 5 t ha^{-1} FYM (2.54 mg kg^{-1}) than rest of the treatments.

Interaction effects of phytase and FYM levels and their combined application were found significant for DTPA Fe. Pots supplied with 3600 IU phytase + 7.5 t ha^{-1} FYM recorded significantly higher (2.85 mg kg^{-1}) DTPA Fe in soil at 50 percent flowering than rest of the treatments.

b) At harvest of the soybean: Data revealed that significantly higher DTPA Fe was recorded in the pots received phytase @ 3600 IU (2.28 mg kg^{-1}) which was followed by 2400 IU (2.26 mg kg^{-1}). Mean lower (2.13 mg kg^{-1}) soil DTPA Fe was observed under P_0F_0 (phytase: 0 IU, FYM: 0 t ha^{-1}).

In case of FYM application, FYM @ 7.5 t ha^{-1} recorded (2.32 mg kg^{-1}) significantly higher DTPA iron. Lower DTPA Fe in soil was noticed with phytase 0 IU and FYM 0 t ha^{-1} treatment (1.99 mg kg^{-1}).

Interaction of phytase and FYM was found significant DTPA Fe in soil at harvest of soybean. Interaction effect of phytase @ 2400 IU + 5 t ha^{-1} FYM recorded significant (2.42 mg kg^{-1}) DTPA Fe in soil. However application of phytase @ 3600 IU + FYM @ 5 t ha^{-1} (2.39 mg kg^{-1}) and phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (2.37 mg kg^{-1}) recorded statistically at par DTPA Fe in soil at harvest of soybean.

Sharma *et al* (2000) observed significant positive correlation of Zn, Cu, Fe, Mn with organic carbon content of soil. Higher availability of DTPA zinc in the pots received FYM might be due to the applied organic matter (through FYM) played an important role in controlling availability of zinc particularly in alkaline soil. Further decomposition of organic matter reduces pH of rhizosphere soil which helps in increasing solubility of zinc from soil.

Table 15: Effect of phytase and FYM levels on DTPA iron in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	2.11	2.19	2.32	2.38	2.25
1200 IU	2.26	2.14	2.44	2.73	2.39
2400 IU	2.3	2.26	2.67	2.54	2.44
3600 IU	2.34	2.39	2.71	2.85	2.57
Mean	2.25	2.25	2.54	2.63	
	P		F		P × F
S.E. ±	0.008		0.008		0.016
CD at 5%	0.023		0.023		0.047

b) At harvest of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	1.99	2.13	2.07	2.32	2.13
1200 IU	2.12	2.10	2.17	2.31	2.17
2400 IU	2.05	2.24	2.42	2.37	2.27
3600 IU	2.28	2.19	2.39	2.27	2.28
Mean	2.11	2.16	2.26	2.32	
	P		F		P × F
S.E. ±	0.08		0.08		0.16
CD at 5%	0.028		0.028		0.055

Initial DTPA iron = 2.4 mgkg^{-1}

Vibha *et al*., (2014) reported significant increase in minor nutrient viz. iron, manganese, zinc and copper availability under mung bean in sandy loam soil for soil inoculated with *Aspergillus* strains than for uninoculated control. Gujar *et al*., (2013) concluded that phytase releases phosphorus from phytate, leading to the loss of ability of phytate to bind or chelate minerals, starch or proteins either directly or via ionic bridges, and consequently amend the availability of nutrients like iron, manganese, zinc, copper. Root exudation of high concentrations of organic acid anions as a result of P deficiency (Hoffland *et al*., 1989) lowers rhizosphere pH, making P (Haynes, 1990; Jones and Darrah, 1994) and micronutrients such as Mn, Fe and Zn to be more available in calcareous soils (Dinkelaker *et al*., 1989).

Kaya *et al.,* (2009) grown different chickpea genotypes on clay loam soil at Suleyman Demirel University research and experimental farm, Isparta-Turkey. They determined zinc, phosphorus, phytic acid, protein concentrations and phytase activity. They concluded that phytases are enzymes that degrade phytate and permit higher availability of Zn and other nutrients such as P and Fe.

4.3.9 DTPA manganese:

DTPA manganese in non calcareous soil as influenced by phytase and FYM levels showed significant variations at 50 percent flowering and at harvest of soybean presented in Table 16 a and b.

Initial DTPA manganese content in soil (2.23 mg kg^{-1}) was sufficient than that of critical level (2 ppm) which was indicated increasing trend at 50 percent flowering than at harvest of soybean.

a) At 50 percent flowering: Soils amended with phytase enzyme were recorded higher availability of DTPA manganese than FYM. The mean DTPA

manganese content in soil was significantly higher (2.64 mg kg^{-1}) with application of 3600 IU phytase. However this treatment was followed by 2400 IU (2.50 mg kg^{-1}). Mean lower DTPA manganese was noticed in control (0 IU phytase) (2.32 mg kg^{-1}).

FYM application @ 5 t ha^{-1} (2.60 mg kg^{-1}) recorded significantly higher manganese content in soil than rest of the treatments. Whereas pots received 2.5, 7.5 and 0 t ha^{-1} FYM recorded 2.55, 2.49, 2.25 mg kg^{-1} DTPA manganese.

Data indicated significant interaction among phytase and FYM levels for DTPA manganese at 50 percent flowering of soybean. Application of 3600 IU phytase + 7.5 t ha^{-1} FYM recorded significantly higher (2.86 mg kg^{-1}) DTPA manganese followed by 2400 IU phytase + 2.5 t ha^{-1} FYM (2.75 mg kg^{-1}).

b) At harvest of soybean: DTPA manganese content in soil at harvest of soybean was ranged between 2.0 and 2.50 mg kg^{-1}. Significantly higher mean available manganese (2.37 mg kg^{-1}) was noticed with 3600 IU phytase. However use of 2400, 1200 and 0 IU phytase recorded 2.25, 2.28 and 2.17 mg kg^{-1} DTPA manganese at harvest of soybean.

The highest soil DTPA manganese was obtained with application of 7.5 t ha^{-1} FYM (2.37 mg kg^{-1}). Whereas use of 5 t ha^{-1} (2.30 mg kg^{-1}) FYM recorded statistically at par results. Lowest soil DTPA manganese was observed in the pots where no FYM was given (2.12 mg kg^{-1}).

Data indicated significant interaction effect of phytase and FYM levels on soil DTPA manganese at harvest of soybean. Combine use of 3600 IU phytase + 7.5 t ha^{-1} FYM (2.50 mg kg^{-1}) recorded significantly higher DTPA manganese content in soil which was statistically at par and followed by

Table 16: Effect of phytase and FYM levels on DTPA manganese in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	2.12	2.36	2.47	2.34	2.32
1200 IU	2.26	2.44	2.61	2.43	2.43
2400 IU	2.26	2.75	2.64	2.35	2.50
3600 IU	2.38	2.64	2.70	2.86	2.64
Mean	2.25	2.55	2.60	2.49	
	P		F		P × F
S.E. ±	0.006		0.006		0.012
CD at 5%	0.017		0.017		0.034

b) At harvest of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	2.00	2.20	2.24	2.26	2.17
1200 IU	2.17	2.35	2.37	2.25	2.28
2400 IU	2.09	2.13	2.31	2.49	2.25
3600 IU	2.22	2.46	2.29	2.50	2.37
Mean	2.12	2.28	2.30	2.37	
	P		F		P × F
S.E. ±	0.009		0.009		0.018
CD at 5%	0.026		0.026		0.053

Initial DTPA manganese: 2.23 mg kg^{-1}

application of phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (2.49 mg kg^{-1}) and phytase @ 3600 IU + FYM @ 2.5 t ha^{-1} (2.46 mg kg^{-1}).

Sharma *et al* (2000) observed significant positive correlation of Zn, Cu, Fe, Mn with organic carbon content of soil. Higher availability of DTPA zinc in the pots received FYM might be due to the applied organic matter (through FYM) played an important role in controlling availability of zinc particularly in alkaline soil. Further decomposition of organic matter reduces pH of rhizosphere soil which helps in increasing solubility of zinc from soil.

Vibha *et al*., (2014) reported significant increase in minor nutrient viz. iron, manganese, zinc and copper availability under mung bean in sandy

loam soil for soil inoculated with *Aspergillus* strains than for uninoculated control. Gujar *et al*., (2013) concluded that phytase releases phosphorus from phytate, leading to the loss of ability of phytate to bind or chelate minerals, starch or proteins either directly or via ionic bridges, and consequently amend the availability of nutrients like iron, manganese, zinc, copper. Root exudation of high concentrations of organic acid anions as a result of P deficiency (Hoffland *et al*., 1989) lowers rhizosphere pH, making P (Haynes, 1990; Jones and Darrah, 1994) and micronutrients such as Mn, Fe and Zn to be more available in calcareous soils (Dinkelaker *et al*., 1989).

4.3.10 DTPA zinc

Soil available zinc extracted by DTPA was assessed at 50 percent flowering and at harvest of soybean as influenced by phytase and FYM levels are presented in Table 17 a and b.

Data indicated significant effects on DTPA zinc by phytase and FYM levels at both the growth stages of soybean. The initial DTPA zinc (1.23 mg kg^{-1}) was 2 times more than soil critical limit was slightly increased during pot culture study. Consistent increase for DTPA zinc in soil was observed by levels of both phytase and FYM but the magnitude of increase was slightly higher with FYM than phytase. DTPA zinc in soil influenced by phytase and FYM levels were ranged from 1.11 to 1.41 mg kg^{-1} and 1.05 to 1.34 mg kg^{-1} at 50 percent flowering and at harvest of soybean respectively.

a) At 50 percent flowering: Significantly higher DTPA zinc in soil was observed with phytase application @ 3600 IU (1.34 mg kg^{-1}) over rest of the treatments which was found statistically at par with application of phytase @ 2400 IU (1.35 mg kg^{-1}). In general higher levels of phytase application found beneficial for more DTPA zinc in soil. However application of 1200 IU (1.28 mg kg^{-1}) and 0 IU (1.23 mg kg^{-1}) recorded mean lower DTPA zinc in soil.

As like phytase, application of FYM significantly influenced DTPA zinc in soil. Data indicated that use of 7.5 t ha^{-1} FYM recorded significantly higher (1.37 mg kg^{-1}) DTPA zinc at 50 percent flowering of soybean followed by the application of 5 t ha^{-1} FYM (1.34 mg kg^{-1}). Mean lower DTPA zinc was obtained in pots amended with no FYM (1.20 mg kg^{-1}).

Significant interaction effect was noticed with the combine application of phytase and FYM on zinc content in soil. Data revealed that application of phytase @ 3600 IU along with FYM @ 7.5 t ha^{-1} (1.42 mg kg^{-1}) recorded significantly higher DTPA zinc which was statistically at par with use of phytase @ 2400 IU along with FYM either @ 7.5 t ha^{-1} (1.40 mg kg^{-1}) or @ 5 t ha^{-1} (1.38 mg kg^{-1}).

Table 17: Effect of phytase and FYM levels on DTPA zinc in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	1.12	1.22	1.28	1.31	1.23
1200 IU	1.19	1.26	1.32	1.36	1.28
2400 IU	1.25	1.31	1.38	1.40	1.34
3600 IU	1.25	1.37	1.37	1.42	1.35
Mean	1.20	1.29	1.34	1.37	
	P		F		P × F
S.E. ±	0.006		0.006		0.011
CD at 5%	0.016		0.016		0.032

b) At harvest of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	1.06	1.16	1.21	1.27	1.18
1200 IU	1.16	1.11	1.24	1.33	1.21
2400 IU	1.22	1.25	1.28	1.30	1.26
3600 IU	1.19	1.30	1.30	1.29	1.27
Mean	1.16	1.21	1.26	1.30	
	P		F		P × F
S.E. ±	0.004		0.004		0.009
CD at 5%	0.013		0.013		0.026

Initial DTPA zinc = 1.23 mg kg^{-1}

b) At harvest of soybean: DTPA extractable zinc content of soil with an initial value of (1.23 mg kg^{-1}) had increased significantly and attained a maximum mean value with the treatment of phytase @ 3600 IU (1.27 mg kg^{-1}) which was statistically at par with the application of phytase @ 2400 IU (1.26 mg kg^{-1}). However application of phytase @ 1200, and 0 IU recorded 1.21 and 1.18 mg kg^{-1} DTPA zinc respectively.

The perusal of data indicated increasing trend for DTPA extractable zinc with increasing graded levels of FYM. While significantly higher DTPA zinc was observed with 7.5 t ha^{-1} FYM (1.30 mg kg^{-1}) followed by 5 t ha^{-1} FYM (1.26 mg kg^{-1}). Lowest (1.16 mg kg^{-1}) value of DTPA zinc was noticed with the pots where 0 IU phytase and 0 t ha^{-1} FYM applied.

Data pertaining to interaction effect of phytase and FYM on DTPA zinc was found significant. Use of phytase @ 1200 IU (1.33 mg kg^{-1}) along with 7.5 t ha^{-1} FYM recorded significantly higher DTPA zinc in soil followed by 2400 IU + 7.5 t ha^{-1} (1.30 mg kg^{-1}) than rest of the treatments. Among the phytase and FYM levels lower zinc availability (1.06 mg kg^{-1}) was recorded with the application of 0 phytase + 0 FYM.

Sharma *et al* (2000) observed significant positive correlation of Zn, Cu, Fe, Mn with organic carbon content of soil. Higher availability of DTPA zinc in the pots received FYM might be due to the applied organic matter (through FYM) played an important role in controlling availability of zinc particularly in alkaline soil. Further decomposition of organic matter reduces pH of rhizosphere soil which helps in increasing solubility of zinc from soil.

Vibha *et al*., (2014) reported significant increase in minor nutrient viz. iron, manganese, zinc and copper availability under mung bean in sandy

loam soil for soil inoculated with *Aspergillus* strains than for uninoculated control. Gujar *et al*., (2013) concluded that phytase releases phosphorus from phytate, leading to the loss of ability of phytate to bind or chelate minerals, starch or proteins either directly or via ionic bridges, and consequently amend the availability of nutrients like iron, manganese, zinc, copper. Root exudation of high concentrations of organic acid anions as a result of P deficiency (Hoffland *et al*., 1989) lowers rhizosphere pH, making P (Haynes, 1990; Jones and Darrah, 1994) and micronutrients such as Mn, Fe and Zn to be more available in calcareous soils (Dinkelaker *et al*., 1989).

Kaya *et al.,* (2009) grown different chickpea genotypes on clay loam soil at Suleyman Demirel University research and experimental farm, Isparta-Turkey. They determined zinc, phosphorus, phytic acid, protein concentrations and phytase activity. They concluded that phytases are enzymes that degrade phytate and permit higher availability of Zn and other nutrients such as P and Fe.

4.3.11 DTPA copper:

Soil available copper extracted by DTPA was assessed at 50 percent flowering and at harvest of soybean as influenced by phytase and FYM levels are depicted in Table 18 a and b.

Data indicated significant effects on DTPA copper by phytase and FYM levels at both the growth stages of soybean. Consistent increase for DTPA copper in soil was observed by levels of both phytase and FYM but the magnitude of increase was slightly higher with FYM than phytase. DTPA copper in soil influenced by phytase and FYM levels were ranged from 3.46 to 5.89 mg kg^{-1} and 3.42 to 4.98 mg kg^{-1} at 50 percent flowering and at harvest of soybean respectively.

a) At 50 percent flowering: With the application of phytase @ 3600 IU significantly higher (5.25 $mgkg^{-1}$) value was noticed for soil DTPA copper. While pots received 7.5 t ha^{-1} FYM recorded significantly higher (5.47 $mgkg^{-1}$) soil DTPA copper. Combined application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (5.89 $mgkg^{-1}$) recorded significantly higher DTPA copper.

b) At harvest of soybean: Application of phytase @ 3600 IU recorded significantly higher (4.66 $mgkg^{-1}$)soil DTPA copper than other treatments. While in relation to FYM application significantly higher copper was noticed with the treatment FYM @ 7.5 t ha^{-1} (4.46 $mgkg^{-1}$). Combined application of phytase @ 3600 IU + FYM @ 5 t ha^{-1} (4.98 $mgkg^{-1}$) recorded significantly higher DTPA copper. However, phytase @ 2400 IU + FYM @ 5 t ha^{-1} (4.92 mg kg^{-1}) found statistically at par value of soil DTPA copper over rest of the treatments.

Sharma *et al* (2000) observed significant positive correlation of Zn, Cu, Fe, Mn with organic carbon content of soil. Higher availability of DTPA zinc in the pots received FYM might be due to the applied organic matter (through FYM) played an important role in controlling availability of zinc particularly in alkaline soil. Further decomposition of organic matter reduces pH of rhizosphere soil which helps in increasing solubility of zinc from soil.

Vibha *et al*., (2014) reported significant increase in minor nutrient viz. iron, manganese, zinc and copper availability under mung bean in sandy loam soil for soil inoculated with *Aspergillus* strains than for uninoculated control. Gujar *et al*., (2013) concluded that phytase releases phosphorus from phytate, leading to the loss of ability of phytate to bind or chelate minerals, starch or proteins either directly or via ionic bridges, and consequently amend the availability of nutrients like iron, manganese, zinc, copper. Root exudation of high concentrations of organic acid anions as a result of P deficiency

Table 18: Effect of phytase and FYM levels on DTPA copper in non calcareous soil

a) At 50 % flowering of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	4.22	4.12	4.00	5.12	4.36
1200 IU	4.31	4.42	4.21	5.38	4.58
2400 IU	3.46	4.83	4.8	5.50	4.64
3600 IU	5.33	4.64	5.16	5.89	5.25
Mean	4.33	4.50	4.54	5.47	
	P		F		P × F
S.E. ±	0.006		0.006		0.013
CD at 5%	0.019		0.019		0.037

b) At harvest of soybean

FYM (F) / Phytase (P)	mgkg^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	3.42	3.08	3.13	4.12	3.43
1200 IU	3.41	3.90	4.30	4.16	3.94
2400 IU	4.13	4.41	4.92	4.84	4.57
3600 IU	4.30	4.61	4.98	4.75	4.66
Mean	3.81	4.00	4.33	4.46	
	P		F		P × F
S.E. ±	0.011		0.011		0.022
CD at 5%	0.032		0.032		0.064

Initial DTPA copper: 4.45 mg kg^{-1}

(Hoffland *et al*., 1989) lowers rhizosphere pH, making P (Haynes, 1990; Jones and Darrah, 1994) and micronutrients such as Mn, Fe and Zn to be more available in calcareous soils (Dinkelaker *et al*., 1989).

4.4 Effect of phytase and FYM levels on morphological characteristics of soybean at 50% flowering in non calcareous soil

4.4.1 Root length

The root length of soybean as influenced by graded levels of phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5 5 and 7.5 t ha^{-1}) in non calcareous soil were assessed and presented in Table 19 a and Fig

The results obtained from the present experiment reported consistent increasing trend for root length of soybean was recorded by the application of phytase and FYM. Data revealed significantly higher and statistically at par value for root length of soybean was observed with the application of phytase 3600 IU (67 cm) over rest of the treatments. The lower mean root length of soybean was noticed with no phytase (0 IU phytase) (48 cm) application.

With the application of FYM @ 7.5 t ha^{-1} (68 cm) significantly higher value of root length of soybean was recorded, followed by 5 t ha^{-1} (63 cm) and 2.5 t ha^{-1} (60 cm) FYM. The lower (53 cm) mean root length was found with no application of FYM.

Combined application of phytase and FYM showed numerical increase in root length of soybean. Application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (74 cm) recorded higher root length. The lowest root length (48 cm) was noticed in the pots receiving 0 IU phytase and 0 t ha^{-1} FYM.

The increase in root length 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 solubility (Yadav and Verma, 2012). Gujar *et.al.,*(2013) studied effect of phytase from *Aspergillus niger* on plant growth & mineral assimilation in wheat (*Triticum aestivum Linn.)* on calcareous soil and its potential for use as a soil amendment at NCL, Pune during 2012 and concluded that phytase isolated from *Aspergillus niger* promoted plant growth (upto 200 %). The length of root was higher in pots supplemented with phytase than uninoculated control. Vibha *et al.,* 2014 studied impact of phosphorus solubilisation of fungi on yield of mung bean on sandy loam soil and concluded significantly higher root length with inoculation of *Aspergillus niger* strains No. 2. Tarafdar, (2008) conducted an experiment on mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He concluded that seed inoculation with *Aspergillus fumigates* and soil inoculation with *Glomus mosseae* increased root length. Muhammad Iqbal *et al.,* (2013) concluded that phosphate solublizing bacteria enhance the growth (plant height, root length, dry weight of shoot and root) through simultaneous exudation of organic acids (by decreasing pH) and/or through releasing phosphatases and ACC-deaminase.

4.4.2 Plant height:

The shoot length of soybean as influenced by graded levels of phytase (0, 1200, 2400 and 3600 IU) and FYM (0, 2.5 5 and 7.5 t ha^{-1}) in non calcareous soil were assessed and presented in Table 19 b.

Data revealed that consistent increasing trend for plant height of soybean was recorded by the application of phytase and FYM. Significantly higher and statistically at par value for shoot length of soybean was observed

Table 19: Effect of phytase and FYM levels on root and shoot length of soybean at 50% flowering grown in non calcareous soil

a. Root length

FYM (F) / Phytase (P)	Root length (cm)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	48	55	58	62	56
1200 IU	52	57	60	68	59
2400 IU	56	61	65	69	63
3600 IU	58	66	69	74	67
Mean	53	60	63	68	
	P	F		P × F	
S.E. ±	0.462	0.462		0.924	
CD at 5%	1.337	1.337		NS	

b. Shoot length/ Plant height

FYM (F) / Phytase (P)	Plant height (cm)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	28	33	37	41	35
1200 IU	31	36	41	43	38
2400 IU	35	37	40	42	38
3600 IU	36	41	42	46	41
Mean	32	37	40	43	
	P	F		P × F	
S.E. ±	0.425	0.425		0.85	
CD at 5%	1.23	1.23		2.459	

with the application of phytase 3600 IU (41 cm) over rest of the treatments. The lower mean valve of shoot length was recorded in the pots supplied with 0 IU phytase (35 cm). Results obtained from this pot culture experiment disclosed that with the application of FYM @ 7.5 t ha^{-1} (43 cm) significantly higher value of shoot length of soybean was obtained. The lower mean value was obtained with the application of 0 t ha^{-1} FYM (32 cm).

Combined application of phytase and FYM showed significant increase in shoot length of soybean. Application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (46 cm) recorded significantly higher shoot length which was statistically at par with the use of 1200 IU phytase + 7.5 t ha^{-1} FYM (43 cm).

The minimum value for shoot length of soybean was noticed with the application of 0 IU phytase + 0 t ha^{-1} FYM (28 cm).

Gujar *et.al.*,(2013) studied effect of phytase from *Aspergillus niger* on plant growth & mineral assimilation in wheat (*Triticum aestivum Linn.)* on calcareous soil and its potential for use as a soil amendment at NCL, Pune during 2012 and concluded that phytase isolated from *Aspergillus niger* promoted plant growth (upto 200%). The length of shoot was higher in pots supplemented with phytase than uninoculated control. Tarafdar, (2008) conducted an experiment on mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He concluded that seed inoculation with *Aspergillus fumigates* and soil inoculation with *Glomus mosseae* increased shoot length. The increase in shoot length 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 solubility (Yadav and Verma, 2012). Muhammad Iqbal *et al.*, (2013) concluded that phosphate solublizing bacteria enhance the growth (plant height, root length, dry weight of shoot and root) through simultaneous

exudation of organic acids (by decreasing pH) and/or through releasing phosphatases and ACC-deaminase. Jain *et al.* (1995) reported that the application of FYM and sugar pressmud had significantly enhanced the plant height, number of leaves per plant, number of branches per plant over control in soybean when grown in medium black soil in Madhya Pradesh.

4.4.3 Root-shoot ratio:

The root shoot ratio of soybean in non calcareous soil as influenced by various levels of phytase and FYM was observed and found significant. The result regarding to the root shoot ratio is noted in Table 20.

The data with respect to root-shoot ratio revealed that significantly higher value was recorded with the application of phytase @ 0 IU (1.76) which was found statistically at par with the application of phytase @ 3600 IU (1.66) over rest of treatments. While with the use of FYM @ 5 t ha^{-1} (1.70) recorded significantly higher root-shoot ratio, however the application of FYM @ 7.5 t ha^{-1} (1.68) registered statistically at par values.

Significant interaction effect was observed with the combined application of phytase and FYM on root-shoot ratio of soybean. Significantly

higher value for root-shoot ratio was noticed with the conjoint application FYM @ 5 t ha^{-1} + phytase @ 0 IU (2.37) over rest of the treatments.

The increase in shoot and root length and root-shoot ratio in the treatments with phytase could be attributed to increase in solubilisation of phosphorus by secretion of enzymes and organic acids by the phosphorus solubilizing fungi that are used for cell division and enlargement by plants (Yadav and Verma, 2012). Similar result was also obtained by Mittal *et al*., 2008 in case of chickpea. Gujar *et al.*, 2013 reported enhanced growth (higher

Table 20: Effect of phytase and FYM levels on root-shoot ratio at 50% flowering of soybean in non calcareous soil

FYM (F) / Phytase (P)	Root-shoot ratio				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	1.49	1.48	2.37	1.70	1.76
1200 IU	1.46	1.50	1.15	2.03	1.53
2400 IU	1.65	1.43	1.69	1.28	1.51
3600 IU	1.57	1.74	1.60	1.73	1.66
Mean	1.54	1.54	1.70	1.68	
	P	F	P × F		
S.E. $\pm$	0.044	0.044	0.088		
CD at 5%	0.127	0.127	0.254		

root and shoots length) of wheat seedlings grown with phytase supplementation as compared to control. Jain *et al.* (1995) reported that the application of FYM and sugar press mud had significantly enhanced the plant height, number of leaves per plant, number of branches per plant over control in soybean when grown in medium black soil in Madhya Pradesh.

4.4.4 Fresh and dry weight of soybean shoot:

The fresh weight of soybean shoot at 50% flowering of soybean in calcareous soil as influenced by graded levels of phytase and FYM is depicted in Table 21 a.

The fresh weight of soybean shoot was significantly affected by graded levels of phytase and FYM. Application of phytase @ 3600 IU recorded significantly higher (21.75 g) mean fresh shoot weight of soybean. FYM application @ 7.5 t ha^{-1} recorded significantly higher (21.89 g) fresh weight of soybean shoot followed by 5 t ha^{-1} FYM (20.39).

The interaction effect comprising graded levels of phytase and FYM noticed non significant in relation to the fresh weight of soybean shoot in non calcareous soil. Among the treatment combinations, application of phytase @ 3600 IU with FYM @ 7.5 t ha^{-1} (24.15 g) recorded numerically higher fresh weight of soybean shoot followed by treatment combinations phytase @ 3600 IU + FYM @ 5 t ha^{-1} (22.52 g) and phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (21.94 g).

The dry weight of soybean shoot at 50% flowering was significantly influenced by graded levels of phytase and FYM imposed in non calcareous soil (Table 21 b). Significantly higher mean dry shoot weight was obtained with application of phytase @ 3600 IU (7.42 g) which was found statistically at par with the use of phytase @ 2400 IU (7.26 g). FYM

Table 21: Effect of phytase and FYM levels on fresh and dry shoot weight of soybean at 50% flowering in non calcareous soil.

a. Fresh weight of shoot

FYM (F) / Phytase (P)	Fresh weight (g)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	16.49	17.44	18.95	21.02	18.47
1200 IU	17.04	18.38	19.76	20.45	18.91
2400 IU	17.87	18.80	20.31	21.94	19.73
3600 IU	19.81	20.52	22.52	24.15	21.75
Mean	17.80	18.78	20.39	21.89	
	P	F	P × F		
S.E. ±	0.203	0.203	0.407		
CD at 5%	0.589	0.589	NS		

b. Dry weight of shoot

FYM (F) / Phytase (P)	Dry weight (g)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	5.28	6.08	7.01	7.88	6.56
1200 IU	5.83	6.81	7.21	7.24	6.77
2400 IU	6.66	6.86	7.42	8.11	7.26
3600 IU	6.60	6.90	7.66	8.50	7.42
Mean	6.09	6.66	7.33	7.93	
	P	F	P × F		
S.E. ±	0.178	0.178	0.356		
CD at 5%	0.515	0.515	NS		

application @ 5 t ha^{-1} recorded significantly higher (7.93 g) dry weight of soybean shoot.

The interaction effect of phytase and FYM levels was found non significant in respect of dry weight of shoot. Among all treatment combinations application of phytase @ 3600 IU with 7.5 t ha^{-1} FYM recorded numerically higher mean dry shoot weight (8.50 g) followed by phytase @ 2400 IU with 7.5 t ha^{-1} FYM (8.11 g).

Vibha *et al*., (2014) studied impact of phosphorus solubilisation of fungi on yield of mung bean on sandy loam soil and concluded significantly higher root length, dry weight and seed yield with inoculation of *Aspergillus niger* strains*., Penicilium citrinum* strains and phosphate solubilising bacteria over uninoculated control. Tarafdar, (2008) conducted an experiment on mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He concluded that seed inoculation with *Aspergillus fumigates* and soil inoculation with *Glomus mosseae* increased shoot length, root length, shoot and root dry weight. Muhammad Iqbal *et al.*, (2013) concluded that phosphate solublizing bacteria enhance the growth (plant height, root length, dry weight of shoot and root) through simultaneous exudation of organic acids (by decreasing pH) and/or through releasing phosphatases and ACC-deaminase.

4.4.5 Fresh and dry weight of soybean root:

The data in relation to the fresh weight of soybean root in non calcareous soil as influenced by graded levels of phytase and FYM is presented in table 22 a.

The perusal of data revealed that fresh weight of soybean root was significantly affected by different levels of phytase and FYM. An

increasing trend in mean root weight was observed with application of phytase @ 0 IU (5.53 g), 1200 IU (6.38 g), 2400 IU (7.44 g) and 3600 IU (8.01 g). Similar trend was noticed with the application of FYM as @ 0 t ha^{-1} (5.53 g), 2.5 t ha^{-1} (5.94 g), 5 t ha^{-1} (6.79 g) and 7.5 t ha^{-1} (7.25 g).

Data indicated that application of phytase @ 3600 IU (7.92 g) recorded higher mean fresh root weight of soybean which was statistically at par with the use of phytase @ 2400 IU (7.66 g). In case of FYM, application of FYM @ 7.5 t ha^{-1} (8.43 g) recorded significantly higher fresh root weight of soybean followed by 5 t ha^{-1} FYM (7.81 g) on non calcareous soil.

The interaction effect of treatment combinations comprising graded levels of phytase and FYM recorded non significant effects on the fresh weight of soybean root in non calcareous soil. Among the treatment combinations, application of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM (8.86 g) recorded numerically higher fresh weight of soybean root followed by

phytase @ 2400 IU + FYM @ 7.5 T ha^{-1} (8.60 g). Data reported that the dry weight of soybean root was significantly influenced by graded levels of phytase and FYM imposed in non calcareous soil (Table 22 b).

An increasing trend in mean dry root weight of soybean was observed with application of phytase @ 0 IU (3.24 g), 1200 IU (4.1 g), 2400 IU (5.16 g) and 3600 IU (5.73 g). Similar trend was noticed with the application of FYM as @ 0 t ha^{-1} (3.24 g), 2.5 t ha^{-1} (3.66 g), 5 t ha^{-1} (4.50 g) and 7.5 t ha^{-1} (4.97 g).

Data showed that application of phytase @ 3600 IU (5.57 g) recorded higher mean dry root weight of soybean which was statistically at par with the use of phytase @ 2400 IU (5.46 g). In case of FYM, significantly

Table 22: Effect of phytase and FYM levels on fresh and dry root weight of soybean at 50% flowering grown in non calcareous soil

a. Fresh weight of root

FYM (F) / Phytase (P)	Fresh weight (g)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	5.53	6.38	7.44	8.01	6.84
1200 IU	5.94	7.06	7.91	8.24	7.29
2400 IU	6.79	7.31	7.94	8.60	7.66
3600 IU	7.25	7.62	7.96	8.86	7.92
Mean	6.38	7.09	7.81	8.43	
	P	F		P × F	
S.E. ±	0.199	0.199		0.397	
CD at 5%	0.575	0.575		NS	

b. Dry weight

FYM (F) / Phytase (P)	Dry weight (g)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	3.24	4.1	5.16	5.73	4.56
1200 IU	3.66	4.77	5.62	5.95	5.00
2400 IU	4.50	5.34	5.66	6.32	5.46
3600 IU	4.97	5.03	5.68	6.58	5.57
Mean	4.09	4.81	5.53	6.15	
	P	F		P × F	
S.E. ±	0.17	0.17		0.34	
CD at 5%	0.492	0.492		NS	

higher value for dry root weight of soybean was noticed with application of FYM @ 7.5 t ha^{-1} (6.15 g) followed by 5 t ha^{-1} FYM (5.53 g).

The interaction effect of treatment combinations comprising graded levels of phytase and FYM recorded non significant effects on the dry weight of soybeanroot in non calcareous soil. Among the treatment combinations, application of phytase @ 3600 IU along with 7.5 t ha^{-1} FYM (6.58 g) recorded numerically higher fresh weight of soybean root followed by phytase @ 2400 IU + FYM @ 7.5 T ha^{-1} (6.32 g).

Vibha *et al*., (2014) studied impact of phosphorus solubilisation of fungi on yield of mung bean on sandy loam soil and concluded significantly higher root length, dry weight and seed yield with inoculation of *Aspergillus niger* strains., *Penicilium citrinum* strains and phosphate solubilising bacteria over uninoculated control. Tarafdar, (2008) conducted an experiment on mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur. He concluded that seed inoculation with *Aspergillus fumigates* and soil inoculation with *Glomus mosseae* increased shoot length, root length, shoot and root dry weight. Muhammad Iqbal *et al.,* (2013) concluded that phosphate solublizing bacteria enhance the growth (plant height, root length, dry weight of shoot and root) through simultaneous exudation of organic acids (by decreasing pH) and/or through releasing phosphatases and ACC-deaminase.

4.4.6 Number of active root nodules:

Effect of phytase and FYM levels on number of active root nodules under soybean cultivation was assessed and stated in Table 23 a and graphically represented in Fig.

The data with respect to count of active root nodules showed increasing trend in number of active root nodules with the application of graded levels of phytase and FYM. The application of phytase and FYM significantly affected the number of active root nodules of soybean.

The data revealed that the application of phytase @ 3600 IU recorded significantly higher (62) number of active root nodules followed by 2400 IU (54), 1200 IU (47). The lower mean number of active root nodules (45) were observed with no phytase treatment (0 IU).

FYM application @ 7.5 t ha^{-1} (65) recorded significantly higher and statistically superior number of active root nodules over rest of the treatments, followed by 5 t ha^{-1} (54), 2.5 t ha^{-1} (49) FYM application.

Combine application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (77) recorded significantly higher number of active root nodules over rest of the treatments. Lower (33) number of active root nodules was noticed at absolute control.

Increase in number of active root nodules might be due to the increased phytase activity in soil and hence increased phosphate utilization by soybean. Similar results were obtained by Saber Kouas *et al*., (2009) who reported that the induction of higher phytase activity under P deficiency as observed in bean roots and nodules may contribute the P utilization from phytate in the plant and contribute to improve its adaptation to low P soils.

Tarafdar and Rao, (2001) reported that root nodulation of clusterbean was increased with inoculation of AMF + Rhizobium and FYM. This could be attributed to a more extensive root system, tapping a large volume of soil for water and nutrients.

Table 23: Effect of phytase and FYM levels on number and weight of active root nodules of soybean at 50% flowering grown in non calcareous soil

a. Number of active root nodules

FYM (F) / Phytase (P)	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	33	43	48	56	45
1200 IU	35	45	48	59	47
2400 IU	40	51	54	70	54
3600 IU	46	59	65	77	62
Mean	38	49	54	65	
	P	F		P × F	
S.E. ±	0.529	0.529		1.057	
CD at 5%	1.53	1.53		3.06	

b. Fresh weight of active root nodules

FYM (F) / Phytase (P)	Weight of active root nodules (g)				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	0.28	0.31	0.33	0.35	0.32
1200 IU	0.40	0.43	0.60	0.70	0.53
2400 IU	0.44	0.52	0.65	0.75	0.59
3600 IU	0.59	0.63	0.70	0.84	0.69
Mean	0.43	0.47	0.57	0.66	
	P	F		P × F	
S.E. ±	0.022	0.022		0.043	
CD at 5%	0.063	0.063		NS	

4.4.7 Fresh weight of active root nodules:

The perusal of data indicated that the fresh weight of active root nodules of soybean as influenced by the graded levels of phytase and FYM application was found significant which is presented in Table 23 b.

The increasing trend was observed with the individual application of phytase or FYM and their combined application. The indivadual application of 0, 1200, 2400 and 3600 IU phytase showed 0.28, 0.40, 0.44 and 0.59 g respectively and FYM @ 0, 2.5, 5 and 7.5 t ha^{-1} FYM recorded 0.28, 0.31, 0.33 and 0.35 g fresh weight of active root nodules respectively.

The fresh weight of active root nodules were found higher in the pot receiving 3600 IU phytase (0.69 g) followed by 2400 IU (0.59 g) and 1200 IU (0.53). The lower mean fresh weight of active root nodules (0.32 g) were noticed in the pot without phytase application.

FYM applied pot @ 7.5 t ha^{-1} (0.66 g) showed significantly higher fresh weight of active root nodules over rest of the treatments, followed by FYM @ 5, 2.5 and 0 t ha^{-1} with the weights 0.57, 0.47 and 0.43 g respectively.

The non significant interaction effect was observed with the application of graded levels of phytase and FYM. With the application of phytase @ 3600 IU phytase + 7.5 t ha^{-1} FYM (0.84 g) recorded numerically higher fresh weight of active root nodules over rest of the treatments.

Tarafdar and Rao, (2001) reported that root nodulation of clusterbean was increased with inoculation of AMF + Rhizobium and FYM. This could be attributed to a more extensive root system, tapping a large volume of soil for water and nutrients.

4.5 Effect of phytase and FYM levels on dry matter production of soybean at 50% flowering grown in non calcareous soil

The dry matter yield of soybean at 50 per cent flowering as influenced by the application of graded levels of phytase and FYM is presented in Table 27 revealed that an increase in dry matter of soybean was observed due to the application of phytase and FYM.

Data indicated that significant increase in dry matter of soybean with the application of phytase was observed. Higher mean dry matter was noticed with the application of 3600 IU phytase (12.86 g pot^{-1}) which was found at par with the treatment of phytase @ 2400 IU (12.83 g pot^{-1}).

Application of FYM also found significant increase in the dry matter of soybean at 50 percent flowering. A continuous increasing trend (8.52, 10.18, 12.17 and 13.36 g pot^{-1}) with increasing levels of FYM (0, 2.5, 5 and 7.5 t ha^{-1}) was recorded. Significantly higher mean value for dry matter was recorded with the application of 7.5 t ha^{-1} (14.08 g pot^{-1}) followed by 5 t ha^{-1} (12.86 g pot^{-1}). Lower mean value (10.19 g pot^{-1}) was recorded with the no FYM application.

Non significant interaction effect was noticed with the application of treatment combinations phytase and FYM. Numerically higher value for dry matter was recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (15.08 g pot^{-1}).

Vibha *et al.*, 2014 studied impact of phosphorus solubilisation of fungi on yield of mung bean on sandy loam soil and concluded significantly higher root length, dry weight and seed yield with inoculation of *Aspergillus niger* strains No. 2 which has more ability to secrete phytase. Tarafdar and Rao, (2001) reported that dry matter production of cluster bean was increased with

Table 24: Effect of phytase and FYM levels on dry matter production at 50% flowering grown in non calcareous soil

FYM (F) / Phytase (P)	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

inoculation of AMF + Rhizobium and FYM. Application of FYM had additive effect on dry matter production and seed yield. This could be attributed to a more extensive root system, tapping a large volume of soil for water and nutrients.

4.6 Effect of phytase and FYM levels on dry matter production of soybean at harvest

The data obtained on grain yield of soybean as influenced by graded levels of phytase and FYM is presented in Table 28 a.

The significant variation in yield of soybean was observed due to the application of various levels of phytase and FYM to soybean in the present study. Application of phytase @ 3600 IU recorded significantly higher grain weight (17.34 g pot^{-1}) which was statistically at par with the application of phytase @ 2400 IU (17.28 g pot^{-1}) over rest of the treatments.

While with the use of FYM @ 7.5 t ha^{-1} significantly higher (17.77 g pot^{-1}) grain weight was noticed, followed by FYM @ 5 t h a^{-1} (17.17 g pot^{-1}) and 2.5 t ha^{-1} (16.41 g pot^{-1}).

The grain yield in the present study ranged from 14.33 to 18.68 g pot^{-1}. The treatment P_3F_3 (phytase @ 3600 IU + FYM @ 7.5 t ha^{-1}) found numerically superior over rest of the treatments with grain yield 18.68 g. The lowest grain yield was observed at absolute control (0 IU phytase + 0 t ha^{-1} FYM) and it was 14.33 g.

` The straw yield data of soybean as influenced by phytase and FYM application is presented in Table 28 b. Data revealed that as like grain yield, straw yield also increased due to application of various levels of phytase and FYM to soybean.

Table 25: Effect of phytase and FYM levels on grain and straw yield of soybean at harvest

a. Grain yield:

FYM (F) / Phytase (P)	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

With the application of phytase @ 3600 IU significantly higher straw yield was noticed (14.31 g pot^{-1}) over rest of the treatments, followed by phytase @ 2400 IU (13.50 g pot^{-1}). In relation to FYM application data indicated that pots received FYM @ 7.5 t ha^{-1} registered significantly higher (14.17 g pot^{-1}) straw yield than rest of the treatments.

Significant interaction effect was noticed on straw yield of soybean with the use of different treatment combinations of phytase ad FYM. The straw yield in present study ranged from 12.37 to 14.84 g pot^{-1}. The significantly higher straw yield (14.84 g pot^{-1}) was recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} which was statistically at par with the application of phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (14.67 g pot^{-1}). The lowest straw yield (12.37 g pot^{-1}) was observed in the pots receiving no phytase and FYM.

Effect of inoculation with phosphatase producing fungi dry matter production and grain yield of cluster bean, chickpea and wheat was studied by Tarafdar *et al.*, 1995, Tarafdar and Rao 1996. Vibha *et al.*, 2014 studied impact of phosphorus solubilisation of fungi on yield of mung bean on sandy loam soil and concluded significantly higher root length, dry weight and seed yield with inoculation of *Aspergillus niger* strains No. 2. The experiment conducted by Yadav and Tarafdar 2010, reported that grain yield of cluster bean was increased by 26 per cent of straw yield by 42 per cent plant phosphorus content by 12 per cent due to inoculation of *Emericella rugulosa* (phosphorus solubilising fungi). Tarafdar and Rao, (2001) reported that dry matter production and grain yield of clusterbean was increased with inoculation of AMF + Rhizobium and FYM. Application of FYM had additive effect on dry matter production and seed yield. This could be attributed to a more extensive root system, tapping a large volume of soil for water and nutrients. Tomar

(1998) showed that the application of phosphate solubilizing bacteria (10 g/kg seed) combined with farm yard manure (5 tonnes ha^{-1}) gave the maximum grain and straw yields than the control.

4.7 Effect of phytase and FYM levels on nutrient uptake by soybean grown in non calcareous soil (at 50 % flowering)

The total uptake of nitrogen by soybean at 50 percent flowering as influenced by the application of graded levels of phytase and FYM are presented in Table 26 a.

It is evident from the data presented in Table 26 a that the application of increasing levels of phytase and FYM significantly increased nitrogen uptake by soybean over the absolute control.

The application of phytase found significant for the uptake of nitrogen by soybean. Statistically significant value for nitrogen uptake was recorded with the application of phytase @ 3600 IU (495.53 mg pot^{-1}) followed by the application of phytase @ 2400 IU (489.11mg pot^{-1}).

The application of FYM also reported significant increase in the nitrogen uptake by soybean at 50 percent flowering. An increasing trend was noticed with the application of graded levels of FYM i.e. 0 t ha^{-1}: 238.03, 2.5 t ha^{-1}: 340.13, 5 t ha^{-1}: 416.27 and 7.5 t ha^{-1}: 566.47 mg pot^{-1} nitrogen uptake by soybean. Significantly higher and statistically superior mean nitrogen uptake was recorded with the application of 7.5 t ha^{-1} (593.44 mg pot^{-1}).

Significant interaction effect on nitrogen uptake by soybean at 50 percent flowering was observed with the application of phytase and FYM. The total uptake of nitrogen in soybean crop ranged from 280.03 to 643.93 mg pot^{-1}. The treatment phytase @ 3600 IU + FYM 7.5 t ha^{-1} (643.93 mg pot^{-1}) recorded

Table 26. Effect of phytase and FYM levels on nitrogen, phosphorus and potassium uptake of soybean at 50 percent flowering

I) 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

II) 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 ±	1.811		1.811		3.623
CD at 5%	5.242		5.242		10.483

III) 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 ±	7.183		7.183		14.366
CD at 5%	20.786		20.786		NS

significantly superior uptake of nitrogen which was statistically at par with the application of phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (584.2 mg pot^{-1}) over rest of the treatments. The minimum nitrogen uptake was noticed in control (phytase @ 0 IU + FYM @ 0 t ha^{-1}) which noticed uptake value as 238.03 mg pot^{-1}.

The data in respect of the total uptake of phosphorus by soybean at 50 percent flowering is presented in Table 26 b.

The data indicated that the application of phytase found significant for the uptake of phosphorus by soybean. An increasing trend was noticed with the application of graded levels of phytase (0 IU: 29.10, 1200 IU: 36.35, 2400 IU: 52.07 and 3600 IU: 53.64 mg pot^{-1}) in phosphorus uptake by soybean. Statistically significant value for phosphorus uptake was recorded with the application of phytase @ 3600 IU (66.43 mg pot^{-1}) which was statistically at par with the application of phytase @ 2400 IU (61.62 mg pot^{-1}) over control and rest of the treatment combinations.

The application of FYM also reported significant increase in the phosphorus uptake by soybean at 50 percent flowering. An increasing trend was noticed with the application of graded levels of FYM i.e. 0 t ha^{-1}: 29.10, 2.5 t ha^{-1}: 42.67, 5 t ha^{-1}: 60.13 and 7.5 t ha^{-1}: 66.61 mg pot^{-1} phosphorus uptake by soybean. Significantly higher and statistically superior mean phosphorus uptake was recorded with the application of 7.5 t ha^{-1} (72.90 mg pot^{-1}).

Significant interaction effect was noticed on total phosphorus uptake by soybean with the combine application of phytase and FYM at 50 percent flowering. The total uptake of phosphorus by soybean was ranged from 29.10 to 82.89 mg pot^{-1}. The maximum phosphorus uptake (82.89 mg pot^{-1}) recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} which

was significant and statistically at par with the treatment combination phytase @ 3600 IU + FYM @ 5 t ha^{-1} (73.98 mg pot^{-1}).

The data revealed that the application graded levels of phytase significantly influenced the potassium uptake by soybean at 50 percent flowering. An increasing trend in uptake of potassium (0 IU: 135.85, 1200 IU: 173.11, 2400 IU: 194.29, and 3600 IU: 242.21 mg pot^{-1}) was noticed with the application of phytase. Statistically significant value for mean potassium uptake was recorded with the application of phytase @ 3600 IU (285.12 mg pot^{-1}) which was statistically at par over control and rest of the treatment combinations.

The application of FYM also reported significant increase in the potassium uptake by soybean at 50 percent flowering. An increasing trend was noticed with the application of graded levels of FYM i.e. 0 t ha^{-1}: 135.85, 2.5 t ha^{-1}: 228.40, 5 t ha^{-1}: 282.84 and 7.5 t ha^{-1}: 303.85 mg pot^{-1} potassium uptake by soybean. Significantly higher and statistically superior mean potassium uptake was recorded with the application of 7.5 t ha^{-1} (321.94 mg pot^{-1}).

It is evident from the data that application of graded levels of phytase and FYM found statistically non significant with an increase in the total potassium uptake. The highest potassium uptake was recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (354.75 mg pot^{-1}) followed by application of either phytase @ 2400 IU (330.51 mg pot^{-1}) or @ 1200 IU (298.64 mg pot^{-1}) with FYM @ 7.5 t ha^{-1}.

Effect of inoculation with phosphatase producing fungi on dry matter production and grain yield of cluster bean, chickpea and wheat was studied by Tarafdar *et al.*, 1995, Tarafdar and Rao, 1996. They concluded higher nitrogen, phosphorus, potassium and calcium uptake by cluster bean,

chickpea and wheat due to inoculation with phosphatase producing fungi. Gujar *et al.*, 2013 concluded that phytase promoted phosphorus mineral assimilation much more efficiently as compared to chemical fertilizer. Reddy *et al.* (1999) reported that the P uptake by soybean and wheat increased with increasing rates of manure and fertilizer P and was relatively larger in soybean than in wheat. The per cent P recovery by the crops from fertilizer P decreased with increasing fertilizer P rate, while it was improved in the presence of manure.

4.8 Effect of phytase and FYM levels on nutrient uptake by soybean (grain) at harvest

The total uptake of nitrogen, phosphorus and potassium by soybean grain at harvest as influenced by the application of graded levels of phytase and FYM are presented in Table 30 a, b and c.

It is evident from the data presented in Table 30 a that the application of increasing levels of phytase and FYM significantly increased nitrogen uptake by soybean grain over the absolute control.

With the application of phytase found significant for the uptake of nitrogen by soybean grain. Statistically significant value for nitrogen uptake was recorded with the application of phytase @ 3600 IU (755.37 mg pot^{-1}).

The application of FYM also reported significant increase in the nitrogen uptake by soybean grain. An increasing trend was noticed with the application of graded levels of FYM i.e. 0 t ha^{-1}: 534.08, 2.5 t ha^{-1}: 587.77, 5 t ha^{-1}: 602.18 and 7.5 t ha^{-1}: 689.09 mg pot^{-1} nitrogen uptake by soybean grain. Significantly higher and statistically superior mean nitrogen uptake was recorded with the application of 7.5 t ha^{-1} (731.29 mg pot^{-1}).

Significant interaction effect on nitrogen uptake by soybean grain at harvest was observed with the application of phytase and FYM. The total

uptake of nitrogen in soybean grain ranged from 534.08 to 801.95 mg pot^{-1}. The treatment phytase @ 3600 IU + FYM 5 t ha^{-1} (801.95 mg pot^{-1}) recorded significantly superior uptake of nitrogen which was statistically at par with phytase either @ 2400 IU + FYM @ 7.5 t ha^{-1} (780.81 mg pot^{-1}) over rest of the treatments.

Table 27.Effect of phytase and FYM levels on nitrogen, phosphorus and potassium uptake of soybean (grain) at harvest

I) 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	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	801.95	757.50	755.37
Mean	623.10	676.76	694.88	731.29	
	P		F		P × F
SE ±	4.029		4.029		8.058
CD at 5%	11.659		11.659		23.318

II) 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

III) 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

The minimum nitrogen uptake was noticed in control (phytase @ 0 IU + FYM @ 0 t ha^{-1}) which noticed uptake values as 534.08 mg pot^{-1}.

The data indicated that with the application of phytase found significant for the uptake of phosphorus by soybean grain. An increasing trend was noticed with the application of graded levels of phytase (0 IU: 50.42, 1200 IU: 68.36, 2400 IU: 83.67 and 3600 IU: 97.17 mg pot^{-1}) phosphorus uptake by soybean grain. Statistically significant value for phosphorus uptake was recorded with the application of phytase @ 3600 IU (111.23 mg pot^{-1}).

The application of FYM also reported significant increase in the phosphorus uptake by soybean grain at harvest. Significantly higher and statistically superior mean phosphorus uptake was recorded with the application of 7.5 t ha^{-1} (110.08 mg pot^{-1}).

The data regarding the uptake of phosphorus by soybean grain revealed that phosphorus uptake was significantly influenced by the conjoint application phytase and FYM. The total uptake of phosphorus by soybean grain ranged from 50.42 to 128.08 mg pot^{-1}. The maximum phosphorus uptake (128.08 mg pot^{-1}) recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} which was significant and statistically at par with the treatment combination phytase @ 3600 IU + FYM @ 5 t ha^{-1} (121.96 mg pot^{-1}) and phytase @ 2400 IU + FYM @ 7.5 t ha^{-1} (115.18 mg pot^{-1}).

The data revealed that the application graded levels of phytase significantly influenced the potassium uptake by soybean at harvest. Statistically significant value for mean potassium uptake was recorded with the application of phytase @ 3600 IU (96.41 mg pot^{-1}).

The application of FYM also reported significant increase in the potassium uptake by soybean grain. Significantly higher and statistically

superior mean potassium uptake was recorded with the application of 7.5 t ha^{-1} (105.47 mg pot^{-1}).

It is evident from the data that combine application of phytase and FYM found statistically significant with an increase in the total potassium uptake. The significantly higher potassium uptake was recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (114.72 mg pot^{-1}) which was statistically at par with the application of phytase @ 2400 IU along with FYM @ 7.5 t ha^{-1} (111.00 mg pot^{-1}).

Khan *et al.*, 2013 concluded positive correlation (r=0.79) between dry matter yield and phosphorus uptake by rye grass in high clay textured soil with 61.8 g kg^{-1} organic carbon. Effect of inoculation with phosphatase producing fungi on dry matter production and grain yield of cluster bean, chickpea and wheat was studied by Tarafdar *et al.*, 1995, Tarafdar and Rao 1996. They concluded higher nitrogen, phosphorus, potassium and calcium uptake by cluster bean, chickpea and wheat due to inoculation with phosphatase producing fungi. Gujar *et al.*, 2013 concluded that phytase promoted phosphorus mineral assimilation much more efficiently as compared to chemical fertilizer. Reddy *et al.* (1999) reported that the P uptake by soybean and wheat increased with increasing rates of manure and fertilizer P and was relatively larger in soybean than in wheat. The per cent P recovery by the crops from fertilizer P decreased with increasing fertilizer P rate, while it was improved in the presence of manure.

4.9 Effect of phytase and FYM levels on nutrient uptake by soybean (straw) at harvest in non calcareous soil

The total uptake of nitrogen, phosphorus and potassium by soybean straw at harvest as influenced by the application of graded levels of phytase and FYM are presented in Table 31 a, b and c.

It is evident from the data presented in Table 31 a that the application of increasing levels of phytase and FYM significantly increased nitrogen uptake by soybean straw over the absolute control.

With the application of phytase found significant for the uptake of nitrogen by soybean straw. Statistically significant value for nitrogen uptake was recorded with the application of phytase @ 3600 IU (212.10 mg pot^{-1}).

The application of FYM also reported significant increase in the nitrogen uptake by soybean straw. An increasing trend was noticed with the application of graded levels of FYM i.e. 0 t ha^{-1}: 70.91, 2.5 t ha^{-1}: 123.28, 5 t ha^{-1}: 150.38 and 7.5 t ha^{-1}: 176.08 mg pot^{-1} nitrogen uptake by soybean straw. Significantly higher and statistically superior mean nitrogen uptake was recorded with the application of 7.5 t ha^{-1} (124.87 mg pot^{-1}).

Significant interaction effect on nitrogen uptake by soybean straw at harvest was observed with the application of phytase and FYM. The total uptake of nitrogen in soybean straw crop ranged from 70.91 to 257.79 mg pot^{-1}. The treatment phytase @ 3600 IU + FYM 7.5 t ha^{-1} (801.95 mg pot^{-1}) recorded significantly superior uptake of nitrogen.

Table 28.Effect of phytase and FYM levels on nitrogen, phosphorus and potassium uptake of soybean (straw) at harvest

I) 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	70.91	123.28	150.38	176.08	130.16
1200 IU	107.22	196.03	204.27	193.10	175.16
2400 IU	127.16	196.51	214.45	232.51	192.66
3600 IU	143.90	215.95	230.76	257.79	212.10
Mean	112.30	182.94	199.96	214.87	
	P		F		P × F
SE ±	3.031		3.031		6.062
CD at 5%	8.771		8.771		17.542

II) 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	19.34	26.03	39.44	38.75	30.89
1200 IU	22.91	41.02	54.62	58.30	44.21
2400 IU	26.33	50.20	54.42	61.24	48.05
3600 IU	27.86	36.44	67.96	68.18	50.11
Mean	24.11	38.42	54.11	56.62	
	P		F		P × F
SE ±	0.906		0.906		1.812
CD at 5%	2.621		2.621		5.243

III) 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	61.54	68.29	80.84	98.61	77.32
1200 IU	68.29	70.57	93.96	95.28	82.03
2400 IU	70.01	74.89	98.34	108.16	87.85
3600 IU	99.45	97.28	116.71	118.48	107.98
Mean	74.82	77.76	97.46	105.13	
	P		F		P × F
SE ±	1.328		1.328		2.656
CD at 5%	3.843		3.843		7.685

The minimum nitrogen uptake was noticed in control (phytase @ 0 IU + FYM @ 0 t ha^{-1}) which noticed uptake values as 70.91 mg pot^{-1}.

The data indicated that with the application of phytase found significant for the uptake of phosphorus by soybean straw. An increasing trend was noticed with the application of graded levels of phytase (0 IU: 19.34, 1200 IU: 22.91, 2400 IU: 26.33 and 3600 IU: 27.86 mg pot^{-1}) phosphorus uptake by soybean straw. Statistically significant value for phosphorus uptake was recorded with the application of phytase @ 3600 IU (50.11 mg pot^{-1}).

The application of FYM also reported significant increase in the phosphorus uptake by soybean straw at harvest. Significantly higher and statistically superior mean phosphorus uptake was recorded with the application of 7.5 t ha^{-1} (56.62 mg pot^{-1}) which was statistically at par with FYM @ 5 t ha^{-1} (54.11 mg pot^{-1}).

The data regarding the uptake of phosphorus by soybean straw revealed that phosphorus uptake was significantly influenced by the conjoint application phytase and FYM. The total uptake of phosphorus by soybean straw ranged from 19.34 to 68.18 mg pot^{-1}. The maximum phosphorus uptake (68.18 mg pot^{-1}) recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} which was significant and statistically at par with the treatment combination phytase @ 3600 IU + FYM @ 5 t ha^{-1} (67.96 mg pot^{-1}).

The data revealed that the application graded levels of phytase significantly influenced the potassium uptake by soybean straw at harvest. Statistically significant value for mean potassium uptake was recorded with the application of phytase @ 3600 IU (107.98 mg pot^{-1}).

The application of FYM also reported significant increase in the potassium uptake by soybean straw. Significantly higher and statistically

superior mean potassium uptake was recorded with the application of 7.5 t ha^{-1} FYM (107.98 mg pot^{-1}).

It is evident from the data that combine application of phytase and FYM found statistically significant with an increase in the total potassium uptake. The significantly higher potassium uptake was recorded with the application of phytase @ 3600 IU + FYM @ 7.5 t ha^{-1} (118.48 mg pot^{-1}) which was statistically at par with the application of phytase @ 3600 IU along with FYM @ 5 t ha^{-1} (116.71 mg pot^{-1}).

Khan *et al.*, 2013 concluded positive correlation (r=0.79) between dry matter yield and phosphorus uptake by rye grass in high clay textured soil with 61.8 g kg^{-1} organic carbon. Effect of inoculation with phosphatase producing fungi on dry matter production and grain yield of cluster bean, chickpea and wheat was studied by Tarafdar *et al.*, 1995, Tarafdar and Rao 1996. They concluded higher nitrogen, phosphorus, potassium and calcium uptake by cluster bean, chickpea and wheat due to inoculation with phosphatase producing fungi. Gujar *et al.*, 2013 concluded that phytase promoted phosphorus mineral assimilation much more efficiently as compared to chemical fertilizer. Reddy *et al.* (1999) reported that the P uptake by soybean and wheat increased with increasing rates of manure and fertilizer P and was relatively larger in soybean than in wheat. The per cent P recovery by the crops from fertilizer P decreased with increasing fertilizer P rate, while it was improved in the presence of manure.

4.10 Effect of phytase and FYM levels on phytin content of soybean grain in non calcareous soil.

The phytin content of soybean seed was assessed at harvest and is presented in table 29.

The data collected revealed that phytase application to soybean on calcareous significantly affected the phytin content of soybean seed as compared to application of FYM. Phytase @ 3600 IU recorded lower phytin content (16.50 mg/g) over other treatments. This can be attributed to the ability of phytase to hydrolyse phytin and release inorganic phosphorus. Significantly lower phytin content was recorded with application of 7.5 t ha^{-1}FYM (19.54 mg/g). The interaction effect was found to be significant, with lower phytin content observed for phytase @ 3600 IU along with 7.5 t ha^{-1} FYM (15.66 mg/g).

Table 29: Effect of phytase and FYM levels on phytin content of soybean grain

FYM (F) / Phytase (P)	mg g^{-1}				
	0 tha^{-1}	2.5 tha^{-1}	5 tha^{-1}	7.5 tha^{-1}	Mean
0 IU	24.06	23.36	23.05	22.21	23.17
1200 IU	23.06	22.30	21.80	21.71	22.22
2400 IU	20.50	19.66	19.50	18.57	19.55
3600 IU	17.63	16.57	16.16	15.66	16.50
Mean	21.31	20.47	20.12	19.54	
	P		F		P × F
S.E. ±	0.055		0.055		0.109
CD at 5%	0.158		0.158		0.315

4.11 Nutrient Use Efficiency

a) At 50 percent flowering: Nitrogen, phosphorus and potassium efficiency was reported as increase in trend with phytase as well as FYM levels at both the growth stages of soybean. Higher nutrient use efficiency for phosphorus (84.32 %) potassium (78.29 %) was recorded with the application of phytase @ 3600 IU and 2400 IU for nitrogen (54.43 %) at 50 % flowering. In the similar way, application of FYM @ 7.5 t ha^{-1} recorded higher nutrient use efficiency for nitrogen (137.98 %), phosphorus (128.90 %) and potassium (123.66 %) than rest of the FYM levels at 50 % flowering. The magnitude of increase in nutrient use efficiency for N, P, K was more pronounced with FYM levels than phytase.

b) At harvest: Higher nutrient use efficiency was recorded with the application of phytase @ 3600 IU for nitrogen (136.1 %) phosphorus (136.77 %). and potassium (108.88 %). In case of FYM application, the trend of nutrient use efficiency for nitrogen, phosphorus and potassium was recorded similar to that of 50 % flowering.

Table 30: Effect of phytase and FYM levels on nutrient use efficiency of soybean at 50 percent flowering and at harvest in non calcareous soil.

a) 50% flowering:

NUE (%)							
Nutrient / Phytase (P)	N	P	K	Nutrient / FYM (F)	N	P	K
1200 IU	11.24	24.91	27.42	2.5 tha^{-1}	42.83	46.63	68.12
2400 IU	54.43	78.93	43.01	5 tha^{-1}	74.88	106.63	108.20
3600 IU	49.02	84.32	78.29	7.5 tha^{-1}	137.98	128.90	123.66

b) At harvest:

NUE (%)							
Nutrient / Phytase (P)	N	P	K	Nutrient / FYM (F)	N	P	K
1200 IU	57.96	54.03	34.98	2.5 tha^{-1}	83.9	89.9	14.68
2400 IU	106.05	102.08	50.03	5 tha^{-1}	124.82	172.38	70.08
3600 IU	136.1	136.77	108.88	7.5 tha^{-1}	177.33	193.14	160.72

5. SUMMARY AND CONCLUSION

SUMMARY:

- Application of phytase @ 2400 IU along with 7.5 t ha^{-1} FYM were found effective to obtain higher soil acid phosphatase, alkaline phosphatase as well as dehydrogenase activity at 50 % flowering and at harvest of soybean.

- Application of phytase @ 2400 IU along with FYM @ 7.5 t ha^{-1} was found beneficial for higher soil available nitrogen, phosphorus and potassium at 50 percent flowering and at harvest of soybean.

- Liquid phytase application @ 2400 IU along with FYM @ 7.5 t ha^{-1} was found superior for higher availability of soil DTPA extractable Fe, Mn, Zn and Cu at harvest of soybean.

- Considerable improvement in soil microbial population (bacterial, fungal and actinomycetes) was found with combine application of phytase @ 2400 IU and 7.5 t ha^{-1} FYM at both growth stages of soybean.

- In case of soybean yield interaction effect among phytase and FYM levels were found non-significant however numerically higher soybean grain (18.65g pot^{-1}) and straw (14.67g pot^{-1}) yield was obtained with the application of phytase @ 2400 IU along with 7.5 t ha^{-1} FYM.

- Significantly higher uptake of phosphorus and potassium by soybean grain was obtained with the application of phytase @ 3600 IU 3600 IU along with 7.5 t ha^{-1} FYM which was found at par with 2400 IU phytase + 7.5 t ha^{-1} FYM. However significantly superior nitrogen uptake was recorded with the combine application of 3600 IU phytase along with either 7.5 or 5 t ha^{-1} FYM.

CONCLUSION

- On the basis of results, it could be concluded that application of 2400 IU phytase along with 7.5 t ha^{-1} FYM was found beneficial for soil enzymes, nutrient availability (major and minor), yield and nutrient uptake of soybean.

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