

**EFFECT OF PHYTASE AND PHOSPHORUS LEVELS ON SOIL  
PHOSPHORUS AVAILABILITY AND YIELD OF FODDER MAIZE  
IN AN INCEPTISOL**

By

**Mr. Tapkeer Prakash Bapurao**  
(Reg. No. Ph.D./ 015/023)

A Thesis submitted to the  
**MAHATMA PHULE KRISHI VIDYAPEETH  
RAHURI – 413 722, DIST. AHMEDNAGAR  
MAHARASHTRA, INDIA**

in partial fulfillment of the requirements for the degree

of

**DOCTOR OF PHILOSOPHY (AGRICULTURE)**

in

**SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**



**DEPARTMENT OF SOIL SCIENCE AND AGRICULTURAL  
CHEMISTRY**

**POST GRADUATE INSTITUTE  
MAHATMA PHULE KRISHI VIDYAPEETH  
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MAHARASHTRA, INDIA.**

**2019**

## **CANDIDATE'S DECLARATION**

I hereby declare that this thesis or part  
thereof has not been submitted  
by me or other person to any  
other University or Institution  
for a Degree or  
Diploma

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Date :     /     /2019

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## CERTIFICATE

This is to certify that the thesis entitled, “**EFFECT OF PHYTASE AND PHOSPHORUS LEVELS ON SOIL PHOSPHORUS AVAILABILITY AND YIELD OF FODDER MAIZE IN AN INCEPTISOL**” submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri Dist. Ahmednagar (M.S.) in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**, embodies the results of a piece of *bona fide* research work carried out by **Mr. PRAKASH BAPURAO TAPKEER**, under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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## **CERTIFICATE**

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Date : / /2019

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## LIST OF ABBREVIATIONS

%	:	Per cent
@	:	At the rate of
µg	:	Micro gram (s)
<sup>0</sup> C	:	Degree Celsius
CD	:	Critical Difference
CEC	:	Cation Exchange Capacity
cm	:	Centimeter (s)
DAI	:	Days After Incubation
DAS	:	Days After Sowing
DAT	:	Days After Transplanting
dS m <sup>-1</sup>	:	Deci Siemens per meter
e.g.	:	Exempli gratia (For example)
EC	:	Electrical Conductivity
<i>et al.</i>	:	And other (etalli)
etc	:	Et cetera
Fig.	:	Figure
FYM	:	Farm Yard Manure
g	:	Gram
GRDF	:	General Recommended Dose of Fertilizer
ha <sup>-1</sup>	:	Per hectare
i.e.	:	Id est (that is)
IU	:	International unit
K	:	Potassium
K <sub>2</sub> O	:	Potassium Oxide
kg	:	Kilogram (s)
kg ha <sup>-1</sup>	:	Kilogram Per hectare
L	:	Litre (s)
M.S.	:	Maharashtra State
max.	:	Maximum
mg	:	Milligram (s)

mg kg <sup>-1</sup>	:	Milligram per kg
min.	:	Minimum
mm	:	Millimetre (s)
MW	:	Meteorological Week
N	:	Nitrogen
N.S.	:	Non Significant
NCIM	:	National Collection of Industrial Microorganisms
NCL	:	National Chemical Laboratory
P	:	Phosphorus
P <sub>2</sub> O <sub>5</sub>	:	Phosphorus Pentoxide
PSB	:	Phosphorus Solubilizing Bacteria
PSF	:	Phosphorus Solubilizing Fungi
PSM	:	Phosphorus Solubilizing Microorganisms
q ha <sup>-1</sup>	:	Quintal per hectare
RDF	:	Recommended Dose of Fertilizer
RH	:	Relative Humidity
SSP	:	Single Superphosphate
t ha <sup>-1</sup>	:	Tonnes per hectare
TCP	:	Tri Calcium Phosphate
VAM	:	Vascular Arbuscular Mycorrhizae
<i>viz.</i>	:	Videlicet (Namely)

## ABSTRACT

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### EFFECT OF PHYTASE AND PHOSPHORUS LEVELS ON SOIL PHOSPHORUS AVAILABILITY AND YIELD OF FODDER MAIZE IN AN INCEPTISOL

By

**PRAKASH BAPURAO TAPKEER**

A candidate for the degree of

**DOCTOR OF PHILOSOPHY (AGRICULTURE)**

in

**SOIL SCIENCE AND AGRICULTURAL CHEMISTRY**

2019

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<b>Research Guide</b>	: <b>Dr. A.G. Durgude</b>
<b>Department</b>	: <b>Soil Science and Agril. Chemistry</b>

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An incubation and pot culture experiment was conducted to study the 'Effect of phytase and phosphorus levels on soil phosphorus availability and yield of fodder maize in an Inceptisol' during *Kharif* season, 2016-17 at Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri. The experiment was laid out in factorial completely randomized design with two replications and seventeen treatments, consisting combinations of phytase @ 1200, 2400, 3600 and 4800 IU and phosphorus @ 0, 25, 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> levels with absolute control to evaluate the phosphorus availability, enzyme activity, forms of phosphorus at 0, 7, 15, 30, 45 and 60 DAI in soil.

The periodical soil available phosphorus was significantly influenced by the phytase and phosphorus levels at 0, 7, 15, 30 and 45 DAI, which were 7.83, 8.07, 8.89, 9.66 and 10.90 mg kg<sup>-1</sup>, respectively and declined at 60 DAI (9.95 mg kg<sup>-1</sup>) by the application of phytase @ 4800 IU. The application of phosphorus @ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased significantly soil available phosphorus (8.01, 8.55, 9.58, 10.53, 11.70 and 10.73 mg kg<sup>-1</sup>), respectively at 0, 7, 15, 30, upto 45 DAI and declined at 60 DAI.

The application of phytase @ 4800 IU increased significantly all forms of phosphorus in soil at 0, 7, 15, 30, 45 and 60 DAI which increased upto 45 DAI and decreased at 60 DAI except organic-P. Saloid-P was (11.53, 12.76, 13.50, 14.34, 16.00 and 14.98 mg kg<sup>-1</sup>), respectively; Al-P (46.88, 56.25, 75.94, 90.94, 104.06 and 96.56 mg kg<sup>-1</sup>), respectively; Fe-P (31.44, 33.19, 36.41, 39.23 and 37.32 mg kg<sup>-1</sup>), respectively except 0 DAI, Ca-P (317.76, 322.42, 324.63, 326.83, 329.86 and 328.09 mg kg<sup>-1</sup>), respectively; Red-P (78.00, 84.50, 85.80, 96.20 and 91.98 mg kg<sup>-1</sup>), respectively except 0 DAI, Occl-P (7.49, 10.99, 11.87, 12.03, 13.67 and 12.91 mg kg<sup>-1</sup>), respectively;

organic-P (246.56, 234.95, 209.54, 201.38, 173.78 and 199.09 mg kg<sup>-1</sup>), respectively and total-P (746.82, 753.16, 767.73, 772.79 and 780.93 mg kg<sup>-1</sup>), respectively and 0 DAI (722.75 mg kg<sup>-1</sup>) by the application of phytase @2400 IU.

The application of phosphorus @ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased significantly all forms of phosphorus in soil at 0, 7, 15, 30, 45 and 60 DAI which increased upto 45 DAI and decreased at 60 DAI except organic-P. Saloid-P (11.95, 13.48, 14.69, 15.64, 17.62 and 16.62 mg kg<sup>-1</sup>), respectively; Al-P (52.50, 61.88, 88.13, 103.75, 118.13 and 110.63 mg kg<sup>-1</sup>), respectively; Fe-P (29.22, 33.54, 36.21, 39.62, 42.88 and 40.97 mg kg<sup>-1</sup>), respectively; Ca-P (318.26, 324.50, 326.20, 330.99, 334.01 and 330.68 mg kg<sup>-1</sup>), respectively; Red-P (64.03, 82.23, 89.05, 92.95, 103.35 and 97.83 mg kg<sup>-1</sup>), respectively; Occl-P (7.60, 11.70, 12.58, 12.85, 14.77 and 13.89 mg kg<sup>-1</sup>), respectively; organic-P (242.35, 227.73, 194.54, 178.26, 148.38 and 175.39 mg kg<sup>-1</sup>), respectively and Total-P (725.92, 755.06, 761.39, 774.06, 779.13 and 786.00 mg kg<sup>-1</sup>), respectively.

The alkaline phosphatase activity was significantly increased upto 30 DAI and declined at 45 and 60 DAI. Application of phytase @ 4800 IU at 0, 7, 15, 30, 45 and 60 DAI showed significant for alkaline phosphatase (15.91, 19.12, 23.12, 24.28, 22.49 and 21.48 µg PNP g<sup>-1</sup> hr<sup>-1</sup>) respectively. The application of phosphorus @ 0 kgP<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> at 0, 7, 15, 30, 45 and 60 DAI was significantly increased in alkaline phosphatase (16.54, 18.89, 22.32, 23.82, 21.80 and 21.23 µg PNP g<sup>-1</sup> hr<sup>-1</sup>), respectively.

In pot culture experiments, the green fodder yield and dry matter yield of maize showed highest in the application of phytase @ 4800 IU (139.90 and 13.50 g plant<sup>-1</sup>, respectively). The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was significantly increased the green fodder yield and dry matter production (160.58 g and 15.66 g plant<sup>-1</sup>), respectively.

The application of phytase @ 4800 IU significantly increased uptake of nitrogen, phosphorus and potassium (12.40 mg g<sup>-1</sup>, 4.95 mg g<sup>-1</sup> and 15.98 mg g<sup>-1</sup>), respectively in green fodder maize. Application of phosphorus @ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the uptake of nitrogen, phosphorus and potassium (13.63, 5.21 and 17.09 mg g<sup>-1</sup>), respectively and apparent P recovery (27.42 mg mg<sup>-1</sup>).

The soil available phosphorus content increased by application of phytase @ 4800 IU at 30 DAS (11.35 mg kg<sup>-1</sup>) and decreased at harvest (9.27 mg kg<sup>-1</sup>). The application of phosphorus @ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased available phosphorus at 30 DAS (11.67 mg kg<sup>-1</sup>) and decreased at harvest (9.82 mg kg<sup>-1</sup>). Available phosphorus at harvest showed significant for soil available phosphorus by the conjoint application of phytase @ 4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (10.27 mg kg<sup>-1</sup>).

All forms of phosphorus in soil such as Saloid-P, Al-P, Fe-P, Ca-P, Red-P, occl-P and total-P were significantly increased and organic-P decreased by the application of phytase @ 4800 IU at 30 DAS (15.29, 83.44, 38.27, 333.32, 86.45, 12.03 and 776.06 mg kg<sup>-1</sup>), respectively, except organic-P (207.26 mg kg<sup>-1</sup>) and at harvest (12.24, 64.69, 30.97, 307.37, 73.45, 9.84 167.45 and 666.00 mg kg<sup>-1</sup>), respectively. The

application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased Saloid-P, Al-P, Fe-P, Ca-P, Red-P, Occl-P and total-P and decreased organic-P (17.15, 98.44, 45.42, 344.09, 95.55, 13.56 173.35 and 787.56 mg kg<sup>-1</sup>), respectively at 30 DAS and slightly decreased all forms of phosphorus at harvest of fodder maize (14.45, 75.47, 37.48, 318.89, 82.88, 11.38, 136.71 and 677.26 mg kg<sup>-1</sup>), respectively.

The application of phytase @ 4800 IU significantly increased soil alkaline phosphatase activity (27.81 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) at 30 DAS and it significantly declined at harvest (23.05 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>). The application of 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased soil alkaline phosphatase activity (29.09 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) at 30 DAS and it significantly declined at harvest (24.38 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>). The significant interaction effect of phytase and phosphorus @ 4800 IU + 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was recorded highest soil alkaline phosphatase activity (29.86 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) at 30 DAS.

Thus, it can be concluded that, the application of phytase @ 4800 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was significantly increased soil available phosphorus and all forms of phosphorus under incubation study and pot culture experiments. The application of phytase @ 4800 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was significantly increased the green fodder yield and dry fodder yield of maize and apparent P recovery under pot culture experiments. However, application of phytase @ 2400 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> showed at par results. Hence, the optimum level of phytase and inorganic phosphorus for fodder maize is phytase @ 2400 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

## 1. INTRODUCTION

Phosphorus is the second most essential major plant nutrient after nitrogen. It is an important nutrient required for the normal growth and metabolic processes occurring in plants (Singh and Satyanarayana, 2011) and also an important structural constituent of many biomolecules. There is no atmospheric source of P that could be made available to plants (Ezawa *et al.*, 2002) and soils generally contain only a small amount of available P (predominantly as  $\text{HPO}_4^{2-}$  and  $\text{H}_2\text{PO}_4^-$ ) that is readily available for plant uptake. Phosphorus is a plant nutrient that is expected to limit the agriculture production of India in the next millennium (Prasad, 2002). Therefore, P is applied as fertilizer to replenish the soil to satisfy plant requirements (Richardson *et al.*, 2009). There is a continuous need to improve the use efficiency of fertilizers in order to achieve more efficient production of crops to minimize the fertilizer related environmental stresses. Both organic and inorganic P accumulates in soil and become unavailable for plant uptake. Only 0.1 per cent of the total phosphorus exists in a soluble form available for plant uptake because of its fixation into an unavailable form.

The inorganic P fractions are bound by precipitation and chemical adsorption, while the organic P is immobilized in soil organic matter (Sharma *et al.*, 2012). In many soils, 30 to 80 per cent of organic P is present, but not properly utilized by plants due to P immobilization (Menezes *et al.*, 2013). Organic P is primarily found in the form of inositol phosphates, and its activity is controlled by environmental factors, like climate, soil type and cultural practices (Richardson *et al.*, 2005). Almost 58 per cent of soil organic P comprises inositol phosphates, while inorganic forms exist as apatites, strengite and variscite (Shen *et al.*, 2011). The availability of P is very low ( $1.0 \text{ mg kg}^{-1}$  soil); moreover, in acidic, calcareous or normal soils, P reacts with reactive metals such as  $\text{Al}^{3+}$ ,  $\text{Fe}^{3+}$  and  $\text{Ca}^{2+}$ , thereby becoming unavailable for plant uptake (Gyaneshwar *et al.*, 2002). Phosphorus is added in soil through application of chemical fertilizers but major part of it gets fixed in the soil, and only 15 to 20 per cent is utilized by the crop. Major limitation of adequate phosphorus supply to plant is phosphorus fixation. Phosphate fertilizers containing small fractions of water soluble phosphorus such as single super phosphate and di-ammonium phosphate are utilized by crops, while remaining portion of

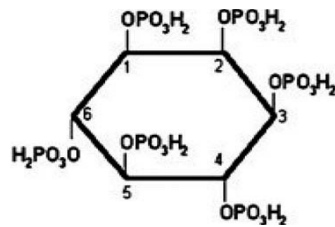
applied phosphorus becomes fixed in the soil or unavailable to crop plants. The transformation of phosphorus in soil is dependent upon a large number of factors *viz.*, soil pH, other nutrients, organic matter, type of clay, soil temperature, aeration, moisture, crop species and time of applications.

The phosphorus deficiency is considered as one of the greatest limitations in the productivity of crops. About 30 per cent of the crop production in the world is affected by lack of macronutrients in soil. Motsara (2002) reported that in India 42 per cent of the districts are in low P category, 38 per cent are in medium P category and 20 per cent districts are high in P category. P deficiency is perhaps one of the most important factors that limit crop yields on many soils. Its availability depends on soil characteristics and contents of labile P fraction. It is estimated that P availability to plant roots is limited in two thirds of the cultivated soil in the world (Batjes, 1997).

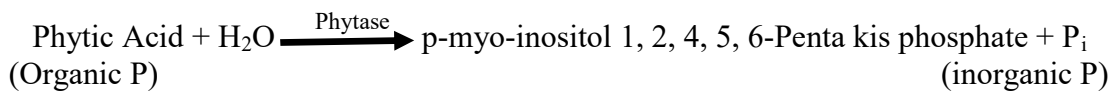
In the past decades, P fertilizer application for crop growth has been increasing rapidly, but P-use efficiency has decreased to a low level of 10-20 per cent. Natural P reserves are limited and sedimentary deposits are the source of 80-90 per cent of the world P production. The economic challenges associated with increasing P fertilizer prices are driving the increased interest in improving P use efficiency. Development of cultivars with improved P-deficiency tolerance is believed to be an effective solution to this problem. Increasing prices of P- fertilizer in developing countries like India and high fixation of phosphorus in soil have led to the search for sustainable way of phosphorus nutrition of crops.

### **Phytic acid or phytate**

Phytic acid or phytate also known as inositol hexakisphosphate (IP6). It is a saturated cyclic acid, discovered in 1903 (Mullaney and Ullah, 2005) and is the principal storage form of P in many soil and plant tissues. Sixty to eighty per cent of organic P exists in the form of phytate in soil and is not directly available to plants. Inositol phosphate can vary in binding sites between one and six phosphate groups and thus results in the formation of isomeric forms – *myo*, *d-chiro*, *scyllo*, and *neo*.



**Fig.1 Structure of phytate or phytic acid (Singh and Satyanarayana 2011)**



### **Phytase:-**

Phytases (myo-inositol hexakisphosphate phosphohydrolases) are enzymes that catalyze mineralization – conversion of organic P from phytate to inorganic P, which can be readily taken up by the plants (Tu *et al.*, 2011). George *et al.*, (2007) demonstrated three discrete categories of phytase on the basis of protein sequencing and the succession of dephosphorylation of phosphate moieties (myo-inositol-hexaphosphate phosphohydrolases, i.e. EC 3.1.3.8, EC 3.1.3.26 and EC 3.1.3.72). P occurs at three and six positions, respectively. Their names were derived from three and six phosphate bonds of myo-IP6. Usually 3-phytases are found in microorganisms and filamentous fungi while 6-phytases are in plant tissues. The enzyme 3-phytase (EC 3.1.3.8) obtained from *Aspergillus sp.* and 6-phytase (EC 3.1.3.26) from wheat.

Phytases can be produced and extracted from different sources including plants, animal tissues and microorganisms (Tu *et al.*, 2011). However, phytase is derived from microbial sources due to relatively higher phytase production potential (De *et al.* 2003). In microorganisms, bacteria, yeast and fungi have been successfully investigated for extracellular phytase activity (Pandey *et al.*, 2001). Crystal structures of phytase when purified revealed different catalytic properties and hydrolyze phytate with different mechanisms. The presence of phytase in the rhizosphere may enhance plant growth under field conditions (Singh and Satyanarayana, 2010). Several studies have shown that increase in yield or plant growth can be achieved through the inoculation of P-solubilizing fungi in either pot experiments or under field conditions.

Maize (*Zea mays* L.) is a member of family Gramineae, and its rank third position after wheat and rice throughout the world. It is widely sown in tropical,

subtropical and temperate areas. Maize is cultivated extensively in various countries of the world. United States, France, Brazil, India and Italy are the countries which produce maize in greater amount. Starch, glucose and corn oil are produced by maize; in addition to this bio fuel such as ethanol is also produced in the world (Ahmad *et al.*, 2007). The crop has a wide range of uses as forage to feed animals. About 60 per cent of the global harvest of maize is fed to livestock (Dowsell *et al.*, 1996). Maize (*Zea mays*) is an ideal forage crop grown throughout the country; it is quick growing, high yielding and supplies palatable and nutritious forage which can be fed at any stage of growth without any risk to animals.

Maize is an exhaustive crop having higher potential than other cereals and absorbs large quantity of nutrients from the soil during different growth stages. Among the essential nutrients, phosphorus is one of the most important nutrients for higher yield in larger quantity. Generally, P is the second most crop-limiting nutrient in most soils. Ali *et al.* (2002) reported significant effect of phosphorus application on grain yield. Whereas, Ayub *et al.* (2002) observed significant effect of P application on dry matter yield and individual plant characteristics like height, number of leaves and leaf area.

With the above background information, the present investigation has planned to evaluate the effect of phytase from *Aspergillus niger* NCIM 563 in mineralization of organic phosphorus to benefit plant nutrition and soil amendment in combination with chemical fertilizers with the following objectives.

1. To study the effect of phytase and phosphorus levels on phosphorus availability and its forms in soil.
2. To study the effect of phytase and phosphorus levels on growth and fodder yield of maize.
3. To study the effect of phytase and phosphorus levels on soil chemical and phosphatase enzyme activity in soil.

## 2. REVIEW OF LITERATURE

Phosphorus as a major essential element plays an important role for sustaining the crop productivity as well as profitability to farmers. Because of these prime facts, scientific management of phosphorus in cultivable soils always remain a major challenge to soil scientists. Being scarce and non-renewable natural resource which is under the threat of rapid depletion as a result of intensive mining across the world more emphasis is being given to increase P use efficiency in soil for successful and sustainable crop production (Jasinski, 2006). The available literature pertaining to ‘Effect of phytase and phosphorus levels on soil phosphorus availability, enzyme activity and yield of fodder maize in an Inceptisol’ has reviewed in this chapter. For the sake of comprehension, the literature on these aspects has reviewed under the following sub-heads, Effect of phytase and phosphorus levels on:

- 2.1 Phosphorus availability in soil
- 2.2 Forms of phosphorus
- 2.3 Soil phosphatase activity
- 2.4 Soil chemical properties
- 2.5 Growth contributing characters of green fodder maize
- 2.6 Yield of green fodder maize
- 2.7 Nutrient uptake in green fodder maize
- 2.8 Soil chemical and enzymatic activities during incubation study

### **2.1 Effect of Phytase and Phosphorus Levels on Phosphorus Availability in Soil**

The available phosphorus found in a biogeochemical cycle in the upper soil profile, while phosphorus found at lower depths is primarily involved in geochemical reactions with secondary minerals. Plant growth depends on the rapid root development and uptake of phosphorus released from dead organic matter in the biochemical cycle.

Gaur *et al.* (1979) tested various microorganisms for solubilization and showed that, solubilizing power of fungi *viz.* *Aspergillus awamori* and *Aspergillus niger* was more than bacterial isolate *Pseudomonas striata*. These microorganisms effectively solubilized tricalcium phosphate than Mussoorie rock phosphate.

Tarafdar (1989) reported that, microorganisms may produce both acid and alkaline phosphatase, but plant can only secrete acid phosphatase. Phosphatase and phytase which catalyse hydrolytic cleavage of the C-O-P ester bond of organic P present in soil and release P as plant available inorganic forms ( $\text{H}_2\text{PO}_4^-$  and  $\text{HPO}_4^{2-}$ ) may originate from plant and soil microorganisms.

Reddy *et al.* (1999) reported that, the integrated use of manure and P fertilizer as a promising strategy to improve soil P availability status and to obtain higher and sustained productivity of soybean-wheat cropping system in Vertisol.

Whitelaw (2000) studied that, soil fungi of the genera *Aspergillus* and *Penicillium* possess the ability to solubilize sparingly soluble phosphates in vitro by secreting inorganic or organic acids.

Kang *et al.* (2002) observed that, microorganisms enhance the phosphorus availability to plants by mineralizing organic P in soil and by solubilizing precipitates phosphates in soil.

Sujatha *et al.* (2004) reported that, phosphate solubilizing microorganisms increase the P nutrition of plants through increased solubility of Ca-phosphates and their solubility increases with a decrease of soil pH.

Chen *et al.* (2006) observed that, microbial community influences soil fertility through soil processes *viz.*, decomposition, mineralization and storage/release of nutrients. Microorganisms enhance the P availability to plants by mineralizing organic P in soil and by solubilizing precipitated phosphates.

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  $\text{H}_2\text{PO}_4$  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  $\text{H}_2\text{PO}_4$  from the adsorption sites (Havlin *et al.*, 2007).

El-Azouni (2008) reported that, the dual inoculation of phosphate solubilizing fungi (*Aspergillus niger* and *Penicillium digitatum*) significantly increased the available P and organic carbon of the treated soil compared to untreated soil.

Kumari *et al.* (2008) reported that, citric acid and oxalic acids were responsible for P solubilization for tri calcium phosphate and Udaipur rock phosphate and observed that, citric acid was the most effective in P solubilization.

Tarafdar (2008) concluded that, inoculation with *Aspergillus* strains significantly increased the phosphorus concentration, total phosphorus uptake as well as soil available phosphorus status in rhizosphere of chickpea and wheat than in bulk soil.

Khan and Joergensen (2009) observed that, release of P by PSB from insoluble and fixed/adsorbed forms is an important aspect regarding P availability in soils. There are strong evidences that soil bacteria are capable of transforming soil P to the forms available to plants.

Pattanayak *et al.* (2009) carried out a study work in relation to the availability of phosphorus at Orissa University of Agriculture and Technology, Bhubaneswar. The results showed that, the rate of P mineralization depends on microbial activity and 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.

Wang *et al.* (2010) reported that, Olsen-P increased linearly with fertilizer P application rates after long-term annual P (superphosphate) application compared with no fertilizer P application. Applications of 20, 39, 59, and 79 kg P ha<sup>-1</sup> increased soil Olsen-P content by 3.7, 5.2, 11.2 and 20.6 mg P kg<sup>-1</sup> soil, respectively.

The field experiment was carried out at Raipur, during 2005 by Sarawgi *et al.* (2012) on effect of phosphorus application along with PSB, Rhizobium and VAM on P fractionation and productivity of soybean. The results revealed that, increases in all inorganic P fractions by applied dose of 30 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> through rock phosphate + PSB+ RI.

Gujar *et al.* (2013) studied on effect of phytase from *Aspergillus niger* on plant growth and mineral assimilation in wheat (*Triticum aestivum* Linn.) on calcareous soil and its potential for use as a soil amendment at NCL, Pune (M.S.) during 2012. The results indicated that, phytase isolated from *Aspergillus niger* showed improvement in phosphorus and nutrient availability. Phosphorus content in soil increased by about 1.18 fold due to phytase application. They further reported that, the phytase was able to degrade about 30 per cent of the phytic acid in the soil.

Ushari *et al.* (2013) 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 be liberated by hydrolysis.

The field experiment was conducted at Dharwad, during *kharif* 2013 by Geetha and Radder (2015) on effect of phosphorus cured with FYM and application of biofertilizers on productivity of soybean and phosphorus transformation in soil. The result showed that, all the P fractions increase by addition of 80 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> cured with FYM+ PSB+ VAM.

Ram *et al.* (2015) reported that, Olsen-P content increased with increasing levels of phosphorus, irrespective of phosphorus solubilizing fungi.

Jadhav *et al.* (2017) studied on effect of phytase and FYM on enzyme activities and nutrient availability of non-calcareous soil and concluded that, significantly higher availability of phosphorus (23.9 kg ha<sup>-1</sup>) recorded with application of phytase 3600 IU + FYM 7.5 t ha<sup>-1</sup>.

## **2.2 Effect of Phytase and Phosphorus Levels on Forms of Phosphorus in Soil**

Phosphorus in soil occurs in organic and inorganic forms. Their relative distribution varies with climate, vegetation, parent material, addition of organic manures, fertilizers, pH of soil and also varies with soil management practices. An inorganic form of phosphorus in soil occurs as saloid bound phosphorus, Al-bound P, Fe -P, reductant soluble P, occluded P, Ca-P and organic P. Since, these forms of phosphorus have different solubilities, the availability of P to crops and uptake depend upon the amount of forms of phosphorus in soil. Manure application has reported to increase soil

concentration of both total and soluble P, as well as concentration of specific P forms, including stable organic P (Waldrip-Dail *et al.*, 2009).

Reddy *et al.* (1983) reported that, top soils were significantly richer in available and inorganic phosphorus fractions except Ca-P than sub soils. Al-P and Fe-P were significantly higher in top soils than in lower depths while Ca-P remained same in both the layers. All three fractions of inorganic P increased as a result of regular P application.

Agrawal *et al.* (1987) reported that, in black soil the saloid-P ranged from 1.5 to 12.0 mg kg<sup>-1</sup> due to application of phosphatic fertilizers.

Sharma *et al.* (1987) observed that, the application of phosphatic fertilizers for a long time resulted in build-up of all forms of phosphorus quite markedly, the Ca-P increased due to continuous manuring and cropping in almost all the treatments except control and 100 per cent N alone.

Subramaniam and Kumarswamy (1989) reported that, increased trends in total phosphorus content due to addition of fertilizer with FYM under continuous cropping and fertilization programme.

Vazquez *et al.* (1991) analyzed soils under different cropping systems for various forms of phosphorus and reported decreasing trends in total-P under continuous cropping. Further, continuous cropping not only decrease total-P, but also reported depletion in more labile P and other inorganic forms of phosphorus.

Dakhore *et al.* (1993) reported that, the application of higher dose (80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) of phosphorus significantly increased all forms of phosphorus Ca- P (287 mg kg<sup>-1</sup>), Al-P (75 mg kg<sup>-1</sup>), Fe- P (24 mg kg<sup>-1</sup>) and Saloid- P (7 mg kg<sup>-1</sup>) as compared to lower dose (40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) of phosphorus and control.

Srivastava and Srivastava (1993) studied various forms of P *viz.* adsorbed P, Al-P, Fe-P, Ca-P and Red-P in relation to varying soil pH, availability of P under air dried and waterlogged condition and response to P application in saline-sodic soil under rice-wheat crop sequence and concluded that, the Ca-P was most predominant P fraction followed by reductant soluble P, Fe-P, Al-P, and adsorbed P.

Singh *et al.* (1993) observed maximum accumulation of Ca-P form followed by RS-P and minimum in Saloid-P form and Al-P form was more than Fe-P. All

the forms were decreased in control plots after 11 cycles of maize-wheat sequence in long term experiment. The results also indicated that, NPK application increased total phosphorus over initial and magnitude of increase was more with higher rate of phosphorus application under that cropping sequence with continuous fertilization.

Bharadwaj *et al.* (1994) found that Ca-P and Fe-P forms were more important than Al-P in rice based cropping system. The tendencies of shift from Ca-P to Fe-P and Al-P were noticed.

Inorganic phosphorus fractions were studied in a long term fertilizer experiment on chromustert soil with soybean-wheat-fodder maize sequence by Dixit *et al.* (1994). The results showed that, contents of Fe-P, Al-P, Saloid bound phosphate and available phosphorus increased at the expence of Ca-P and RSP. Application of 100 % NPK increased the Saloid bound P but maximum available P and RSP were found in 150 % NPK. The treatment receiving 100 % NPK+ Zn gave the highest Al-P and highest Ca-P in the treatment of 100 % N alone.

Bahl and Singh (1997) in a greenhouse experiments on ten soils (eight alkaline and two acids) in Punjab found that, extractable P was higher in green manured soil and phosphorus fertilization resulted in increase in all the estimated phosphorus fractions. In alkaline soils, added phosphorus transformed into saloid-P and Ca-P while in acid soils, higher amount of Al-P and Saloid-P was formed. Due to green manure addition, Saloid-P increased at the expense of other phosphorus fractions. Cropping with maize increased Saloid-P and Ca-P but decreased Fe-P and Al-P.

Prasad *et al.* (1997) in a long term fertilizer experiment with 19 years of soybean-wheat cropping sequence on acid Alfisol and found that, amount of Al-P, Fe-P and saloid-P increased with increased dose of added phosphate.

Bhakare (1998) reported that, the application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> recorded significantly increased all forms of phosphorus such as Saloid-P, Al-P, Fe-P, Ca-P, red-P, organic-P and total-P (11.9, 36.7, 28.1, 363, 84.4, 463 and 984 mg kg<sup>-1</sup>), respectively at harvest of the soybean.

Santhy *et al.* (1998) in a long term fertilizer experiment with finger millet-maize-cowpea cropping sequence observed that, there was an appreciable build-up of all the fractions of phosphorus under 150 per cent NPK, followed by NPK +FYM. The

concentration of Al-P was greater than saloid-P but was relatively lower as compared to Ca-P due to the calcareous nature of the experimental soil. Continuous application of 150 per cent of optimal NPK recorded the highest total phosphorus in all the three crops. A positive balance of phosphorus was recorded in all the treatments except in N alone and control.

Sihag *et al.* (2005) concluded that, significant increase in the saloid-P, Al-P and Ca-P forms with the application of inorganic fertilizers along with organic materials.

Singh *et al.* (2010) studied on maize-wheat sequence of 40 years experiment and they reported that, the inorganic P- fraction increased saloid-P ( $9.2 \text{ mg kg}^{-1}$ ), Al-P ( $136.1 \text{ mg kg}^{-1}$ ), Fe-P ( $8.9 \text{ mg kg}^{-1}$ ), Ca-P ( $522 \text{ mg kg}^{-1}$ ) and total-P ( $701 \text{ mg kg}^{-1}$ ).

Wang *et al.* (2010) reported that, the long-term application of superphosphate would facilitate the accumulation of soil Ca-P and explained that this effect was due to the calcareous nature of soil.

Hemalatha and Chellamuthu (2011) reported that, all the P fractions increased with increasing levels of fertilizer doses and were higher under continuous application of 150 per cent NPK followed by 100 per cent NPK+FYM.

Chang *et al.* (2014) studied on improving phosphorus availability in an acid soil using organic amendments produced from agro-industrial wastes and reported that, increasing available P, soluble-P, Al-P, Fe-P, Ca-P, reductant soluble-P and total-P in an incubation study at 30, 60 and 90 days of interval.

Dotaniya *et al.* (2014) reported that, the mean Al-P content in soil increased with increasing levels of P application.

Shrilatha and Sharma (2015) concluded that, the continuous addition of P fertilizers in combination with nitrogen and potassium raised the soil-P content in all the fractions and increased more at higher rates of P addition due to build-up of phosphate in soil which got transformed into different inorganic-P fractions.

Nayak *et al.* (2015) conducted a permanent field experiment to study the long-term fertilization effects in Vertisol under rice crop. They reported that, the highest value of saloid-P, red-P and Ca-P in soil with 150 % NPK only and Fe-P and Al-P in soil

with 100 % NPK + FYM is due to high chelation of Fe and Al with organic acids from FYM which is not favoured for formation of other P fractions.

Shelke (2017) reported that, saloid-P and Al-P (18.39 and 27.15 mg kg<sup>-1</sup>) increased at harvest of soybean by the application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> over rest of application of 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### **2.3 Effect of Phytase and Phosphorus Levels on Phosphatase Activity in Soil**

Both acid and alkaline phosphatases are known to hydrolyze a variety of phosphomonoesters. The hydrolysis of organic P compounds using specific phosphatase enzymes both in soils and biological systems is vital for energy metabolisms, metabolic regulations, and cellular signal transductions pathways. Low P availability in soils may trigger phosphatase secretions to the rhizosphere; however, soil phosphatases are sometimes derived from i) intracellular enzymes or enzymes bound to cell components and ii) abiotic or extracellular enzymes leaking from intact cells or released from dead or lysed cells that originate from the cell membrane (Acosta *et al.* 2014). The acid phosphatases in soils are of both plant and microbial origins, while alkaline phosphatase is mostly of microbial origin (Tarafdar and Maschner, 1994).

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

Goyal *et al.* (1999) in their long term field experiment studies reported significantly higher values of alkaline phosphatase activity in soils treated with long term (11 years) application of inorganic fertilizers with organic amendments like farmyard manure, wheat straw and *Sesbania bispinosa* green manure.

Yadav and Tarafdar (2003) concluded that, phytase has a slight edge over phosphatases in hydrolysis of different organic P compounds.

Parham *et al.* (2004) reported that, significant higher values of phosphomonoesterase, phosphodiesterase, inorganic pyrophosphatase and dehydrogenase in soil treated with cattle manure, however, the activity of acid phosphatase was observed significantly higher in soils treated with chemical fertilizers.

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

Kumar *et al.* (2007) concluded that, cropping pattern significantly increased the activity of acid phosphatase, alkaline phosphatase in the soil as compared to their activities in fallow soils. The maximum acid phosphatase (126.8 %) and alkaline phosphatase (145.6 %) activities were observed in moth bean-pearl millet crop rotation.

Manna *et al.* (2007) studied the effect of FYM and fertilizer P on soil enzymes and microbial activities in soybean-wheat rotation and they reported that, an increase in alkaline phosphatase activity with an advancement of growth stages of both the crops and they also observed significant increase in activity of alkaline phosphatase in the treatment received both the manures and P fertilizer but decreased with maturation of crop and also observed that root acid phosphatase activity was maximum at the early growth stage of wheat and significantly increased with manure P application but decreased as crop matured.

Tarafdar (2008) studied mobilization of native phosphorus for plant nutrition at Central Arid Zone Research Institute, Jodhpur and 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. Further, inoculation with *Aspergillus* strains significantly increased 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) 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 and concluded that, the utilization of phytate by plant was limited by low rates of hydrolysis. The results showed that *Trichoderma spp.* was found to be most efficient for hydrolysis of organic P by secreting phosphatases and phytase enzymes.

Kaya and Ibrahim (2009) concluded that, phytase are enzymes that degrade phytate and permit higher availability of Zn and other nutrients such as P and Fe.

Further, N and Zn fertilization showed different effect on phytase activity for different varieties.

Extracellular phytase activity of buffel grass genotypes grown on nutrient solution under P deficient ( $5 \mu\text{g P L}^{-1}$ ) and P sufficient ( $250 \text{ mg P L}^{-1}$ ) sterile conditions were compared by Yadav and Tarafdar (2009) and concluded that, the 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 per cent 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 that, the increase in acid phosphatase (15%), alkaline phosphatase (12 %) and phytase (71 %) as against without inoculated organic matter.

Influence of organic and inorganic nutrient source on soil enzyme activities was studied by Rai and Yadav (2011) on sandy loam soil at BHU, Varanasi by conducting lysimetric, pot culture experiment and incubation study during 2004-05 and 2005-06. The result showed that, soil dehydrogenase, alkaline phosphates were higher with application of farm yard manure, carpet waste, press mud, digested sludge, and poultry manure equilibrium to  $120 \text{ kg N ha}^{-1}$  at different days of incubation. Application of 100 per cent nitrogen through press mud recorded higher alkaline phosphate activity at harvest of wheat.

Jones and Oburger (2011) reported that, the PSB and PSF may mobilize soil P by the acidification of soil, the release of enzymes (such as phosphatases and phytases), or the production of carboxylates such as gluconate, citrate and oxalate.

Ramesh *et al.* (2011) revealed that, phosphatase and phytase activity increased with inoculation of Bacillus isolates might have been responsible for increased P mobilization and acquisition by plant.

Mohammadi (2012) studied the activities of enzymes varied significantly in different fertilization treatment. The alkaline and acid phosphatase generally increase with compost application increase phosphatase activity could be responsible for hydrolysis of organically bound phosphate in to free long, which are taken by plant. The

highest phosphate activity was observed in compost and FYM with chemical fertilizer treatment compare to chemical fertilizer and control.

Adnane *et al.* (2012) conducted a 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*. The result revealed that, 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.

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 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, the phosphatases play a key role in phosphorus cycle by solubilizing organic and inorganic phosphates into available forms that support growth of crop plants.

Dotaniya *et al.* (2014) concluded that, the maximum acid phosphatase activities were observed at 75 DAT (days after transplanting) in rice (0.206 mg PNP g<sup>-1</sup> h<sup>-1</sup>), 90 DAS in sorghum (0.194 mg PNP g<sup>-1</sup> h<sup>-1</sup>) and pearl millet (0.201 mg PNP g<sup>-1</sup> h<sup>-1</sup>) at 50 DAS in soybean (0.127 mg PNP g<sup>-1</sup> h<sup>-1</sup>). 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.

Jadhav *et al.* (2017) reported that, application of phytase @ 3600 IU recorded significantly higher acid phosphatase (17.0 and 20.6 μM PNP g<sup>-1</sup> soil h<sup>-1</sup>) and alkaline phosphatase activity (30.9 and 32.3 μM PNP g<sup>-1</sup> soil h<sup>-1</sup>), respectively at 50 % flowering and at harvest of soybean.

## 2.4 Effect of Phytase and Phosphorus Levels on Chemical Properties in Soil

Bhakare (1998) reported that, the application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> recorded significantly decreased pH (8.1), electrical conductivity (0.22 dS m<sup>-1</sup>) and significantly increased organic carbon (7.8 g kg<sup>-1</sup>) and available potassium (420 kg ha<sup>-1</sup>) as compared to control and other levels of phosphate fertilizers.

Hinsinger (2001) reported that, PSM through secretion of different organic acids lower the soil rhizosphere pH resulting in the dissociation of bound form of phosphate like Ca<sub>3</sub>(PO<sub>4</sub>)<sub>2</sub>.

El-Azouni (2008) reported that, the dual inoculation of phosphate solubilizing fungi (*Aspergillus niger* and *Penicillium digitatum*) significantly increased the organic carbon of the treated soil compared to untreated soil.

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 and concluded that, significantly increases available nitrogen, phosphorus and potassium after harvest of the crop.

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 and nitrogen contents varied from 0.10 to 1.40 per cent and 0.05 to 0.55 per cent respectively, in a salt affected soil through amendment of soil with green and farm yard manure.

Gujar *et al.* (2013) reported that, nitrogen availability in soil significantly increased by 12 per cent, whereas, potassium availability decreased by 3 per cent and increase in phosphorus content of soil by about 1.18 fold due to phytase application. The phytase was able to degrade about 30 per cent of the phytic acid in the soil. Increase in the nitrogen availability can be attributed to release of ammonium ions bound to phytate following degradation by phytase. Phytase 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 chelate minerals and consequently amend the availability of nutrients (N, Ca, Mg, Zn, and Fe) in soil.

Vibha *et al.* (2014) reported that, the availability of nitrogen, potassium and phosphorus significantly improved for inoculated soil than un-inoculated soil.

The pot culture experiment was conducted by Jadhav *et al.* (2017) to assess effect of phytase and FYM levels on nutrient availability of non-calcareous soil under soybean cultivation. Significantly higher available N ( $289.3 \text{ kg ha}^{-1}$ ) and available K ( $295.8 \text{ kg ha}^{-1}$ ) was recorded with application of phytase @  $3600 \text{ IU} + 7.5 \text{ t ha}^{-1}$  and reduction trend was obtained for calcium carbonate content from 4.23 to 3.68 % at flowering and from 4.20 to 3.60 % at harvest of soybean.

Shelke (2017) concluded that, significantly increased available nitrogen and phosphorus ( $224.47$  and  $22.06 \text{ kg ha}^{-1}$ ) by the application of  $100 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  as compared to  $50$  and  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ .

## **2.5 Effect of Phytase and Phosphorus Levels on Growth Contributing Characters of Green Fodder Maize**

Mohamed (2010) reported that, combination of organic fertilizers with urea gave the highest number of leaves and plant height. The combination of chicken manure with urea recorded highest number of (16.03) leaves and plant height (154.0 cm) in maize. Whereas, combined application of cattle manure with urea showed higher leaves(15.03), plant height (138.9 cm) followed by single addition of chicken and cattle manure.

Khaliq *et al.* (2012) reported that, fodder maize plant height (120.95 cm) and number of leaves per plant (16.60) enhanced with the application of recommendation of fertilizer  $100:50 \text{ kg NP ha}^{-1} + \text{manure } 5 \text{ t ha}^{-1}$ .

Kumar and Bohra (2014) reported that, growth attributes like plant height (213.3 cm), number of green leaves (14.3), were increased significantly with application of 125 % RDF (recommended dose of fertilizer) over 100 % RDF in fodder maize .

Farooq *et al.* (2016) reported that, the higher plant height (194.47 cm) and number of leaves plant<sup>-1</sup> (16.00) was recorded in the application of  $80 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  as compared to the  $40 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  in fodder maize.

Hussain *et al.* (2016) reported that, number of leaves (12.67) and plant height (208.20 cm) significantly influenced by application of nitrogen and phosphorus

levels of 150 :90 kg ha<sup>-1</sup> respectively as compared to other levels and control treatments in maize.

## 2.6 Effect of Phytase and Phosphorus Levels on Yield of Crop

Wani *et al.* (1978) studied the effect of phosphorus solubilizing culture (*Aspergillus awamori*, *Bacillus polymixa*, *Pseudomonas striata*, and *Penicillium digitatum*) on dry matter yield of gram. *Aspergillus awamori* and *Bacillus polymixa*, were the most efficient isolates in increasing dry matter yield of gram.

Findenegg and Nelemans (1993) studied on the effect of phytase on the availability of P from myo-inositol hexakisphosphate (phytate) for maize roots. They concluded that, the addition of phytase (10 enzyme units per kg sand) increased the plant dry weight yield by 32 per cent.

Pasricha *et al.* (1995) concluded that, pigeonpea showed a significant and consistent yield response to residual P. Thus, result of experiment revealed that in pigeonpea-wheat rotation, direct application of recommended level of P to wheat can meet the P requirement of both the crops.

Bhakare (1998) concluded that, significantly increased grain (32.1 q ha<sup>-1</sup>) and straw yield (64.9 q ha<sup>-1</sup>) of soybean by the application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as compare to control and 25, 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

Sharma and Vyas (2001) reported that, significant increase in grain yield of wheat was due to residual effect of increased levels of P fertilization at 90 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> through rock phosphate.

Sharar *et al.* (2003) concluded that, in maize the application of NP @ 180-130 kg ha<sup>-1</sup> produced significantly higher grain yield (4.62 t ha<sup>-1</sup>) than other NP combinations.

Idapuganti and Ahlawat (2007) revealed that, the application of P<sub>2</sub>O<sub>5</sub> to pigeon pea at 34.4 kg ha<sup>-1</sup> through single super phosphate + phosphorus solubilizing bacteria (PSB) recorded higher grain yield (1.49 t ha<sup>-1</sup>) of pigeon pea and also yield (4.49 t ha<sup>-1</sup>) of succeeding wheat over no P and 17.2 kg ha<sup>-1</sup> through SSP alone or rock phosphate + PSB to pigeon pea.

El-Azouni (2008) reported that, the dual inoculation of phosphate solubilizing fungi (*Aspergillus niger* and *Penicillium digitatum*) significantly increased dry matter and yield of soybean compared to control.

Tarafdar (2008) concluded that, inoculation with *Aspergillus* strains significantly increased phosphorus concentration, total phosphorus uptake, available phosphorus (25-62 per cent) 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.

Onasanya *et al.* (2009) reported that, in soybean application of 120 kg N ha<sup>-1</sup> + 40 kg P ha<sup>-1</sup> significantly enhanced grain yield (7.13 t ha<sup>-1</sup>) as compared to control and other levels of NP.

Amanullah *et al.* (2010) reported that, increased in grain yield might be due to increase in yield and yield components of maize by increased level of P.

Singh *et al.* (2010) conducted a field experiment on effect of long-term differential fertilization on distribution of inorganic-P fractions and P nutrition of wheat under maize-wheat sequence. The highest grain and straw yield was recorded in application of higher dose of phosphorus fertilizers.

Yadav and Tarafdar (2010) reported that, grain yield of cluster bean increased by 26 per cent, straw yield by 42 per cent due to inoculation of *Emericella rugulosa* (Phosphorus solubilizing fungi).

Dadhich *et al.* (2011) concluded that, the application of phosphorus levels, FYM and biofertilizers to soybean significantly increased seed yield of soybean and their residual effect significantly enhanced grain yield of subsequent wheat.

Massod *et al.* (2011) reported that, in maize the application of P @ 100 kg ha<sup>-1</sup> resulted in maximum plant height (158 cm), grain yield (2415 kg ha<sup>-1</sup>) and biological yield (7999 kg ha<sup>-1</sup>) as compared to the control plots.

A field experiment was conducted by Virkar and Tumbare (2011) to study the effect of integrated nutrient management on growth and yield of soybean-wheat cropping sequence. The application of recommended dose of fertilizer (50 kg N + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) + 5 t FYM ha<sup>-1</sup> + biofertilizers (Rhizobium + PSB) to preceding soybean crop

recorded significantly higher seed ( $30.86 \text{ q ha}^{-1}$ ) and straw ( $32.12 \text{ q ha}^{-1}$ ) yield of soybean.

Ali *et al.* (2012) reported that, in combination of nitrogen and phosphorus @  $180:90 \text{ kg ha}^{-1}$ , there was a significant increase in green fodder yield ( $35.09 \text{ t ha}^{-1}$ ) and dry matter yields ( $35.09 \text{ t ha}^{-1}$ ) through their interactive effect in maize.

Khaliq *et al.* (2012) reported that, the higher green fodder yield ( $19971.5 \text{ kg ha}^{-1}$ ) was recorded in the application of recommended fertilizer  $100:50 \text{ kg NP ha}^{-1} + 5 \text{ t ha}^{-1}$  manure than fodder yield of maize ( $18349.1 \text{ kg ha}^{-1}$ ) produced by applying recommended fertilizer  $100:50 \text{ kg NP ha}^{-1}$ .

Sharma *et al.* (2012) reported that, combined application of  $60 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  with *Aspergillus awamori* recorded significantly higher grain and straw yield of wheat crop.

Sarawgi *et al.* (2012) reported that, in soybean increase the seed yield ( $18.8 \text{ g plant}^{-1}$ ) and straw ( $4.08 \text{ t ha}^{-1}$ ) yield by applied dose of  $30 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  through rock phosphate + PSB+ RI+ VAM.

Gujar *et al.* (2013) concluded that, phytase isolated from *Aspergillus niger* promoted plant growth (upto 200 per cent) in wheat. The length of shoot, root and shoot: root ratio was higher in pot supplemented with phytase than uninoculated control.

Muhammad *et al.* (2013) concluded that, the phosphate solubilizing 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.

Khan *et al.* (2014) reported that, in maize the highest number of leaves per plant (11), highest fresh weight ( $1764.6 \text{ kg ha}^{-1}$ ) and highest dry weight ( $827.1 \text{ kg ha}^{-1}$ ) was recorded by application of  $120 \text{ kg P ha}^{-1}$  as compared to  $90 \text{ kg P ha}^{-1}$  and  $60 \text{ kg P ha}^{-1}$  respectively.

Vibha *et al.* (2014) concluded that, in soybean the significantly higher root length, dry weight and seed yield with inoculation of *Aspergillus niger* strains, *Penicillium citrinum* strains and phosphate solubilizing bacteria over uninoculated control.

Amanullah and Khan (2015) reported that, compost applied at sowing time and phosphorus applied at the two higher rates (75 and 100 kg P ha<sup>-1</sup>) had significantly increased yield and yield components of maize under semi-arid condition.

Geetha and Radder (2015) reported that, the increased grain yield of soybean (30.80 q ha<sup>-1</sup>) and straw (45.40 q ha<sup>-1</sup>) yield by addition of highest dose of phosphorus i.e 80 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> with FYM+ PSB+ VAM.

Ram *et al.* (2015) reported that, combination of 50 % P fertilizer dose along with phosphorus solubilizing fungi produced wheat yield similar to that obtained with 100 % of P fertilizer dose.

Farooq *et al.* (2016) reported that, highest green fodder yield of maize (59.45 t ha<sup>-1</sup>) and dry matter (17.83 t ha<sup>-1</sup>) was recorded in the application of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> which was at par with the application of 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as compared to the 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

Hussain *et al.* (2016) reported that, the grain yield of maize (6356.06 kg ha<sup>-1</sup>) significantly influenced by application of nitrogen and phosphorus levels of 150:90 kg ha<sup>-1</sup> respectively as compared to other levels and control treatments.

Kanduri *et al.* (2016) reported that, green fodder yield of maize (44.97 t ha<sup>-1</sup>) and dry matter percentage (22.38 %) were influenced significantly by the application of nitrogen and phosphorus. Maximum green fodder yield was obtained at nitrogen-phosphorus level of 160-50 kg ha<sup>-1</sup>.

Shelke (2017) reported that, application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was recorded significantly increased grain and straw yield of soybean (27.87 and 31.41 q ha<sup>-1</sup>) as compared to application of 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

## **2.7 Effect of Phytase and Phosphorus Levels on Nutrient Uptake by Different Crops**

Motsara and Dutta (1971) claimed that, higher P content of rice was resulted from the application of single superphosphate rather than MRP (mussoorie rock phosphate) under the same level of applied phosphorus.

Findenegg and Nelemans (1993) studied on the effect of phytase on the availability of P from myo-inositol hexakisphosphate (phytate) for maize roots. They

reported that, the addition of phytase in soil was significantly increased P-uptake of maize plants (from 90.8 to 196.5  $\mu\text{ mol pot}^{-1}$ ) respectively.

Bhakare (1998) concluded that, application of 100 kg  $\text{P}_2\text{O}_5\text{ ha}^{-1}$  significantly increased NPK uptake in soybean grain (208, 12.9 and 41 kg  $\text{ha}^{-1}$ ), respectively and straw (113, 14.6 and 155 kg  $\text{ha}^{-1}$ ), respectively as compared to control and 25, 50 and 75 kg  $\text{P}_2\text{O}_5\text{ ha}^{-1}$ .

Chuang *et al.* (2007) studied on solubilization of inorganic phosphates and plant growth promotion by *Aspergillus niger* and they concluded that, *Aspergillus niger* 6A was significantly increased dry weight, N and P content of *Brassica chinensis* with addition of small amount of organic fertilizer and rock phosphate in pot culture experiments.

Srikanth *et al.* (2009) reported that, application of 250:125:125 NPK kg  $\text{ha}^{-1}$  significantly increased NPK uptake due to build-up of vigorous growth and higher photosynthetic rate, P uptake increased might be due to increased growth and dry matter production with the application of N and also due to increased N uptake. K uptake increased due to synergistic effect of N and K and also due to better foraging capacity of roots due to increased NP application which resulted in increased dry matter production.

Sharma *et al.* (2012) reported that, the increasing levels of P application (0 to 90 kg  $\text{P}_2\text{O}_5\text{ ha}^{-1}$ ) and inoculation with *Aspergillus awamori* and *Aspergillus niger* significantly increased uptake of N, P and K in wheat at all stages of crop growth. The maximum nitrogen, phosphorus and potassium uptake was recorded at harvest (grain and straw) followed by ear emergence and tillering when seeds were inoculated with *Aspergillus awamori*.

Ram *et al.* (2015) reported that, phosphorus content (0.31 %) in wheat leaves increased significantly with fertilizer P application over no P at all growth stages of wheat as well as phosphorus uptake (25.7 kg  $\text{ha}^{-1}$ ) due to higher dose of phosphorus along with phosphorus solubilizing fungi.

Effect of various levels of NP fertilizers on maize (*Zea mays* L.) under different moisture conditions by Hussain *et al.* (2016) and they reported that, application of 150 kg nitrogen and 90 kg phosphorus  $\text{ha}^{-1}$  were significantly increased total phosphorus content (3.46  $\mu\text{ g}^{-1}$ ) and total nitrogen content (0.45  $\mu\text{ g}^{-1}$ ), respectively.

## 2.8 Effect of Phytase and Phosphorus Levels on Soil Chemical and Enzymatic Activities during Incubation Study

Deshmukh *et al.* (1993) studied that, there was significant increase in the nitrogen availability due to the increase in the rates of press mud cake application. The availability of N increases at all levels of press mud cake up to 45 days of incubation followed by small decrease upto 75 days of incubation. Phosphorus availability was significantly increased over control with the application of press mud cake at different period of incubation.

Santhy *et al.* (1998) studied on long-term effects of continuous cropping and fertilization on sandy clay loam soil at Coimbatore. They reported that, available K content of the soil was increased (765, 605 and 590 kg ha<sup>-1</sup> in 1982, 1987 and 1992, respectively) over the initial value (490 kg ha<sup>-1</sup>) under 100% NPK +FYM treatment.

Akhtar and Alam (2001) showed that, increasing the time of incubation, P availability in soil decreased for both organic and inorganic P sources.

Weerasinghe *et al.* (2010) reported that, Olsen-P content was first increased and then declined with incubation upto 15 weeks. This is common behavior of P under moist incubation. Increase in Olsen-P content during the early phase of incubation with rock phosphate. Application in the absence of organic manures may be the result of the exposure of the apatite surface due to fine grinding. The subsequent decreased in Olsen-P at later stages of incubation.

Deraoui *et al.* (2015) reported that, adding of P fertilizers caused the enrichment of soil available phosphorus; they showed that the values of fertilized samples are higher compared to the control by addition of single super phosphate.

Shelke (2017) concluded that, application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> at 7, 45, 60, 90, 120 and 150 days of incubation recorded significantly increased available phosphorus (17.55, 23.40, 23.64, 23.78, 23.92 and 23.98 kg ha<sup>-1</sup>) and increased all forms of phosphorus such as saloid-P, Al-P, Fe-P, Ca-P, red-P, occl-P, Org-P and total-P in 45 days of incubation (17.86, 27.35, 20.60, 343.45, 61.06, 10.61, 338.74 and 819.67 mg kg<sup>-1</sup>) and 150 days of incubation (18.44, 26.46, 19.91, 351.47, 59.39, 10.74, 340.91 and 827.32 mg kg<sup>-1</sup>) as compared to 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### 3. MATERIAL AND METHODS

The present investigations entitled ‘Effect of phytase and phosphorus levels on soil phosphorus availability and yield of fodder maize in an Inceptisol’. was conducted during *Kharif* 2016-2017 at Micronutrient Research Scheme, Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri to study under pot culture and incubation experiments on nutrient availability, forms of phosphorus, enzyme activity and yield of fodder maize. The incubation study was carried out to assess the effect of phytase and phosphorus levels on various soil chemical properties, forms of phosphorus and enzyme activity.

The details of the material used and analytical methods adopted for this investigation are presented in this chapter.

3.1 Details of pot culture experiment

3.2 Details of incubation study

3.3 Methods used for soil and plant analysis

#### 3.1 Details of Pot Culture Experiment

##### 3.1.1 Location

Geographically the central campus of Mahatma Phule Krishi Vidyapeeth, Rahuri is situated 30 km away from Ahmednagar district (MS) on Ahmednagar – Manmad State Highway. It lies between 19° 57’ N latitude and 74° 19’ E longitudes. The altitude varied from 495 to 569 meters above mean sea level. This tract is lying on the eastern side of western ghat and falls under rain shadow area.

##### 3.1.2 Soils

The required quantity of surface soil of 0-15 cm depth was collected from Post Graduate Institute Farm, Mahatma Phule Krishi Vidyapeeth, Rahuri. The soil was grouped under Inceptisol order which comprises of montmorillonite type of clay mineral having swell-shrink property. The important chemical and biological properties of soils were determined by using standard methods (Table 3.14)

##### 3.1.3 Climate

The climatic conditions during crop growth *viz.*, average maximum and minimum temperature, rainfall, average morning and evening humidity are recorded in (Table 3.1). The maximum and minimum temperature was ranged between 31.8° C and 10.1 °C during the months of July to December 2016.

Table 3.1. Meteorological data of Kharif 2016

Met. week	Temp. ( <sup>0</sup> C)		RH (%)		Rainfall (mm)	No. of rainy days
	Max.	Min.	Morn.	Even.		
<b>Jul-16</b>						
27	29.6	23.6	79.1	69.6	9.0	1.0
28	28.2	22.9	82.7	72.6	22.6	2.0
29	29.6	22.8	75.9	66.9	2.0	0.0
30	29.2	22.7	84.3	65.7	127.8	5.0
31	28.3	22.7	82.1	73.7	50.6	4.0
	<b>29.0</b>	<b>22.9</b>	<b>80.8</b>	<b>69.7</b>	<b>212.0</b>	<b>12.0</b>
<b>Aug-16</b>						
32	30.1	23.0	69.9	65.4	1.0	0.0
33	30.6	22.0	71.6	59.0	0.0	0.0
34	31.8	22.0	71.7	55.9	0.0	0.0
35	31.7	22.8	74.4	55.9	1.6	0.0
	<b>31.0</b>	<b>22.5</b>	<b>71.9</b>	<b>59.0</b>	<b>2.6</b>	<b>0.0</b>
<b>Sep-16</b>						
36	30.9	20.3	69.7	53.1	0.0	0.0
37	31.4	21.8	75.7	58.0	62.0	2.0
38	28.8	22.3	89.1	77.1	163.6	4.0
39	30.1	21.1	82.0	63.9	60.8	5.0
	<b>30.3</b>	<b>21.4</b>	<b>79.1</b>	<b>63.0</b>	<b>286.4</b>	<b>11.0</b>
<b>Oct-16</b>						
40	28.6	21.5	87.3	70.1	60.6	3.0
41	31.6	20.6	80.4	53.3	0.0	0.0
42	31.6	17.2	66.6	43.3	0.0	0.0
43	31.4	16.4	65.9	35.6	0.0	0.0
	<b>30.8</b>	<b>18.9</b>	<b>75.0</b>	<b>50.6</b>	<b>60.6</b>	<b>3.0</b>
<b>Nov-16</b>						
44	30.1	13.6	52.4	39.6	0.0	0.0
45	29.6	11.7	52.0	25.3	0.0	0.0
46	29.5	12.6	64.1	40.7	0.0	0.0
47	29.1	10.1	56.0	26.7	0.0	0.0
	<b>29.6</b>	<b>12.0</b>	<b>56.1</b>	<b>33.1</b>	<b>0.0</b>	<b>0.0</b>
<b>Dec-16</b>						
48	30.2	10.5	61.3	26.0	0.0	0.0
49	28.9	10.6	63.7	34.4	0.0	0.0
50	28.7	11.7	51.7	36.9	0.0	0.0
51	29.2	10.2	54.0	34.4	0.0	0.0
52	28.9	8.7	56.5	28.5	0.0	0.0
	<b>29.2</b>	<b>10.3</b>	<b>57.4</b>	<b>32.0</b>	<b>0.0</b>	<b>0.0</b>

### 3.1.4 Seeds

The good quality maize seeds of variety African tall were obtained from the Central Seed Cell, Mahatma Phule Krishi Vidyapeeth, Rahuri.

### 3.1.5 FYM

The quantity of farm yard manure required for experiment was procured from the Cattle Project, Department of Animal Husbandry and Dairy Science, Mahatma Phule Krishi Vidyapeeth, Rahuri.

### 3.1.6 Phytase Enzyme

The phytase enzyme isolated from *Aspergillus niger* strain NCIM 563 was obtained from National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL) Pune, (MS) India. The procured phytase solution @ 27, 54, 81 and 108 mL was diluted in 250 mL volumetric flask and applied uniformly to the soil at field capacity after sowing of green fodder maize for pot culture and 5.4, 10.8, 16.2 and 21.6 mL for incubation study to supply 1200, 2400, 3600 and 4800 IU phytase. In 1 mL solution contain 45 IU of phytase activity. This application was on the soil weight basis for 100 gm soil added 12 IU of phytase (Plate-1).

**Table 3.2. Initial chemical and biological properties of experimental soils**

Sr. No.	Parameters	Value
<b>A.</b>	<b>Enzyme activity</b>	
1.	Acid phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ )	6.72
2.	Alkaline phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ )	11.93
<b>B.</b>	<b>Chemical properties</b>	
1.	pH (1:2.5)	8.36
2.	EC ( $\text{dS m}^{-1}$ )	0.35
3.	Organic carbon (%)	0.67
4.	$\text{CaCO}_3$ (%)	5.98
5.	Available nitrogen ( $\text{mg kg}^{-1}$ )	84.70
6.	Available phosphorus ( $\text{mg kg}^{-1}$ )	6.84
7.	Available potassium ( $\text{mg kg}^{-1}$ )	162.00
8.	Saloid-P ( $\text{mg kg}^{-1}$ )	8.19
9.	Al-P ( $\text{mg kg}^{-1}$ )	41.68
10.	Fe-P ( $\text{mg kg}^{-1}$ )	26.68
11.	Ca-P ( $\text{mg kg}^{-1}$ )	316.15
12.	RS-P ( $\text{mg kg}^{-1}$ )	63.70
13.	Occl-P ( $\text{mg kg}^{-1}$ )	7.66
14.	Organic-P ( $\text{mg kg}^{-1}$ )	253.34
14.	Total-P ( $\text{mg kg}^{-1}$ )	719.39

**Table 3.3. Experimental details: Pot culture**

1.	Location of the experiment	Wire house, Dept. of Soil Science and Agril. Chemistry, Post Graduate Institute, MPKV. Rahuri
2.	Crop	Fodder maize
3.	Variety	African Tall
4.	Season	<i>Kharif</i> , 2016
5.	No. of treatments	17
a.	Phytase levels	04 (1200, 2400, 3600 and 4800 IU)
b.	Phosphorus fertilizer levels	04 (0, 25, 50 and 75 Kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )
6.	No. of replications	02
7.	Design of experiment	FCRD
8.	Weight of soil	10 kg pot <sup>-1</sup>
9.	General recommended dose	100:50:50 N: P <sub>2</sub> O <sub>5</sub> : K <sub>2</sub> O Kg ha <sup>-1</sup> + FYM @ 10 t ha <sup>-1</sup>
10.	Type of pot	Earthen pots (Plastic cover inner side)
11.	Capacity of pot	12 kg

**Table 3.4: Treatment Details**

Factor A- Phytase levels (IU)	Factor B- P fertilizer levels (kg ha <sup>-1</sup> )
1200	0
2400	25
3600	50
4800	75

**Table 3.5. Treatment combinations: (Common to pot culture experiment and incubation study)**

1	Phytase @ 1200 IU+ 00 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	10	Phytase @ 3600 IU+ 25 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
2	Phytase @ 1200 IU+ 25 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	11	Phytase @ 3600 IU+ 50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
3	Phytase @ 1200 IU+ 50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	12	Phytase @ 3600 IU+ 75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
4	Phytase @ 1200 IU+ 75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	13	Phytase @ 4800 IU+ 00 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
5	Phytase @ 2400 IU+ 00 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	14	Phytase @ 4800 IU+ 25 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
6	Phytase @ 2400 IU+ 25 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	15	Phytase @ 4800 IU+ 50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
7	Phytase @ 2400 IU+ 50 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	16	Phytase @ 4800 IU+ 75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>
8	Phytase @ 2400 IU+ 75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>	17	Absolute control
9	Phytase @ 3600 IU+ 00 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup>		

**Note:**

- Recommended dose of N and K<sub>2</sub>O @ (100: 50) kg ha<sup>-1</sup> and FYM @ 10 t ha<sup>-1</sup> was common to all the treatment except absolute control.
- Seed treatment with PSB @ 25 g kg<sup>-1</sup> was common to all treatment except absolute control.
- IU- International Unit as amount of enzyme that liberates 1 μmol phosphorus min<sup>-1</sup>

**Table 3.6 Phytase application methodology (for pot culture)**

Phytase levels (IU)	Soil taken (kg)	Quantity of stock solution taken (mL)	distilled water added (mL)	Total volume made (mL)	Number of pots	Volume added per pot (mL)
1200	10	27	223	250	08	250
2400	10	54	196	250	08	250
3600	10	81	169	250	08	250
4800	10	108	142	250	08	250

**3.1.7 Pots**

Earthen pots of 12 kg capacity were used for experiment. Due care was taken to ensure enough aeration and one small hole was kept at bottom of the pots for drainage purpose.

**3.1.8 Fertilizer**

Required quantity of recommended dose of nitrogen, phosphorus and potassium through fertilizer urea, single superphosphate and muriate of potash were applied in the soil.

**Table 3.7. Details of P fertilizer used**

P fertilizer levels ( $P_2O_5$ kg ha <sup>-1</sup> )	Single superphosphate added for 10 kg soil (g)	Number of pots	Total quantity required (g)
0	0	08	0
25	0.69	08	5.52
50	1.39	08	11.12
75	2.09	08	16.72

**3.1.9 Filling of Pots with Soil**

Earthen pots were washed with water and used for experiment. Thirty four pots each with ten kg (2 mm sieved) soil were filled. At the time of filling the pots, treatment wise required quantity of FYM and fertilizer dose was thoroughly mixed with soil. Before sowing, treatment wise recommended dose of nitrogen and potassium was applied through urea and muriate of potash. Application of phosphorus (*viz.*, 0, 25, 50 and 75 kg  $P_2O_5$  ha<sup>-1</sup>, respectively) through single superphosphate was given to crop as per treatments and thereafter, pots were irrigated. Dibbling of maize seed equidistantly in the pot were carried out on the same day at field capacity.

### 3.1.10 Sowing

The maize seed were sown on 23<sup>rd</sup> August, 2016. Sixteen pots each with P<sub>2</sub>O<sub>5</sub> and phytase as per levels and one pot of absolute control was maintained and three plants were sown per pot equidistantly.

### 3.1.11 Irrigation

At field capacity, moisture of soil was maintained by irrigating the pots at an interval of four to six days starting from 2 to 3 days after sowing till the tasseling stage by using moisture meter.

### 3.1.12 Harvesting and yield

The maize plants after flowering were harvested for green fodder yield and nutrient uptake studies. Maize green fodder yield from each pot was recorded at harvest.

### 3.1.13 Phosphorus efficiency

Beneficial effects of the different sources and levels of phosphorus were assessed by calculating the following parameters according to Mengel and Kirkby (2001).

$$\text{Apparent P recovery (\%)} = \frac{\text{P uptake F} - \text{P uptake C}}{\text{Fertilizer P applied}} \times 100$$

Where,

F = Fertilizer applied

C = Control (without fertilizer)

### 3.1.14 Preparation of Plant and Soil Samples for Analysis

#### a. Plant

Plant samples were collected, cleaned with deionized and air dried under shade and subsequently in oven dried at 65<sup>o</sup>C till constant weight and ground well to maximum fineness stainless steel Willey mill. The processed plant samples were analyzed for concentration and uptake of macronutrients.

#### b. Analysis of Soil samples

The rhizosphere soil samples were used for assessing the enzyme activities (acid and alkaline phosphatase) at initial, 30 DAS and at harvest of maize. For chemical analysis, soil samples were air dried in shade, pound in wooden mortar and pestle and

passed through 2 mm sieve prior to analysis and used for chemical analysis by adopting standard analytical methods table 3.14.

### **3.1.15 Fractionation of phosphorus in Soil**

For determination of inorganic forms of phosphorus in soil, the method of Peterson and Corey (1966) was used. Each of the P fractions was extracted from 1 g soil (0.15 mm) by extracting it successively with different extractants developing colour by using acid molybdate and reducing agents and the colour intensity was recorded on spectronic-20. The following methods were used for determination of fractionation of phosphorus.

#### **a. Saloid bound phosphate (Sal-P)**

Sal-P in the soil was extracted by 1 M  $\text{NH}_4\text{Cl}$  and after shaking for 30 minutes, the phosphorus content in the extract was estimated by chlorostannous reduced molybdophosphoric blue colour method in hydrochloric acid system.

#### **b. Aluminium bound phosphate (Al-P)**

The soil residue was extracted with 0.5 M  $\text{NH}_4\text{F}$  pH 8.2 and after shaking for one hour, P in the extract was determined by chlorostannous reduced molybdophosphoric blue colour method in hydrochloric acid system.

#### **c. Iron bound phosphate (Fe-P)**

The soil residue was extracted with 0.1 M  $\text{NaOH}$  for 17 hours to extract Fe-P. The phosphorus in the extract was determined by chlorostannous reduced molybdophosphoric blue colour method in sulphuric acid system.

#### **d. Calcium bound phosphate (Ca-P)**

The soil residue left after estimation of Fe-P was treated with 0.25 M  $\text{H}_2\text{SO}_4$  and after shaking for one hour, the phosphorus in the extract was determined by using chlorostannous reduced molybdophosphoric blue colour method in sulphuric acid system.

#### **e. Reductant soluble phosphate (red-P)**

The soil residue was suspended in 0.3 M sodium citrate and shaken for 15 minutes with 0.5 g sodium dithionate. The red-P was determined by using chlorostannous reduced molybdophosphoric blue colour method in sulphuric acid system.

**f. Occluded phosphate (occl-P)**

After estimation of RS-P, the soil residue left was treated with 0.25 ml 0.1 M NaOH and shaken for one hour. After centrifugation, a supernatant solution was used for the determination of occluded-P by chlorostannous reduced molybdophosphoric blue colour method, in sulphuric acid system.

**g. Total phosphorus**

A fresh soil sample passed through 0.25 mm sieve was digested with HClO<sub>4</sub> and the Total-P was determined by vanadomolybdate yellow colour method as described by Jackson (1973).

**3.1.16 Methodology of Enzyme Assay**

**a. Acid and alkaline phosphatase activity**

The assay method for phosphomonoesterases EC 3.1.3.1 and EC 3.1.3.2 (alkaline and acid phosphatase) was given by Tabatabai and Bremner, (1969).

**Principles of phosphatase enzyme**

Phosphatase in the sample catalyzes the hydrolysis of colourless *p*-nitrophenyl phosphate (*p*-NPP) to give *p*-nitrophenol and inorganic phosphate. At the pH of the phosphatase assay, the *p*-nitrophenol is the yellow phenoxide forms. The rate of absorbance increase at 404 nm is directly proportional to the phosphatase activity.

**Table 3.8. Observation recorded in the pot culture experiment**

Sr. No.	Particular	Parameter	Sample taken periods
<b>A.</b>	<b>Soil analysis</b>		
I	Chemical properties	pH, EC, organic carbon, CaCO <sub>3</sub> , available N, P and K	Initial, 30 DAS and at harvest
		P- fractions and total-P	Initial, 30 DAS and at harvest
III	Biological properties	<b>Soil enzyme activity</b> Acid phosphatase activity and alkaline phosphatase activity	Initial, 30 DAS and at harvest
<b>B.</b>	<b>Plant analysis</b>		
I	Nutrient content	Total N, P, K	30 DAS and after harvest
II	Yield	Fodder	After harvest
III	Morphological observation	Plant height	At harvest
		Root biomass, dry matter	At harvest
		Root volume and weight	At harvest
		Root CEC	At harvest

### 3.1.17 Details of Operations:

**Table 3.9. Details of operations in the pot culture experiment**

<b>Particulars</b>	<b>Maize( Date of work)</b>
Filling of pots	12.08.2016
Addition of FYM	16.08.2016
Fertilizer application	22.08.2016
Sowing	23.08.2016
Phytase application	24.08.2016
Gap filling	06.09.2016
Weeding	22.09.2016
Irrigations (For maintaining field capacity)	1) 22.08.2016 2) 26.08.2016 3) 31.08.2016 4) 06.09.2016 4) 12.09.2016 5) 18.09.2016 6) 24.09.2016 7) 30.10.2016 8) 06.10.2016 9) 11.10.2016 10) 18.10.2016 11) 24.10.2016 12) 29.10.2016
Harvesting	03.11.2016

### 3.2 Incubation Study

The incubation study was conducted in laboratory under ambient condition. The soil for incubation study was collected from field of experimental area. The collected soil samples were processed in the laboratory. Two kilogram processed and (< 2 mm) sieved soil was filled in plastic bowl. The sufficient quantity of deionised water was added in soil and then allowed to evaporate the moisture till to attain the moisture at field capacity (40.21%). At field capacity phytase with superphosphate as per levels were mixed as per treatment and mixed thoroughly with the help of glass rod. The treatment wise 100 g soil was filled in the plastic bottles of capacity 130 ml. The moisture content of soil in bottles was maintained at field capacity by adding deionised water gravimetrically. The soil samples were analyzed periodically to assess the soil biochemical changes *viz* available N, P, K, forms of phosphorus, pH, EC, organic carbon, CaCO<sub>3</sub> and soil enzyme activity.

**Table 3.10. Experimental details: Incubation study**

Location of the experiment	Micronutrient Research Laboratory, Dept. of Soil Science and Agril. Chemistry, MPKV, Rahuri
Design	FCRD
No. of factors	Factor A = 4 levels of phytase (1200, 2400, 3600 and 4800 IU) Factor B = 4 levels of phosphorus (0, 25, 50 and 75 kg P <sub>2</sub> O <sub>5</sub> ha <sup>-1</sup> )
No. of treatments	17
No. of replications	02
Weight of soil	2 kg pot <sup>-1</sup>
Soil type	Inceptisol (Medium deep black)
Incubation period	0, 07,15,30,45 and 60 days

**Table 3.11 Phytase application methodology (for incubation study)**

Phytase levels (IU)	Soil Taken (kg)	Quantity of stock solution taken (mL)	Distilled water added (mL)	Total volume made (mL)	Number of pots	Volume added per pot (mL)
1200	2	5.4	44.6	50	08	50
2400	2	10.8	39.2	50	08	50
3600	2	16.2	33.8	50	08	50
4800	2	21.6	28.4	50	08	50

**Table 3.12 Details of P fertilizer used (for incubation study)**

P fertilizer levels (P <sub>2</sub> O <sub>5</sub> kg ha <sup>-1</sup> )	Single superphosphate added for 2 kg soil (g)	Number of pots	Total quantity required (g)
0	0	08	0
25	0.14	08	1.12
50	0.27	08	2.16
75	0.41	08	3.28

**Table 3.13. Observations recorded in incubation study**

Sr. No.	Parameter	Methods	Incubation periods
1.	Chemical properties	pH, EC, organic carbon, CaCO <sub>3</sub> , available N, P and K, P-fraction and total-P	At 0, 7, 15, 30, 45 and 60 days.
2.	Enzyme activity	Acid phosphatase activity and alkaline phosphatase activity	At 0, 7, 15, 30, 45 and 60 days.

**Table 3.14. Methods Used for Soil and Plant Analysis**

<b>Sr. No.</b>	<b>Parameter</b>	<b>Method used</b>	<b>Reference</b>
<b>A.</b>	<b>Soil analysis</b>		
<b>I</b>	<b>Chemical properties</b>		
1.	pH (1:2.5)	Potentiometric	Jackson (1973)
2.	EC (1:2.5)	Conductometry	Jackson (1973)
3.	Organic carbon	Wet oxidation	Nelson and Sommer (1982)
4.	CaCO <sub>3</sub>	Acid neutralization	Allison and Moodier(1965)
5.	Available nitrogen	Alkaline permanganate	Subbiah and Asija (1956)
6.	Available phosphorous	0.5 M NaHCO <sub>3</sub> (pH 8.5)	Watanable and Olsen (1965)
7.	Available potassium	N N NH <sub>4</sub> OAc	Jackson (1973)
8.	Forms of phosphorus	Modified procedure of Chang and Jackson (1957)	Peterson and Corey (1966)
9.	Total-P	HClO <sub>4</sub> digestion	Jackson (1973)
<b>II</b>	<b>Biological properties</b>		
	<b>Soil enzymes</b>		
1.	Acid phosphatase	Spectrophotometry	Tabatabai and Bremner (1969)
2.	Alkaline phosphatase	Spectrophotometry	Tabatabai and Bremner (1969)
<b>B</b>	<b>Plant analysis</b>		
1.	Total N	Micro- Kjeldahl	Parkinson and Allen (1975)
2.	Total P	Digestion 9:4 ratio (HNO <sub>3</sub> : HClO <sub>4</sub> ) (Vanadomolybdate yellow colour method in nitric acid)	Jackson (1973)
3.	Total K	Digestion 9:4 ratio (HNO <sub>3</sub> : HClO <sub>4</sub> ) Flame photometry	Chapman and Pratt (1961)
4.	Root CEC	Potentiometric titration	Dontsov (1976)

### 3.3 Statistical Analysis

Statistical analysis was done by following standard procedures and data generated from present experiment was statistically analysed by using FCRD (Factorial Completely Randomized Design) Panse and Sukhatme (1985).

## 4. RESULTS AND DISCUSSION

### 4.1 Incubation Study

The incubation study was conducted under ambient condition to assess the effect of phytase and phosphorus levels and their interactions on periodical soil chemical properties, enzyme activity, available nitrogen, phosphorus, potassium and forms of phosphorus in soil. The results obtained are discussed as below and presented in (Table 4.1 to 4.27 and depicted in Fig. 4.1 to 4.25).

#### 4.1.1 Effect of Phytase and Phosphorus Levels on Periodical Soil Chemical Properties in Incubation Study

##### 4.1.1.1 Effect of phytase and phosphorus levels on periodical soil pH in incubation

The periodical soil pH in an incubation study under ambient condition in laboratory did not influence by the phytase and phosphorus levels are presented in (Table 4.1).

The application of phytase and phosphorus levels found non significant soil pH at all the periods of incubation study. The interactions between phytase and phosphorus levels found non significant for soil pH at all the periods of incubations. In respect of treated vs control, the periodical soil pH at 0, 7, 15, 30, 45 and 60 DAI were also found non significant.

##### 4.1.1.2 Effect of phytase and phosphorus levels on periodical soil electrical conductivity in incubation

The periodical soil electrical conductivity in an incubation study under ambient condition in laboratory did not influenced by the phytase and phosphorus levels are presented in (Table 4.2).

Soil electrical conductivity did not influenced by the application of phytase and phosphorus levels at all the periods of incubations. The interactions between phytase and phosphorus levels found non significant for electrical conductivity at all the periods of incubations.

In respect of treated vs. control, the periodical results of soil electrical conductivity at 30, 45 and 60 days (0.36, 0.33 and 0.33  $\text{dSm}^{-1}$ ), respectively found

**Table 4.1. Effect of Phytase and Phosphorus Levels on Periodical Soil pH at Various Days of Incubation**

Treatment	Soil pH (1:2.5)					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	8.35	8.36	8.34	8.33	8.33	8.32
P <sub>2</sub> (2400 IU)	8.36	8.34	8.33	8.34	8.34	8.33
P <sub>3</sub> (3600 IU)	8.33	8.35	8.32	8.34	8.33	8.33
P <sub>4</sub> (4800 IU)	8.34	8.35	8.32	8.34	8.33	8.32
<b>SEm ±</b>	<b>0.01</b>	<b>0.008</b>	<b>0.006</b>	<b>0.006</b>	<b>0.006</b>	<b>0.006</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	8.33	8.34	8.33	8.33	8.32	8.32
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	8.34	8.35	8.32	8.33	8.33	8.32
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	8.35	8.36	8.32	8.34	8.33	8.33
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	8.36	8.35	8.34	8.35	8.34	8.33
<b>SEm ±</b>	<b>0.01</b>	<b>0.008</b>	<b>0.006</b>	<b>0.006</b>	<b>0.006</b>	<b>0.006</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	8.33	8.35	8.34	8.33	8.32	8.33
P <sub>1</sub> F <sub>2</sub>	8.34	8.36	8.33	8.31	8.33	8.31
P <sub>1</sub> F <sub>3</sub>	8.36	8.37	8.34	8.34	8.32	8.32
P <sub>1</sub> F <sub>4</sub>	8.38	8.36	8.35	8.35	8.34	8.34
P <sub>2</sub> F <sub>1</sub>	8.34	8.33	8.33	8.32	8.32	8.33
P <sub>2</sub> F <sub>2</sub>	8.36	8.34	8.32	8.34	8.34	8.32
P <sub>2</sub> F <sub>3</sub>	8.38	8.36	8.32	8.34	8.34	8.33
P <sub>2</sub> F <sub>4</sub>	8.37	8.34	8.35	8.35	8.35	8.34
P <sub>3</sub> F <sub>1</sub>	8.33	8.36	8.33	8.33	8.32	8.32
P <sub>3</sub> F <sub>2</sub>	8.31	8.36	8.32	8.34	8.32	8.33
P <sub>3</sub> F <sub>3</sub>	8.32	8.36	8.32	8.33	8.33	8.33
P <sub>3</sub> F <sub>4</sub>	8.37	8.34	8.33	8.35	8.35	8.34
P <sub>4</sub> F <sub>1</sub>	8.34	8.33	8.32	8.34	8.33	8.31
P <sub>4</sub> F <sub>2</sub>	8.35	8.34	8.30	8.33	8.32	8.33
P <sub>4</sub> F <sub>3</sub>	8.34	8.36	8.32	8.35	8.33	8.33
P <sub>4</sub> F <sub>4</sub>	8.34	8.35	8.33	8.34	8.34	8.33
<b>SEm ±</b>	<b>0.023</b>	<b>0.016</b>	<b>0.013</b>	<b>0.013</b>	<b>0.012</b>	<b>0.012</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	8.34	8.35	8.33	8.34	8.33	8.32
Control	8.38	8.37	8.35	8.34	8.35	8.35
<b>SEm ±</b>	<b>0.024</b>	<b>0.016</b>	<b>0.014</b>	<b>0.014</b>	<b>0.013</b>	<b>0.012</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>Initial</b>	<b>8.36</b>					

**Table 4.2. Effect of Phytase and Phosphorus Levels on Periodical Soil Electrical Conductivity at Various Days of Incubation**

Treatment	Electrical conductivity (dS m <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	0.35	0.35	0.36	0.37	0.33	0.32
P <sub>2</sub> (2400 IU)	0.36	0.35	0.36	0.37	0.33	0.34
P <sub>3</sub> (3600 IU)	0.35	0.36	0.35	0.36	0.34	0.33
P <sub>4</sub> (4800 IU)	0.34	0.35	0.36	0.36	0.34	0.33
<b>SEm ±</b>	<b>0.006</b>	<b>0.007</b>	<b>0.008</b>	<b>0.005</b>	<b>0.004</b>	<b>0.005</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	0.34	0.34	0.34	0.36	0.33	0.32
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	0.35	0.36	0.37	0.37	0.34	0.33
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	0.35	0.35	0.36	0.37	0.33	0.34
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	0.36	0.36	0.36	0.36	0.34	0.34
<b>SEm ±</b>	<b>0.006</b>	<b>0.007</b>	<b>0.008</b>	<b>0.005</b>	<b>0.004</b>	<b>0.005</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	0.34	0.33	0.35	0.36	0.32	0.32
P <sub>1</sub> F <sub>2</sub>	0.35	0.36	0.37	0.37	0.34	0.31
P <sub>1</sub> F <sub>3</sub>	0.35	0.35	0.36	0.38	0.34	0.33
P <sub>1</sub> F <sub>4</sub>	0.35	0.37	0.37	0.37	0.32	0.33
P <sub>2</sub> F <sub>1</sub>	0.34	0.35	0.32	0.35	0.33	0.32
P <sub>2</sub> F <sub>2</sub>	0.36	0.35	0.38	0.38	0.32	0.35
P <sub>2</sub> F <sub>3</sub>	0.36	0.34	0.37	0.37	0.34	0.35
P <sub>2</sub> F <sub>4</sub>	0.37	0.36	0.38	0.37	0.35	0.35
P <sub>3</sub> F <sub>1</sub>	0.35	0.35	0.35	0.36	0.34	0.32
P <sub>3</sub> F <sub>2</sub>	0.35	0.36	0.37	0.38	0.35	0.33
P <sub>3</sub> F <sub>3</sub>	0.35	0.36	0.35	0.37	0.33	0.35
P <sub>3</sub> F <sub>4</sub>	0.37	0.36	0.34	0.36	0.36	0.34
P <sub>4</sub> F <sub>1</sub>	0.34	0.33	0.35	0.36	0.32	0.32
P <sub>4</sub> F <sub>2</sub>	0.34	0.36	0.37	0.37	0.35	0.32
P <sub>4</sub> F <sub>3</sub>	0.35	0.36	0.36	0.36	0.34	0.34
P <sub>4</sub> F <sub>4</sub>	0.36	0.37	0.37	0.36	0.34	0.33
<b>SEm ±</b>	<b>0.01</b>	<b>0.01</b>	<b>0.02</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	0.35	0.35	0.36	0.36	0.33	0.33
Control	0.34	0.34	0.33	0.30	0.31	0.29
<b>SEm ±</b>	<b>0.012</b>	<b>0.014</b>	<b>0.016</b>	<b>0.012</b>	<b>0.009</b>	<b>0.011</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>0.034</b>	<b>0.026</b>	<b>0.033</b>
<b>Initial</b>	<b>0.35</b>					

significant as compare to control (0.30, 0.31 and 0.29 dS m<sup>-1</sup>), respectively, and showed non significant at 0, 7 and 15 DAI.

#### **4.1.1.3 Effect of phytase and phosphorus levels on periodical soil organic carbon content in incubation**

The periodical effects of phytase, phosphorus levels and their interactions on organic carbon content in soil are presented in (Table 4.3).

The organic carbon content in soil showed non significant by the application of phytase levels at all periods of incubations. The phosphorus application in an incubation study, the soil organic carbon content significantly increased in all the periods of incubations except 7 DAI. Significantly, the highest soil organic carbon content recorded in the application of 25, 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.69 %), respectively at 0 DAI which was at par with the application of 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.67 %). At 15 DAI, the significantly higher soil organic carbon content recorded in the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.73 %).

The soil organic carbon content significantly recorded higher in the application of 25 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.73 %) at 30 DAI which was at par with the application of 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.72 and 0.70 %), respectively. At 45 DAI, the significantly higher soil organic carbon content recorded in the application of 25 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.70 %) which was at par with the application of 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.69 and 0.67 %), respectively. At 60 DAI, the significantly higher soil organic carbon content recorded in the application of 25 and 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.68 %) which was at par with the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (0.66 %).

The interactions between phytase and phosphorus levels found non significant for soil organic carbon content at all the periods of incubation study. In respect of treated vs. control, the periodical result of soil organic carbon content was non significant at 0, 7, 45 and 60 DAI. At 15 and 30 DAI, the organic carbon content was significantly higher in the treated (0.71 %) as compare to control (0.64 and 0.63 %), respectively.

The increase in soil organic carbon content in all treatment combinations might be due to addition of organic matter through FYM. Similar results were reported by Aseri, (2009) and Tarafdar, (2008). El-Azouni (2008) reported that, the dual

**Table 4.3. Effect of Phytase and Phosphorus Levels on Periodical Soil Organic Carbon Content at Various Days of Incubation**

Treatment	Organic carbon (%)					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	0.68	0.68	0.69	0.71	0.68	0.67
P <sub>2</sub> (2400 IU)	0.68	0.72	0.72	0.70	0.67	0.66
P <sub>3</sub> (3600 IU)	0.69	0.72	0.71	0.71	0.68	0.66
P <sub>4</sub> (4800 IU)	0.69	0.73	0.72	0.70	0.67	0.66
<b>SEm ±</b>	<b>0.004</b>	<b>0.015</b>	<b>0.006</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	0.67	0.68	0.68	0.68	0.65	0.63
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	0.69	0.72	0.70	0.73	0.70	0.68
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	0.69	0.72	0.71	0.72	0.69	0.68
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	0.69	0.73	0.73	0.70	0.67	0.66
<b>SEm ±</b>	<b>0.004</b>	<b>0.015</b>	<b>0.006</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>
<b>C.D at 5 (%)</b>	<b>0.014</b>	<b>NS</b>	<b>0.018</b>	<b>0.03</b>	<b>0.03</b>	<b>0.03</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	0.67	0.63	0.64	0.65	0.62	0.60
P <sub>1</sub> F <sub>2</sub>	0.69	0.70	0.70	0.74	0.71	0.69
P <sub>1</sub> F <sub>3</sub>	0.68	0.69	0.69	0.75	0.72	0.71
P <sub>1</sub> F <sub>4</sub>	0.69	0.72	0.72	0.71	0.68	0.66
P <sub>2</sub> F <sub>1</sub>	0.66	0.71	0.70	0.69	0.66	0.64
P <sub>2</sub> F <sub>2</sub>	0.67	0.71	0.69	0.74	0.71	0.69
P <sub>2</sub> F <sub>3</sub>	0.69	0.74	0.75	0.72	0.69	0.67
P <sub>2</sub> F <sub>4</sub>	0.68	0.73	0.73	0.68	0.65	0.63
P <sub>3</sub> F <sub>1</sub>	0.68	0.69	0.67	0.71	0.68	0.66
P <sub>3</sub> F <sub>2</sub>	0.70	0.74	0.73	0.72	0.69	0.68
P <sub>3</sub> F <sub>3</sub>	0.69	0.73	0.71	0.69	0.66	0.65
P <sub>3</sub> F <sub>4</sub>	0.70	0.73	0.73	0.72	0.69	0.67
P <sub>4</sub> F <sub>1</sub>	0.67	0.69	0.70	0.66	0.63	0.62
P <sub>4</sub> F <sub>2</sub>	0.69	0.73	0.70	0.71	0.68	0.66
P <sub>4</sub> F <sub>3</sub>	0.70	0.74	0.71	0.73	0.70	0.69
P <sub>4</sub> F <sub>4</sub>	0.69	0.75	0.75	0.71	0.68	0.66
<b>SEm ±</b>	<b>0.01</b>	<b>0.03</b>	<b>0.01</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	0.68	0.71	0.71	0.71	0.68	0.66
Control	0.67	0.68	0.64	0.63	0.63	0.62
<b>SEm ±</b>	<b>0.01</b>	<b>0.03</b>	<b>0.013</b>	<b>0.02</b>	<b>0.023</b>	<b>0.02</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>0.037</b>	<b>0.06</b>	<b>NS</b>	<b>NS</b>
<b>Initial</b>	<b>0.67</b>					

inoculation of phosphorus solubilizing fungi (*Aspergillus niger* and *Penicillium digitarium*) significantly increased the organic carbon levels of the treated soil when compared to untreated soil. 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 and nitrogen contents ranging from 0.10 to 1.40 per cent and 0.05 to 0.55 per cent, respectively were achieved in a salt affected soil through amendment of soil with green and farm yard manure.

#### **4.1.1.4 Effect of phytase and phosphorus levels on periodical soil calcium carbonate content (CaCO<sub>3</sub>) in incubation**

The periodical effects of phytase, phosphorus levels and their interactions on calcium carbonate content in soil are presented in (Table 4.4).

The soil calcium carbonate content did not influenced by the application of phytase at all the periods of incubations.

The application of phosphorus did not influenced initially on calcium carbonate content in soil. At 7 DAI, the significantly higher soil calcium carbonate content recorded by the application of 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.11 %) which was at par with the application of 25 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.08 %). At 15 DAI, the significantly higher soil calcium carbonate content recorded by the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.18 %). At 30 DAI, the significantly higher calcium carbonate content recorded by the application of 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.19 %) which was at par with the application of 25 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.17 %), respectively. At 45 DAI, the significantly higher soil calcium carbonate content recorded in the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.14 %) which was at par with the application of 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.12 %), respectively. At 60 DAI, the significantly higher soil calcium carbonate content recorded in the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.08 %) which was at par with the application of 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (6.06 %).

The increase in soil calcium carbonate content in soil upto 30 DAI, which might be due to the application of superphosphate and later on decreased due to that some chemical reactions occurs in soil they get fixed and some part of calcium carbonate utilized by the microorganisms.

The interactions between phytase and phosphorus levels were non significant for soil calcium carbonate content at all the periods of incubation study. In

**Table 4.4. Effect of Phytase and Phosphorus Levels on Periodical Soil Calcium Carbonate Content at Various Days of Incubation**

Treatment	CaCO <sub>3</sub> (%)					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	5.96	6.10	6.15	6.16	6.11	6.05
P <sub>2</sub> (2400 IU)	5.96	6.10	6.13	6.16	6.12	6.06
P <sub>3</sub> (3600 IU)	5.93	6.07	6.14	6.17	6.08	6.02
P <sub>4</sub> (4800 IU)	5.94	6.09	6.14	6.18	6.11	6.05
<b>SEm ±</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.009</b>	<b>0.01</b>	<b>0.01</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	5.94	6.07	6.11	6.13	6.06	6.00
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	5.93	6.08	6.12	6.17	6.09	6.03
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	5.96	6.11	6.14	6.19	6.12	6.06
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	5.96	6.11	6.18	6.19	6.14	6.08
<b>SEm ±</b>	<b>0.01</b>	<b>0.01</b>	<b>0.01</b>	<b>0.009</b>	<b>0.01</b>	<b>0.01</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>0.03</b>	<b>0.03</b>	<b>0.026</b>	<b>0.034</b>	<b>0.034</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	5.95	6.06	6.12	6.13	6.09	6.03
P <sub>1</sub> F <sub>2</sub>	5.97	6.12	6.15	6.17	6.10	6.04
P <sub>1</sub> F <sub>3</sub>	5.98	6.13	6.15	6.18	6.12	6.06
P <sub>1</sub> F <sub>4</sub>	5.94	6.09	6.18	6.17	6.13	6.07
P <sub>2</sub> F <sub>1</sub>	5.96	6.08	6.10	6.13	6.08	6.02
P <sub>2</sub> F <sub>2</sub>	5.94	6.09	6.12	6.15	6.12	6.06
P <sub>2</sub> F <sub>3</sub>	5.96	6.11	6.14	6.18	6.14	6.08
P <sub>2</sub> F <sub>4</sub>	5.98	6.13	6.19	6.20	6.16	6.10
P <sub>3</sub> F <sub>1</sub>	5.95	6.08	6.12	6.13	6.03	5.97
P <sub>3</sub> F <sub>2</sub>	5.86	6.01	6.13	6.18	6.04	5.98
P <sub>3</sub> F <sub>3</sub>	5.95	6.10	6.13	6.19	6.11	6.05
P <sub>3</sub> F <sub>4</sub>	5.95	6.10	6.19	6.20	6.13	6.07
P <sub>4</sub> F <sub>1</sub>	5.90	6.05	6.12	6.14	6.05	5.99
P <sub>4</sub> F <sub>2</sub>	5.94	6.09	6.11	6.19	6.12	6.06
P <sub>4</sub> F <sub>3</sub>	5.97	6.12	6.14	6.20	6.13	6.07
P <sub>4</sub> F <sub>4</sub>	5.96	6.11	6.19	6.21	6.14	6.08
<b>SEm ±</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	5.95	6.09	6.14	6.17	6.10	6.04
Control	5.99	6.01	6.02	6.01	6.00	5.97
<b>SEm ±</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.024</b>	<b>0.024</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>0.067</b>	<b>0.06</b>	<b>0.055</b>	<b>0.07</b>	<b>0.07</b>
<b>Initial</b>	<b>5.98</b>					

respect of treated vs control, the periodical soil calcium carbonate content at 7, 15, 30, 45 and 60 DAI found significantly highest in the treated except 0 DAI (6.09, 6.14, 6.17, 6.10 and 6.04 %), respectively as compare to control (6.01, 6.02, 6.01, 6.00 and 5.97 %), respectively.

#### **4.1.1.5 Effect of phytase and phosphorus levels on periodical soil available nitrogen in incubation**

The periodical soil available nitrogen content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels presented in (Table 4.5).

##### **4.1.1.5.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil available nitrogen at all the periods of incubation study. It was increased with an increased level of phytase and significantly higher by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (85.47, 88.13, 90.30, 100.31, 98.46 and 94.57 mg kg<sup>-1</sup>, respectively). However, the soil available nitrogen was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 30 days of incubation and decreased at 45 and 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of organically bound phosphorus by phytase enzyme. Simultaneously, the carbon also released from organic matter in which phosphorus was bound. These released organic carbon act as source of energy to microorganisms responsible for nitrogen release and fixing of atmospheric nitrogen in soil. The increase in nitrogen availability in soil can be attributed to release of ammonium ions bound to organic phosphate degradation by phytase. Similar results were reported by Gujar *et al.* (2013).

##### **4.1.1.5.2 Phosphorus levels**

The phosphorus levels were found to release the soil available nitrogen during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (85.79, 89.15, 92.30, 101.89, 99.37 and 96.57 mg kg<sup>-1</sup> of soil, respectively). The soil available nitrogen was increased with an

**Table 4.5. Effect of Phytase and Phosphorus Levels on Periodical Soil Available Nitrogen at Various Days of Incubation**

Treatment	Available nitrogen (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	84.95	87.61	89.60	99.61	97.65	93.73
P <sub>2</sub> (2400 IU)	85.09	87.75	89.74	99.79	97.63	93.99
P <sub>3</sub> (3600 IU)	85.37	87.96	90.02	100.10	98.25	94.27
P <sub>4</sub> (4800 IU)	85.47	88.13	90.30	100.31	98.46	94.57
<b>SEm ±</b>	<b>0.025</b>	<b>0.008</b>	<b>0.025</b>	<b>0.016</b>	<b>0.016</b>	<b>0.037</b>
<b>C.D at 5 (%)</b>	<b>0.07</b>	<b>0.02</b>	<b>0.07</b>	<b>0.04</b>	<b>0.04</b>	<b>0.10</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	84.67	86.52	87.50	97.83	96.60	91.67
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	84.88	86.87	87.82	98.46	97.20	92.02
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	85.54	88.90	92.05	101.64	99.12	96.32
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	85.79	89.15	92.30	101.89	99.37	96.57
<b>SEm ±</b>	<b>0.025</b>	<b>0.008</b>	<b>0.025</b>	<b>0.016</b>	<b>0.016</b>	<b>0.037</b>
<b>C.D at 5 (%)</b>	<b>0.07</b>	<b>0.02</b>	<b>0.07</b>	<b>0.04</b>	<b>0.04</b>	<b>0.10</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	84.49	86.31	87.08	97.51	96.11	91.07
P <sub>1</sub> F <sub>2</sub>	84.63	86.73	87.64	98.07	96.67	91.63
P <sub>1</sub> F <sub>3</sub>	85.19	88.55	91.70	101.29	98.77	95.97
P <sub>1</sub> F <sub>4</sub>	85.47	88.83	91.98	101.57	99.05	96.25
P <sub>2</sub> F <sub>1</sub>	84.63	86.45	87.36	97.79	96.39	91.56
P <sub>2</sub> F <sub>2</sub>	84.77	86.87	87.64	98.21	97.23	91.91
P <sub>2</sub> F <sub>3</sub>	85.33	88.69	91.84	101.43	98.91	96.11
P <sub>2</sub> F <sub>4</sub>	85.61	88.97	92.12	101.71	99.19	96.39
P <sub>3</sub> F <sub>1</sub>	84.77	86.59	87.64	97.93	96.81	91.84
P <sub>3</sub> F <sub>2</sub>	85.05	86.87	87.78	98.63	97.37	92.05
P <sub>3</sub> F <sub>3</sub>	85.75	89.11	92.26	101.85	99.33	96.53
P <sub>3</sub> F <sub>4</sub>	85.89	89.25	92.40	101.99	99.47	96.67
P <sub>4</sub> F <sub>1</sub>	84.77	86.73	87.92	98.07	97.09	92.19
P <sub>4</sub> F <sub>2</sub>	85.05	87.01	88.20	98.91	97.51	92.47
P <sub>4</sub> F <sub>3</sub>	85.89	89.25	92.40	101.99	99.47	96.67
P <sub>4</sub> F <sub>4</sub>	86.17	89.53	92.68	102.27	99.75	96.95
<b>SEm ±</b>	<b>0.05</b>	<b>0.02</b>	<b>0.05</b>	<b>0.03</b>	<b>0.03</b>	<b>0.07</b>
<b>C.D at 5 (%)</b>	<b>0.14</b>	<b>0.04</b>	<b>0.14</b>	<b>0.10</b>	<b>0.10</b>	<b>NS</b>
Tr. Vs Control						
Treated	85.22	87.86	89.92	99.95	98.07	94.14
Control	84.56	84.98	85.19	85.75	85.61	85.33
<b>SEm ±</b>	<b>0.052</b>	<b>0.017</b>	<b>0.052</b>	<b>0.035</b>	<b>0.035</b>	<b>0.077</b>
<b>C.D at 5 (%)</b>	<b>0.15</b>	<b>0.05</b>	<b>0.15</b>	<b>0.10</b>	<b>0.10</b>	<b>0.22</b>
<b>Initial</b>	<b>84.70</b>					

advanced period of incubation and numerically it was the highest at 30 days (101.89 mg kg<sup>-1</sup>) of incubation and decreased at 45 and 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil available nitrogen in an incubation study. This might be because of levels of phosphorus provides the optimum concentration of phosphorus as a substrate upon which the phytase enzyme acted to release nitrogen in soil due to mineralization of organic matter.

#### **4.1.1.5.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for soil available nitrogen periodically in incubation study. The interactions of increased levels of phytase and phosphorus were significant for periodical release of soil available nitrogen in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the soil available nitrogen also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to release the nitrogen in soil. Similar results were reported by Gujar *et al.* (2013).

In respect of treated vs. control, the periodical results of available nitrogen content in soil at 0, 7, 15, 30, 45 and 60 DAI significantly recorded the highest in the treated (85.22, 87.86, 89.92, 94.14, 98.07 and 99.95 mg kg<sup>-1</sup>), respectively as compare to control (84.56, 84.98, 85.19, 85.33, 85.61 and 85.75 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of nitrogen in soil. However, it is necessary to optimize the levels of phytase to release the soil available nitrogen.

#### **4.1.1.6 Effect of phytase and phosphorus levels on periodical soil available phosphorus in incubation**

The periodical soil available phosphorus content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.6 and depicted in Fig. 4.1).

**Table 4.6. Effect of Phytase and Phosphorus Levels on Periodical Soil Available Phosphorus at Various Days of Incubation**

Treatment	Available phosphorus (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	7.06	7.64	8.49	9.35	10.22	9.36
P <sub>2</sub> (2400 IU)	7.20	7.75	8.61	9.61	10.42	9.52
P <sub>3</sub> (3600 IU)	7.50	8.01	8.69	9.67	10.70	9.69
P <sub>4</sub> (4800 IU)	7.83	8.07	8.89	9.66	10.90	9.95
<b>SEm ±</b>	<b>0.042</b>	<b>0.058</b>	<b>0.059</b>	<b>0.063</b>	<b>0.046</b>	<b>0.052</b>
<b>C.D at 5 (%)</b>	<b>0.12</b>	<b>0.16</b>	<b>0.17</b>	<b>0.18</b>	<b>0.13</b>	<b>0.15</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	6.75	7.12	7.64	8.59	9.15	8.50
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	7.20	7.63	8.39	9.26	10.29	9.26
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	7.63	8.18	9.06	9.90	11.12	10.02
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	8.01	8.55	9.58	10.53	11.70	10.73
<b>SEm ±</b>	<b>0.042</b>	<b>0.058</b>	<b>0.059</b>	<b>0.063</b>	<b>0.046</b>	<b>0.052</b>
<b>C.D at 5 (%)</b>	<b>0.12</b>	<b>0.16</b>	<b>0.17</b>	<b>0.18</b>	<b>0.13</b>	<b>0.15</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	6.46	7.07	7.63	8.49	9.04	8.24
P <sub>1</sub> F <sub>2</sub>	6.89	7.44	8.18	8.98	9.96	9.04
P <sub>1</sub> F <sub>3</sub>	7.26	7.87	8.86	9.78	10.70	9.84
P <sub>1</sub> F <sub>4</sub>	7.63	8.18	9.29	10.15	11.19	10.33
P <sub>2</sub> F <sub>1</sub>	6.46	7.13	7.50	8.55	9.10	8.49
P <sub>2</sub> F <sub>2</sub>	7.01	7.44	8.43	9.35	9.96	9.23
P <sub>2</sub> F <sub>3</sub>	7.44	8.00	8.98	9.96	11.01	9.78
P <sub>2</sub> F <sub>4</sub>	7.87	8.43	9.53	10.58	11.62	10.58
P <sub>3</sub> F <sub>1</sub>	6.89	7.07	7.69	8.67	9.16	8.61
P <sub>3</sub> F <sub>2</sub>	7.26	7.87	8.36	9.41	10.58	9.29
P <sub>3</sub> F <sub>3</sub>	7.75	8.36	9.16	9.90	11.25	10.02
P <sub>3</sub> F <sub>4</sub>	8.12	8.73	9.53	10.70	11.81	10.82
P <sub>4</sub> F <sub>1</sub>	7.20	7.20	7.75	8.67	9.29	8.67
P <sub>4</sub> F <sub>2</sub>	7.63	7.75	8.61	9.29	10.64	9.47
P <sub>4</sub> F <sub>3</sub>	8.06	8.49	9.23	9.96	11.50	10.46
P <sub>4</sub> F <sub>4</sub>	8.43	8.86	9.96	10.70	12.18	11.19
<b>SEm ±</b>	<b>0.08</b>	<b>0.12</b>	<b>0.12</b>	<b>0.13</b>	<b>0.09</b>	<b>0.11</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>0.27</b>	<b>NS</b>
Tr. Vs Control						
Treated	7.40	7.87	8.67	9.57	10.56	9.63
Control	6.09	6.33	6.83	6.52	6.70	7.01
<b>SEm ±</b>	<b>0.086</b>	<b>0.12</b>	<b>0.12</b>	<b>0.13</b>	<b>0.095</b>	<b>0.10</b>
<b>C.D at 5 (%)</b>	<b>0.25</b>	<b>0.34</b>	<b>0.35</b>	<b>0.37</b>	<b>0.27</b>	<b>0.31</b>
<b>Initial</b>	<b>6.84</b>					

#### **4.1.1.6.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil available phosphorus at all the periods of incubation study. It was increased with an increased level of phytase and significantly increased by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (7.83, 8.07, 8.89, 9.67, 10.90 and 9.95 mg kg<sup>-1</sup>, respectively). However, the soil available phosphorus was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of organically bound phosphorus by phytase enzyme. Simultaneously, the carbon also released from organic matter in which phosphorus was bound. These released organic carbon act as source of energy to microorganisms responsible for phosphorus mineralization in soil. These results were accordance with the observations of Gujar *et al.* (2013). The increase in soil available phosphorus may be due to presence of microorganisms which play a key role in P dynamics (Richardson, 2001).

#### **4.1.1.6.2 Phosphorus levels**

The phosphorus levels were found to release the soil available phosphorus during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (8.01, 8.55, 9.58, 10.53, 11.70 and 10.73 mg kg<sup>-1</sup> of soil respectively). The soil available phosphorus was increased with an advanced period of incubation and numerically it was the highest at 45 days (11.70 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil available phosphorus in an incubation study. Similar results also obtained by Deraoui *et al.* (2015) and reported that, adding of phosphorus fertilizers caused the enrichment of soil available phosphorus; and showed that, the values of fertilized samples are higher compared to control by addition of single superphosphate during incubation.

#### **4.1.1.6.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for soil available phosphorus periodically in incubation study. The interactions

of increased levels of phytase and phosphorus were significant for periodical release of soil available phosphorus in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the soil available phosphorus also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to release the phosphorus in soil. Similar results found by Gujar *et al.* (2013).

In respect of treated vs control, the periodical available phosphorus content in soil recorded significantly higher (7.40, 7.87, 8.67, 9.57, 10.56 and 9.63 mg kg<sup>-1</sup>), respectively at 0, 7, 15, 30, 45 and 60 DAI in the treated as compare to control (6.09, 6.33, 6.83, 6.52, 6.70 and 7.01 mg kg<sup>-1</sup>), respectively. Similar results were also obtained by Dotaniya *et al.* (2014) and reported that, available P in soil solution increased with higher levels of organic residues as well as increasing incubation time. Shelke (2017) concluded that application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased the soil available phosphorus (17.55, 23.40, 23.64, 23.78, 23.92 and 23.98 kg ha<sup>-1</sup>, respectively) at 7, 45, 60, 90, 120 and 150 days of incubation.

Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of phosphorus in soil. However, it is necessary to optimize the levels of phytase to release the soil available phosphorus.

#### **4.1.1.7 Effect of phytase and phosphorus levels on periodical soil available potassium in incubation**

The periodical soil available potassium content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.7).

##### **4.1.1.7.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil available potassium at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest increased by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (201.01, 209.76, 217.31 and 241.85, 233.60 and 223.60 mg kg<sup>-1</sup>, respectively). However, the soil available potassium was numerically increased with an advanced period of incubation over 0 days of incubation.

But, it was numerically the highest at 30 days of incubation and decreased at 45 and 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of organically bound potassium by phytase enzyme. Simultaneously, the carbon also released from organic matter in which potassium was bound. These released organic carbon act as source of energy to microorganisms responsible for potassium mineralization in soil. These results were accordance with the observations of Gujar *et al.* (2013). The increased in soil available potassium which might be due to solubilization of fixed and non-exchangeable potassium by secretion of organic acids by microorganisms.

#### **4.1.1.7.2 Phosphorus levels**

The phosphorus levels were found to release the soil available potassium during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly higher in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30 and 60 days of incubation (200.61, 209.36, 216.94, 241.36 and 223.11 mg kg<sup>-1</sup>, respectively). The soil available phosphorus was increased with an advanced period of incubation and numerically it was the highest at 30 days (241.36 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation. At 45 days of incubation showed non significant. The levels of phytase and phosphorus showed the similar trend for soil available phosphorus in an incubation study. Similar results obtained by Santhy *et al.* (1998) and reported that, available potassium content of soil was increased over the initial value under 100 % NPK+ FYM.

#### **4.1.1.7.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for soil available potassium periodically in incubation study, except 45 and 60 days of incubation. The interactions of increased levels of phytase and phosphorus were significant for periodical release of soil available potassium in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the soil available potassium also increased. Similar results found by Gujar *et al.* (2013).

**Table 4.7. Effect of Phytase and Phosphorus Levels on Periodical Soil Available Potassium at Various Days of Incubation**

Treatment	Available potassium (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	196.60	205.35	212.93	237.35	229.10	219.10
P <sub>2</sub> (2400 IU)	199.04	208.93	216.52	240.96	232.71	222.71
P <sub>3</sub> (3600 IU)	200.55	209.31	216.83	241.36	233.11	223.11
P <sub>4</sub> (4800 IU)	201.01	209.76	217.31	241.85	233.60	223.60
<b>SEm ±</b>	<b>0.03</b>	<b>0.12</b>	<b>0.26</b>	<b>0.09</b>	<b>0.38</b>	<b>0.35</b>
<b>C.D at 5 (%)</b>	<b>0.088</b>	<b>0.34</b>	<b>0.76</b>	<b>0.27</b>	<b>1.17</b>	<b>1.03</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	197.89	207.81	215.46	239.98	231.73	221.73
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	199.30	208.05	215.55	240.05	231.80	221.80
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	199.39	208.14	215.64	240.14	231.89	221.89
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	200.61	209.36	216.94	241.36	233.11	223.11
<b>SEm ±</b>	<b>0.03</b>	<b>0.12</b>	<b>0.26</b>	<b>0.09</b>	<b>0.38</b>	<b>0.35</b>
<b>C.D at 5 (%)</b>	<b>0.088</b>	<b>0.34</b>	<b>0.76</b>	<b>0.27</b>	<b>NS</b>	<b>1.03</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	195.40	204.15	211.65	236.15	227.90	217.90
P <sub>1</sub> F <sub>2</sub>	195.45	204.20	211.70	236.20	227.95	217.95
P <sub>1</sub> F <sub>3</sub>	195.50	204.25	211.75	236.25	228.00	218.00
P <sub>1</sub> F <sub>4</sub>	200.05	208.80	216.63	240.80	232.55	222.55
P <sub>2</sub> F <sub>1</sub>	195.40	208.75	216.58	240.85	232.60	222.60
P <sub>2</sub> F <sub>2</sub>	200.15	208.90	216.40	240.90	232.65	222.65
P <sub>2</sub> F <sub>3</sub>	200.25	209.00	216.50	241.00	232.75	222.75
P <sub>2</sub> F <sub>4</sub>	200.35	209.08	216.60	241.10	232.85	222.85
P <sub>3</sub> F <sub>1</sub>	200.20	209.00	216.58	241.20	232.95	222.95
P <sub>3</sub> F <sub>2</sub>	200.55	209.30	216.80	241.30	233.05	223.05
P <sub>3</sub> F <sub>3</sub>	200.65	209.40	216.90	241.40	233.15	223.15
P <sub>3</sub> F <sub>4</sub>	200.80	209.55	217.05	241.55	233.30	223.30
P <sub>4</sub> F <sub>1</sub>	200.58	209.33	217.03	241.70	233.45	223.45
P <sub>4</sub> F <sub>2</sub>	201.05	209.80	217.30	241.80	233.55	223.55
P <sub>4</sub> F <sub>3</sub>	201.15	209.90	217.40	241.90	233.65	223.65
P <sub>4</sub> F <sub>4</sub>	201.25	210.00	217.50	242.00	233.75	223.75
<b>SEm ±</b>	<b>0.06</b>	<b>0.24</b>	<b>0.53</b>	<b>0.19</b>	<b>0.78</b>	<b>0.72</b>
<b>C.D at 5 (%)</b>	<b>0.18</b>	<b>0.70</b>	<b>1.53</b>	<b>0.56</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	199.30	208.34	215.90	240.38	232.13	222.13
Control	162.25	162.75	165.00	166.50	167.75	166.00
<b>SEm ±</b>	<b>0.06</b>	<b>0.24</b>	<b>0.54</b>	<b>0.2</b>	<b>0.8</b>	<b>0.73</b>
<b>C.D at 5 (%)</b>	<b>0.18</b>	<b>0.71</b>	<b>1.57</b>	<b>0.57</b>	<b>2.30</b>	<b>2.12</b>
<b>Initial</b>	<b>162.00</b>					

In respect of treated vs. control, the periodical results of available potassium content in soil at 0, 7, 15, 30, 45 and 60 DAI significantly recorded the highest in treatment of treated (199.30, 208.34, 215.90, 240.38, 232.13 and 222.13 mg kg<sup>-1</sup>), respectively as compare to control (162.25, 162.75, 165.00, 166.50, 167.75 and 166.00 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of potassium in soil. However, it is necessary to optimize the levels of phytase to release the soil available potassium.

#### **4.1.2 Effect of Phytase and Phosphorus Levels on Periodical Forms of Phosphorus in Soil in Incubation Study**

##### **4.1.2.1 Saloid-P**

The periodical soil Saloid-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.8 and depicted in Fig. 4.2).

##### **4.1.2.1.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil Saloid-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest increased by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (11.53, 12.76, 13.50, 14.34, 16.00 and 14.98 mg kg<sup>-1</sup>, respectively). However, the soil Saloid-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of organically bound phosphate by phytase enzyme. This might be due to hydrolysis of organically bound phosphate and release of soluble phosphorus in the form of Saloid-P.

##### **4.1.2.1.2 Phosphorus levels**

The phosphorus levels were found to release the soil Saloid-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (11.95, 13.48, 14.69, 15.64, 17.62 and

**Table 4.8. Effect of Phytase and Phosphorus Levels on Periodical Saloid-P in Soil at Various Days of Incubation**

Treatment	Saloid- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	11.05	12.22	12.84	13.45	14.81	14.10
P <sub>2</sub> (2400 IU)	11.12	12.36	13.06	13.72	15.24	14.34
P <sub>3</sub> (3600 IU)	11.31	12.53	13.24	13.95	15.57	14.64
P <sub>4</sub> (4800 IU)	11.53	12.76	13.50	14.34	16.00	14.98
<b>SEm ±</b>	<b>0.044</b>	<b>0.038</b>	<b>0.032</b>	<b>0.024</b>	<b>0.043</b>	<b>0.031</b>
<b>C.D at 5 (%)</b>	<b>0.12</b>	<b>0.10</b>	<b>0.09</b>	<b>0.06</b>	<b>0.12</b>	<b>0.09</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	10.64	11.29	11.62	11.91	13.05	12.41
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	10.79	11.95	12.14	12.76	13.81	12.93
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	11.62	13.14	14.19	15.14	17.15	16.10
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	11.95	13.48	14.69	15.64	17.62	16.62
<b>SEm ±</b>	<b>0.044</b>	<b>0.038</b>	<b>0.032</b>	<b>0.024</b>	<b>0.043</b>	<b>0.031</b>
<b>C.D at 5 (%)</b>	<b>0.12</b>	<b>0.10</b>	<b>0.09</b>	<b>0.06</b>	<b>0.12</b>	<b>0.09</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	10.38	10.95	11.43	11.62	12.48	12.10
P <sub>1</sub> F <sub>2</sub>	10.57	11.62	11.92	12.38	13.05	12.48
P <sub>1</sub> F <sub>3</sub>	11.33	12.86	13.72	14.67	16.67	15.62
P <sub>1</sub> F <sub>4</sub>	11.91	13.43	14.29	15.13	17.03	16.19
P <sub>2</sub> F <sub>1</sub>	10.48	11.14	11.62	11.81	12.86	12.29
P <sub>2</sub> F <sub>2</sub>	10.57	11.81	12.05	12.57	13.62	12.67
P <sub>2</sub> F <sub>3</sub>	11.53	13.05	14.10	15.05	17.05	16.00
P <sub>2</sub> F <sub>4</sub>	11.91	13.43	14.48	15.43	17.43	16.38
P <sub>3</sub> F <sub>1</sub>	10.76	11.33	11.62	12.00	13.24	12.48
P <sub>3</sub> F <sub>2</sub>	10.86	12.10	12.19	12.76	14.00	13.14
P <sub>3</sub> F <sub>3</sub>	11.72	13.24	14.29	15.24	17.24	16.19
P <sub>3</sub> F <sub>4</sub>	11.91	13.43	14.86	15.81	17.81	16.76
P <sub>4</sub> F <sub>1</sub>	10.95	11.72	11.81	12.19	13.62	12.76
P <sub>4</sub> F <sub>2</sub>	11.14	12.29	12.38	13.34	14.57	13.43
P <sub>4</sub> F <sub>3</sub>	11.91	13.43	14.67	15.62	17.62	16.57
P <sub>4</sub> F <sub>4</sub>	12.10	13.62	15.12	16.19	18.19	17.15
<b>SEm ±</b>	<b>0.09</b>	<b>0.08</b>	<b>0.06</b>	<b>0.05</b>	<b>0.09</b>	<b>0.06</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>0.19</b>	<b>0.14</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	11.25	12.47	13.16	13.86	15.41	14.51
Control	10.20	10.15	10.19	10.26	10.27	10.21
<b>SEm ±</b>	<b>0.091</b>	<b>0.078</b>	<b>0.066</b>	<b>0.049</b>	<b>0.090</b>	<b>0.065</b>
<b>C.D at 5 (%)</b>	<b>0.26</b>	<b>0.22</b>	<b>0.19</b>	<b>0.14</b>	<b>0.26</b>	<b>0.18</b>
<b>Initial</b>	<b>8.19</b>					

16.62 mg kg<sup>-1</sup> of soil respectively). The soil Saloid-P was increased with an advanced period of incubation and numerically it was the highest at 45 days (17.62 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil Saloid-P in an incubation study. Saloid-P increased might be due to solubilization of added superphosphate by the various microorganisms. Similar results also obtained by Shelke (2017) the adding of phosphorus fertilizers caused the enrichment of soil saloid-P; and the values of fertilized samples are higher compared to control by addition of single superphosphate during incubation.

#### **4.1.2.1.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for soil Saloid-P at 15 and 30 days of incubation. The interactions of increased levels of phytase and phosphorus were significant for periodical release of Saloid-P in an incubation study at 15 and 30 days. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the Saloid-P also increased. This might be due to the levels of phytase concentration and levels of phosphorus as concentration of substrate were optimum to release the phosphorus in soil.

In respect of treated vs control, the periodical results of Saloid-P at 0, 7, 15, 30, 45 and 60 DAI significantly recorded the highest in the treated (11.25, 12.47, 13.16, 13.86, 15.41 and 14.51 mg kg<sup>-1</sup>), respectively as compare to control (10.20, 10.15, 10.19, 10.26, 10.27 and 10.21 mg kg<sup>-1</sup>), respectively. In general Saloid-P at incubation condition increased upto 45 DAI in all treatments later on decreased at 60 DAI. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of Saloid-P in soil.

#### **4.1.2.2 Aluminium bound-P**

The periodical soil Al-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.9 and depicted in Fig. 4.3).

#### 4.1.2.2.1 Phytase levels

The levels of phytase enzyme significantly increased the soil Al-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (46.88, 56.25, 75.94, 90.94, 104.06 and 96.56 mg kg<sup>-1</sup>, respectively) which was at par with the application of 3600 IU phytase (55.31, 75.00 and 102.19 mg kg<sup>-1</sup>), respectively at 7, 15 and 30 DAI. However, the soil Al-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of Al-P from organically bound phosphate by phytase enzyme. This might be due to hydrolysis of organically bound phosphate and release of Al-P.

#### 4.1.2.2.2 Phosphorus levels

The phosphorus levels were found to release the soil Al-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (52.50, 61.88, 88.13, 103.75, 118.13 and 110.63 mg kg<sup>-1</sup> of soil respectively). The soil Al-P was increased with an advanced period of incubation and numerically it was the highest at 45 days (118.13 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation.

The levels of phytase and phosphorus showed the similar trend for soil Al-P in an incubation study. Similar results also obtained by Dotaniya *et al.* (2014) that the mean Al-P content in soil increased with increasing levels of phosphorus application.

#### 4.1.2.2.3 Phytase and phosphorus interactions

The interactions between levels of phytase and phosphorus were non significant for soil Al-P except 30 days of incubation. The interactions of increased levels of phytase and phosphorus were non significant for periodical release of Al-P in an incubation study, except 30 days.

**Table 4.9. Effect of Phytase and Phosphorus Levels on Periodical Al-P in Soil at Various Days of Incubation**

Treatment	Al- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	43.13	51.56	68.91	80.94	93.75	86.25
P <sub>2</sub> (2400 IU)	42.88	52.50	70.31	86.81	97.97	90.47
P <sub>3</sub> (3600 IU)	45.94	55.31	75.00	89.75	102.19	94.69
P <sub>4</sub> (4800 IU)	46.88	56.25	75.94	90.94	104.06	96.56
<b>SEm ±</b>	<b>0.31</b>	<b>0.47</b>	<b>0.63</b>	<b>0.36</b>	<b>0.84</b>	<b>0.85</b>
<b>C.D at 5 (%)</b>	<b>0.92</b>	<b>1.35</b>	<b>1.82</b>	<b>1.04</b>	<b>2.44</b>	<b>2.46</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	37.50	46.41	56.25	69.38	80.63	73.13
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	40.31	49.22	61.41	75.00	85.78	78.28
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	48.50	58.13	84.38	100.31	113.44	105.94
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	52.50	61.88	88.13	103.75	118.13	110.63
<b>SEm ±</b>	<b>0.31</b>	<b>0.47</b>	<b>0.63</b>	<b>0.36</b>	<b>0.84</b>	<b>0.85</b>
<b>C.D at 5 (%)</b>	<b>0.92</b>	<b>1.35</b>	<b>1.82</b>	<b>1.04</b>	<b>2.44</b>	<b>2.46</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	35.63	43.13	50.63	61.88	73.13	65.63
P <sub>1</sub> F <sub>2</sub>	39.38	46.88	56.25	65.63	76.88	69.38
P <sub>1</sub> F <sub>3</sub>	46.88	56.25	82.50	95.63	108.75	101.25
P <sub>1</sub> F <sub>4</sub>	50.63	60.00	86.25	100.63	116.25	108.75
P <sub>2</sub> F <sub>1</sub>	35.63	45.00	54.38	69.38	78.75	71.25
P <sub>2</sub> F <sub>2</sub>	39.38	48.75	58.13	76.88	84.38	76.88
P <sub>2</sub> F <sub>3</sub>	45.88	56.25	82.50	99.38	112.50	105.00
P <sub>2</sub> F <sub>4</sub>	50.63	60.00	86.25	101.63	116.25	108.75
P <sub>3</sub> F <sub>1</sub>	39.38	48.75	60.00	73.13	84.38	76.88
P <sub>3</sub> F <sub>2</sub>	39.38	48.75	63.75	76.88	88.13	80.63
P <sub>3</sub> F <sub>3</sub>	50.63	60.00	86.25	103.13	116.25	108.75
P <sub>3</sub> F <sub>4</sub>	54.38	63.75	90.00	105.88	120.00	112.50
P <sub>4</sub> F <sub>1</sub>	39.38	48.75	60.00	73.13	86.25	78.75
P <sub>4</sub> F <sub>2</sub>	43.13	52.50	67.50	80.63	93.75	86.25
P <sub>4</sub> F <sub>3</sub>	50.63	60.00	86.25	103.13	116.25	108.75
P <sub>4</sub> F <sub>4</sub>	54.38	63.75	90.00	106.88	120.00	112.50
<b>SEm ±</b>	<b>0.64</b>	<b>0.94</b>	<b>1.27</b>	<b>0.72</b>	<b>1.70</b>	<b>1.71</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>2.09</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	44.70	53.91	72.54	87.11	99.49	91.99
Control	41.13	41.85	41.72	41.88	41.71	41.66
<b>SEm ±</b>	<b>0.65</b>	<b>0.96</b>	<b>1.30</b>	<b>0.74</b>	<b>1.75</b>	<b>1.76</b>
<b>C.D at 5 (%)</b>	<b>1.89</b>	<b>2.79</b>	<b>3.75</b>	<b>2.14</b>	<b>5.04</b>	<b>5.07</b>
<b>Initial</b>	<b>41.68</b>					

In respect of treated vs control, the periodical results of Al-P (44.70, 53.91, 72.54, 87.11, 99.49 and 91.99 mg kg<sup>-1</sup>) at 0, 7, 15, 30, 45 and 60 DAI found significantly the highest in the treated as compare to control (41.13, 41.85, 41.72, 41.88, 41.71 and 41.66 mg kg<sup>-1</sup>), respectively. In general Al-P at incubation condition increased upto 45 DAI in all treatments later on decreased at 60 DAI. Thus, the levels of phytase and phosphorus alone were found beneficial for enhancing the availability of Al-P in soil.

#### **4.1.2.3 Iron bound-P (Fe-P)**

The periodical soil Fe-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.10 and depicted in Fig. 4.4)

##### **4.1.2.3.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil Fe-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly highest by the application of 4800 IU phytase in 7, 15, 30, 45 and 60 days of incubation (31.44, 33.19, 36.41, 39.23 and 37.32 mg kg<sup>-1</sup>, respectively) which was at par with the application of 3600 IU phytase (32.87 and 38.75 mg kg<sup>-1</sup>), respectively at 15 and 45 DAI. However, the soil Fe-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of Fe-P from organically bound phosphate by phytase enzyme. This might be due to hydrolysis of organically bound phosphate and release of Fe-P. This could be attributed to the formation of Fe-P due to the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> resulting from the release of organic acids from the decomposition of organic materials are observed by Jaggi (1991).

##### **4.1.2.3.2 Phosphorus levels**

The phosphorus levels were found to release the soil Fe-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (29.22, 33.54, 36.21, 39.62, 42.88 and 40.87 mg kg<sup>-1</sup> of soil, respectively).

**Table 4.10. Effect of Phytase and Phosphorus Levels on Periodical Fe-P in Soil at Various Days of Incubation**

Treatment	Fe- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	27.00	29.54	31.29	34.24	37.16	34.94
P <sub>2</sub> (2400 IU)	27.79	30.28	31.92	34.77	37.80	35.89
P <sub>3</sub> (3600 IU)	27.95	30.81	32.87	35.27	38.75	36.84
P <sub>4</sub> (4800 IU)	27.95	31.44	33.19	36.41	39.23	37.32
<b>SEm ±</b>	<b>0.27</b>	<b>0.14</b>	<b>0.22</b>	<b>0.13</b>	<b>0.22</b>	<b>0.22</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>0.40</b>	<b>0.64</b>	<b>0.38</b>	<b>0.64</b>	<b>0.64</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	26.20	27.55	28.27	30.36	33.35	31.29
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	27.00	28.90	29.38	31.95	34.78	32.72
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	28.27	32.08	35.42	38.75	41.93	40.02
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	29.22	33.54	36.21	39.62	42.88	40.97
<b>SEm ±</b>	<b>0.27</b>	<b>0.14</b>	<b>0.22</b>	<b>0.13</b>	<b>0.22</b>	<b>0.22</b>
<b>C.D at 5 (%)</b>	<b>0.80</b>	<b>0.40</b>	<b>0.64</b>	<b>0.38</b>	<b>0.64</b>	<b>0.64</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	26.05	26.57	27.95	29.59	32.40	29.86
P <sub>1</sub> F <sub>2</sub>	26.05	28.59	28.59	31.13	33.67	31.13
P <sub>1</sub> F <sub>3</sub>	27.32	31.13	33.67	37.48	40.66	38.75
P <sub>1</sub> F <sub>4</sub>	28.59	31.90	34.94	38.75	41.93	40.02
P <sub>2</sub> F <sub>1</sub>	26.05	26.47	27.95	30.00	33.03	31.13
P <sub>2</sub> F <sub>2</sub>	26.68	28.59	29.22	31.13	34.30	32.40
P <sub>2</sub> F <sub>3</sub>	28.59	32.40	34.94	38.75	41.93	40.02
P <sub>2</sub> F <sub>4</sub>	29.86	33.67	35.57	39.19	41.93	40.02
P <sub>3</sub> F <sub>1</sub>	26.05	28.59	28.59	30.75	34.30	32.40
P <sub>3</sub> F <sub>2</sub>	27.95	28.59	29.86	31.90	35.57	33.67
P <sub>3</sub> F <sub>3</sub>	28.59	32.40	36.21	38.75	41.93	40.02
P <sub>3</sub> F <sub>4</sub>	29.22	33.67	36.84	39.69	43.20	41.29
P <sub>4</sub> F <sub>1</sub>	26.68	28.59	28.59	31.13	33.67	31.76
P <sub>4</sub> F <sub>2</sub>	27.32	29.86	29.86	33.67	35.57	33.67
P <sub>4</sub> F <sub>3</sub>	28.59	32.40	36.84	40.02	43.20	41.29
P <sub>4</sub> F <sub>4</sub>	29.22	34.94	37.48	40.83	44.47	42.56
<b>SEm ±</b>	<b>0.56</b>	<b>0.28</b>	<b>0.45</b>	<b>0.27</b>	<b>0.45</b>	<b>0.45</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>0.81</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	27.67	30.52	32.32	35.17	38.23	36.25
Control	26.05	26.05	26.82	26.51	26.46	26.37
<b>SEm ±</b>	<b>0.57</b>	<b>0.29</b>	<b>0.46</b>	<b>0.27</b>	<b>0.46</b>	<b>0.46</b>
<b>C.D at 5 (%)</b>	<b>1.64</b>	<b>0.83</b>	<b>1.33</b>	<b>0.80</b>	<b>1.32</b>	<b>1.33</b>
<b>Initial</b>	<b>26.68</b>					

The soil Fe-P was increased with an advanced period of incubation and numerically it was the highest at 45 days (42.88 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil Fe-P in an incubation study.

#### **4.1.2.3.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were non significant for soil Fe-P except 7 days of incubation. The interactions of increased levels of phytase and phosphorus were non significant for periodical release of Fe-P in an incubation study, except 7 days.

In respect of treated vs control, the periodical results of Fe-P in soil at 0, 7, 15, 30, 45 and 60 DAI found significantly the highest in treatment of treated (27.67, 30.52, 32.32, 35.17, 38.23 and 36.25 mg kg<sup>-1</sup>), respectively as compare to control (26.05, 26.05, 26.82, 26.51, 26.46 and 26.37 mg kg<sup>-1</sup>), respectively. In general, Fe-P at incubation condition increased upto 45 DAI in all treatment later on decreased at 60 DAI. Thus, the levels of phytase and phosphorus alone were found beneficial for enhancing the availability of Fe-P in soil.

#### **4.1.2.4 Calcium bound-P (Ca-P)**

The periodical soil Ca-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.11 and depicted in Fig. 4.5).

##### **4.1.2.4.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil Ca-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest increased by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (317.76, 322.42, 324.63, 326.83, 329.86 and 328.09 mg kg<sup>-1</sup>, respectively). However, the soil Ca-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme. This might be associated with the release of Ca-P from organically bound tricalcium phosphate by phytase enzyme. This might be due to hydrolysis of organically bound phosphate and release of Ca-P.

**Table 4.11. Effect of Phytase and Phosphorus Levels on Periodical Ca-P in Soil at Various Days of Incubation**

Treatment	Ca- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	317.32	320.22	321.67	322.80	325.82	323.93
P <sub>2</sub> (2400 IU)	317.19	320.72	322.55	323.81	326.83	325.07
P <sub>3</sub> (3600 IU)	317.51	321.67	323.68	325.32	328.34	326.83
P <sub>4</sub> (4800 IU)	317.76	322.42	324.63	326.83	329.86	328.09
<b>SEm ±</b>	<b>0.04</b>	<b>0.16</b>	<b>0.09</b>	<b>0.06</b>	<b>0.06</b>	<b>0.24</b>
<b>C.D at 5 (%)</b>	<b>0.12</b>	<b>0.48</b>	<b>0.26</b>	<b>0.18</b>	<b>0.17</b>	<b>0.7</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	316.63	318.39	320.09	318.39	321.41	321.29
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	317.00	318.64	320.53	318.89	321.92	321.79
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	317.89	323.49	325.70	330.49	333.51	330.17
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	318.26	324.50	326.20	330.99	334.01	330.68
<b>SEm ±</b>	<b>0.04</b>	<b>0.16</b>	<b>0.09</b>	<b>0.06</b>	<b>0.06</b>	<b>0.24</b>
<b>C.D at 5 (%)</b>	<b>0.12</b>	<b>0.48</b>	<b>0.26</b>	<b>0.18</b>	<b>0.17</b>	<b>0.7</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	316.50	318.01	319.02	317.51	320.53	320.03
P <sub>1</sub> F <sub>2</sub>	316.50	318.01	319.52	318.01	321.04	320.53
P <sub>1</sub> F <sub>3</sub>	318.01	321.79	323.81	327.59	330.61	327.34
P <sub>1</sub> F <sub>4</sub>	318.26	323.05	324.31	328.09	331.12	327.84
P <sub>2</sub> F <sub>1</sub>	316.50	318.01	319.78	318.01	321.04	321.04
P <sub>2</sub> F <sub>2</sub>	317.00	318.52	320.28	318.52	321.54	321.54
P <sub>2</sub> F <sub>3</sub>	317.51	322.55	324.82	329.10	332.12	328.60
P <sub>2</sub> F <sub>4</sub>	317.76	323.81	325.32	329.60	332.63	329.10
P <sub>3</sub> F <sub>1</sub>	316.50	318.52	320.53	318.52	321.54	321.79
P <sub>3</sub> F <sub>2</sub>	317.00	319.02	321.04	319.02	322.04	322.30
P <sub>3</sub> F <sub>3</sub>	318.01	324.06	326.33	331.62	334.64	331.37
P <sub>3</sub> F <sub>4</sub>	318.52	325.07	326.83	332.12	335.15	331.87
P <sub>4</sub> F <sub>1</sub>	317.00	319.02	321.04	319.52	322.55	322.30
P <sub>4</sub> F <sub>2</sub>	317.51	319.02	321.29	320.03	323.05	322.80
P <sub>4</sub> F <sub>3</sub>	318.01	325.57	327.84	333.64	336.66	333.38
P <sub>4</sub> F <sub>4</sub>	318.52	326.08	328.34	334.14	337.16	333.89
<b>SEm ±</b>	<b>0.08</b>	<b>0.33</b>	<b>0.18</b>	<b>0.12</b>	<b>0.12</b>	<b>0.49</b>
<b>C.D at 5 (%)</b>	<b>0.24</b>	<b>0.96</b>	<b>0.52</b>	<b>0.35</b>	<b>0.35</b>	<b>1.40</b>
Tr. Vs Control						
Treated	317.44	321.26	323.13	324.69	327.71	325.98
Control	316.01	317.00	317.51	317.51	318.01	317.76
<b>SEm ±</b>	<b>0.086</b>	<b>0.34</b>	<b>0.18</b>	<b>0.12</b>	<b>0.12</b>	<b>0.5</b>
<b>C.D at 5 (%)</b>	<b>0.24</b>	<b>0.98</b>	<b>0.53</b>	<b>0.36</b>	<b>0.36</b>	<b>1.44</b>
<b>Initial</b>	<b>316.15</b>					

#### 4.1.2.4.2 Phosphorus levels

The phosphorus levels were found to release the soil Ca-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (318.26, 324.50, 326.20, 330.99, 334.01 and 330.68 mg kg<sup>-1</sup> of soil, respectively). The soil Ca-P was increased with an advanced period of incubation and numerically it was the highest at 45 days (334.01 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation.

The levels of phytase and phosphorus showed the similar trend for soil Ca-P in an incubation study. It was observed that among all the fractions of inorganics P, Ca-P was dominant in Vertisol, which may be attributed to the presence of higher amount of exchangeable and soluble Ca which on reaction might have resulted in the formation of Ca-phosphates. The similar results were also reported by Vishwanatha and Doddamani (1991) and Sarawgi *et al.* (2012). Subramanian and Kumaraswamy (1989) and concluded that in soils with calcareous and alkaline pH, bulk of the fixed P was recovered as Ca-P, which is stable under calcareous and alkaline conditions.

#### 4.1.2.4.3 Phytase and phosphorus interactions

The interactions between levels of phytase and phosphorus were found significant for soil Ca-P periodically in incubation study. The interactions of increased levels of phytase and phosphorus were significant for periodical release of Ca-P in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the Ca-P also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to release the Ca-P in soil.

In respect of treated vs control, the periodical results of Ca-P content in soil (317.44, 321.26, 323.13, 324.69, 327.71 and 325.98 mg kg<sup>-1</sup>), respectively at 0, 7, 15, 30, 45 and 60 DAI found significantly the highest in treatment of treated as compare to control (316.01, 317.00, 317.51, 317.51, 318.01 and 317.76 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for

enhancing the availability of Ca-P in soil. However, it is necessary to optimize the levels of phytase to release the Ca-P.

#### **4.1.2.5 Reductant soluble-P (Red-P)**

The periodical soil Red-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.12 and depicted in Fig. 4.6).

##### **4.1.2.5.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil Red-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly highest by the application of 4800 IU phytase in 7, 15, 30, 45 and 60 days of incubation (78.00, 84.50, 85.80, 96.20 and 91.98 mg kg<sup>-1</sup>, respectively). However, the soil Red-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme.

##### **4.1.2.5.2 Phosphorus levels**

The phosphorus levels were found to release the soil Red-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (64.03, 82.23, 89.05, 92.95, 103.35 and 97.83 mg kg<sup>-1</sup> of soil, respectively). The soil Red-P was increased with an advanced period of incubation and numerically it was the highest at 45 days (103.35 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil Red-P in an incubation study. The similar results were also reported by Shelke (2017).

##### **4.1.2.5.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for soil Red-P periodically in incubation study, except 0 days. The interactions of increased levels of phytase and phosphorus were significant for periodical release of Red-P in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the Red-P also increased. This might be because of the levels

**Table 4.12 Effect of Phytase and Phosphorus Levels on Periodical Red-P in Soil at Various Days of Incubation**

Treatment	Red- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	60.13	69.55	74.75	77.03	84.50	80.28
P <sub>2</sub> (2400 IU)	61.10	69.55	77.03	78.00	88.40	83.20
P <sub>3</sub> (3600 IU)	60.45	72.80	79.30	81.90	92.30	87.10
P <sub>4</sub> (4800 IU)	61.43	78.00	84.50	85.80	96.20	91.98
<b>SEm ±</b>	<b>0.50</b>	<b>0.67</b>	<b>0.28</b>	<b>0.47</b>	<b>0.16</b>	<b>0.21</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>1.93</b>	<b>0.81</b>	<b>1.36</b>	<b>0.47</b>	<b>0.62</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	58.18	63.70	68.90	68.90	77.35	73.45
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	59.48	65.33	71.18	70.53	79.95	75.73
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	61.43	78.65	86.45	90.35	100.75	95.55
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	64.03	82.23	89.05	92.95	103.35	97.83
<b>SEm ±</b>	<b>0.50</b>	<b>0.67</b>	<b>0.28</b>	<b>0.47</b>	<b>0.16</b>	<b>0.21</b>
<b>C.D at 5 (%)</b>	<b>1.44</b>	<b>1.93</b>	<b>0.81</b>	<b>1.36</b>	<b>0.47</b>	<b>0.62</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	58.50	63.70	66.30	68.90	71.50	68.90
P <sub>1</sub> F <sub>2</sub>	58.50	65.00	68.90	67.60	74.10	70.20
P <sub>1</sub> F <sub>3</sub>	61.10	74.10	80.60	84.50	94.90	89.70
P <sub>1</sub> F <sub>4</sub>	62.40	75.40	83.20	87.10	97.50	92.30
P <sub>2</sub> F <sub>1</sub>	58.50	63.70	68.90	66.30	76.70	71.50
P <sub>2</sub> F <sub>2</sub>	61.10	63.70	70.20	68.90	79.30	74.10
P <sub>2</sub> F <sub>3</sub>	61.10	71.50	83.20	87.10	97.50	92.30
P <sub>2</sub> F <sub>4</sub>	63.70	79.30	85.80	89.70	100.10	94.90
P <sub>3</sub> F <sub>1</sub>	57.20	61.10	67.60	68.90	79.30	74.10
P <sub>3</sub> F <sub>2</sub>	58.50	63.70	70.20	71.50	81.90	76.70
P <sub>3</sub> F <sub>3</sub>	61.10	81.90	88.40	92.30	102.70	97.50
P <sub>3</sub> F <sub>4</sub>	65.00	84.50	91.00	94.90	105.30	100.10
P <sub>4</sub> F <sub>1</sub>	58.50	66.30	72.80	71.50	81.90	79.30
P <sub>4</sub> F <sub>2</sub>	59.80	68.90	75.40	74.10	84.50	81.90
P <sub>4</sub> F <sub>3</sub>	62.40	87.10	93.60	97.50	107.90	102.70
P <sub>4</sub> F <sub>4</sub>	65.00	89.70	96.20	100.10	110.50	104.00
<b>SEm ±</b>	<b>1.00</b>	<b>1.34</b>	<b>0.57</b>	<b>0.95</b>	<b>0.33</b>	<b>0.43</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>3.87</b>	<b>1.64</b>	<b>2.72</b>	<b>0.96</b>	<b>1.24</b>
Tr. Vs Control						
Treated	60.78	72.48	78.89	80.68	90.35	85.64
Control	58.50	61.10	63.70	63.70	63.67	63.70
<b>SEm ±</b>	<b>1.03</b>	<b>1.38</b>	<b>0.58</b>	<b>0.97</b>	<b>0.34</b>	<b>0.44</b>
<b>C.D at 5 (%)</b>	<b>2.97</b>	<b>3.98</b>	<b>1.68</b>	<b>2.80</b>	<b>0.98</b>	<b>1.28</b>
<b>Initial</b>	<b>63.70</b>					

of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to release the Red-P in soil.

In respect of treated vs control, the periodical results of Red-P found significantly the highest (60.78, 72.48, 78.89, 80.68, 90.35 and 85.64 mg kg<sup>-1</sup>), respectively at 0, 7, 15, 30, 45 and 60 DAI in the treated as compare to control (58.50, 61.10, 63.70, 63.70, 63.67 and 63.70 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of Red-P in soil.

#### **4.1.2.6 Occluded-P (Occl-P)**

The periodical soil Occl-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.13 and depicted in fig. 4.7).

##### **4.1.2.6.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil Occl-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest increased by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (7.49, 10.99, 11.87, 12.03, 13.67 and 12.91 mg kg<sup>-1</sup>, respectively). However, the soil Occl-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 45 days of incubation and decreased at 60 days of incubation in all the levels of phytase enzyme.

##### **4.1.2.6.2 Phosphorus levels**

The phosphorus levels were found to release the soil Occl-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (7.60, 11.70, 12.58, 12.85, 14.77 and 13.89 mg kg<sup>-1</sup> of soil, respectively). The soil Occl-P was increased with an advanced period of incubation and numerically it was the highest at 45 days (103.35 mg kg<sup>-1</sup>) of incubation and decreased at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil Occl-P in an incubation study. The similar results were also reported by Shelke (2017).

**Table 4.13. Effect of Phytase and Phosphorus Levels on Periodical Occl-P in Soil at Various Days of Incubation**

Treatment	Occl- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	6.89	8.75	9.52	9.41	11.43	10.50
P <sub>2</sub> (2400 IU)	7.00	9.63	10.39	10.50	12.25	11.38
P <sub>3</sub> (3600 IU)	7.33	10.39	11.27	11.38	13.13	12.25
P <sub>4</sub> (4800 IU)	7.49	10.99	11.87	12.03	13.67	12.91
<b>SEm ±</b>	<b>0.075</b>	<b>0.060</b>	<b>0.059</b>	<b>0.085</b>	<b>0.061</b>	<b>0.029</b>
<b>C.D at 5 (%)</b>	<b>0.21</b>	<b>0.17</b>	<b>0.17</b>	<b>0.24</b>	<b>0.17</b>	<b>0.08</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	6.73	8.20	8.97	8.75	10.50	9.63
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	7.00	8.53	9.30	9.13	10.94	10.06
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	7.38	11.32	12.20	12.58	14.27	13.45
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	7.60	11.70	12.58	12.85	14.77	13.89
<b>SEm ±</b>	<b>0.075</b>	<b>0.060</b>	<b>0.059</b>	<b>0.085</b>	<b>0.061</b>	<b>0.029</b>
<b>C.D at 5 (%)</b>	<b>0.21</b>	<b>0.17</b>	<b>0.17</b>	<b>0.24</b>	<b>0.17</b>	<b>0.08</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	6.56	7.66	8.31	8.09	9.84	8.97
P <sub>1</sub> F <sub>2</sub>	6.78	8.09	8.75	8.32	10.28	9.41
P <sub>1</sub> F <sub>3</sub>	7.22	9.41	10.28	10.72	12.69	11.59
P <sub>1</sub> F <sub>4</sub>	7.00	9.84	10.72	10.50	12.91	12.03
P <sub>2</sub> F <sub>1</sub>	6.56	8.09	8.75	8.53	10.28	9.41
P <sub>2</sub> F <sub>2</sub>	6.78	8.53	9.19	8.97	10.72	9.84
P <sub>2</sub> F <sub>3</sub>	7.22	10.72	11.59	12.03	13.78	12.91
P <sub>2</sub> F <sub>4</sub>	7.44	11.16	12.03	12.47	14.22	13.34
P <sub>3</sub> F <sub>1</sub>	6.78	8.53	9.41	8.97	10.72	9.84
P <sub>3</sub> F <sub>2</sub>	7.22	8.53	9.41	9.41	11.16	10.28
P <sub>3</sub> F <sub>3</sub>	7.44	12.03	12.91	13.34	15.09	14.22
P <sub>3</sub> F <sub>4</sub>	7.88	12.47	13.34	13.78	15.53	14.66
P <sub>4</sub> F <sub>1</sub>	7.00	8.53	9.41	9.41	11.16	10.28
P <sub>4</sub> F <sub>2</sub>	7.22	8.97	9.84	9.84	11.59	10.72
P <sub>4</sub> F <sub>3</sub>	7.66	13.13	14.00	14.22	15.53	15.09
P <sub>4</sub> F <sub>4</sub>	8.09	13.34	14.22	14.66	16.41	15.53
<b>SEm ±</b>	<b>0.15</b>	<b>0.12</b>	<b>0.12</b>	<b>0.17</b>	<b>0.12</b>	<b>0.06</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>0.35</b>	<b>0.34</b>	<b>0.49</b>	<b>0.36</b>	<b>0.17</b>
Tr. Vs Control						
Treated	7.18	9.94	10.76	10.83	12.62	11.76
Control	7.00	7.22	7.33	7.51	7.63	7.47
<b>SEm ±</b>	<b>0.15</b>	<b>0.12</b>	<b>0.12</b>	<b>0.17</b>	<b>0.12</b>	<b>0.060</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>0.36</b>	<b>0.35</b>	<b>0.50</b>	<b>0.36</b>	<b>0.17</b>
<b>Initial</b>	<b>7.66</b>					

#### 4.1.2.6.3 Phytase and phosphorus interactions

The interactions between levels of phytase and phosphorus were found significant for soil Occl-P periodically in incubation study, except 0 days. The interactions of increased levels of phytase and phosphorus were significant for periodical release of Occl-P in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the Occl-P also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to release the Occl-P in soil.

In respect of treated vs control, the periodical results found significantly the highest of Occl-P in soil (9.94, 10.76, 10.83, 12.62 and 11.76 mg kg<sup>-1</sup>), respectively at 7, 15, 30, 45 and 60 DAI in the treated as compare to control (7.00, 7.22, 7.33, 7.51, 7.63 and 7.47 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of Occl-P in soil.

#### 4.1.2.7 Organic P

The periodical soil Org-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.14 and depicted in Fig. 4.8).

##### 4.1.2.7.1 Phytase levels

The levels of phytase enzyme significantly decreased the soil Org-P at all the periods of incubation study. It was decreased with an increased level of phytase and significantly the highest decreased by the application of 4800 IU phytase in 0, 7, 15, 30, 45 and 60 days of incubation (246.56, 234.95, 209.54, 201.38, 173.78 and 199.09 mg kg<sup>-1</sup>, respectively). However, the soil Org-P was numerically decreased with an advanced period of incubation over 0 days of incubation. But, it was numerically the lowest at 45 days of incubation and slightly increased at 60 days of incubation in all the levels of phytase enzyme. This might be due to dissociation of organic matter and release of soluble phosphorus from the organic phosphate ions in the soil.

**Table 4.14. Effect of Phytase and Phosphorus Levels on Periodical Org-P in Soil at Various Days of Incubation**

Treatment	Organic- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	261.09	243.58	221.53	213.40	188.85	210.66
P <sub>2</sub> (2400 IU)	255.67	243.55	218.40	209.99	184.17	207.92
P <sub>3</sub> (3600 IU)	249.73	238.88	212.09	202.55	174.91	198.44
P <sub>4</sub> (4800 IU)	246.56	234.95	209.54	201.38	173.78	199.09
<b>SEm ±</b>	<b>3.25</b>	<b>0.85</b>	<b>0.87</b>	<b>1.15</b>	<b>0.97</b>	<b>1.10</b>
<b>C.D at 5 (%)</b>	<b>9.38</b>	<b>2.47</b>	<b>2.52</b>	<b>3.32</b>	<b>2.80</b>	<b>3.16</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	266.28	251.02	237.52	236.60	213.07	233.14
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	256.74	247.78	231.50	231.09	207.27	227.89
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	247.67	234.44	198.00	181.37	153.02	179.70
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	242.35	227.73	194.54	178.26	148.38	175.39
<b>SEm ±</b>	<b>3.25</b>	<b>0.85</b>	<b>0.87</b>	<b>1.15</b>	<b>0.97</b>	<b>1.10</b>
<b>C.D at 5 (%)</b>	<b>9.38</b>	<b>2.47</b>	<b>2.52</b>	<b>3.32</b>	<b>2.80</b>	<b>3.16</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	288.94	254.64	246.08	239.74	222.51	239.98
P <sub>1</sub> F <sub>2</sub>	259.28	249.00	238.33	239.33	218.45	237.41
P <sub>1</sub> F <sub>3</sub>	250.26	236.85	202.88	189.55	160.92	186.54
P <sub>1</sub> F <sub>4</sub>	245.87	233.84	198.82	184.99	153.53	178.73
P <sub>2</sub> F <sub>1</sub>	263.34	252.24	238.35	240.90	217.33	238.98
P <sub>2</sub> F <sub>2</sub>	260.61	249.83	235.72	233.03	211.20	233.23
P <sub>2</sub> F <sub>3</sub>	252.84	240.99	201.38	183.79	155.38	181.04
P <sub>2</sub> F <sub>4</sub>	245.90	231.16	198.14	182.24	152.77	178.43
P <sub>3</sub> F <sub>1</sub>	260.39	250.37	234.51	232.67	206.51	228.11
P <sub>3</sub> F <sub>2</sub>	256.14	251.57	230.88	228.53	202.26	223.94
P <sub>3</sub> F <sub>3</sub>	244.64	228.90	193.21	175.88	147.47	172.88
P <sub>3</sub> F <sub>4</sub>	237.76	224.71	189.78	173.14	143.41	168.81
P <sub>4</sub> F <sub>1</sub>	252.47	246.82	231.15	233.12	205.91	225.51
P <sub>4</sub> F <sub>2</sub>	250.94	240.72	221.05	223.46	197.08	216.96
P <sub>4</sub> F <sub>3</sub>	242.93	231.03	194.52	176.27	148.30	178.34
P <sub>4</sub> F <sub>4</sub>	239.88	221.23	191.43	172.67	143.80	175.57
<b>SEm ±</b>	<b>6.52</b>	<b>1.72</b>	<b>1.75</b>	<b>2.31</b>	<b>1.94</b>	<b>2.20</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>4.95</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>6.34</b>
Tr. Vs Control						
Treated	253.26	240.24	215.39	206.83	180.43	204.03
Control	255.78	263.82	262.46	254.76	259.44	252.97
<b>SEm ±</b>	<b>6.71</b>	<b>1.76</b>	<b>1.80</b>	<b>2.37</b>	<b>2.0</b>	<b>2.26</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>5.09</b>	<b>5.20</b>	<b>6.84</b>	<b>5.77</b>	<b>6.53</b>
<b>Initial</b>	<b>253.34</b>					

#### 4.1.2.7.2 Phosphorus levels

The phosphorus levels were found to release the soil Org-P during incubation study in the laboratory under ambient condition. It was decreased with increased levels of phosphorus and significantly the lowest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (242.35, 227.73, 194.54, 178.26, 148.38 and 175.39 mg kg<sup>-1</sup> of soil, respectively). The soil Org-P was decreased with an advanced period of incubation and numerically it was the lowest at 45 days (148.38 mg kg<sup>-1</sup>) of incubation and slightly increased at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil Org-P in an incubation study. The similar results were also reported by Shelke (2017).

#### 4.1.2.7.3 Phytase and phosphorus interactions

The interactions between levels of phytase and phosphorus were non significant for soil Org-P periodically in incubation study, except 7 and 60 days. The interactions of increased levels of phytase and phosphorus were non significant for periodical release of Org-P in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increases the Org-P also decreased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to reduce the Org-P in soil.

In respect of treated vs control, the periodical results were significantly decreased the organic-P in soil (240.24, 215.39, 206.83, 180.43 and 204.03 mg kg<sup>-1</sup>), respectively at 7, 15, 30, 45 and 60 DAI in the treated as compare to control (263.82, 262.46, 254.76, 259.44 and 252.97 mg kg<sup>-1</sup>), respectively except 0 DAI. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for reducing the Org-P in soil and increasing inorganic-P.

#### 4.1.2.8 Total-P

The periodical soil Total-P content in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.15 and depicted in Fig. 4.9).

**Table 4.15. Effect of Phytase and Phosphorus Levels on Periodical Total-P in Soil at Various Days of Incubation**

Treatment	Total- P (mg kg <sup>-1</sup> )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	720.22	735.42	740.49	751.26	756.32	760.66
P <sub>2</sub> (2400 IU)	722.75	738.59	743.66	757.59	762.66	768.26
P <sub>3</sub> (3600 IU)	720.22	742.39	747.46	760.13	765.19	770.79
P <sub>4</sub> (4800 IU)	719.59	746.82	753.16	767.73	772.79	780.93
<b>SEm ±</b>	<b>0.55</b>	<b>0.55</b>	<b>1.00</b>	<b>0.97</b>	<b>0.98</b>	<b>1.08</b>
<b>C.D at 5 (%)</b>	<b>1.59</b>	<b>1.59</b>	<b>2.90</b>	<b>2.81</b>	<b>2.81</b>	<b>3.12</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	715.78	726.55	731.62	744.29	749.36	754.32
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	718.32	730.35	735.42	749.36	754.42	759.39
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	722.75	751.26	756.32	768.99	774.06	780.93
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	725.92	755.06	761.39	774.06	779.13	786.00
<b>SEm ±</b>	<b>0.55</b>	<b>0.55</b>	<b>1.00</b>	<b>0.97</b>	<b>0.98</b>	<b>1.08</b>
<b>C.D at 5 (%)</b>	<b>1.59</b>	<b>1.59</b>	<b>2.90</b>	<b>2.81</b>	<b>2.81</b>	<b>3.12</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	717.05	724.65	729.72	737.32	742.39	745.46
P <sub>1</sub> F <sub>2</sub>	717.05	727.19	732.25	742.39	747.46	750.52
P <sub>1</sub> F <sub>3</sub>	722.12	742.39	747.46	760.13	765.19	770.79
P <sub>1</sub> F <sub>4</sub>	724.65	747.46	752.52	765.19	770.26	775.86
P <sub>2</sub> F <sub>1</sub>	717.05	724.65	729.72	744.92	749.99	755.59
P <sub>2</sub> F <sub>2</sub>	722.12	729.72	734.79	749.99	755.06	760.66
P <sub>2</sub> F <sub>3</sub>	724.65	747.46	752.52	765.19	770.26	775.86
P <sub>2</sub> F <sub>4</sub>	727.19	752.52	757.59	770.26	775.33	780.93
P <sub>3</sub> F <sub>1</sub>	717.05	727.19	732.25	744.92	749.99	755.59
P <sub>3</sub> F <sub>2</sub>	717.05	732.25	737.32	749.99	755.06	760.66
P <sub>3</sub> F <sub>3</sub>	722.12	752.52	757.59	770.26	775.33	780.93
P <sub>3</sub> F <sub>4</sub>	724.65	757.59	762.66	775.33	780.40	786.00
P <sub>4</sub> F <sub>1</sub>	711.98	729.72	734.79	749.99	755.06	760.66
P <sub>4</sub> F <sub>2</sub>	717.05	732.25	737.32	755.06	760.13	765.73
P <sub>4</sub> F <sub>3</sub>	722.12	762.66	767.73	780.40	785.46	796.13
P <sub>4</sub> F <sub>4</sub>	727.19	762.66	772.79	785.46	790.53	801.20
<b>SEm ±</b>	<b>1.11</b>	<b>1.11</b>	<b>2.02</b>	<b>1.96</b>	<b>1.96</b>	<b>2.17</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>3.19</b>	<b>5.80</b>	<b>NS</b>	<b>NS</b>	<b>6.26</b>
Tr. Vs Control						
Treated	720.69	740.81	746.19	759.17	764.24	770.16
Control	724.65	727.19	729.72	722.12	727.19	720.12
<b>SEm ±</b>	<b>1.14</b>	<b>1.14</b>	<b>2.07</b>	<b>2.01</b>	<b>2.01</b>	<b>2.24</b>
<b>C.D at 5 (%)</b>	<b>3.28</b>	<b>3.28</b>	<b>5.98</b>	<b>5.80</b>	<b>5.80</b>	<b>6.45</b>
<b>Initial</b>	<b>719.39</b>					

#### **4.1.2.8.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil total-P at all the periods of incubation study. It was increased with an increased level of phytase and significantly highest by the application of 4800 IU phytase in 7, 15, 30, 45 and 60 days of incubation (746.82, 753.16, 767.73, 772.79 and 780.93 mg kg<sup>-1</sup>, respectively) and total-P increased by the application of 2400 IU phytase (722.75 mg kg<sup>-1</sup>) at 0 DAI. However, the soil Total-P was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 60 days of incubation in all the levels of phytase enzyme. This might be due to dissociation of organic matter and release of inorganic and organic phosphate ions in the soil.

#### **4.1.2.8.2 Phosphorus levels**

The phosphorus levels were found to release the soil total-P during incubation study in the laboratory under ambient condition. It was increased with increased levels of phosphorus and significantly highest in 75 kg ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (725.92, 755.06, 761.39, 774.06, 779.13 and 786.00 mg kg<sup>-1</sup> of soil, respectively). The soil Total-P was increased with an advanced period of incubation and numerically it was the highest at 60 days of incubation. The levels of phytase and phosphorus showed the similar trend for soil total-P in an incubation study. The similar results were also reported by Shelke (2017).

#### **4.1.2.8.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for soil total-P periodically in incubation study, except 0, 30 and 45 days. The interactions of increased levels of phytase and phosphorus were significant for periodical release of total-P in an incubation study. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increases the Total-P also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to increase the total-P in soil.

In respect of treated vs control, the periodical results were found significantly the highest total-P in soil (740.81, 746.19, 759.17, 764.24 and 770.16 mg kg<sup>-1</sup>), respectively during the incubation period at 7, 15, 30, 45 and 60 DAI in the treated

as compare to control (727.19, 729.72, 722.12, 727.19 and 720.12 mg kg<sup>-1</sup>), respectively except 0 DAI. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for increasing the Total-P in soil.

Shelke (2017) concluded that application of 100 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> at 7, 45, 60, 90, 120 and 150 DAI recorded significantly increased all forms of phosphorus such as saloid-P, Al-P, Fe-P, Ca-P, red-P, occl-P, org-P and total-P in 45 DAI (17.86, 27.35, 20.60, 343.45, 61.06, 10.61, 338.74 and 819.67 mg kg<sup>-1</sup>), respectively and 150 days of incubation (18.44, 26.46, 19.91, 351.47, 59.39, 10.74, 340.91 and 827.32 mg kg<sup>-1</sup>), respectively, as compared to 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### **4.1.3 Effect of Phytase and Phosphorus Levels on Periodical Soil Phosphatase Activity in Incubation Study**

#### **4.1.3.1 Soil acid phosphatase activity**

The periodical soil acid phosphatase activity in an incubation study under ambient condition in laboratory was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.16 and depicted in Fig. 4.10).

##### **4.1.3.1.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil acid phosphatase activity at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest soil acid phosphatase activity (8.70, 9.15, 11.66, 13.44, 11.14 and 10.46 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>, respectively) in 0, 7, 15, 30, 45 and 60 days of incubation by the application of 4800 IU phytase. However, the soil acid phosphatase activity was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 30 days of incubation and decreased at 45 and 60 days of incubation in all the levels of phytase enzyme. These kinds of trend of activation in enzymes activities was due to addition of organic sources acting as the sole source of carbon and energy for the heterotrophs as also reported previously by Selvi *et al.* (2004) and Qureshi *et al.* (2005). Chhonkar and Tarafdar (1981) found that the activities of enzymes were significantly and positively correlated with organic carbon, fungal, bacterial and actinomycetes population in the soil.

**Table 4.16. Effect of Phytase and Phosphorus Levels on Periodical Soil Acid Phosphatase Activity at Various Days of Incubation**

Treatment	Acid phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	7.86	8.35	11.34	12.91	10.70	9.74
P <sub>2</sub> (2400 IU)	8.10	8.38	11.44	13.16	10.81	10.01
P <sub>3</sub> (3600 IU)	8.10	8.55	11.53	13.30	11.07	10.07
P <sub>4</sub> (4800 IU)	8.70	9.15	11.66	13.44	11.14	10.46
<b>SEm<math>\pm</math></b>	<b>0.11</b>	<b>0.09</b>	<b>0.04</b>	<b>0.07</b>	<b>0.04</b>	<b>0.11</b>
<b>C.D at 5 (%)</b>	<b>0.33</b>	<b>0.27</b>	<b>0.12</b>	<b>0.22</b>	<b>0.13</b>	<b>0.33</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	8.92	9.19	12.01	13.89	11.44	10.61
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	8.32	8.88	11.62	13.44	11.13	10.24
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	7.89	8.44	11.31	12.97	10.76	9.86
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	7.62	7.92	11.02	12.51	10.39	9.58
<b>SEm<math>\pm</math></b>	<b>0.11</b>	<b>0.09</b>	<b>0.04</b>	<b>0.07</b>	<b>0.04</b>	<b>0.11</b>
<b>C.D at 5 (%)</b>	<b>0.33</b>	<b>0.27</b>	<b>0.12</b>	<b>0.22</b>	<b>0.13</b>	<b>0.33</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	8.54	8.88	11.84	13.67	11.17	10.27
P <sub>1</sub> F <sub>2</sub>	8.05	8.57	11.52	13.18	10.90	9.95
P <sub>1</sub> F <sub>3</sub>	7.55	8.27	11.17	12.65	10.47	9.55
P <sub>1</sub> F <sub>4</sub>	7.32	7.68	10.84	12.13	10.28	9.22
P <sub>2</sub> F <sub>1</sub>	8.98	9.12	11.89	13.96	11.33	10.55
P <sub>2</sub> F <sub>2</sub>	8.05	8.74	11.57	13.47	11.01	10.30
P <sub>2</sub> F <sub>3</sub>	7.83	8.03	11.30	12.86	10.61	9.81
P <sub>2</sub> F <sub>4</sub>	7.54	7.63	11.00	12.35	10.30	9.40
P <sub>3</sub> F <sub>1</sub>	8.67	9.03	12.00	13.94	11.54	10.61
P <sub>3</sub> F <sub>2</sub>	8.03	8.78	11.66	13.47	11.28	10.11
P <sub>3</sub> F <sub>3</sub>	7.96	8.35	11.37	13.08	10.88	9.89
P <sub>3</sub> F <sub>4</sub>	7.73	8.07	11.09	12.72	10.57	9.67
P <sub>4</sub> F <sub>1</sub>	9.51	9.74	12.33	13.98	11.72	11.01
P <sub>4</sub> F <sub>2</sub>	9.15	9.44	11.75	13.66	11.33	10.62
P <sub>4</sub> F <sub>3</sub>	8.22	9.13	11.43	13.30	11.08	10.20
P <sub>4</sub> F <sub>4</sub>	7.91	8.30	11.15	12.84	10.42	10.03
<b>SEm<math>\pm</math></b>	<b>0.23</b>	<b>0.19</b>	<b>0.09</b>	<b>0.15</b>	<b>0.09</b>	<b>0.23</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	8.19	8.61	11.49	13.20	10.93	10.07
Control	7.07	7.09	7.14	7.16	7.11	7.13
<b>SEm<math>\pm</math></b>	<b>0.23</b>	<b>0.19</b>	<b>0.09</b>	<b>0.15</b>	<b>0.09</b>	<b>0.23</b>
<b>C.D at 5 (%)</b>	<b>0.68</b>	<b>0.56</b>	<b>0.26</b>	<b>0.45</b>	<b>0.26</b>	<b>0.68</b>
<b>Initial</b>	<b>6.72</b>					

#### **4.1.3.1.2 Phosphorus levels**

The phosphorus levels were found to release the soil acid phosphatase activity during incubation study in the laboratory under ambient condition. It was decreased with an increased level of phosphorus and significantly the highest in 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (8.92, 9.19, 12.01, 13.89, 11.44 and 10.61 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>, respectively). The soil acids phosphatase activity was increased with an advanced period of incubation and numerically it was the highest at 30 days of incubation and decreased at 45 and 60 days of incubation. Yadav and Tarafdar (2004) reported that phosphorus deficiency increases the activity of both phosphatases and phytase. Similar results were obtained by Yadav and Tarafdar (2009).

#### **4.1.3.1.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus were non significant for soil acid phosphatase activity in incubation study. This might be due to addition of superphosphate in soil in that connection acid phosphatase activity showed non significant at incubation study.

In respect of treated vs control, the periodical results of acid phosphatase activity at 0, 7, 15, 30, 45 and 60 DAI found significantly the highest in the treated (8.19, 8.61, 10.07, 13.20, 11.49 and 10.93 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>), respectively as compare to control (7.07, 7.09, 7.13, 7.16, 7.14 and 7.11 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>), respectively. Thus, the levels of phytase alone were found beneficial for enhancing the acid phosphatase activity in soil. However, it is necessary to optimize the level of phytase to release the soil acid phosphatase activity.

#### **4.1.3.2 Alkaline phosphatase**

The periodical soil alkaline phosphatase activity in an incubation study under ambient condition in laboratory was significantly influenced by the phytase levels are presented in (Table 4.17 and depicted Fig. 4.11).

##### **4.1.3.2.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil alkaline phosphatase activity at all the periods of incubation study. It was increased with an increased level of phytase and significantly the highest soil alkaline phosphatase activity

(19.12, 23.12, 24.28, 22.49 and 21.48  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ , respectively) in 7, 15, 30, 45 and 60 days of incubation by the application of 4800 IU phytase which was at par with (18.49, 22.64, 23.99 and 21.12  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$  respectively) in 7, 15, 30 and 60 days of incubation by the application of 3600 IU phytase and (15.91  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ) in 0 days of incubation by the application of 3600 IU phytase. However, the soil alkaline phosphatase activity was numerically increased with an advanced period of incubation over 0 days of incubation. But, it was numerically the highest at 30 days of incubation and decreased at 45 and 60 days of incubation in all the levels of phytase enzyme. The increase in soil alkaline phosphatase activity might be due to alkaline nature of soil. These kinds of trend of activation in enzymes activities was due to addition of organic sources acting as the sole source of carbon and energy for the heterotrophs as also reported previously by Selvi *et al.* (2004) and Qureshi *et al.* (2005). Chhonkar and Tarafdar (1981) found that the activities of enzymes were significantly and positively correlated with organic carbon, fungal, bacterial and actinomycetes population in the soil.

#### **4.1.3.2.2 Phosphorus levels**

The phosphorus levels were found to release the soil alkaline phosphatase activity during incubation study in the laboratory under ambient condition. It was decreased with an increased level of phosphorus and significantly the highest in 0 kg phosphorus levels at 0, 7, 15, 30, 45 and 60 days of incubation (16.54, 18.89, 22.32, 23.82, 21.80 and 21.23  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ , respectively) which was at par with the application of 25 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ . The soil acids phosphatase activity was increased with an advanced period of incubation and numerically it was the highest at 30 days of incubation and decreased at 45 and 60 days of incubation. Yadav and Tarafdar (2004) reported that phosphorus deficiency increases the activity of both phosphatases and phytase. Similar results were obtained by Yadav and Tarafdar (2009).

#### **4.1.3.2.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus were non significant for soil alkaline phosphatase activity in incubation study. This might be due to addition of superphosphate in soil in that connection alkaline phosphatase activity showed non significant at incubation study.

**Table 4.17. Effect of Phytase and Phosphorus Levels on Periodical Alkaline Phosphatase Activity in Soil at Various Days of Incubation**

Treatment	Alkaline phosphatase ( $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ )					
	0 Days	7 Days	15 Days	30 Days	45 Days	60 Days
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	15.52	16.89	18.94	20.14	18.86	18.07
P <sub>2</sub> (2400 IU)	15.38	17.51	20.83	22.67	20.31	19.66
P <sub>3</sub> (3600 IU)	15.91	18.49	22.64	23.99	21.52	21.12
P <sub>4</sub> (4800 IU)	15.64	19.12	23.12	24.28	22.49	21.48
<b>SEm<math>\pm</math></b>	<b>0.12</b>	<b>0.27</b>	<b>0.20</b>	<b>0.26</b>	<b>0.32</b>	<b>0.38</b>
<b>C.D at 5 (%)</b>	<b>0.34</b>	<b>0.80</b>	<b>0.60</b>	<b>0.76</b>	<b>0.94</b>	<b>1.09</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	16.54	18.89	22.32	23.82	21.80	21.23
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	15.95	18.18	21.90	23.10	21.25	20.59
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	15.24	17.92	21.33	22.56	20.52	19.75
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	14.72	17.01	19.38	21.60	19.59	18.77
<b>SEm<math>\pm</math></b>	<b>0.12</b>	<b>0.27</b>	<b>0.20</b>	<b>0.26</b>	<b>0.32</b>	<b>0.38</b>
<b>C.D at 5 (%)</b>	<b>0.34</b>	<b>0.80</b>	<b>0.60</b>	<b>0.76</b>	<b>0.94</b>	<b>1.09</b>
<b>In. (P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	16.11	17.48	19.48	21.11	19.50	19.06
P <sub>1</sub> F <sub>2</sub>	15.80	17.14	19.21	20.83	19.09	18.29
P <sub>1</sub> F <sub>3</sub>	15.38	16.74	18.89	19.92	18.70	18.01
P <sub>1</sub> F <sub>4</sub>	14.80	16.19	18.19	18.70	18.14	16.94
P <sub>2</sub> F <sub>1</sub>	16.33	18.60	22.24	23.85	21.45	20.85
P <sub>2</sub> F <sub>2</sub>	15.59	17.45	21.48	22.92	20.65	20.12
P <sub>2</sub> F <sub>3</sub>	15.04	17.26	20.84	22.61	20.34	19.82
P <sub>2</sub> F <sub>4</sub>	14.55	16.74	18.75	21.29	18.79	17.86
P <sub>3</sub> F <sub>1</sub>	16.84	19.68	23.63	25.09	22.94	22.68
P <sub>3</sub> F <sub>2</sub>	16.63	18.64	23.31	24.25	21.97	21.99
P <sub>3</sub> F <sub>3</sub>	15.30	18.57	22.77	23.75	20.91	19.97
P <sub>3</sub> F <sub>4</sub>	14.87	17.07	20.85	22.87	20.28	19.85
P <sub>4</sub> F <sub>1</sub>	16.89	19.82	23.92	25.24	23.34	22.33
P <sub>4</sub> F <sub>2</sub>	15.79	19.50	23.61	24.39	23.31	21.97
P <sub>4</sub> F <sub>3</sub>	15.25	19.11	22.85	23.97	22.12	21.22
P <sub>4</sub> F <sub>4</sub>	14.66	18.06	22.12	23.53	21.18	20.43
<b>SEm<math>\pm</math></b>	<b>0.24</b>	<b>0.56</b>	<b>0.42</b>	<b>0.53</b>	<b>0.65</b>	<b>0.76</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control						
Treated	15.61	18.00	21.38	22.77	20.79	20.08
Control	11.95	11.94	11.91	11.94	11.97	11.57
<b>SEm<math>\pm</math></b>	<b>0.25</b>	<b>0.57</b>	<b>0.43</b>	<b>0.54</b>	<b>0.67</b>	<b>0.78</b>
<b>C.D at 5 (%)</b>	<b>0.72</b>	<b>1.66</b>	<b>1.24</b>	<b>1.57</b>	<b>1.93</b>	<b>2.26</b>
<b>Initial</b>	<b>11.93</b>					

In respect of treated vs control, the periodical result of alkaline phosphatase activity at 0, 7, 15, 30, 45 and 60 DAI found significantly highest in the treated (15.61, 18.00, 21.38, 22.77, 20.79 and 20.08  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ), respectively as compare to control (11.95, 11.94, 11.91, 11.94, 11.97 and 11.57  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ), respectively. Thus, the levels of phytase alone were found beneficial for enhancing the alkaline phosphatase activity in soil. However, it is necessary to optimize the level of phytase to release the soil alkaline phosphatase activity.

## **4.2 Pot Culture Study**

### **4.2.1 Effect of Phytase and Phosphorus Levels on Growth Contributing Characters at 30 DAS and at Harvest of Green Fodder Maize**

#### **4.2.1.1 Plant height**

The plant height of fodder maize in pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.18).

##### **4.2.1.1.1 Phytase levels**

The levels of phytase enzyme significantly increased the plant height at harvest of fodder maize. It was increased with an increased level of phytase and significantly the highest in application of 4800 IU phytase (154.04 cm) which was at par with the application of phytase @ 3600 IU (149.75 cm). Similar results were reported by Gujar *et al.* (2013) and concluded that, phytase isolated from *Aspergillus niger* promoted plant growth (upto 200 %).

##### **4.2.1.1.2 Phosphorus levels**

The phosphorus levels found to increase plant height in pot culture experiment. It was increased with an increased level of phosphorus and significantly the highest in 75 kg  $\text{P}_2\text{O}_5 \text{ ha}^{-1}$  at 30 days after sowing and at harvest plant height (46.19 and 161.00 cm) over rest of all the treatments. Farooq *et al.* (2016) reported that, higher the plant height (194.47 cm) was recorded in the application of higher level of phosphorus fertilizer.

**Table 4.18. Effect of Phytase and Phosphorus Levels on Growth Contributing Characters of Green Fodder Maize**

Treatment	Plant height (cm)		Number of leaves	
	30 DAS	At harvest	30 DAS	At harvest
<b>Phytase levels (P)</b>				
P <sub>1</sub> (1200 IU)	43.28	138.46	6.66	11.75
P <sub>2</sub> (2400 IU)	43.97	147.75	7.19	13.00
P <sub>3</sub> (3600 IU)	44.31	149.75	7.09	13.33
P <sub>4</sub> (4800 IU)	43.69	154.04	7.38	13.79
<b>SEm ±</b>	<b>0.29</b>	<b>1.62</b>	<b>0.06</b>	<b>0.17</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>4.69</b>	<b>0.16</b>	<b>0.51</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>				
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	41.16	131.25	6.47	10.33
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	43.22	144.67	6.94	12.67
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	44.69	153.08	7.25	13.96
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	46.19	161.00	7.66	14.92
<b>SEm ±</b>	<b>0.29</b>	<b>1.62</b>	<b>0.06</b>	<b>0.17</b>
<b>C.D at 5 (%)</b>	<b>0.84</b>	<b>4.69</b>	<b>0.16</b>	<b>0.51</b>
<b>In. (P x F)</b>				
P <sub>1</sub> F <sub>1</sub>	41.25	128.83	6.13	9.33
P <sub>1</sub> F <sub>2</sub>	42.38	138.50	6.63	11.00
P <sub>1</sub> F <sub>3</sub>	44.13	139.17	6.63	12.50
P <sub>1</sub> F <sub>4</sub>	45.38	147.33	7.25	14.17
P <sub>2</sub> F <sub>1</sub>	41.25	129.67	6.38	10.17
P <sub>2</sub> F <sub>2</sub>	43.63	146.83	7.13	12.50
P <sub>2</sub> F <sub>3</sub>	45.13	154.33	7.50	14.33
P <sub>2</sub> F <sub>4</sub>	45.88	160.17	7.75	15.00
P <sub>3</sub> F <sub>1</sub>	41.25	135.67	6.63	11.17
P <sub>3</sub> F <sub>2</sub>	44.00	145.17	6.88	13.17
P <sub>3</sub> F <sub>3</sub>	45.75	156.50	7.25	14.17
P <sub>3</sub> F <sub>4</sub>	46.25	161.67	7.63	14.83
P <sub>4</sub> F <sub>1</sub>	40.88	130.83	6.75	10.67
P <sub>4</sub> F <sub>2</sub>	42.88	148.17	7.13	14.00
P <sub>4</sub> F <sub>3</sub>	43.75	162.33	7.63	14.83
P <sub>4</sub> F <sub>4</sub>	47.25	174.83	8.00	15.67
<b>SEm ±</b>	<b>0.58</b>	<b>3.26</b>	<b>0.11</b>	<b>0.35</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control				
Treated	43.81	147.50	7.08	12.97
Control	30.63	92.33	5.13	7.83
<b>SEm ±</b>	<b>0.60</b>	<b>3.35</b>	<b>0.11</b>	<b>0.37</b>
<b>C.D at 5 (%)</b>	<b>1.73</b>	<b>9.67</b>	<b>0.34</b>	<b>1.06</b>

#### 4.2.1.1.3 Phytase and phosphorus interactions

The interaction between levels of phytase and phosphorus was non significant for plant height at 30 days after sowing and at harvest of fodder maize.

The plant height significantly recorded the highest (43.81 cm) in treated as compared to control (30.63 cm) at 30 DAS and at harvest, The highest plant height recorded in treated pots (147.50 cm) as compared to control pot (92.33 cm). Similar results were found by Massod *et al.* (2011) and Hussain *et al.* (2016). Khaliq *et al.* (2012) reported that fodder maize plant height (120.95 cm) enhanced with the application of recommendation of fertilizer 100:50 kg NP ha<sup>-1</sup> + FYM 5 t ha<sup>-1</sup>.

The length of shoot was higher in pots supplemented with phytase than uninoculated control. Tarafdar, (2008) 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 cause higher solubility (Yadav and Verma, 2012). Muhammad *et al.* (2013) and concluded that, phosphorus solubilizing 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.

#### 4.2.1.2 Number of leaves

The number of leaves of fodder maize in pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.18).

##### 4.2.1.2.1 Phytase levels

The levels of phytase enzyme significantly increased the number of leaves at 30 days after sowing and at harvest (7.38 and 13.79) of fodder maize by the application of 4800 IU phytase which was at par with the application of phytase @ 3600 IU (13.33).

##### 4.2.1.2.2 Phosphorus levels

The phosphorus levels were found to increase the number of leaves during pot culture experiment. It was increased with increased levels of phosphorus and significantly the higher in 75 kg ha<sup>-1</sup> phosphorus levels at 30 days after sowing and at harvest (7.66 and 14.92, respectively) over rest of all the treatments.

#### 4.2.1.2.3 **Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus found non significant for number of leaves in pot culture experiment. In respect of treated with phytase and phosphorus levels, the number of leaves were significantly registered higher (7.08 and 12.97) as compared to control (5.13 and 7.83), respectively. Similar results were reported by Hussain *et al.* (2016) that the number of leaves (12.67) significantly influenced by application of nitrogen and phosphorus levels of 150:90 kg ha<sup>-1</sup> in maize. In fodder maize Khaliq *et al.* (2012) reported that, number of leaves per plant (16.60) enhanced with the application of recommendation of fertilizer 100:50 kg NP ha<sup>-1</sup> + manure 5 t ha<sup>-1</sup>. Farooq *et al.* (2016) reported that, higher the plant height (194.47 cm) and number of leaves plant<sup>-1</sup> (16.00) was recorded in the application of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as compared to the 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> in green fodder maize.

#### 4.2.2 **Effect of phytase and phosphorus levels on root volume, root cation exchange capacity and root biomass of green fodder maize at harvest**

##### 4.2.2.1 **Root volume**

The root volume of fodder maize in pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.19).

##### 4.2.2.1.1 **Phytase levels**

The levels of phytase enzyme significantly increased the root volume of the root rhizosphere of green fodder maize and showed higher (20.75 ml) in the application of phytase @ 4800 IU which was at par with the application of phytase @ 3600 IU (20.00 ml). This might be due to increasing level of phosphorus in soil also improving the root development.

##### 4.2.2.1.2 **Phosphorus levels**

The phosphorus levels were found to increasing root volume significantly. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels at harvest (25.25 ml) over rest of all the treatments. This might be because of level of phosphorus provide the optimum concentration of phosphorus for root development.

**Table 4.19. Effect of Phytase and Phosphorus Levels on Root Volume, Root Cation Exchange Capacity and Root Biomass of Green Fodder Maize**

Treatment	Root volume (ml)	Root CEC (cmol kg <sup>-1</sup> )	Root biomass (g plant <sup>-1</sup> )
<b>Phytase levels (P)</b>	<b>At harvest</b>		
P <sub>1</sub> (1200 IU)	17.50	7.20	5.05
P <sub>2</sub> (2400 IU)	18.00	7.58	5.24
P <sub>3</sub> (3600 IU)	20.00	7.70	5.40
P <sub>4</sub> (4800 IU)	20.75	7.85	5.63
<b>SEm ±</b>	<b>0.80</b>	<b>0.13</b>	<b>0.11</b>
<b>C.D at 5 (%)</b>	<b>2.32</b>	<b>0.38</b>	<b>0.34</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>			
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	12.13	6.10	3.61
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	18.88	6.73	4.65
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	20.00	8.13	5.76
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	25.25	9.38	7.29
<b>SEm ±</b>	<b>0.80</b>	<b>0.13</b>	<b>0.11</b>
<b>C.D at 5 (%)</b>	<b>2.32</b>	<b>0.38</b>	<b>0.34</b>
<b>In. (P x F)</b>			
P <sub>1</sub> F <sub>1</sub>	11.00	5.20	3.10
P <sub>1</sub> F <sub>2</sub>	17.50	6.10	4.35
P <sub>1</sub> F <sub>3</sub>	19.00	8.30	6.30
P <sub>1</sub> F <sub>4</sub>	22.50	9.20	6.45
P <sub>2</sub> F <sub>1</sub>	11.50	6.30	3.90
P <sub>2</sub> F <sub>2</sub>	18.00	6.70	4.65
P <sub>2</sub> F <sub>3</sub>	19.00	8.20	5.40
P <sub>2</sub> F <sub>4</sub>	23.50	9.10	7.00
P <sub>3</sub> F <sub>1</sub>	12.50	6.50	3.65
P <sub>3</sub> F <sub>2</sub>	19.00	6.80	4.90
P <sub>3</sub> F <sub>3</sub>	20.50	8.00	5.50
P <sub>3</sub> F <sub>4</sub>	28.00	9.50	7.55
P <sub>4</sub> F <sub>1</sub>	13.50	6.40	3.80
P <sub>4</sub> F <sub>2</sub>	21.00	7.30	4.70
P <sub>4</sub> F <sub>3</sub>	21.50	8.00	5.85
P <sub>4</sub> F <sub>4</sub>	27.00	9.70	8.15
<b>SEm ±</b>	<b>1.61</b>	<b>0.26</b>	<b>0.23</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>0.68</b>
Tr. Vs Control			
Treated	19.06	7.58	5.33
Control	7.50	4.40	1.75
<b>SEm ±</b>	<b>1.66</b>	<b>0.27</b>	<b>0.24</b>
<b>C.D at 5 (%)</b>	<b>4.78</b>	<b>0.79</b>	<b>0.70</b>

#### **4.2.2.1.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus were non significant. In respect of treated with phytase and phosphorus fertilizer levels, the root volume showed significantly higher (19.06 ml) as compared to control (7.50 ml), respectively. Thus, the levels of phytase and phosphorus alone were found beneficial for enhancing the root length.

#### **4.2.2.2 Root cation exchange capacity**

The root cation exchange capacity of fodder maize in pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.19).

##### **4.2.2.2.1 Phytase levels**

The levels of phytase enzyme significantly increased the root cation exchange capacity of fodder maize. It was increased with an increased levels of phytase and significantly the highest (7.85 cmol kg<sup>-1</sup>) in application of phytase @ 4800 IU which was at par with application of phytase @ 2400 IU and Phytase @ 3600 IU (7.58 and 7.70 cmol kg<sup>-1</sup>), respectively at harvest.

##### **4.2.2.2.2 Phosphorus levels**

The phosphorus levels were found to increasing root cation exchange capacity. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels f (9.38 cmol kg<sup>-1</sup>) over rest of all the treatments at harvest.

##### **4.2.2.2.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus levels were non significant for root cation exchange capacity. In respect of treated with phytase and phosphorus levels, the root CEC found significantly the highest (7.58 cmol kg<sup>-1</sup>) as compared to control (4.40 cmol kg<sup>-1</sup>) per plant, respectively. Similar results were reported by Dontsov (1996).

##### **4.2.2.3 Root biomass**

The root biomass of fodder maize in pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.19).

#### **4.2.2.3.1 Phytase levels**

The levels of phytase enzyme significantly increased the root cation exchange capacity of fodder maize. It was increased with an increased levels of phytase and significantly the highest (5.63 g plant<sup>-1</sup>) in 4800 IU phytase which was at par with 3600 IU phytase (5.40 g plant<sup>-1</sup>), respectively at harvest.

#### **4.2.2.3.2 Phosphorus levels**

The phosphorus levels were found to increasing root cation exchange capacity. It was increased with increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels (7.29 g plant<sup>-1</sup>) over rest of all the treatments at harvest.

#### **4.2.2.3.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus levels were found significant for root biomass. The interactions of increased levels of phytase and phosphorus were significant for increasing root biomass. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increased the root biomass also increased. This might be because of the levels of phytase and phosphorus (as concentration of enzyme) and levels of phosphorus (as concentration of substrate) were optimum in developing the root biomass.

In respect of treated with phytase and phosphorus levels, the root biomass yield found significantly higher (5.33 g plant<sup>-1</sup>) as compared to control (1.75 g plant<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the root biomass. However, it is necessary to optimize the level of phytase for increasing root biomass.

### **4.2.3 Effect of Phytase and Phosphorus Levels on Green and Dry Fodder Yield of Maize in Pot Culture**

#### **4.2.3.1 Green fodder yield**

The green fodder yield of maize was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.20 and depicted in fig.4.12).

#### 4.2.3.1.1 Phytase levels

The levels of phytase enzyme significantly increased the green fodder yield of maize. It was increased with an increased levels of phytase and significantly the highest (141.71 g plant<sup>-1</sup>) in the application of 4800 IU phytase which was at par with application of phytase @ 2400 IU and 3600 IU (134.64 and 140.44 g plant<sup>-1</sup>), respectively. This might be associated with release of essential nutrient by the phytase enzyme and increasing the yield of fodder maize.

#### 4.2.3.1.2 Phosphorus levels

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly recorded the highest green fodder yield (164.45 g plant<sup>-1</sup>) over rest of all the treatments. This might be because of fulfilment of phosphorus requirement of crops and improving the root and shoot development as well as increasing the yield of maize. Ram *et al.* (2015) reported that combination of 50 per cent P fertilizer dose along with phosphorus solubilizing fungi produced wheat yield compare to 100 % of P fertilizer dose.

#### 4.2.3.1.2 Phytase and phosphorus interactions

The interaction of phytase and phosphorus levels, the highest green fodder yield (182.75 g plant<sup>-1</sup>) recorded in the application of phytase @ 4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> over all the treatments. However, application of phytase @ 4800 U + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> was at par with the application of phytase @ 3600 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (175.00 g plant<sup>-1</sup>), respectively. In respect of treated with phytase and phosphorus levels, the significantly higher green fodder yield of maize (136.03 g plant<sup>-1</sup>) observed in treated as compared to control (53.10 g plant<sup>-1</sup>), respectively.

#### 4.2.3.2 Dry fodder yield

The dry matter yield of maize was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.20 and depicted in Fig. 4.13).

##### 4.2.3.2.1 Phytase levels

The dry fodder yield of maize found significantly higher (13.50 g plant<sup>-1</sup>) in the application of phytase @ 4800 IU over application of phytase @ 1200 IU. However, phytase 4800 IU was at par with phytase @ 2400 IU and 3600 IU (12.82 and 13.38 g plant<sup>-1</sup>), respectively. Similar results were reported by Findenegg and Nelemans (1993) and concluded that, the addition of phytase (10 enzyme units per kg sand) increased the plant dry weight yield by 32 per cent.

**Table 4.20. Effect of Phytase and Phosphorus Levels on Green Fodder and Dry Fodder Yield of Maize**

Treatment	Green fodder yield (g plant <sup>-1</sup> )	Dry matter yield (g plant <sup>-1</sup> )
<b>Phytase levels (P)</b>		
P <sub>1</sub> (1200 IU)	127.31	12.13
P <sub>2</sub> (2400 IU)	134.64	12.82
P <sub>3</sub> (3600 IU)	140.44	13.38
P <sub>4</sub> (4800 IU)	141.71	13.50
<b>SEm ±</b>	<b>2.63</b>	<b>0.25</b>
<b>C.D at 5 (%)</b>	<b>7.59</b>	<b>0.72</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>		
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	115.00	10.95
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	127.45	12.14
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	137.20	13.07
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	164.45	15.66
<b>SEm ±</b>	<b>2.63</b>	<b>0.25</b>
<b>C.D at 5 (%)</b>	<b>7.59</b>	<b>0.72</b>
<b>In. (P x F)</b>		
P <sub>1</sub> F <sub>1</sub>	111.50	10.62
P <sub>1</sub> F <sub>2</sub>	121.00	11.52
P <sub>1</sub> F <sub>3</sub>	130.25	12.40
P <sub>1</sub> F <sub>4</sub>	146.50	13.95
P <sub>2</sub> F <sub>1</sub>	117.25	11.17
P <sub>2</sub> F <sub>2</sub>	129.05	12.29
P <sub>2</sub> F <sub>3</sub>	138.70	13.21
P <sub>2</sub> F <sub>4</sub>	153.55	14.62
P <sub>3</sub> F <sub>1</sub>	115.75	11.02
P <sub>3</sub> F <sub>2</sub>	136.75	13.02
P <sub>3</sub> F <sub>3</sub>	134.25	12.79
P <sub>3</sub> F <sub>4</sub>	175.00	16.67
P <sub>4</sub> F <sub>1</sub>	115.50	11.00
P <sub>4</sub> F <sub>2</sub>	123.00	11.71
P <sub>4</sub> F <sub>3</sub>	145.60	13.87
P <sub>4</sub> F <sub>4</sub>	182.75	17.40
<b>SEm ±</b>	<b>5.27</b>	<b>0.50</b>
<b>C.D at 5 (%)</b>	<b>15.18</b>	<b>1.45</b>
Tr. Vs Control		
Treated	136.03	12.95
Control	53.10	5.06
<b>S.Em. ±</b>	<b>5.43</b>	<b>0.51</b>
<b>C.D at 5 (%)</b>	<b>15.67</b>	<b>1.49</b>

#### 4.2.3.2.2 Phosphorus levels

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> recorded significantly higher dry matter (15.66 g plant<sup>-1</sup>) over rest of all the treatments. Khan *et al.* (2014) reported that, the highest dry weight (827.1 kg ha<sup>-1</sup>) was recorded by application of 120 kg P ha<sup>-1</sup> which was compared to 90 kg P ha<sup>-1</sup> and 60 kg P ha<sup>-1</sup>, respectively.

#### 4.2.3.2.3 Phytase and phosphorus interactions

The interaction between levels of phytase and phosphorus were found significant for dry matter yield of fodder maize. The highest dry matter yield (17.40 g plant<sup>-1</sup>) recorded in the application of phytase @ 4800 IU + phosphorus 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> which was at par with the application of phytase @ 3600 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (16.67 g plant<sup>-1</sup>).

In respect of treated phytase and phosphorus levels, the dry matter yield was found significantly higher (12.95 g plant<sup>-1</sup>) as compared to control (5.06 g plant<sup>-1</sup>). Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the green and dry fodder of maize.

Kanduri *et al.* (2016) reported that, green fodder yield (44.97 t ha<sup>-1</sup>) and dry matter percentage (22.38 %) were influenced significantly by the application of nitrogen and phosphorus. Maximum green fodder yield was obtained at nitrogen-phosphorus level of 160-50 kg ha<sup>-1</sup>. Farooq *et al.* (2016) reported that, the highest green fodder yield (59.45 t ha<sup>-1</sup>) and dry matter (17.83 t ha<sup>-1</sup>) was recorded in the application of 80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> which was at par with the application of 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as compared to the 40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. 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). Wani *et al.* (1978) studied the effect of phosphorus solubilizing culture (*Aspergillus awamori*, *Bacillus polymixa*, *Pseudomonas striata*, and *Penicillium digitatum*) on dry matter yield of gram and found that *Aspergillus awamori* and *Bacillus polymixa*, were the most efficient isolates in increasing dry matter yield of gram. El-Azouni (2008) reported that, the dual inoculation of phosphorus solubilizing fungi (*Aspergillus niger* and *Penicillium digitatum*) significantly increased dry matter and yield of soybean compared to control.

#### **4.2.4 Effect of Phytase and Phosphorus Levels on Total Nutrient Uptake and Apparent P Recovery of Green Fodder Maize at Harvest**

The total uptake of nitrogen, phosphorus and potassium by green fodder maize at harvest as influenced by the application of graded levels of phytase and phosphorus and their interactions are presented in (Table 4.21).

##### **4.2.4.1 Nitrogen uptake**

The total uptake of nitrogen content in a pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.21).

##### **4.2.4.1.1 Phytase levels**

The levels of phytase enzyme significantly increased the total uptake of nitrogen at harvest of fodder maize. It was increased with an increased levels of phytase and significantly the highest ( $12.40 \text{ mg g}^{-1}$ ) was recorded by the application of 4800 IU phytase level which was at par with the application of phytase @ 2400 IU and phytase 3600 IU ( $12.28$  and  $12.33 \text{ mg g}^{-1}$ ), respectively.

##### **4.2.4.1.2 Phosphorus levels**

The application of phosphorus also reported significant increase in the nitrogen uptake by fodder maize. An increasing trend of nitrogen uptake was noticed with the application of graded levels of phosphorus 0, 25, 50 and  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $9.61$ ,  $11.78$ ,  $12.93$  and  $13.63 \text{ mg g}^{-1}$ ) respectively, in fodder maize. Significantly higher nitrogen uptake was recorded with the application of  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $13.63 \text{ mg g}^{-1}$ ) over rest of all treatments except application of  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $12.93 \text{ mg g}^{-1}$ ).

##### **4.2.4.1.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus was non significant for total uptake of nitrogen. In respect of phytase and phosphorus levels, the nitrogen uptake was significantly recorded the higher value in treated ( $11.98 \text{ mg g}^{-1}$ ) as compared to control ( $5.50 \text{ mg g}^{-1}$ ). Similar result was recorded by Tarafdar and Rao (1996) that the higher nitrogen, phosphorus and potassium uptake by cluster bean, chickpea and wheat due to inoculation with phosphatase producing fungi.

**Table 4.21. Effect of Phytase and Phosphorus Levels on Total Nutrient Uptake and Apparent P Recovery of Green Fodder Maize**

Treatment	Total nutrient uptake (mg g <sup>-1</sup> )			Apparent P recovery (mg mg <sup>-1</sup> )
	Nitrogen	Phosphorus	Potassium	
<b>Phytase levels (P)</b>				
P <sub>1</sub> (1200 IU)	10.94	4.52	14.08	-
P <sub>2</sub> (2400 IU)	12.28	4.73	14.24	-
P <sub>3</sub> (3600 IU)	12.33	4.78	15.26	-
P <sub>4</sub> (4800 IU)	12.40	4.95	15.98	-
<b>SEm ±</b>	<b>0.33</b>	<b>0.04</b>	<b>0.45</b>	-
<b>C.D at 5 (%)</b>	<b>0.96</b>	<b>0.11</b>	<b>1.31</b>	-
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>				
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	9.61	4.29	12.61	-
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	11.78	4.58	14.36	25.98
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	12.93	4.90	15.49	27.32
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	13.63	5.21	17.09	27.42
<b>SEm ±</b>	<b>0.33</b>	<b>0.04</b>	<b>0.45</b>	
<b>C.D at 5 (%)</b>	<b>0.96</b>	<b>0.11</b>	<b>1.31</b>	
<b>In. (P x F)</b>				
P <sub>1</sub> F <sub>1</sub>	8.35	4.17	12.25	-
P <sub>1</sub> F <sub>2</sub>	9.90	4.35	13.45	16.13
P <sub>1</sub> F <sub>3</sub>	12.40	4.66	14.80	21.95
P <sub>1</sub> F <sub>4</sub>	13.10	4.90	15.80	21.80
P <sub>2</sub> F <sub>1</sub>	9.80	4.29	12.10	-
P <sub>2</sub> F <sub>2</sub>	12.40	4.66	13.60	33.15
P <sub>2</sub> F <sub>3</sub>	13.10	4.90	14.85	27.33
P <sub>2</sub> F <sub>4</sub>	13.80	5.08	16.40	23.59
P <sub>3</sub> F <sub>1</sub>	10.00	4.29	12.75	-
P <sub>3</sub> F <sub>2</sub>	13.10	4.59	14.85	26.88
P <sub>3</sub> F <sub>3</sub>	13.10	4.90	16.10	27.33
P <sub>3</sub> F <sub>4</sub>	13.10	5.33	17.35	31.06
P <sub>4</sub> F <sub>1</sub>	10.30	4.41	13.35	-
P <sub>4</sub> F <sub>2</sub>	11.70	4.72	15.55	27.78
P <sub>4</sub> F <sub>3</sub>	13.10	5.15	16.20	33.15
P <sub>4</sub> F <sub>4</sub>	14.50	5.51	18.80	32.85
<b>SEm ±</b>	<b>0.67</b>	<b>0.08</b>	<b>0.91</b>	
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	
Tr. Vs Control				
Treated	11.98	4.74	14.89	
Control	5.50	2.39	5.90	
<b>S.Em. ±</b>	<b>0.68</b>	<b>0.08</b>	<b>0.93</b>	
<b>C.D at 5 (%)</b>	<b>1.98</b>	<b>0.24</b>	<b>2.70</b>	

#### **4.2.4.2 Phosphorus uptake**

The total uptake of phosphorus content in a pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.21).

##### **4.2.4.2.1 Phytase levels**

The levels of phytase enzyme significantly increased the total uptake of phosphorus at harvest of fodder maize. It was increased with an increased levels of phytase and significantly the highest ( $4.95 \text{ mg g}^{-1}$ ) by the application of 4800 IU phytase. This might be associated with levels of phytase with the release of organically bound phosphorus by phytase enzyme and uptake by the plants. Similar result was found by Tarafdar and Rao (1996).

##### **4.2.4.2.2 Phosphorus levels**

The application of phosphorus levels also observed significantly increase in the phosphorus uptake by fodder maize at harvest. Significantly higher mean phosphorus uptake ( $5.21 \text{ mg g}^{-1}$ ) recorded with the application of  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ . 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.

##### **4.2.4.2.3 Phytase and phosphorus levels**

The interaction between levels of phytase and phosphorus was non significant for total phosphorus uptake. In respect of phosphorus uptake significantly higher value was found in treated ( $4.74 \text{ mg g}^{-1}$ ) over control ( $2.39 \text{ mg g}^{-1}$ ). Khan *et al.* (2013) concluded positive correlation between dry matter yield and phosphorus uptake by rye grass in high clay textured soil with  $61.8 \text{ g kg}^{-1}$  organic carbon.

#### **4.2.4.3 Potassium uptake**

The total uptake of potassium content in a pot culture experiment was significantly influenced by the phytase and phosphorus levels are presented in (Table 4.21).

#### 4.2.4.3.1 Phytase levels

The data revealed that, the application of graded levels of phytase significantly influenced the potassium uptake by fodder maize at harvest. The maximum potassium uptake ( $15.98 \text{ mg g}^{-1}$ ) recorded with the application of phytase @ 4800 IU which was at par with the application of phytase @ 3600 IU ( $15.26 \text{ mg g}^{-1}$ ).

#### 4.2.4.3.2 Phosphorus levels

The application of phosphorus levels also reported significant increase in the potassium uptake by fodder maize. Significantly the higher potassium uptake ( $15.26 \text{ mg g}^{-1}$ ) recorded with the application of  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$ .

#### 4.2.4.3.3 Phytase and phosphorus interactions

It is evident from the data, the interaction between levels of phytase and phosphorus was found non significant. The potassium uptake was significantly recorded the higher value in treated ( $14.89 \text{ mg g}^{-1}$ ) over control ( $5.90 \text{ mg g}^{-1}$ ). Similar result was found by Tarafdar and Rao (1996) and concluded that higher nitrogen, phosphorus, potassium and calcium uptake by cluster bean, chickpea and wheat due to inoculation with phosphatase producing fungi.

#### 4.2.4.4 Apparent P recovery

The apparent recovery of green fodder maize as influenced by the application of graded levels of phosphorus and their combination with phytase enzyme are presented in table 4.21.

##### 4.2.4.4.1 Phosphorus levels

The higher apparent P recovery was recorded in the application of  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $27.47 \text{ mg mg}^{-1}$ ) followed by the  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $27.23 \text{ mg mg}^{-1}$ ) and the lowest was recorded in the application of the  $25 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $25.98 \text{ mg mg}^{-1}$ ). This was might be due to the application of higher dose of phosphorus and which was easily taken by the plants.

##### 4.2.4.4.2 Phytase and phosphorus interactions

The interaction between levels of phytase and phosphorus was recorded higher apparent P recovery by the combined application phytase @ 2400 IU +  $25 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  and phytase @ 4800 IU +  $50 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $33.15 \text{ mg mg}^{-1}$ ) followed by the phytase @ 4800 IU +  $75 \text{ kg P}_2\text{O}_5 \text{ ha}^{-1}$  ( $32.85 \text{ mg mg}^{-1}$ ) and the lowest was recorded in phytase @

1200 IU + 25 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (16.13 mg mg<sup>-1</sup>). The apparent P recovery was increased might be due to the increasing availability of the water soluble phosphorus and which was utilized by the crop plant due to solubilization of inorganic phosphorus added in soil and mineralization of the organic bound phosphate by the application of phytase.

#### **4.2.5 Effect of Phytase and Phosphorus Levels on Soil Chemical Properties at 30 DAS and at Harvest of Green Fodder Maize**

##### **4.2.5.1 Soil pH**

Soil pH as influenced by different phytase and phosphorus levels imposed to fodder maize assessed at 30 DAS and at harvest are presented in (Table 4.22).

Effect of phytase levels (1200, 2400, 3600, 4800 IU) and phosphorus levels (0, 25, 50, 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) on soil pH under fodder maize cultivation assessed at 30 DAS and at harvest showed non significant.

The reduction in soil pH over initial and during experimentation of fodder maize on 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 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 phosphorus 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 solubilizing microbes, in which hydroxyl (OH<sup>-</sup>) and carboxyl (COOH<sup>-</sup>) groups of acid chelates cations (Ca<sup>2+</sup> in calcareous soil) and decrease pH in basic soils (Kpombrekou 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<sub>2</sub>/ CO<sub>2</sub>) 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). 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).

#### **4.2.5.2 Soil Electrical conductivity**

Electrical conductivity of soil as influenced by different phytase and phosphorus levels imposed to fodder maize assessed at 30 DAS and at harvest are presented in (Table 4.22).

The electrical conductivity of soil as influenced by different phytase and phosphorus levels and their interactions imposed to fodder maize was assessed at 30 DAS and at harvest showed non significant.

The increase in electrical conductivity or total soluble salt content of soil was reported either with successive application of phytase or phosphorus levels 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 released phytase.

#### **4.2.5.3 Soil Organic Carbon**

The data by the application of phytase and phosphorus levels on soil organic carbon content in soil are presented in (Table 4.23).

Effect of phytase levels (1200, 2400, 3600, 4800 IU) and phosphorus levels (0, 25, 50, 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) on soil organic carbon content in soil under fodder maize cultivation was assessed at 30 DAS and at harvest showed non significant.

In respect of treated vs control, the treated pots were significantly recorded higher soil organic carbon content (0.71 %) compared to control (0.60 %) at 30 DAS.

The increase in organic carbon content in all treatment combinations might be due to organic matter addition through FYM. 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

**Table 4.22. Effect of Phytase and Phosphorus Levels on Soil pH and Electrical Conductivity of Soil at 30 DAS and At Harvest of Fodder Maize**

Treatment	Soil pH (1:2.5)		Electrical conductivity (dS m <sup>-1</sup> )	
	30 DAS	At harvest	30 DAS	At harvest
<b>Phytase levels (P)</b>				
P <sub>1</sub> (1200 IU)	8.32	8.29	0.31	0.31
P <sub>2</sub> (2400 IU)	8.30	8.29	0.32	0.30
P <sub>3</sub> (3600 IU)	8.31	8.30	0.32	0.30
P <sub>4</sub> (4800 IU)	8.32	8.29	0.34	0.31
<b>SEm ±</b>	<b>0.009</b>	<b>0.009</b>	<b>0.01</b>	<b>0.008</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>				
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	8.30	8.28	0.31	0.29
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	8.30	8.29	0.31	0.31
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	8.32	8.30	0.32	0.31
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	8.33	8.31	0.34	0.32
<b>SEm ±</b>	<b>0.009</b>	<b>0.009</b>	<b>0.01</b>	<b>0.008</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>In. (P x F)</b>				
P <sub>1</sub> F <sub>1</sub>	8.29	8.27	0.30	0.30
P <sub>1</sub> F <sub>2</sub>	8.31	8.29	0.31	0.31
P <sub>1</sub> F <sub>3</sub>	8.33	8.31	0.32	0.33
P <sub>1</sub> F <sub>4</sub>	8.34	8.32	0.33	0.32
P <sub>2</sub> F <sub>1</sub>	8.31	8.28	0.32	0.28
P <sub>2</sub> F <sub>2</sub>	8.29	8.28	0.31	0.32
P <sub>2</sub> F <sub>3</sub>	8.29	8.29	0.32	0.29
P <sub>2</sub> F <sub>4</sub>	8.33	8.30	0.33	0.32
P <sub>3</sub> F <sub>1</sub>	8.30	8.29	0.32	0.28
P <sub>3</sub> F <sub>2</sub>	8.29	8.30	0.31	0.30
P <sub>3</sub> F <sub>3</sub>	8.32	8.30	0.32	0.31
P <sub>3</sub> F <sub>4</sub>	8.33	8.31	0.35	0.32
P <sub>4</sub> F <sub>1</sub>	8.31	8.28	0.31	0.29
P <sub>4</sub> F <sub>2</sub>	8.33	8.30	0.34	0.31
P <sub>4</sub> F <sub>3</sub>	8.34	8.31	0.35	0.32
P <sub>4</sub> F <sub>4</sub>	8.32	8.30	0.36	0.33
<b>SEm ±</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>	<b>0.02</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control				
Treated	8.32	8.29	0.32	0.31
Control	8.35	8.33	0.29	0.28
<b>S.Em. +</b>	<b>0.02</b>	<b>0.18</b>	<b>0.02</b>	<b>0.18</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>Initial</b>	<b>8.36</b>		<b>0.35</b>	

**Table 4.23. Effect of Phytase and Phosphorus Levels on Organic Carbon and CaCO<sub>3</sub> in Soil at 30 DAS and at Harvest of Fodder Maize**

Treatment	Organic carbon (%)		CaCO <sub>3</sub> (%)	
	30 DAS	At harvest	30 DAS	At harvest
<b>Phytase levels (P)</b>				
P <sub>1</sub> (1200 IU)	0.71	0.65	5.80	5.72
P <sub>2</sub> (2400 IU)	0.71	0.66	5.78	5.72
P <sub>3</sub> (3600 IU)	0.71	0.66	5.80	5.77
P <sub>4</sub> (4800 IU)	0.72	0.64	5.85	5.80
<b>S.Em. ±</b>	<b>0.006</b>	<b>0.010</b>	<b>0.023</b>	<b>0.03</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>				
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	0.69	0.65	5.73	5.67
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	0.71	0.66	5.80	5.72
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	0.72	0.66	5.83	5.78
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	0.72	0.65	5.88	5.84
<b>S.Em. ±</b>	<b>0.006</b>	<b>0.010</b>	<b>0.023</b>	<b>0.03</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>0.067</b>	<b>0.09</b>
<b>In. (P x F)</b>				
P <sub>1</sub> F <sub>1</sub>	0.67	0.65	5.73	5.62
P <sub>1</sub> F <sub>2</sub>	0.70	0.66	5.77	5.69
P <sub>1</sub> F <sub>3</sub>	0.72	0.66	5.83	5.75
P <sub>1</sub> F <sub>4</sub>	0.73	0.64	5.88	5.83
P <sub>2</sub> F <sub>1</sub>	0.69	0.63	5.73	5.64
P <sub>2</sub> F <sub>2</sub>	0.70	0.66	5.76	5.70
P <sub>2</sub> F <sub>3</sub>	0.72	0.66	5.79	5.76
P <sub>2</sub> F <sub>4</sub>	0.72	0.67	5.86	5.79
P <sub>3</sub> F <sub>1</sub>	0.70	0.66	5.74	5.69
P <sub>3</sub> F <sub>2</sub>	0.71	0.66	5.79	5.74
P <sub>3</sub> F <sub>3</sub>	0.71	0.66	5.83	5.80
P <sub>3</sub> F <sub>4</sub>	0.72	0.65	5.85	5.85
P <sub>4</sub> F <sub>1</sub>	0.71	0.66	5.72	5.74
P <sub>4</sub> F <sub>2</sub>	0.71	0.64	5.87	5.77
P <sub>4</sub> F <sub>3</sub>	0.72	0.65	5.90	5.82
P <sub>4</sub> F <sub>4</sub>	0.72	0.64	5.93	5.89
<b>S.Em. ±</b>	<b>0.01</b>	<b>0.02</b>	<b>0.05</b>	<b>0.06</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control				
Treated	0.71	0.65	5.81	5.75
Control	0.60	0.63	5.92	5.88
<b>S.Em. ±</b>	<b>0.012</b>	<b>0.021</b>	<b>0.048</b>	<b>0.063</b>
<b>C.D at 5 (%)</b>	<b>0.036</b>	<b>NS</b>	<b>0.13</b>	<b>NS</b>
<b>Initial</b>	<b>0.67</b>		<b>5.98</b>	

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 phosphorus solubilizing fungi (*Aspergillus niger* and *Penicillium digitarium*) significantly increased the organic carbon levels of the treated soil when compared to untreated soil. 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 and nitrogen contents ranging from 0.10 to 1.40 per cent and 0.05 to 0.55 per cent, respectively were achieved in a salt affected soil through amendment of soil with green and farm yard manure.

#### **4.2.5.4 Calcium carbonate**

The soil calcium carbonate content was significantly influenced by phosphorus levels applied to fodder maize at both the growth stages are presented in (Table 4.23).

##### **4.2.5.4.1 At 30 days after sowing**

The levels of phytase enzyme in relation to soil calcium carbonate content showed non significant at 30 days after sowing of green fodder maize.

The application of phosphorus found significantly higher calcium carbonate contents of soil by the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (5.88 %) which was at par with the application of 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (5.83 %).

The interaction between levels of phytase and phosphorus was non significant for calcium carbonate content in soil. In respect of treated vs control, the treated significantly reported the lower soil calcium carbonate content (5.81 %) compared to control (5.92 %). It was also showed that application of phytase and phosphorus levels declined the calcium carbonate content in the soil at 30 DAS of green fodder maize.

##### **4.2.5.4.2 At harvest of fodder maize**

At harvest of fodder maize, calcium carbonate content of soil declined as compared to the 30 days after sowing of the fodder maize. Incorporation of various levels of phytase found non significant for calcium carbonate content of soil at harvest.

The phosphorus levels were found to increase the soil calcium carbonate content at harvest of fodder maize. It was increased with increased levels of phosphorus and significantly the highest (5.84 %) in 75 kg ha<sup>-1</sup> phosphorus levels which was at par with the application of 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (5.78 %).

The interaction between levels of phytase and phosphorus was non significant for soil calcium carbonate content. In respect of treated vs. control, the treated reported non significant for soil calcium carbonate content. Similar trend of results for reduction of CaCO<sub>3</sub> content to the tune of 37 per cent were observed by Gujar *et al.* (2013) due to application of phytase. The decrease in CaCO<sub>3</sub> content may be attributed to dissolution of CaCO<sub>3</sub> content with organic acids released during decomposition of organic matter added to soil (Zheng *et al.*, 2005). Markovacki *et al.* (2008) reported that, the higher microbial population in soil decrease CaCO<sub>3</sub> content due to secretion of organic acid and respiration of CO<sub>2</sub> leading to formation of carbonic acid. The reduction in CaCO<sub>3</sub> 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<sub>4</sub>.

#### **4.2.5.5 Soil available nitrogen**

The soil available nitrogen content was significantly influenced by the phytase and phosphorus levels at 30 days after sowing and at harvest are presented in (Table 4.24).

##### **4.2.5.5.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil available nitrogen at 30 days after sowing and at harvest of fodder maize. It was increased with an increased levels of phytase and significantly the highest in 4800 IU phytase (103.08 and 87.15 mg kg<sup>-1</sup>). However, the soil available nitrogen was numerically increased upto 30 days after sowing and decreased at harvest in all the levels of phytase enzyme. This might be associated with release of organically bound phosphorus by phytase enzyme, simultaneously the carbon also released from organic matter in which phosphorus was bound. These released organic carbon act as source of energy to microorganisms responsible for nitrogen release and fixing the atmospheric nitrogen in soil. The increase in nitrogen availability in soil can be attributed to release of ammonium ions bound to organic phosphate degradation by phytase. Similar results were reported by Gujar *et al.* (2013). The decreases in soil available nitrogen might be because of uptake of nitrogen by crops for growth and development.

**Table 4.24. Effect of Phytase and Phosphorus Levels on Available Nitrogen, Phosphorus and Potassium in Soil at 30 DAS and at Harvest of Fodder Maize**

Treatment	Available N (mg kg <sup>-1</sup> )		Available P (mg kg <sup>-1</sup> )		Available K (mg kg <sup>-1</sup> )	
	30 DAS	At harvest	30 DAS	At harvest	30 DAS	At harvest
<b>Phytase levels (P)</b>						
P <sub>1</sub> (1200 IU)	97.65	82.78	10.16	8.52	177.38	168.63
P <sub>2</sub> (2400 IU)	99.05	84.70	10.58	8.92	181.38	172.63
P <sub>3</sub> (3600 IU)	101.33	86.10	10.79	8.95	183.88	174.63
P <sub>4</sub> (4800 IU)	103.08	87.15	11.35	9.27	185.63	176.00
<b>SEm ±</b>	<b>0.44</b>	<b>0.47</b>	<b>0.069</b>	<b>0.025</b>	<b>0.35</b>	<b>0.32</b>
<b>C.D at 5 (%)</b>	<b>1.29</b>	<b>1.35</b>	<b>0.19</b>	<b>0.07</b>	<b>1.03</b>	<b>0.92</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>						
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	93.45	81.38	9.03	7.60	176.00	167.25
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	96.25	82.95	10.82	8.87	181.56	172.81
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	105.0	87.50	11.36	9.36	184.19	174.50
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	106.40	88.90	11.67	9.82	186.50	177.31
<b>SEm ±</b>	<b>0.44</b>	<b>0.47</b>	<b>0.069</b>	<b>0.025</b>	<b>0.35</b>	<b>0.32</b>
<b>C.D at 5 (%)</b>	<b>1.29</b>	<b>1.35</b>	<b>0.19</b>	<b>0.07</b>	<b>1.03</b>	<b>0.92</b>
<b>In.( P x F)</b>						
P <sub>1</sub> F <sub>1</sub>	90.30	79.10	8.43	7.26	174.50	165.75
P <sub>1</sub> F <sub>2</sub>	93.10	79.80	10.15	8.49	177.00	168.25
P <sub>1</sub> F <sub>3</sub>	102.90	85.40	10.82	8.92	178.00	169.25
P <sub>1</sub> F <sub>4</sub>	104.30	86.80	11.25	9.41	180.00	171.25
P <sub>2</sub> F <sub>1</sub>	91.70	81.20	8.92	7.50	175.25	166.50
P <sub>2</sub> F <sub>2</sub>	94.50	82.60	10.64	8.79	180.75	172.00
P <sub>2</sub> F <sub>3</sub>	104.30	86.80	11.19	9.47	183.50	174.75
P <sub>2</sub> F <sub>4</sub>	105.70	88.20	11.56	9.90	186.00	177.25
P <sub>3</sub> F <sub>1</sub>	94.50	82.60	9.10	7.75	177.00	168.25
P <sub>3</sub> F <sub>2</sub>	98.00	84.00	10.95	8.98	183.25	174.50
P <sub>3</sub> F <sub>3</sub>	105.70	88.20	11.38	9.35	186.50	175.75
P <sub>3</sub> F <sub>4</sub>	107.10	89.60	11.75	9.72	188.75	180.00
P <sub>4</sub> F <sub>1</sub>	97.30	82.60	9.66	7.87	177.25	168.50
P <sub>4</sub> F <sub>2</sub>	99.40	85.40	11.56	9.23	185.25	176.50
P <sub>4</sub> F <sub>3</sub>	107.10	89.60	12.05	9.72	188.75	178.25
P <sub>4</sub> F <sub>4</sub>	108.50	91.00	12.12	10.27	191.25	180.75
<b>SEm ±</b>	<b>0.90</b>	<b>0.94</b>	<b>0.13</b>	<b>0.05</b>	<b>0.72</b>	<b>0.64</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>0.14</b>	<b>2.07</b>	<b>1.86</b>
Tr. Vs Control						
Treated	100.28	85.18	10.72	8.91	182.06	172.97
Control	78.40	72.80	4.37	3.44	151.75	143.00
<b>SEm ±</b>	<b>0.92</b>	<b>0.97</b>	<b>0.14</b>	<b>0.052</b>	<b>0.74</b>	<b>0.66</b>
<b>C.D at 5 (%)</b>	<b>2.66</b>	<b>2.79</b>	<b>0.41</b>	<b>0.15</b>	<b>2.13</b>	<b>1.91</b>
<b>Initial</b>	<b>84.70</b>		<b>6.84</b>		<b>162.00</b>	

#### 4.2.5.5.2 Phosphorus levels

The phosphorus levels were found to increasing the soil available nitrogen during 30 days after sowing and slightly decreasing at harvest of fodder maize. It was increased with an increased levels of phosphorus and significantly the highest in 75 kg ha<sup>-1</sup> phosphorus levels (106.40 and 88.90 mg kg<sup>-1</sup>, respectively). The soil available phosphorus was increased numerically at 30 days after sowing and decreased at harvest. The levels of phytase and phosphorus showed the similar trend for soil available nitrogen.

#### 4.2.5.5.3 Phytase and phosphorus interactions

The interaction between levels of phytase and phosphorus was non significant for soil available nitrogen in pot culture experiment.

In respect of treated vs. control, the significant increase in soil available nitrogen was observed at 30 days after sowing and at harvest in treated (100.28 and 85.18 mg kg<sup>-1</sup>) compared to control (85.18 and 72.80 mg kg<sup>-1</sup>). The magnitude of reduction in soil available nitrogen was more pronounced with no phosphorus and lower levels of phosphorus application at 30 DAS and at harvest. However, reduction in soil available nitrogen at 30 DAS and at harvest in all treatment combinations might be due to the crop uptake and evaporative losses. Reduction in soil available nitrogen may be due to microbial immobilization, denitrification losses and leaching losses. Similar results were also reported by (Suresh and Suryaprabha, 2005).

#### 4.2.5.6 Available phosphorus

The soil available phosphorus was significantly influenced by graded levels of phytase (1200, 2400, 3600 and 4800 IU) and phosphorus (0, 25, 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) at 30 days after sowing and at harvest are presented in (Table 4.24 and depicted in Fig. 4.14 and 4.15).

##### 4.2.5.6.1 Phytase levels

The levels of phytase enzyme significantly increased the soil available phosphorus at 30 days after sowing and at harvest. It was increased with an increased levels of phytase and significantly higher in 30 days after sowing (11.35 mg kg<sup>-1</sup>) and at harvest (9.27 mg kg<sup>-1</sup>) in the application of 4800 IU phytase. However, the soil available phosphorus was numerically increased with an advanced period of sowing upto 30 days. This might be associated with the release of organically bound phosphorus by phytase

enzyme. These enzymes breakdown organic phosphorus and thus, making more phosphorus available. Similar trends of results were reported by Richardson (2001).

#### **4.2.5.6.2 Phosphorus levels**

The phosphorus levels were found to increase the soil available phosphorus at 30 days after sowing and at harvest. It was increased with an increased levels of phosphorus and significantly the highest (11.67 and 9.82 mg kg<sup>-1</sup>) in 75 kg ha<sup>-1</sup> phosphorus levels at both stages. The soil available phosphorus was increased an advanced periods of sowing and it was highest at 30 days after sowing and decreased at harvest. The levels of phytase and phosphorus showed the similar trend for soil available phosphorus in pot culture experiment. The increasing soil available phosphorus might be due to addition of phosphorus fertilizer and decreased at harvest by the plants.

#### **4.2.5.6.3 Phytase and phosphorus interactions**

The interaction between levels of phytase and phosphorus was non significant at 30 days after sowing and significant at harvest for soil available phosphorus. At harvest, the interactions of increased levels of phytase and phosphorus was significant for increasing the soil available phosphorus at harvest. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the soil available phosphorus also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to increase the phosphorus in soil.

In respect of treated with phytase and phosphorus levels, at 30 days after sowing and at harvest the soil available phosphorus found significantly higher (10.72 and 8.91 mg kg<sup>-1</sup>) as compared to control (4.37 and 3.44 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of phosphorus in soil.

Higher availability of phosphorus in soil with individual phytase, phosphorus levels and their combine application to pots might be due to plants and micro-organisms increases exudation of phosphorus hydrolyzing enzymes. These enzymes breakdown organic phosphorus thus, making more phosphorus available. Further, phytase catalyzes the organic phosphorus present in soil in the form of inositol

hexaphosphate (major form of organic phosphorus in soil). Application of phytase along with phosphorus levels might have played key role in enhancing microbial population and their activity thereby exudation of microbial phytase causing higher availability of soil phosphorus.

Higher build-up of soil available phosphorus at harvest of fodder maize might be due to acid phosphatase 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. Phosphorus availability in soils with low organic matter is very low. The organic carbon in the soil increase P availability by formation of organophosphorus 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) 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 striata*, *Bacillus polymixa*, *bacillus megatherium*, *penicillium digitatum*, *Aspergillus awamori*) due to secretion of organic acids which prevents fixation of phosphorus 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 phosphorus 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 phosphorus (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. 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 phosphorus by acid anion or can chelate Fe, Al and Ca ions associated with P (Omar 1998). 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. 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 phosphorus 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 phosphorus into available forms that support growth of crop plants. Similar results were also reported by Dotaniya *et al.* (2014).

#### **4.2.5.7 Available potassium**

The soil available potassium was significantly influenced by phytase and phosphorus levels at 30 days after sowing and at harvest are presented in (Table 4.24).

#### 4.2.5.7.1 Phytase levels

The levels of phytase enzyme significantly increased the soil available potassium at 30 days after sowing and at harvest. It was increased with an increased levels of phytase and significantly the highest (185.63 and 176.00 mg kg<sup>-1</sup>) in application of 4800 IU phytase at both the stages. However, the soil available phosphorus was numerically increased with an advanced period of sowing upto 30 days then decreased at harvest. This might be associated with the release of fixed and non exchangeable potassium ions in the soil solution by phytase enzyme. Similar results were reported by 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.

#### 4.2.5.7.1 Phosphorus levels

The phosphorus levels were found to increase the soil available potassium at 30 days after sowing and at harvest. It was increased with an increased levels of phosphorus and significantly the highest (186.50 and 177.31 mg kg<sup>-1</sup>) in 75 kg ha<sup>-1</sup> phosphorus levels at both stages. The soil available potassium was increased an advanced periods of sowing and it was highest at 30 days after sowing and decreased at harvest. The levels of phytase and phosphorus showed the similar trend for soil available potassium in pot culture experiment.

#### 4.2.5.7.1 Phytase and phosphorus interactions

The interaction between levels of phytase and phosphorus was found significant at 30 days after sowing and at harvest for soil available phosphorus. The interactions of increased levels of phytase and phosphorus were significant for increasing the soil available potassium at both the stages. The results of interactions between levels of phytase and phosphorus were showed the synergism between phytase and phosphorus levels as the levels increase the soil available potassium also increased. This might be because of the levels of phytase as concentration of enzyme and levels of phosphorus as concentration of substrate were optimum to increase the potassium in soil.

In respect of treated with phytase and phosphorus levels, at 30 days after sowing and at harvest the soil available potassium found significantly higher (182.06 and 172.97 mg kg<sup>-1</sup>) as compared to control (151.75 and 143.00 mg kg<sup>-1</sup>), respectively. Thus,

the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of potassium in soil. Similar results were reported by Santhy *et al.* (1998).

#### **4.2.6 Effect of Phytase and Phosphorus Levels on Forms of Phosphorus in Soil at 30 DAS and at Harvest of Green Fodder Maize**

##### **4.2.6.1 At 30 Days after sowing**

The forms of phosphorus were significantly influenced by the phytase and phosphorus levels at 30 days after sowing of fodder maize are presented in (Table 4.25 and depicted in 4.16 to 4.23).

##### **4.2.6.1.1 Phytase levels**

The levels of phytase enzyme significantly increased all forms of phosphorus at 30 days after sowing. It was increased with an increased levels of phytase and significantly higher the saloid-P, Al-P, Fe-P, Red-P, Occl-P and Ca-P (15.29, 83.44, 38.27, 86.45, 12.03 and 333.32 mg kg<sup>-1</sup>, respectively) in the application of phytase @ 4800 IU over rest of all the treatments. The application of phytase showed non significant for organic-P and total-P.

##### **4.2.6.1.2 Phosphorus levels**

The phosphorus levels were found to increase the all inorganic forms of phosphorus in 30 days after sowing. It was increased with an increased levels of phosphorus and significantly the highest in 75 hg ha<sup>-1</sup> phosphorus levels of saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P and total-P (17.15, 98.44, 45.42, 95.55, 13.56, 344.09 and 787.56 mg kg<sup>-1</sup>), respectively, over rest of all the treatments at 30 days after sowing. This might be because of addition of higher levels of phosphorus increases the all inorganic forms of phosphorus.

##### **4.2.6.1.3 Phytase and phosphorus interactions**

The interactions between levels of phytase and phosphorus were found significant for inorganic forms of phosphorus in soil. Conjoint application of phytase @ 4800 IU along with 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> recorded significantly increased saloid-P, Al-P, Red-P and Ca-P (18.29, 110.63, 105.30, 350.27 and mg kg<sup>-1</sup>), respectively, which was found to be statistically superior over rest of all the treatments except the application of phytase @ 4800 IU along with 50 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> which was at par with saloid-P (17.81 mg kg<sup>-1</sup>).

**Table 4.25. Effect of Phytase and Phosphorus Levels on Forms of Phosphorus in Soil at 30 DAS of Green Fodder Maize**

Treatment	Forms of phosphorus (mg kg <sup>-1</sup> )							
	Saloid-P	Al- P	Fe- P	Ca- P	Red- P	Occl- P	Organic-P	Total-P
<b>Phytase levels (P)</b>	<b>30 DAS</b>							
P <sub>1</sub> (1200 IU)	13.53	66.56	33.99	326.27	74.10	10.72	245.12	770.28
P <sub>2</sub> (2400 IU)	14.10	72.19	35.89	327.65	77.35	11.27	206.38	744.82
P <sub>3</sub> (3600 IU)	14.60	75.94	37.16	329.73	80.60	11.65	224.40	774.07
P <sub>4</sub> (4800 IU)	15.29	83.44	38.27	333.32	86.45	12.03	207.26	776.06
<b>S.Em. ±</b>	<b>0.090</b>	<b>0.88</b>	<b>0.20</b>	<b>0.35</b>	<b>0.31</b>	<b>0.086</b>	<b>12.41</b>	<b>12.06</b>
<b>C.D at 5 (%)</b>	<b>0.26</b>	<b>2.53</b>	<b>0.60</b>	<b>1.03</b>	<b>0.90</b>	<b>0.25</b>	<b>NS</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>								
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	10.22	42.19	25.09	310.33	61.10	9.02	276.55	734.50
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	13.76	68.44	31.76	321.60	70.85	10.39	249.95	766.76
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	16.38	89.06	43.04	340.94	91.00	12.69	183.30	776.42
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	17.15	98.44	45.42	344.09	95.55	13.56	173.35	787.56
<b>S.Em. ±</b>	<b>0.090</b>	<b>0.88</b>	<b>0.20</b>	<b>0.35</b>	<b>0.31</b>	<b>0.086</b>	<b>12.41</b>	<b>12.06</b>
<b>C.D at 5 (%)</b>	<b>0.26</b>	<b>2.53</b>	<b>0.60</b>	<b>1.03</b>	<b>0.90</b>	<b>0.25</b>	<b>35.76</b>	<b>34.76</b>
<b>In. (P x F)</b>								
P <sub>1</sub> F <sub>1</sub>	9.62	39.38	22.23	308.94	58.50	8.53	309.95	757.15
P <sub>1</sub> F <sub>2</sub>	13.24	61.88	29.86	319.78	66.30	9.41	265.33	765.79
P <sub>1</sub> F <sub>3</sub>	15.24	76.88	41.29	336.41	84.50	12.03	206.45	772.79
P <sub>1</sub> F <sub>4</sub>	16.00	88.13	42.56	339.94	87.10	12.91	198.77	785.40
P <sub>2</sub> F <sub>1</sub>	10.10	43.13	25.41	309.70	61.10	8.97	302.36	760.76
P <sub>2</sub> F <sub>2</sub>	13.62	65.63	31.13	320.78	68.90	10.28	254.95	765.29
P <sub>2</sub> F <sub>3</sub>	15.91	84.38	41.93	338.93	87.10	12.47	191.65	772.36
P <sub>2</sub> F <sub>4</sub>	16.76	95.63	45.10	341.20	92.30	13.34	176.55	780.89
P <sub>3</sub> F <sub>1</sub>	10.38	43.13	26.05	310.70	61.10	9.19	299.92	760.46
P <sub>3</sub> F <sub>2</sub>	13.91	69.38	32.40	322.04	71.50	10.72	248.30	768.24
P <sub>3</sub> F <sub>3</sub>	16.57	91.88	43.83	341.20	92.30	12.91	183.08	781.76
P <sub>3</sub> F <sub>4</sub>	17.53	99.38	46.37	344.98	97.50	13.78	166.29	785.83
P <sub>4</sub> F <sub>1</sub>	10.76	43.13	26.68	311.96	63.70	9.41	293.98	759.62
P <sub>4</sub> F <sub>2</sub>	14.29	76.88	33.67	323.81	76.70	11.16	231.23	767.73
P <sub>4</sub> F <sub>3</sub>	17.81	103.13	45.10	347.24	100.10	13.34	152.04	778.77
P <sub>4</sub> F <sub>4</sub>	18.29	110.63	47.64	350.27	105.30	14.22	151.79	798.13
<b>S.Em. ±</b>	<b>0.18</b>	<b>1.76</b>	<b>0.42</b>	<b>0.72</b>	<b>0.63</b>	<b>0.17</b>	<b>24.83</b>	<b>24.13</b>
<b>C.D at 5 (%)</b>	<b>0.52</b>	<b>5.07</b>	<b>NS</b>	<b>2.06</b>	<b>1.82</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control								
Treated	14.38	74.53	36.33	329.24	79.63	11.42	220.79	766.31
Control	8.57	39.38	20.96	304.40	53.30	6.78	263.69	697.08
<b>S.Em. ±</b>	<b>0.18</b>	<b>1.81</b>	<b>0.43</b>	<b>0.73</b>	<b>0.65</b>	<b>0.17</b>	<b>25.59</b>	<b>24.87</b>
<b>C.D at 5 (%)</b>	<b>0.53</b>	<b>5.22</b>	<b>1.24</b>	<b>2.12</b>	<b>1.87</b>	<b>0.51</b>	<b>73.72</b>	<b>NS</b>
<b>Initial</b>	<b>8.19</b>	<b>41.68</b>	<b>26.68</b>	<b>316.15</b>	<b>63.70</b>	<b>7.66</b>	<b>253.34</b>	<b>719.39</b>

The interaction effect of phytase and phosphorus levels, the Fe-P Occl-P, organic-P and total-P showed non significant at 30 DAS of fodder maize.

In respect of treated and control, the treated pots significantly recorded the higher value of all forms of phosphorus such as saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P, organic-P and total-P (14.38, 74.53, 36.33, 79.63, 11.42, 329.24, 220.79 and 766.31 mg kg<sup>-1</sup>), respectively, compared to control pots saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P and total-P (8.57, 39.38, 20.96, 53.30, 6.78, 304.40, 263.69 and 697.08 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of all the forms of phosphorus.

#### **4.2.6.2 At harvest of fodder maize**

##### **4.2.6.2.1 Phytase levels**

The application of phytase @ 4800 IU significantly increased the saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P and organic-P (12.24, 64.69, 30.97, 73.45, 9.84, 307.37 and 167.45 mg kg<sup>-1</sup>), respectively over rest of all the treatments. The total-P showed non significant by the application of phytase.

##### **4.2.6.2.2 Phosphorus levels**

The application of phosphorus @ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P and total-P (14.45, 75.47, 37.48, 82.88, 11.38, 318.89 and 742.39 mg kg<sup>-1</sup>), respectively, over rest of all the treatments. The application of 00 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the organic-P (250.91 mg kg<sup>-1</sup>) and significantly decrease in the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (136.71 mg kg<sup>-1</sup>).

##### **4.2.6.2.3 Phytase and phosphorus interactions**

Interaction effect reported non significant from the present experimental data with the combined application of phytase and phosphorus levels, at harvest of the fodder maize in some fractions of phosphorus like saloid-P, Al-P, Fe-P, Occl-P and total-P. but the conjoint application of phytase @ 4800 IU along with 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly recorded the higher Red-P and Ca-P (92.30 and 325.07 mg kg<sup>-1</sup>) over rest of all the treatments.

In respect of treated and control the treated pots were significantly recorded the higher values of all forms of phosphorus such as saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P, organic-P and total-P (11.87, 58.71, 28.98, 66.71, 9.23, 303.62, 188.05

**Table 4.26. Effect of Phytase and Phosphorus Levels on Forms of Phosphorus in Soil at Harvest of Green Fodder Maize**

Treatment	Forms of phosphorus (mg kg <sup>-1</sup> )							
	Saloid-P	Al- P	Fe- P	Ca- P	Red- P	Occl- P	Organic-P	Total-P
<b>Phytase levels (P)</b>	<b>At harvest</b>							
P <sub>1</sub> (1200 IU)	11.53	53.91	27.32	300.81	61.43	8.53	204.98	668.50
P <sub>2</sub> (2400 IU)	11.76	56.72	27.79	302.26	64.35	9.08	195.84	667.80
P <sub>3</sub> (3600 IU)	11.95	59.53	29.86	304.03	67.60	9.46	183.92	666.35
P <sub>4</sub> (4800 IU)	12.24	64.69	30.97	307.37	73.45	9.84	167.45	666.00
<b>S.Em. ±</b>	<b>0.085</b>	<b>0.74</b>	<b>0.31</b>	<b>0.37</b>	<b>0.18</b>	<b>0.086</b>	<b>2.71</b>	<b>2.03</b>
<b>C.D at 5 (%)</b>	<b>0.24</b>	<b>2.14</b>	<b>0.91</b>	<b>1.08</b>	<b>0.52</b>	<b>0.25</b>	<b>7.80</b>	<b>NS</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>								
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	8.12	37.97	18.10	285.25	48.10	6.84	250.91	655.29
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	11.24	52.50	25.41	294.58	57.85	8.20	214.00	663.78
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	13.67	68.91	34.94	315.74	78.00	10.50	150.57	672.33
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	14.45	75.47	37.48	318.89	82.88	11.38	136.71	677.26
<b>S.Em. ±</b>	<b>0.085</b>	<b>0.74</b>	<b>0.31</b>	<b>0.37</b>	<b>0.18</b>	<b>0.086</b>	<b>2.71</b>	<b>2.03</b>
<b>C.D at 5 (%)</b>	<b>0.24</b>	<b>2.14</b>	<b>0.91</b>	<b>1.08</b>	<b>0.52</b>	<b>0.25</b>	<b>7.80</b>	<b>5.85</b>
<b>In. (P x F)</b>								
P <sub>1</sub> F <sub>1</sub>	8.00	33.75	17.15	283.74	45.50	6.34	261.99	656.48
P <sub>1</sub> F <sub>2</sub>	10.95	46.88	23.50	293.57	53.30	7.22	227.72	663.14
P <sub>1</sub> F <sub>3</sub>	13.14	61.88	33.03	311.21	71.50	9.84	171.51	672.12
P <sub>1</sub> F <sub>4</sub>	14.00	73.13	35.57	314.74	75.40	10.72	158.70	682.25
P <sub>2</sub> F <sub>1</sub>	7.91	35.63	17.79	285.00	48.10	6.78	251.49	652.69
P <sub>2</sub> F <sub>2</sub>	11.14	50.63	24.77	294.32	55.90	8.09	217.12	661.98
P <sub>2</sub> F <sub>3</sub>	13.72	67.50	33.03	313.73	74.10	10.28	163.57	675.93
P <sub>2</sub> F <sub>4</sub>	14.29	73.13	35.57	316.00	79.30	11.16	151.18	680.62
P <sub>3</sub> F <sub>1</sub>	8.19	39.38	18.42	285.50	48.10	7.00	247.48	654.07
P <sub>3</sub> F <sub>2</sub>	11.33	54.38	26.05	294.83	58.50	8.53	213.33	666.94
P <sub>3</sub> F <sub>3</sub>	13.72	69.38	36.21	316.00	79.30	10.72	145.93	671.25
P <sub>3</sub> F <sub>4</sub>	14.57	75.00	38.75	319.78	84.50	11.59	128.96	673.15
P <sub>4</sub> F <sub>1</sub>	8.38	43.13	19.06	286.76	50.70	7.22	242.69	657.94
P <sub>4</sub> F <sub>2</sub>	11.53	58.13	27.32	295.58	63.70	8.97	197.83	663.05
P <sub>4</sub> F <sub>3</sub>	14.10	76.88	37.48	322.04	87.10	11.16	121.27	670.02
P <sub>4</sub> F <sub>4</sub>	14.95	80.63	40.02	325.07	92.30	12.03	108.01	673.01
<b>S.Em. ±</b>	<b>0.17</b>	<b>1.49</b>	<b>0.64</b>	<b>0.76</b>	<b>0.37</b>	<b>0.17</b>	<b>5.42</b>	<b>4.06</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>	<b>2.18</b>	<b>1.06</b>	<b>NS</b>	<b>NS</b>	<b>NS</b>
Tr. Vs Control								
Treated	11.87	58.71	28.98	303.62	66.71	9.23	188.05	667.16
Control	7.14	30.00	15.88	275.20	39.60	4.59	285.36	657.77
<b>S.Em. ±</b>	<b>0.17</b>	<b>1.53</b>	<b>0.65</b>	<b>0.77</b>	<b>0.37</b>	<b>0.17</b>	<b>5.58</b>	<b>4.18</b>
<b>C.D at 5 (%)</b>	<b>0.50</b>	<b>4.42</b>	<b>1.89</b>	<b>2.24</b>	<b>1.09</b>	<b>0.51</b>	<b>16.09</b>	<b>12.06</b>
<b>Initial</b>	<b>8.19</b>	<b>41.68</b>	<b>26.68</b>	<b>316.15</b>	<b>63.70</b>	<b>7.66</b>	<b>253.34</b>	<b>719.39</b>

and 667.16 mg kg<sup>-1</sup>), respectively, compared to control saloid-P, Al-P, Fe-P, Red-P, Occl-P, Ca-P and total-P (7.14, 30.00, 15.88, 39.60, 4.59, 309.20, 285.36 and 657.77 mg kg<sup>-1</sup>), respectively. Thus, the levels of phytase and phosphorus alone or in combinations were found beneficial for enhancing the availability of all the forms of phosphorus.

Reddy *et al.* (1983) reported that, Al-P and Fe-P were significantly higher in top soils than in lower depths while, Ca-P remained same in both the layers. All three fractions of inorganic P increased as a result of regular P application. Similar result by Sharma *et al.* (1987) in a long term fertilizer experiment with maize-wheat-cowpea rotation and they reported that, application of phosphatic fertilizers for a long time resulted in build-up of all forms of phosphorus quite markedly, the Ca-P increased due to continuous manuring and cropping in almost all the treatments except control and 100 per cent N alone. Subramaniam and Kumarswamy (1989) reported that, increased trends in total phosphorus content due to addition of fertilizer with FYM under continuous cropping and fertilization programme. Dakhore *et al.* (1993) studied on inorganic P fractions in an Entisol under continuous cropping and fertilizer use. They reported that, application of higher dose (80 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) of phosphorus fertilizer significantly increased the all forms of phosphorus Ca- P (287 mg kg<sup>-1</sup>), Al- P (75 mg kg<sup>-1</sup>), Fe- P (24 mg kg<sup>-1</sup>) and saloid- P (7 mg kg<sup>-1</sup>) as compared to lower dose (40 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) of phosphorus and control.

Singh *et al.* (1993) observed maximum accumulation of Ca-P form followed by RS-P and minimum was noticed in Saloid-P form and Al-P form was more than Fe-P. All the forms were decreased in control plots after 11 cycles of maize-wheat sequence in long term experiment. Further, NPK application increased total phosphorus over initial and magnitude of increase was more with higher rate of phosphorus application under cropping sequence with continuous fertilization.

#### **4.2.7 Effect of Phytase and Phosphorus Levels on Phosphatase Activity in Soil at 30 DAS and at Harvest of Green Fodder Maize**

##### **4.2.7.1 Soil acid phosphatase activity**

The soil acid phosphatase activity was significantly influenced at 30 days after sowing and at harvest by the phytase and phosphorus levels are presented in (Table 4.27 and depicted in Fig. 4.24).

**Table 4.27. Effect of Phytase and Phosphorus Levels on Enzyme Activity in Soil at 30 DAS and at Harvest of Fodder Maize**

Treatment	Acid phosphatase		Alkaline phosphatase	
	(µg PNP g <sup>-1</sup> soil hr <sup>-1</sup> )			
	30 DAS	At harvest	30 DAS	At harvest
<b>Phytase levels (P)</b>				
P <sub>1</sub> (1200 IU)	13.69	9.35	25.61	22.08
P <sub>2</sub> (2400 IU)	13.84	9.66	26.17	22.19
P <sub>3</sub> (3600 IU)	14.34	10.33	27.08	22.31
P <sub>4</sub> (4800 IU)	14.74	10.72	27.81	23.05
<b>S.Em. ±</b>	<b>0.15</b>	<b>0.07</b>	<b>0.058</b>	<b>0.14</b>
<b>C.D at 5 (%)</b>	<b>0.43</b>	<b>0.20</b>	<b>0.16</b>	<b>0.42</b>
<b>P<sub>2</sub>O<sub>5</sub> levels (F)</b>				
F <sub>1</sub> (00 kg ha <sup>-1</sup> )	15.83	11.84	29.09	24.38
F <sub>2</sub> (25 kg ha <sup>-1</sup> )	14.40	10.35	27.14	22.83
F <sub>3</sub> (50 kg ha <sup>-1</sup> )	13.55	9.28	26.18	21.77
F <sub>4</sub> (75 kg ha <sup>-1</sup> )	12.82	8.59	24.26	20.66
<b>S.Em. ±</b>	<b>0.15</b>	<b>0.07</b>	<b>0.058</b>	<b>0.14</b>
<b>C.D at 5 (%)</b>	<b>0.43</b>	<b>0.20</b>	<b>0.16</b>	<b>0.42</b>
<b>In. (P x F)</b>				
P <sub>1</sub> F <sub>1</sub>	15.11	11.08	28.20	24.26
P <sub>1</sub> F <sub>2</sub>	13.69	9.62	25.83	23.01
P <sub>1</sub> F <sub>3</sub>	13.47	8.56	25.26	21.16
P <sub>1</sub> F <sub>4</sub>	12.49	8.13	23.14	19.92
P <sub>2</sub> F <sub>1</sub>	15.67	11.40	28.97	24.73
P <sub>2</sub> F <sub>2</sub>	14.04	10.10	26.26	21.99
P <sub>2</sub> F <sub>3</sub>	13.21	9.00	25.92	21.46
P <sub>2</sub> F <sub>4</sub>	12.44	8.17	23.54	20.57
P <sub>3</sub> F <sub>1</sub>	16.08	12.16	29.32	23.78
P <sub>3</sub> F <sub>2</sub>	14.67	10.84	27.78	22.56
P <sub>3</sub> F <sub>3</sub>	13.57	9.50	26.44	22.00
P <sub>3</sub> F <sub>4</sub>	13.05	8.82	24.78	20.92
P <sub>4</sub> F <sub>1</sub>	16.48	12.71	29.86	24.76
P <sub>4</sub> F <sub>2</sub>	15.23	10.84	28.70	23.75
P <sub>4</sub> F <sub>3</sub>	13.98	10.08	27.10	22.46
P <sub>4</sub> F <sub>4</sub>	13.30	9.25	25.60	21.24
<b>S.Em. ±</b>	<b>0.30</b>	<b>0.14</b>	<b>0.12</b>	<b>0.30</b>
<b>C.D at 5 (%)</b>	<b>NS</b>	<b>NS</b>	<b>0.33</b>	<b>NS</b>
Tr. Vs Control				
Treated	14.15	10.01	26.67	22.41
Control	7.76	7.59	16.86	14.48
<b>S.Em. ±</b>	<b>0.31</b>	<b>0.14</b>	<b>0.11</b>	<b>0.30</b>
<b>C.D at 5 (%)</b>	<b>0.89</b>	<b>0.42</b>	<b>0.34</b>	<b>0.87</b>
<b>Initial</b>	<b>6.72</b>		<b>11.93</b>	

#### 4.2.7.1.1 Phytase levels

The levels of phytase enzyme significantly increased the soil acid phosphatase activity at 30 days after sowing and at harvest. It was increased with an increased levels of phytase and significantly the highest in 4800 IU phytase (14.74 and 10.72  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ) which was at par with the application of phytase @ 3600 IU (14.34  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ) at 30 days after sowing.

#### 4.2.7.1.2 Phosphorus levels

The phosphorus levels were found to increase the soil acid phosphatase activity in the lowest levels of phosphorus. It was increased with an decreased levels of phosphorus and significantly the highest in 00  $\text{kg ha}^{-1}$  phosphorus levels (15.83 and 11.84  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ) over rest of all the treatments at 30 days after sowing and at harvest. The soil acid phosphatase activity was increased with an advanced periods of sowing. It was highest at 30 days after sowing and decreased at harvest. It might be because of the soil acid phosphatase activity was highest in phosphorus deficient soil.

#### 4.2.7.1.3 Phytase and phosphorus interactions

The interactions between levels of phytase and phosphorus was non significant for soil acid phosphatase activity at 30 days after sowing and at harvest.

In respect of treated vs control, the treated levels of phytase and phosphorus was recorded significantly higher soil acid phosphatase activity at 30 days after sowing and at harvest (14.15 and 10.01  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ) compared to control (7.76 and 7.59  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ). Thus, the levels of phytase alone were found beneficial for enhancing the soil acid phosphatase activity.

The acid phosphatase activity was higher in no phosphorus application, application of phytase, FYM and phosphorus 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 (2012). Higher soil acid phosphatase activity in FYM amended pots might be ascribed to enhanced microbial population and activity. Further these secret acid phosphatases which have positive effect on native P solubilization (Yadav and Verma, 2012). Dotaniya *et al.* (2014) concluded that maximum acid phosphatase activities were observed at 75 DAT (days after transplanting) in rice (0.206  $\text{mg PNP g}^{-1} \text{ h}^{-1}$ ), 90 DAS in sorghum (0.194  $\text{mg PNP g}^{-1} \text{ h}^{-1}$ )

and pearl millet (0.201 mg PNP g<sup>-1</sup> h<sup>-1</sup>) 50 DAS in soybean (0.127 mg PNP g<sup>-1</sup> h<sup>-1</sup>). 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.

Martens *et al.* (1992) 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) 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.

#### **4.2.7.2 Soil alkaline phosphatase activity**

The soil acid phosphatase activity was significantly influenced at 30 days after sowing and at harvest by the phytase and phosphorus levels are presented in (Table 4.27 and depicted in Fig. 4.25).

##### **4.2.7.2.1 Phytase levels**

The levels of phytase enzyme significantly increased the soil alkaline phosphatase activity at 30 days after sowing and at harvest. It was increased with an increased levels of phytase and significantly the highest in 4800 IU phytase (27.81 and 23.05 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) at 30 days after sowing and at harvest.

##### **4.2.7.2.2 Phosphorus levels**

The phosphorus levels were found to increase the soil alkaline phosphatase activity in the lowest levels of phosphorus. It was increased with an decreased levels of phosphorus and significantly the highest in 00 kg ha<sup>-1</sup> phosphorus levels (29.09 and 24.38 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) over rest of all the treatments at 30 days after sowing and at harvest. The soil alkaline phosphatase activity was increased with an

advanced periods of sowing. It was highest at 30 days after sowing and decreased at harvest. It might be because of the soil alkaline phosphatase activity was highest in phosphorus deficient soil.

#### 4.2.7.2.3 Phytase and phosphorus levels

The interactions between levels of phytase and phosphorus were found significant at 30 days after sowing and non significant at harvest for soil alkaline phosphatase activity.

In respect of treated vs control, the treated levels of phytase and phosphorus was recorded significantly higher soil alkaline phosphatase activity at 30 days after sowing and at harvest (26.67 and 22.41  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ) compared to control (16.86 and 14.48  $\mu\text{g PNP g}^{-1} \text{ soil hr}^{-1}$ ). Thus, the levels of phytase alone were found beneficial for enhancing the soil alkaline phosphatase activity.

Yadav *et al.* (2009) reported 15 per cent increase in acid phosphatase, 12 per cent increase in alkaline phosphatase, 71 per cent 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, 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. 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).

Martens *et al.* (1992) 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<sup>-1</sup>) 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.

## 5. SUMMARY AND CONCLUSION

The incubation and pot culture experiment entitled “Effect of phytase and phosphorus levels on soil phosphorus availability and yield of fodder Maize in an Inceptisol.” was conducted during *Kharif* season of 2016-2017 at Department of Soil Science and Agricultural Chemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri. The experiment was laid out in factorial completely randomized design with two replications and seventeen treatments, involving combination of two factors *viz.*, phytase and phosphorus levels and sixteen treatment combination with one absolute control. The treatment comprised of four levels of phytase @ 1200, 2400, 3600 and 4800 IU and four levels of phosphorus @ 0, 25, 50 and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> and absolute control to evaluate the phosphorus availability in soil, available nutrients, soil enzyme activity, forms of phosphorus, nutrient uptake and yield of fodder maize. The salient findings of the present investigation are summarized as below

### 5.1 Incubation Study

The effect of phytase and phosphorus levels and their interactions on periodical soil pH, electrical conductivity, organic carbon, calcium carbonate, available nitrogen, phosphorus and potassium, forms of phosphorus and enzyme activity at 0, 7, 15, 30, 45 and 60 DAI were studied. The results obtained are summarized in this chapter.

#### 5.1.1 Effect of Phytase and Phosphorus Levels on Periodical Soil pH and Electrical Conductivity

The application of phytase and phosphorus levels and their interactions showed non significant at all the stages of incubation periods in respect of soil pH and electrical conductivity.

#### 5.1.2 Effect of Phytase and Phosphorus Levels on Periodical Soil Organic Carbon and Calcium Carbonate Content

The application of phytase showed non significant for soil organic carbon content and calcium carbonate content at 0, 7, 15, 30, 45 and 60 DAI.

The application of phosphorus levels showed non significant for soil organic carbon content at all the stages of incubation.

The application of phosphorus @ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased calcium carbonate content at 7, 15 and 30 DAI (6.11, 6.18 and 6.19 %), respectively and significantly decreased at 45 and 60 DAI (6.14 and 6.08 %), respectively, except 0 DAI.

The interaction effect of phytase and phosphorus levels showed non significant for organic carbon and calcium carbonate content in incubation study.

### **5.1.3 Effect of Phytase and Phosphorus Levels on Periodical Soil Available Nitrogen, Phosphorus and Potassium**

The levels of phytase enzyme significantly increased in available nitrogen, phosphorus and potassium content in soil was observed during the incubation period. The application of phytase @ 4800 IU significantly recorded the higher soil available nitrogen (85.47, 88.13, 90.30 and 100.31 mg kg<sup>-1</sup>), respectively at 0, 7, 15 and 30 DAI then declined in the 45 DAI (98.46 mg kg<sup>-1</sup>) and 60 DAI (94.57 mg kg<sup>-1</sup>), available potassium content in soil increased significantly (201.01, 209.76, 217.31, 241.85 mg kg<sup>-1</sup>), respectively at 0, 7, 15 and 30 DAI then declined in the 45 and 60 DAI (233.60 and 223.60 mg kg<sup>-1</sup>), respectively. The available phosphorus content in soil increased significantly (7.83, 8.07, 8.89, 9.66 and 10.90 mg kg<sup>-1</sup>), respectively at 0, 7, 15, 30 and 45 DAI, which were declined in 60 DAI (9.95 mg kg<sup>-1</sup>) by the application of phytase @ 4800 IU.

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased significantly in available nitrogen, phosphorus and potassium content in soil at 0, 7, 15, 30, 45 and 60 DAI. The available nitrogen (85.79, 89.15, 92.30, 101.89, 99.37 and 96.57 mg kg<sup>-1</sup>), respectively, available phosphorus (8.01, 8.55, 9.58, 10.53, 11.70 and 10.73 mg kg<sup>-1</sup>), respectively and available potassium (200.61, 209.36, 216.94, 241.36, 233.11 and 223.11 mg kg<sup>-1</sup>), respectively.

The interaction between phytase and phosphorus levels showed significant for increase in available nitrogen at 0, 7, 15 and 30 DAI and decreased at 45 DAI (86.17, 89.53, 92.68, 102.27 and 99.75 mg kg<sup>-1</sup>), respectively by the combine application of phytase @ 4800 IU + 75 P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Available phosphorus showed non significant due to interaction of phytase and phosphorus levels at 0, 7, 15, 30 and 60 DAI. At 45 DAI showed significant increase in available phosphorus (12.18 mg kg<sup>-1</sup>) by the application of

phytase @4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. Available potassium showed significant increased at 0, 7, 15 and 30 DAI (201.25, 210.00, 217.50 and 242.00 mg kg<sup>-1</sup>), respectively.

#### **5.1.4 Effect of Phytase and Phosphorus Levels on Periodical Forms of Phosphorus in Soil**

The application of phytase @ 4800 IU increased significantly the all forms of phosphorus at 0, 7, 15, 30, 45 and 60 DAI, which increased upto 45 DAI and decreased at 60 DAI, except organic-P and total-P in which all forms of phosphorus increased upto 60 DAI. The saloid-P (11.53, 12.76, 13.50, 14.34, 16.00 and 14.98 mg kg<sup>-1</sup>), respectively. Al-P (46.88, 56.25, 75.94, 90.94, 104.06 and 96.56 mg kg<sup>-1</sup>), respectively. Fe-P (31.44, 33.19, 36.41, 39.23 and 37.32 mg kg<sup>-1</sup>), respectively except 0 DAI. Ca-P (317.76, 322.42, 324.63, 326.83, 329.86 and 328.09 mg kg<sup>-1</sup>), respectively. Red-P (78.00, 84.50, 85.80, 96.20 and 91.98 mg kg<sup>-1</sup>), respectively except 0 DAI. Occl-P (7.49, 10.99, 11.87, 12.03, 13.67 and 12.91 mg kg<sup>-1</sup>), respectively. organic-P (246.56, 234.95, 209.54, 201.38, 173.78 and 199.09 mg kg<sup>-1</sup>), respectively and total-P (746.82, 753.16, 767.73, 772.79 and 780.93 mg kg<sup>-1</sup>), respectively and 0 DAI (722.75 mg kg<sup>-1</sup>) by the application of phytase @2400 IU.

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> increased significantly the all forms of phosphorus at 0, 7, 15, 30, 45 and 60 DAI, except organic-P in which it increased upto 45 DAI and thereafter decreased. The saloid-P (11.95, 13.48, 14.69, 15.64, 17.62 and 16.62 mg kg<sup>-1</sup>), respectively. Al-P (52.50, 61.88, 88.13, 103.75, 118.13 and 110.63 mg kg<sup>-1</sup>), respectively. Fe-P (29.22, 33.54, 36.21, 39.62, 42.88 and 40.97 mg kg<sup>-1</sup>), respectively. Ca-P (318.26, 324.50, 326.20, 330.99, 334.01 and 330.68 mg kg<sup>-1</sup>), respectively. Red-P (64.03, 82.23, 89.05, 92.95, 103.35 and 97.83 mg kg<sup>-1</sup>), respectively. Occl-P (7.60, 11.70, 12.58, 12.85, 14.77 and 13.89 mg kg<sup>-1</sup>), respectively. organic-P ( 242.35, 227.73, 194.54, 178.26, 148.38 and 175.39 mg kg<sup>-1</sup>), respectively and total-P (725.92, 755.06, 761.39, 774.06, 779.13 and 786.00 mg kg<sup>-1</sup>), respectively.

The interaction effect of phytase and phosphorus levels on saloid-P increased significantly at 15 DAI (15.12 mg kg<sup>-1</sup>) and 30 DAI (16.19 mg kg<sup>-1</sup>). Al-P significantly increased at 30 DAI (106.88 mg kg<sup>-1</sup>) only and Fe significantly increased at 7 DAI (34.94 mg kg<sup>-1</sup>). Ca-P significantly increased by the application of phytase @4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> at 0, 7, 15, 30, 45 and 60 DAI (318.52, 326.08, 328.34, 334.14,

337.16 and 333.89 mg kg<sup>-1</sup>), respectively. Red-P showed significant (89.70, 96.20, 100.10, 110.50 and 104.00 mg kg<sup>-1</sup>), respectively. Occl-P significantly increased (13.34, 14.22, 14.66, 16.41 and 15.53 mg kg<sup>-1</sup>), respectively at 7, 15, 30, 45 and 60 DAI. Organic-P significantly increased at 7 DAI (221.23 mg kg<sup>-1</sup>) and 60 DAI (175.57 mg kg<sup>-1</sup>). Total-P showed significantly increased at 7, 15 and 60 DAI (762.66, 772.79 and 801.20 mg kg<sup>-1</sup>), respectively.

### **5.1.5 Effect of Phytase and Phosphorus Levels on Periodical Soil Phosphatase Activity in Soil**

The application phytase significantly increased soil acid and alkaline phosphatase activity at various days of incubation. The enzyme activity increased significantly upto 30 DAI then declined at the 45 and 60 DAI. Application of phytase @ 4800 IU at 0, 7, 15, 30, 45 and 60 DAI showed significantly increased acid phosphatase (8.70, 9.15, 11.66, 13.44, 11.14 and 10.46 µg PNP g<sup>-1</sup> hr<sup>-1</sup>), respectively, and alkaline phosphatase (15.91, 19.12, 23.12, 24.28, 22.49 and 21.48 µg PNP g<sup>-1</sup> hr<sup>-1</sup>), respectively.

Application of 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the enzyme activity as compared to other levels of phosphorus application. The application of 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> at 0, 7, 15, 30, 45 and 60 DAI significantly increased acid phosphatase (8.92, 9.19, 12.01, 13.89, 11.44 and 10.61 µg PNP g<sup>-1</sup> hr<sup>-1</sup>), respectively and alkaline phosphatase (16.54, 18.89, 22.32, 23.82, 21.80 and 21.23 µg PNP g<sup>-1</sup> hr<sup>-1</sup>), respectively. The same trend observed in the phosphorus application i.e. enzyme activity increased upto 30 DAI and declined in 45 and 60 DAI.

## **5.2 Pot Culture Study**

### **5.2.1 Effect of Phytase and Phosphorus Levels on Plant Height and Number of Leaves at 30 Days After Sowing and at Harvest of Green Fodder Maize**

The levels of phytase and phosphorus significantly increased in plant height and number of leaves at 30 days after sowing and at harvest.

The plant height of the fodder maize increased significantly at harvest (154.04 cm) by the application of phytase @ 4800 IU. The number of leaves also significantly increased (7.38 and 13.79) at 30 DAS and at harvest, respectively by the application of phytase @ 4800 IU.

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the plant height and number of leaves (46.19 cm and 7.66) at 30 DAS and at harvest (161.00 cm and 14.92), respectively in green fodder maize.

### **5.2.2 Effect of Phytase and Phosphorus Levels on Root Volume, Root Cation Exchange Capacity and Root Biomass at Harvest of Green Fodder Maize**

The application phytase @ 4800 IU significantly increased in the root volume (20.75 ml), root CEC (7.85 cmol kg<sup>-1</sup>) and root biomass (5.63 g plant<sup>-1</sup>) followed by application of phytase 3600 IU.

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the root volume (25.25 ml), root CEC (9.38 cmol kg<sup>-1</sup>) and root biomass (7.29 g plant<sup>-1</sup>) at harvest.

The interaction effect of phytase and phosphorus levels significantly increased root biomass (8.15 g plant<sup>-1</sup>) by the application of phytase @ 4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### **5.2.3 Effect of Phytase and Phosphorus Levels on Green Fodder and Dry Fodder Yield at Harvest of Green Fodder Maize**

The green fodder yield and dry matter yield of maize was highest in the application of phytase @ 4800 IU (141.71 and 13.50 g plant<sup>-1</sup>).

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the green fodder yield and dry matter production (164.45 g and 15.66 g plant<sup>-1</sup>), respectively.

The interaction effect of phytase and phosphorus levels significantly increased green fodder yield and dry matter production in combined application of phytase @ 4800 IU+ 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (182.75 and 17.40 g plant<sup>-1</sup>), respectively.

### **5.2.4 Effect of Phytase and Phosphorus Levels on Nutrient Uptake and Apparent P Recovery at Harvest of Green Fodder Maize**

The application of phytase @ 4800 IU significantly increased uptake of nitrogen, phosphorus and potassium (12.40, 4.95 and 15.98 mg g<sup>-1</sup>), respectively, in green fodder maize. The application of phosphorus fertilizer 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the uptake of nitrogen, phosphorus and potassium (13.63, 5.21 and 17.09 mg g<sup>-1</sup>), respectively and apparent P recovery (27.42 mg mg<sup>-1</sup>).

### **5.2.5 Effect of Phytase and Phosphorus Levels on Soil Chemical Properties at 30 Days After Sowing and at Harvest of Green Fodder Maize**

The effect of phytase levels showed non significant for soil pH, electrical conductivity and soil organic carbon and soil calcium carbonate content at 30 DAS and at harvest of the green fodder maize.

The application of phosphorus levels showed non significant for soil pH, electrical conductivity and soil organic carbon content. The application of phosphorus found significantly higher calcium carbonate content of soil at 30 DAS and at harvest by the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (5.88 and 5.84 %), respectively.

### **5.2.6 Effect of Phytase and Phosphorus Levels on Available Nitrogen, Phosphorus and Potassium at 30 Days After Sowing and at Harvest of Green Fodder Maize**

The available nutrient status of nitrogen, phosphorus and potassium content of soil increased by the application of phytase @ 4800 IU at 30 DAS (103.08, 11.35 and 185.63 mg kg<sup>-1</sup>), respectively at 30 days after sowing and decreased at harvest (87.15, 9.27 and 176.00 mg kg<sup>-1</sup>), respectively of the fodder maize.

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased available nitrogen, phosphorus and potassium at 30 DAS (106.40, 11.67 and 186.50 mg kg<sup>-1</sup>), respectively and significantly decreased at harvest (88.90, 9.82 and 177.31 mg kg<sup>-1</sup>), respectively.

The interaction effect of phytase and phosphorus levels significantly increased available phosphorus at harvest by the application of phytase @ 4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (10.27 mg kg<sup>-1</sup>). The highest available potassium recorded in the application of phytase @ 4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> (191.25 and 180.75 mg kg<sup>-1</sup>), respectively at 30 DAS and at harvest.

### **5.2.7 Effect of Phytase and Phosphorus Levels on Forms of Phosphorus at 30 Days after Sowing and at Harvest of Green Fodder Maize**

All forms of phosphorus such as saloid-P, Al-P, Fe-P, Ca-P, Red-P, Occl-P and total-P were significantly increased and organic-P decreased in application of phytase @ 4800 IU at 30 DAS (15.29, 83.44, 38.27, 333.32, 86.45, 12.03 and 776.06 mg

kg<sup>-1</sup>), respectively, and at harvest (12.24, 64.69, 30.97, 307.37, 73.45, 9.84 167.45 and 666.00 mg kg<sup>-1</sup>), respectively.

The application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased saloid-P, Al-P, Fe-P, Ca-P, Red-P, Occl-P and total-P and decreased organic-P (17.15, 98.44, 45.42, 344.09, 95.55, 13.56 173.35 and 787.56 mg kg<sup>-1</sup>), respectively at 30 DAS and slightly decreased all forms of phosphorus at harvest (14.45, 75.47, 37.48, 318.89, 82.88, 11.38, 136.71 and 677.26 mg kg<sup>-1</sup>), respectively.

The combine application of phytase and phosphorus levels at 30 DAS significantly increased saloid-P, Al-P, Ca-P and Red-P (18.29, 110.63, 350.27 and 105.30 mg kg<sup>-1</sup>), respectively by the application of phytase @ 4800 IU + 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>. The interaction between levels of phytase and phosphorus significantly increased Ca-P (325.07 mg kg<sup>-1</sup>) and Red-P (92.30 mg kg<sup>-1</sup>) which was recorded highest in the application of phytase @ 4800 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### **5.2.8 Effect of Phytase and Phosphorus Levels on Soil Phosphatase Activity at 30 Days after Sowing and at Harvest of Green Fodder Maize**

The application of phytase @ 4800 IU significantly increased acid and alkaline phosphatase activity (14.74 and 27.81 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>), respectively at 30 DAS. The acid and alkaline phosphatase activity was significantly decreased (10.72 and 23.05 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) at harvest.

The acid and alkaline phosphatase activity (15.83 and 29.09 and µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>), respectively were significantly increased by 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> as compared to the other levels of phosphorus at 30 DAS. At harvest, significantly decreased, but highest acid and alkaline phosphatase activity (11.84 and 24.38 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>), respectively recorded in the no application of phosphorus.

The interaction between levels of phytase and phosphorus significantly increased the soil alkaline phosphatase activity (29.86 µg PNP g<sup>-1</sup> soil hr<sup>-1</sup>) at 30 days after sowing by the application of phytase @ 4800 IU and 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

### **Incubation Study**

1. The application of phytase @ 4800 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the soil available nitrogen and potassium content upto 30 DAI and slightly declined at 45 and 60 DAI.

2. The application of phytase @ 4800 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the soil available phosphorus content upto 45 DAI and slightly declined at 60 DAI.
3. The application of phytase @ 4800 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased different forms of phosphorus upto 45 DAI and declined at 60 DAI.
4. The application of phytase @ 4800 IU and 00 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased acid and alkaline phosphatase activity upto 30 DAI and declined at 45 and 60 DAI.

#### **Pot Culture Study:**

1. The application of phytase @ 4800, 3600 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the root volume, root CEC and root biomass at harvest.
2. The application of phytase @ 2400, 3600 and 4800 IU significantly increased green fodder yield and dry fodder yield of maize and apparent P recovery along with the application of 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.
3. The application of phytase @ 4800 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the available nitrogen, available phosphorus and potassium upto 30 DAS and declined at harvest.
4. The application of phytase @ 4800 IU and 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased different forms of phosphorus at 30 DAS and declined at harvest.
5. The application of phytase @ 4800 IU and 00 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the acid and alkaline phosphatase activity at 30 DAS and declined at harvest.

#### **Conclusion**

The application of phytase @ 4800 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> found significantly increased soil available phosphorus and all forms of phosphorus under incubation study and pot culture experiments. The application of phytase @ 4800 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> significantly increased the green fodder yield, dry fodder yield and growth attributing characters and apparent P recovery in green fodder maize under pot culture experiments. However, application of phytase @ 2400 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> showed at par results. Hence, the optimum level of phytase and inorganic phosphorus for fodder maize is phytase @ 2400 IU along with the 75 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>.

**Future Research Needs**

- On the basis of this incubation study and pot culture experiment, there is a need to study in field by using various organic manures.
- It is also essential to evaluate phytase activity in the rhizospheral soil of different crops to assess the phosphorus availability.
- It is also need to evaluate phytase activity in acidic soil with incubation and field experiment.

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## 7. VITAE

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**PRAKASH BAPURAO TAPKEER**

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of

**DOCTOR OF PHILOSOPHY (AGRICULTURE)  
IN  
SOIL SCIENCE AND AGRICULTURAL CHEMISTRY  
2019**

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