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A comparison of phytase efficiency originated from plant and fungal sources

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Abstract

The phytase enzyme was collected from plants (cereal, legumes and oilseeds) and fungal sources and compared their efficiency to release inorganic P from organic P compounds. In general, fungi released more phytase after 21 days of growth and their extracellular activity was more than their intra-cellular counterpart. Plants are releasing 4 to 43% more phytase under P deficient condition as compared to the sufficient P condition. Oilseed crops releases higher phytase than legumes and cereal crops. But phytase released from legumes are discovered to be more efficient to hydrolyze organic phosphorus. Phytase release by *Chaetomium globosum* was exposed to be most efficient on as compared to the phytase produced by other tested fungi. Phytase originated from fungal sources are described to be more efficient than similar amount of phytase originated from plant sources.

Keywords: Phytase; Efficiency; Plant and fungal sources; Adenosine phosphate; Glycerophosphate; Phytin

1. Introduction

Phytase (myo-inositol hexakisphosphate phosphohydrolase) catalyzes the hydrolysis of myo-inositol hexa kisphosphate, commonly known as phytic acid, which is the principal storage form of phosphorus (P) in many seeds and pollen grains (Mega, 1982; Reddy et al., 1982). Phytase can hydrolyzed all types of ester bond (Turner et al., 2002) and consistently produced the greatest degree of hydrolysis and improve the utilization of phosphate (Greiner and Konietzny, 2006). Phytic acid was considered to be an anti-nutritive factor, which formed insoluble complexes with nutritionally important metals such as calcium, zinc and magnesium, as well as protein, thereby decreasing their bioavailability (Mega, 1982; Fox and Tao, 1989). Phytase is widely distributed in plant and animal tissues as well as many fungi and bacteria (Cosgrove, 1966). Hong et al. (2004) identified the two highly active bacterial phytases, the appA and the $SrPF_6$ in the germinated transgenic rice seeds, have high activity over broad pH ranges of 3-5.5 and 2-6.0 respectively. Hydrolysis of precipated phytase by three distinct families of phytate was reported by Tang et al. (2006) i..e. histidine acid phosphatase (HAPs), beta propeller phytase (BPPs) and purple acid phosphatases (PAPs), secreted by genetically modified plants. All three phytases were able to hydrolyse Ca^{+2}, Mg^{+2} and Mn^{+2} phytates. Lung and Lim (2006) reported that the availability of soluble phytate affected by assimilation of phytate phosphorus by the extracellular phytase activity of tobacco (*Niwtaiana tabacum*). Hayes et al. (2000) found a small use of phytin by sterile growing germinating plants, which were increased by *Aspergillus* (fungus) phosphatase. It is conceivable, that P-deficiency not only causes changes in phosphatase quantity and activity, but also changes in isoenzyme composition. It could be possible, that P deficient plants not only release unspecific acid phosphatases, but also phytase (Li et al., 1997). The importance of soil organic P as a source of plant available P depends on its rate of solubilization and the rate of inorganic P release. In soil, the hydrolysis of organic P is predominantly mediated by the activity of soil microorganisms, although plant roots also posses' phosphatase and phytase activity (Tarafdar and Jungk, 1987; Li et al., 1997). Secretion of beta-propeller phytase from tobacco and Arabidopsis roots enhanced the phosphorus utilization by mobilization of soil phytate i.e. phytic acid and improve the accumulation of shoot biomass and shoot P concentration (Lung et al., 2005).

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Phytase is considered to be a special species of phosphatases and plays an important role in mobilizing the P reserves for growing seedlings (Mandal et al., 1974) and for pollen germination (Jackson and Linskens, 1982; Helsper et al., 1984). Although, there have been many studies on the phytase, most of them were focused on the germinating seeds of pollen, while the phytase in roots and other tissues of plant was overlooked. We don't have the systematic study so far about the comparison of the efficiency of phytases produced from plants and microbes. Therefore, the present study was undertaken to differentiate the efficiency of phytase enzymes generated from plant and fungal sources were compared.

2. Material and methods

2.1. Isolation and identification of efficient Phytase producing fungi

Fungi were isolated from thirty diversified soils, using dilution plate technique (Tarafdar and Chhonkar, 1979) on Martin's Rose Bengal agar containing streptomycin sulphate (Allen, 1959). Twenty one different fungi were isolated based on differences in type of colonies (color, shape, growth, spore formation etc.) purified from the single spore in slants, and the pure culture were maintained on a potato dextrose agar (PDA) medium. Based on their intra and extra cellular phytase activity five fungi Aspergillus *candidus, Aspergillus ustus, Chaetomium globosum, Curvularia lunata* and *Phoma species* were selected and identified by Agharkar Research Institute, Pune, India.

2.2. Intra and extracellular phytase activity of fungi

For determination of phytase enzyme activity, fungi were grown in 100 mL (Zapek-Dox broth in 250 mL Erlenmeyer flasks). The medium was inoculated with 8 mm discs of 4-days old fungal growth (on potato dextrose agar medium) and the flasks were incubated at 30±1°C. There were 12 flasks of each fungal species during start of the experiment. At the end of the 7, 14, 21 and 28 days of incubation, three flasks of each fungal culture were harvested. The flasks were chilled in ice and the content was filtered through Whatman No.1 filter paper into another flask kept in ice. The final volume of each filtrate was made up to 100 mL using sterilized cold distilled water. The filtrate was used for assaying the extracellular phytase activity. For determination of intra cellular activity, fungal mat at each sampling time were washed at least 10 times with ice cold distilled water (10 mL each time) to remove traces of extra cellular enzymes. Washed fungal mat were weight after lightly pressing them between sheets of filter paper. A small part of the mat was dried to express the results on dry weight basis. The remaining part was ground with acid washed quartz sand in a mortar. Ice-cold sterilized distilled water was added to obtain a fine suspension of fungal mat. The extract obtained was centrifuged at 12,000 rpm for 20 minutes to settle the fungal debris. A clear supernatant containing the intra cellular enzymes was obtained and made up to a known volume.

2.3. Solution culture experiment for plants

To get phytase from plantsa solution culture experiment was conducted using hundred seeds of wheat (*Triticum aestivum* L., cultivar RAJ-3077), sorghum (*Sorghum bicolor*; cultivar: SSG-1000) as cereal, moong bean (*Phaseolus radiatus*; K-851), moth bean (*Vigna radiata*; cultivar: RMO-435) as legume, mustard (*Brassica compestris*; cultivar: T-59) and sesame (*Sesamum indicum*; cultivar: RJ-125) as oilseed were surface sterilized using 1:1 (v/v) mixture of H_2O_2 and absolute ethanol for 2 minutes, followed by 0.05% $HgCl_2$ with traces of HCl for 2 minutes. The sterilizing agents were drained off completely, and the seeds were washed 10-12 times in sterilized deionized water to remove all traces of the chemicals. The seeds were kept for 5 days, for germination, after wrapping them in a moist filter paper. The germinated seeds were transferred to nutrient solution (Tarafdar and Claassen, 1988). All treatments received solution containing 250 mg P L^{-1} as KH_2PO_4, except for the P-deficient treatment which received only 5µg P L^{-1} as KH_2PO_4. The treatment without any P was excluded because growth of plant was severely affected. Under aseptic condition the plants were grown for three weeks in a sterilized growth chamber and the activity of phytase, released by plant roots, in the nutrient solution was measured after every week. The nutrient solution was changed every week and fresh solution was introduced. All experiments were conducted in an aseptic growth chamber with 14h day light (2500 – 3000 lux) at 65±5% relative humidity. The day temperature was 30°C and the night temperature was 20°C during the experiment. Plants were harvested at three times at 7 days intervals till 21 days after germination.

2.4. Phytase activity

Two mL aliquot from solution culture or fungal culture experiments was placed in 15 mL capacity screw cap test tubes. Four mL of 100 M sodium acetate buffer (pH 4.5), and 1 mL of sodium phytate (1µM) was added to it and incubated at 37°C for 1 h. After 1 h incubation, the reaction was terminated by the addition of 0.5 mL 10% Trichloro acetic acid (TCA) (CCl_3COOH). Proteins precipitated by TCA were removed by centrifugation at 10,000 rpm for 10 minute and the supernatant was analyzed for liberated inorganic P, using chlorostannous reduced molybdophosphoric hydrochloric

acid method as described by Jackson (1967). One unit of phytase activity was defined as the amount of enzyme, which liberated 1 μ mole inorganic P (Pi) per second from sodium phytate.

2.5. Phytin P in soil

The phytate phosphorous was estimated by the procedure described by Mega (1982). In this method one g of soil sample was taken in 50 mL beaker and 10 mL of 15% Trichloro-acetic acid CCl_3-COOH was added in each sample. The suspension was incubated for 16 h at room temperature. The liberated phytate then estimated, using chlorostannous-reduced molybdophosphoric blue colour method as desribed by Jackson (1967) and measured spectrophotometrically.

2.6. Efficiency of phytase towards different organic P compounds

Phytase enzymes generated from plant and fungal sources were tested towards hydrolysis of different organic P compounds. One mL of 300 μg of P from organic sources either in the form of Adenosine di-phosphate, Adenosine tri-phosphate , Glycerophosphate and phytin, were added into 150 mL of Erlenmeyer flasks containing 50 mL of phytase of known concentration (1 EU) originated from plant or fungal sources in citrate buffer (pH 4.5). The flasks were incubated at 30°C for 24 h and the release of inorganic P was determined at 2, 4, 6, 10 and 24 h of incubation by standard method (Jackson, 1967).

2.7. Efficiency of phytase to release P from native sources

Phytase enzymes generated from plant and fungal sources were tested towards hydrolysis of phytin present in soil. One hundred gram of air dried soil was added into 150 mL of Erlenmeyor flasks containing 50 mL of phytase of known concentration (1 EU) originated from plant or fungal sources. The flasks were incubated at 30 °C for 24 h and the release of inorganic P was determined at different time intervals (2, 4, 6, 10, 24 h) by standard method (Jackson, 1967).

3. Results

Table 1 Secretion of phytase by selected fungal species at different time intervals (± represent standard error of mean)

Phytase (EU × 10^{-3}) g^{-1} fungal mat								
Fungal species	**7 DAI**		**14 DAI**		**21 DAI**		**28 DAI**	
	Intra cellular	**Extra cellular**	**Intra cellular**	**Extra cellular**	**Intra cellular**	**Extra cellular**	**Intra cellular**	**Extra cellular**
Aspergillus candidus	99.07± 5.37	1980.99± 250.66	212.00± 22.08	3086.72± 316.08	396.44± 38.18	590.99± 428.93	275.15±30.10	4701.00± 329.55
Aspergillus ustus	102.0± 6.61	1896.70± 213.00	339.66± 30.18	4500.49± 380.11	845.75± 60.19	9497.77± 526.18	419.70±38.19	4399.11± 316.00
Chaetomium globosum	446.59±16.53	6763.07± 369.70	1075.85±114.39	10274.34 ± 590.14	1225.07± 127.13	12520.31±693.50	695.31±55.78	11650.49±416.80
Curvularia lunata	65.99± 3.08	1380.23± 160.19	113.52± 8.91	1676.69± 198.71	276.85± 25.81	4113.99±370.14	217.11±28.71	3870.12±271.10
Phoma species	134.09± 8.50	1583.11± 48.13	246.22± 25.13	2484.51± 80.17	450.45±42.19	5412.81±403.14	381.65±33.67	3588.72±260.18
DAI: Days after incubation								

All the fungal strains differ in their enzyme activities (Table 1). In general, highest phytase (intra and extra) release by different fungi was observed after 21 days of growth. All the fungi had higher extracellular phytase activity (4114×10-3 to 12520×10-3 EU g-1) than the enzyme released by the fungi intracellularly (277×10-3 to 1225× 10-3 EU g-1) at 21

days of growth. After 28 days of growth highest intra - cellular (695.3 × 10-3 EU g-1) and extracellular (11650.49 × 10^{-3} EU g^{-1})phytase activity was observed in *Chaetomium globosum* followed by *Aspergillus ustus* (419.70 × 10-3 EU g^{-1} intra- cellularly and 4399.11×10-3 EU g-1 extra- cellularly). The extra- cellular phytase release was 9 to 21 times higher than their intracellular counterpart.

Table 2 Phytase release by plant species under P deficient and sufficient condition at different times of plant growth (± represent standard errors of mean)

Plant species	Phytase (EU ×10^{-5}) activity (Release by 10 plants of each species)					
	7 DAS		DAS		21 DAS	
	+ P	-P	+ P	-P	+P	-P
Wheat	2.12±0.021	2.20±0.031(3.7)*	2.30±0.032	2.41±0.021(4.9)	2.42±0.032	2.52±0.020(4.1)
Sorghum	2.30±0.031	2.44±0.030(6.0)	2.33±0.022	2.58±.041(10.7)	2.38±0.031	2.61±0.025(9.7)
Moong bean	1.90±0.016	2.10±0.018(10.5)	2.75±0.022	2.95±0.025(7.3)	2.81±0.026	3.10±0.028(10.3)
Moth bean	2.09±0.018	2.35±0.021(12.4)	2.40±0.022	2.79±0.025(19.0)	3.32±0.027	3.63±0.029(9.3)
Musterd	3.02±0.025	3.75±0.028(24.2)	3.08±0.028	3.79±0.031(23.0)	3.31±0.022	3.91±0.040(18.1)
Sesame	2.60±0.021	3.30±0.040(26.9)	2.72±0.021	3.75±0.035(37.9)	2.80±0.018	4.01±0.036(43.2)
DAS: Day after sowing; * Figures in parenthesis represent the percent increase under P deficient condition						

Table 3 Phytase secretion by cereals, legumes and oilseeds (± represents standard errors of mean)

Plants	Phytase release (EU ×10^{-6})		% increase over P sufficient condition
	P-deficiency	P- sufficiency	
Cereals	2.55±0.13	2.25±0.12	3.7 to 10.7
Legumes	4.10±0.21	3.70±0.16	7.3 to 19
Oil seeds	3.90±0.21	2.89±0.13	18 to 43

The release of phytase both under P deficient and sufficient P condition with selected plant species was presented as Table 2. Phytase activity of selected plant species were more under P deficient than sufficient P condition. A wide variation of phytase release was observed among the cereals, legumes and oil seeds as well as P deficient and sufficient P condition (Table 2). The phytase activity under P sufficient condition ranges from 1.90 to 3.02 EU × 10-5 at 7 days which was further increased 2.10 to 3.75 EU ×10^{-5} under P deficient condition. The similar trend was observed after 14 days of plant growth when the variation in phytase activity was observed from 2.30 to 3.08 EU × 10-5 under P sufficient condition that increased to 2.41 to 3.79 EU ×10^{-5} under deficient condition. After 21 day of plant growth 2.42 to 3.32 EU ×10-5 phytase activity was observed in sufficient P condition and 2.52 to 4.01 EU ×10^{-5} in P deficient growth stage. Among the cereals, legumes and oil seeds maximum phytase activity observed in oilseed (4.01 EU × 10-5) under P deficient condition after 21 days of growth. The phytase activity under P deficient condition increases 3.7to 10.7 % more for cereals, 7.3 to19 % more for legumes and 18 to 43% more for oilseeds over P sufficient condition (Table 3).

3.1. A comparison between plant and fungal phytase

The release of inorganic P after hydrolysis from Adenosine-5' diphosphate sodium salt (ADP), Adenosine-5' tri-phosphate sodium salt (ATP), glycerophosphate (NAG) and phytin (300 μg) at different time intervals after incubation with one EU of phytase of plant sources (Table 4) showed maximum inorganic P release from ADP (32.4%) followed by ATP (28.6%) while least release from phytin (7.6%) after 24 hour of incubation. Phytin seems to be most difficult organic P compound to be hydrolyzed by plant phytase. With increase in time of incubation, there was more release of Pi but, in general, there was no change in Pi concentration after 6h of incubation till 24 h (Fig.1).

Table 4 Hydrolysis of organic –P (300 µg) by per enzyme unit of plant phytase (± represent standard error of mean)

Organic-P compound	Time interval				
	2h	4h	6h	10h	24h
ADP	40.54±3.06(13.5)	76.35±4.66(25.4)	95.94±5.52(32.0)	96.91±3.71(32.3)	97.29±3.29(32.4)
ATP	35.13±3.52(11.7)	59.46±4.59(19.8)	83.78±5.12(27.9)	85.81±4.59(28.6)	85.81±4.09(28.6)
NAG	10.13±2.72(3.4)	19.59±3.67(6.5)	27.32±3.54(9.1)	28.43±3.66(9.5)	30.40±4.51(10.1)
Phytin	7.77±1.72(2.6)	14.19±3.09(4.7)	20.94±3.22(7.0)	21.66±3.6(7.2)	22.97±3.0(7.6)

ADP: Adenosine¬ –5'-diphosphate sodium salt;ATP:Adenosine – 5'- tri phosphate sodium salt;NAG: Glycerophosphate * Figures in parenthesis represent the percent of totalP hydrolyzed

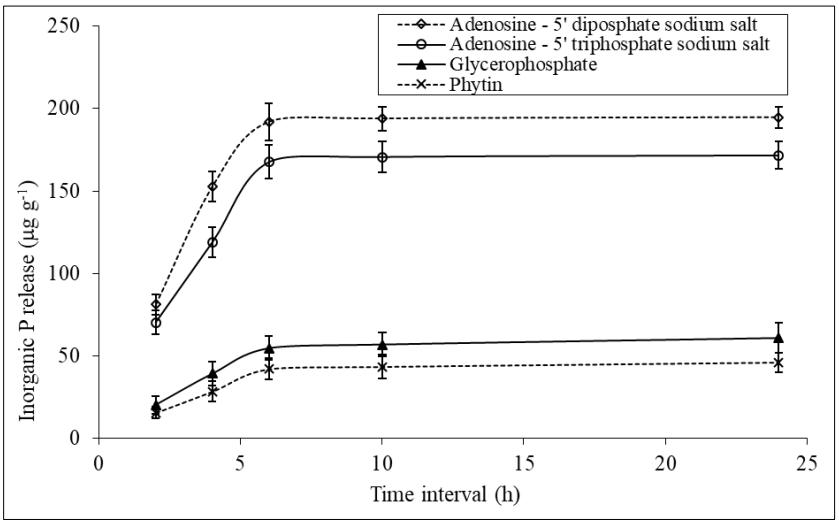

Figure 1 Release of inorganic P from organic P sources by one enzyme unit of plant phytase

Table 5 Hydrolysis of organic –P (300 µg) by per enzyme unit of fungal extra and intra cellular phytase (± represent standard error of mean)

Organic P Compound	Time interval (h)									
	2 h		4 h		6 h		10 h		24 h	
	Intra cellular	Extra cellular	Intra cellular	Extra cellular	Intra cellular	Extra cellular	Intra cellular	Extra cellular	Intra cellular	Extra cellular
ADP	33.15±1.9 (11.0)	57.42±2.5 (19.1)	53.10±2.8 (17.7)	72.96±2.7 (24.3)	63.81±3.4 (21.3)	85.76±4.6 (28.6)	65.92±4.1 (22.0)	93.73±4.7 (31.2)	67.98±3.9 (22.7)	98.35±4.9 (32.8)
ATP	24.93±1.5 (8.3)	49.10±2.9 (16.4)	41.31±2.0 (13,7)	69.30±3.0 (23.1)	59.52±4.2 (19.8)	79.40±3.7 (26.5)	60.35±3.1 (20.1)	88.70±5.2 (29.6)	60.80±4.1 (20.3)	94.20±5.1 (31.4)
NAG	21.72±1.2 (7.2)	43.16±2.8 (14.4)	34.86±2.3 (11.6)	61.98±2.7 (20.7)	54.10±3.5 (18.0)	67.30±3.0 (22.4)	55.17±2.4 (18.4)	73.10±3.8 (24.4)	56.35±3.2 (18.8)	85.94±4.1 (28.6)
Phytin	19.82±1.3 (6.6)	40.10±3.7 (13.4)	31.83±1.5 (10.6)	57.13±2.5 (19.0)	47.34±1.9 (15.8)	61.31±3.3 (20.4)	52.88±2.8 (17.6)	69.82±3.4 (23.3)	55.16±2.4 (18.4)	78.74±3.8 (26.2)

ADP: Adenosine¬ –5'-diphosphate sodium salt; ATP:Adenosine – 5'- tri phosphate sodium salt; NAG: Glycerophosphate; * Figures in parenthesis represent the percent of total phytin P hydrolyzed

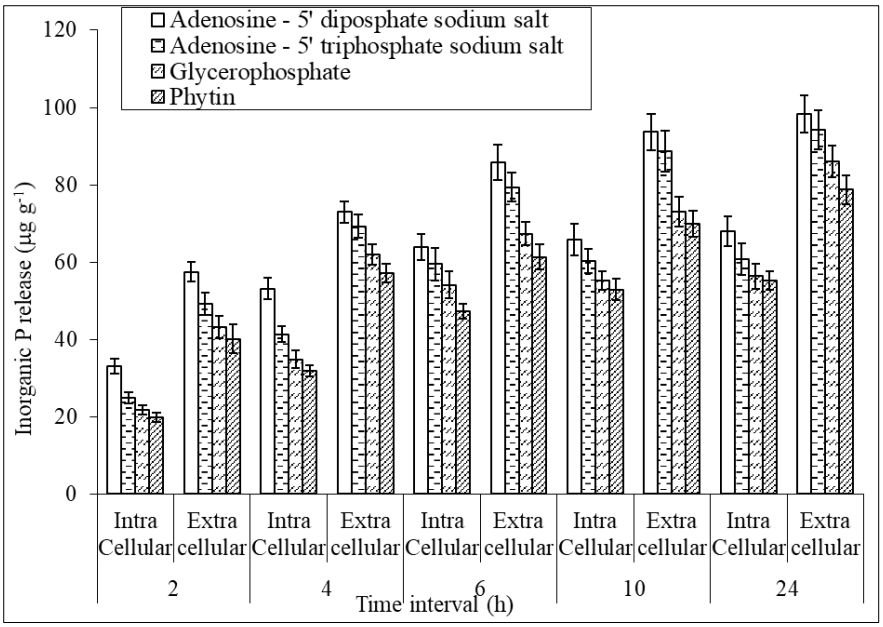

Figure 2 Release of inorganic P with time from organic P sources by one enzyme unit of fungal phytase

The efficiency of fungal extra- and intra- cellular enzyme on four different organic P compounds (Fig.2) demonstrated that both intra– and extra- cellular enzymes were most efficient to hydrolyse ADP, whereas minimum release of P was observed from phytin. It is observed that after 24 h of incubation one enzyme unit (EU) of intra- cellular fungal phytase could release only 55.16 µg inorganic P from phytin, 56.35 µg from glycerophosphate, 60.80 µg from ATP, 67.98 µg from ADP. The similar concentration of extra- cellular fungal phytase could release 78.74 µg, 85.94 µg, 94.2 µg and 98.35 µg of inorganic P from phytin, glycerophosphate, ATP and ADP, respectively. The results clearly demonstrated that extra-cellular phytase was more efficient (Table 5) than intra- cellular counterpart. The phytase release extracellularly was in general 43% more efficient to release Pi from phytin and 55% more efficient to release Pi from ATP. In general, 49% higher efficiency of extra- cellular fungal phytase than intra- cellular counterpart was observed to hydrolyze different organic P compounds.

3.2. Efficiency of plant phytase to hydrolyze native phytin P in soil solution

The release of inorganic P by the action of plant phytase to hydrolyze phytin P present in soil solution at different time intervals (Fig. 3) showed legumes hydrolyze more soil solution phytin-P (14.45 µg g^{-1} in 24h) which was 29% higher than cereals (11.22 µg g^{-1} in 24 h) and 8% higher than oilseeds (13.37 µg g^{-1} in 24h). The total hydrolysis of phytin P was also increased with increase in incubation time but the rate of hydrolysis was consistently lower till 24h. The maximum hydrolysis of the total phytin P in soil (Table 6) solution was found by the phytase released from legumes (58%) followed by oilseeds (53%) and cereals (45%). In general, phytase released by leguminous plants was found to be most efficient one.

Table 6 Soil Phytin P (50 µg g^{-1}) hydrolyzed by per enzyme unit of plant phytase at different time interval (± represent standard error of mean)

Plants	**P release (µg g^{-1} soil)**				
	2h	**4h**	**6h**	**10h**	**24h**
Cereals	6.02±0.31 (24.1)*	8.25±0.39 (33.0)	10.70±0.51 (43.7)	10.92±0.46 (43.7)	11.22±0.54 (44.9)
Legumes	7.31±0.36 (29.2)	10.33±0.44 (41.3)	12.57±0.51 (50.3)	13.56±0.46 (54.2)	14.45±0.60 (57.8)
Oil seeds	6.57±0.22 (26.3)	9.76±0.33 (39.0)	11.26±0.42 (45.0)	12.44±0.51 (49.8)	13.37±0.50 (53.4)

* Figures in parenthesis represent the percent of total phytin P hydrolyzed

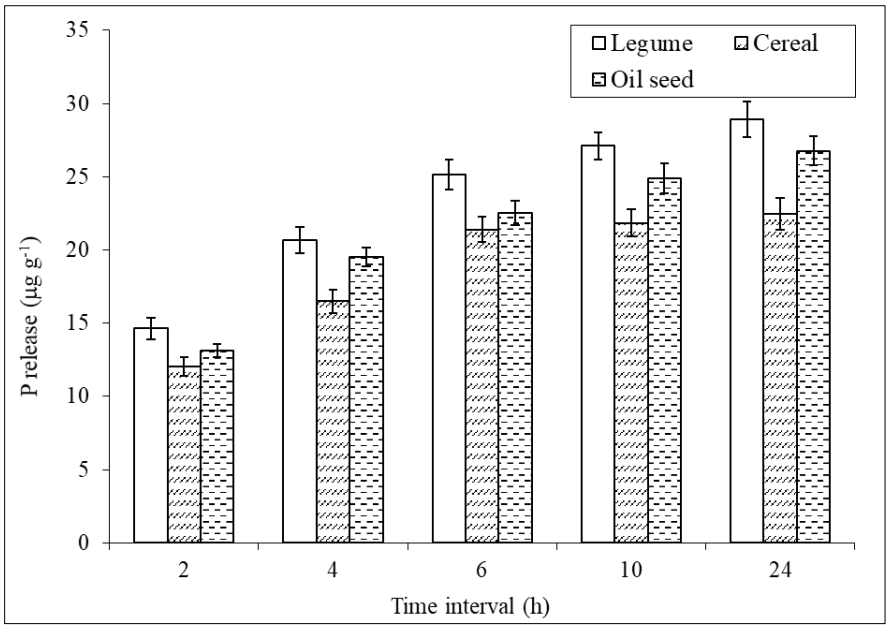

Figure 3 Efficiency of one enzyme unit of phytase from plant sources towards hydrolysis of phytin P (50 µg g-1) in soil. Vertical bars represent standard error of mean

3.3. Efficiency of fungal phytase to hydrolyze native phytin P in soil solution

The efficiency of phytase generated from fungi towards the hydrolysis of phytin P present in soil solution (Fig. 4) revealed that after 24h of incubation *Chaetomium globosum* hydrolyzed maximum (47%) of the available phytin P present in soil solution followed by *Aspergillus flavus* (35%) and *Aspergillus ustus* (33%). There was significant increase in release of phytin P with increase in incubation time in all the fungi tested. In general, Phoma species was found to be the least efficient among all the isolated fungi. In general, 29-47% of phytin P in soil solution was hydrolyzed by one unit fungal phytase within 24 h. The result (Table7) clearly demonstrated *Chaetomium globosum* is the most efficient fungi to exploit native unavailable P compounds for plant nutrition.

Table 7 Soil Phytin P (50 µg g^{-1}) hydrolysed by per enzyme unit of fungal phytase at different time interval (± represent standard error of mean)

Fungi	**P release (µg g^{-1} soil)**				
	2h	**4h**	**6h**	**10h**	**24h**
Chaetomium globosum	9.93±0.336 (19.9)*	14.75±0.49 (29.5)	18.57±0.65 (37.1)	22.55±0.52 (45.1)	23.55±0.70 (47.1)
Aspergillus flavus	8.55±0.51 (17.1)	12.36±0.75 (24.7)	14.05±0.65 (28.1)	16.7±0.52 (33.4)	17.55±0.46 (35.1)
Aspergillus ustus	8.29±0.54 (16.6)	11.91±0.54 (23.8)	15.4±0.66 (30.8)	15.0±0.64 (30.0)	16.58±0.50 (33.2)
Curvularia lunata	8.15±0.60 (16.3)	10.41±0.59 (20.8)	12.95±0.60 (25.9)	14.07±0.59 (28.1)	15.17±0.62 (30.3)
Phoma species	7.99±0.55 (15.9)	10.85±0.54 (21.7)	11.91±0.61 (23.8)	13.67±0.57 (27.3)	14.59±0.65 (29.2)

* Figures in parenthesis represent the percent of total phytin P hydrolyzed

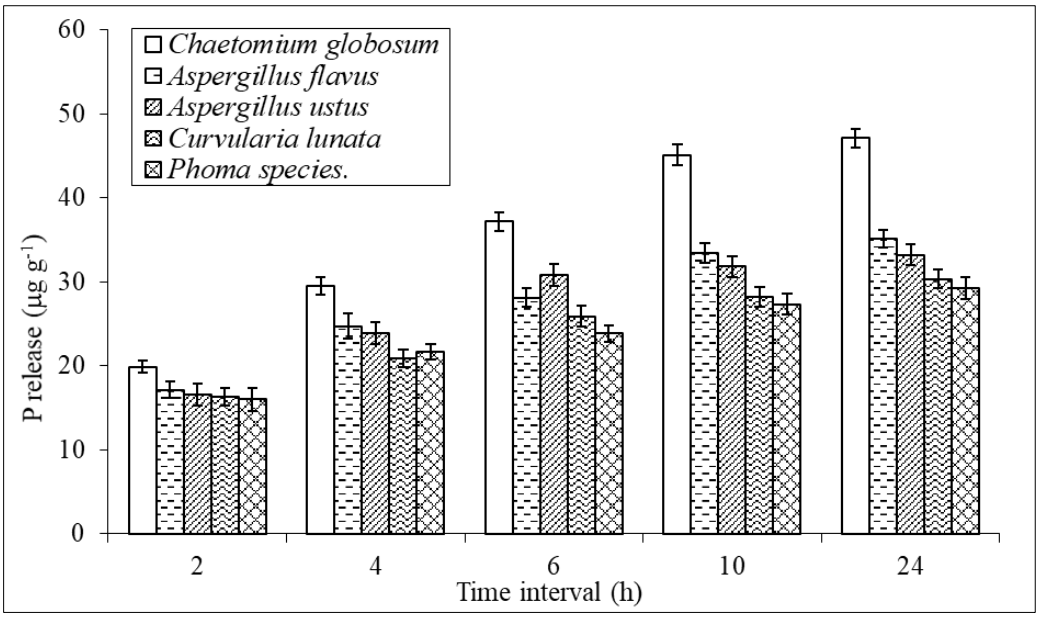

Figure 4 Efficiency of one unit of phytase from fungal sources towards the hydrolysis of phytin (50µg g-1) in soil. Vertical bars represent standard error of mean

4. Discussion

The rate of mineralisation of organic P, rather than its total amount, is the main factor determining the availability of organic P to plants. Many research findings have very clearly demonstrated the potential of soil fungi to hydrolyze and solubilise phosphorus and help in plant P availability under field conditions (Tarafdar and Yadav 2011). Tarafdar and Marschner (1995) showed the importance of soil fungi in increasing the available P from organic P like phytate and glycerophosphate to plant roots. Organic P compounds such as glycerophatae and phytin are hydrolysed by plant roots (Tarafdar and Jungk, 1987; Yadav and Tarafdar, 2001). This hydrolysis is mediated by root born phosphatases and phytase, indicated by a gradient in activity in the rhizosphere. The limiting factor for phosphatase mediated P mobilization is the low mobility of the hydrolytic enzyme (acid phosphatase, phytase) mainly associated with the root cell wall and with mucilage in the apical root zone (Eltrops, 1993). An alternative function of root secondary phytase may be rapid retrieval of P by hydrolysis of organic P, which is permanently lost by diffusion or from damage root cell. The variation in hydrolyzing efficiency of different plant species would be attributed to the difference in isoenzyme of secreted phytase and therefore, their efficiency per unit enzyme to hydrolyze different organic P compounds were also different. The phytin hydrolytic cleavage by phytase controlled the P availability from phytin sources (Findenegg and Neiemans 1993) and ultimately from organic sources. In general, significantly higher inorganic P release from ADP as compared to other organic P compounds tested, due to action of phytase originated from plant and microorganisms (Fig. 1 and 2). The least inorganic P was released from phytin compound and the fungal phytase was found to be more efficient than phytase originated from plants. The rate of P mineralization depends on microbial activity and on the activity of the phosphatases (Dalal, 1978), which is controlled by the solution P concentration (Yadav and Tarafdar, 2001). Plants and micro-organisms were greatly differing in their phytase activity and efficiency towards inorganic P hydrolysis. Probably, it would be attributed to difference in morphological, physiological and bio-chemical characteristics of the organisms, resulting to different isoenzyme types. In general, 49% higher efficiency of extracellular fungal phytase than intracellular fungal phytase was observed to hydrolyze different organic P compounds. The higher efficiency of extracellular phytase may be due to the higher phytase secretion extracellularly, which may act efficiently on organic P compounds. Between extra- and intracellular fungal enzymes, extracellular fungal enzymes were more active than their intracellular counterpart especially in respect to the release of P from phytin sources.The extracellular phytase released by the organisms was 12.7 times more than their intracellular counterpart. Among the phytase-producing fungi, extracellular phytase activity was more in *Emericella rugulosa*, whereas intracellular phytase activity was higher in *Tricoderma harzianum* (Yadav et al. 2010). In addition, the proportion of fungal intra- and extra cellular phytase activity found may differ with the plant phytase content. Asmer (1997) found 10 times higher root associated phytase than root released phytase. Measurements of mineralisation rates have been made by monitoring the change with time of inorganic P or organic P in soil after incubation. The balance between mineralisation and immobilisation is controlled by the population dynamics and activities of microorganisms. The release of inorganic P by the action of plant phytase to hydrolyze phytin P present in soil (Fig. 3) showed legumes hydrolyze maximum phytin P

followed by oilseeds and cereals. The variation in hydrolyzing efficiency of different plant species would be attributed to the differences in isoenzyme of secreted phytase and therefore, their efficiency per unit enzyme to hydrolyze different organic P compound were also different. The present observation (Table 6 and 7) gives a definite evidence of differences in hydrolyzing efficiency of organic P by the phytase originated from plant and microorganisms. The results clearly indicate that phytase originate from fungal species were more efficient to hydrolyze organic P compounds present in the soil (Fig. 4).

5. Conclusion

Phytase enzyme was collected from native plant and fungal sources to compare their efficiency towards hydrolysis of different organic P compound as present in the soil. The result suggested that phytase released from fungal sources were most efficient. Among the fungi tested, phytase released by *Chaetomium globosum* extracellularly was the best one to mobilize soil organic phosphorus for plant nutrition. In general, extracellular phytase was more active than their intracellular counterpart. The result suggested using *Chaetomium globosum* phytase for future P mobilization from the native sources.

Compliance with ethical standards

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Disclosure of conflict of interest

Both the authors declare that there is no conflict of interest regarding the publication of this paper.

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