ISSN Online: 2349-7785

Phytate Mineralizing Fungi for Phosphorus Recovery from Organic Wastes

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Received 14 July 2016; accepted in revised form 19 August 2016

Abstract: Microbial recovery of organic phosphorus (P) from crops and animals residue can be an effective technology for sustainable P management. The present investigation was an attempt to prepare compost from substrate mixture of paddy straw (P.S) and animal manures such as cattle manure (CM), farm yard manure (FYM) and poultry manure (PM) each added separately as source of P and nitrogen. A consortium of phytate mineralizing fungi developed by including *Aspergillus niger* ITCC 6719, *Aspergillus flavus* ITCC 6720 and *Trichoderma harzianum* ITCC 6721 was inoculated for recovery of P from a mixture of P.S + manure. The chemical analysis of mature compost (obtained after 120d of aerobic decomposition) for total and different P fractions revealed that fungal inoculation improved the sodium bicarbonate extractable-P content of CM and FYM supplemented P.S compost by 32.3 % and 23.5 % respectively, compared with their respective un-inoculated counterpart. Sodium hydroxide extractable organic P content was more than inorganic P content and was highest in CM-P.S compost. The fraction of P extracted with HCl ranged from 8.51 % to 14.39 % of total P. Application of P-enriched compost to soil can reduce the input of chemical P and offer potential environment and economic benefits to farmers under sustainable agriculture.

Key words: Phosphorus, compost, manure.

Introduction

Global demand for providing food security to the growing population predicts the higher consumption of phosphate fertilizers in near future. However, modern agriculture relies mainly on the non-renewable resource of phosphorus (P) such as rock phosphate. With current rates of extraction, global commercial phosphate reserves are likely to be depleted in another 50-100 years [24]. Thus, one of the sustainable P management options lies in the recovery of P from existing resources such as agriculture waste and livestock manure whose huge production needs some effective disposal strategies. Animal manures have sufficient amount of nitrogen (N) and P. The direct land application of fresh animal manures results in excessive input of these nutrients in soils and contamination of water bodies via run off [15]. High concentration of N and P in the soil profile is undesirable as excess manure adds salts in soils that decrease crop yields [18]. The effective management of livestock manure for reduced loss of P and improved environment quality is important while recycling farm wastes as soil amendments. On the other hand rice straw is a ligno-cellulosic residue that cannot be used as a resource material without prior pre-treatment. Its high silica content makes it undesirable as an animal feed. Therefore, farmer gets rid of this residue by burning it in the field itself. Burning causes loss of nutrients such as C, N, P, and K, besides polluting the quality of ambient air and reduction of biological activity [6]. For environment friendly management of these residues, composting is one of the effective strategies that convert labile organic compounds into stabilized products for their use as

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an organic fertilizer.

Conventionally produced compost has low P content ranging between 0.2- 0.3 %. The P-enriched compost is generally prepared by supplementing the composting substrates with rock phosphate and its subsequent inoculation with rock phosphate solubilizing microorganisms [5]. However, with depleting rock phosphate reserves there is need to develop P-enriched compost using alternate strategy. The bulking agent with high C:N ratio can be mixed with low C:N ratio animal manure to bring down the C:N ratio of composting mixture to initiate decomposition. The organically bound P fractions present in paddy straw (84 % of the total P) and animal manures (30-40 % of total P) can be de-phosphorylated to inorganic P (Pi) by inoculation of phytate mineralizing microorganisms. Co-composting of paddy straw and animal manure mixture and its bio-augmentation with P-mineralizing fungi may result in organic fertilizer with high P availability. Release of organic P present in crop residue and livestock manure (60-65 % feed P is excreted) can improve the P content of finished compost. The knowledge of total P content in compost or the increase in initial and final concentration of total P does not provide any information of the chemical forms of P present in compost. Thus, fractionation of compost-P is an approach that can give insight of availability, solubility and stability of different P fractions [22]. Hence, the objectives of the present investigation included (i) evaluation of phytate mineralizing fungi to improve the P content of straw-manure compost and (ii) estimation of P- fractions in co-composted rice-straw-animal manure mixture. The study will help to estimate the P supply capacity of the mature compost when used as soil amendment

Materials and methods

Phytate mineralizing fungi

Three phytate mineralizing fungi *Aspergillus niger* ITCC 6719, *Aspergillus flavus* ITCC 6720 and *Trichoderma harzianum* ITCC 6721, isolated from rhizosphere of *Glycine max* and *Pisum sativum* and compost respectively using phytate screening (PS) broth [12] were evaluated for their respective production of cellulase [25], phytase [3] and phosphatase activity [23]. The pure cultures were maintained on potato dextrose agar (PDA) slants and stored in cold room at 4^{o}C.

Inoculum development

Inoculum of selected fungi was raised by immobilizing the respective strain on wheat bran. Flasks containing sterilized wheat bran moistened with mineral salt solution ($MgSO_4$, NH_4NO_3 and NaCl) were inoculated with 6 mm disc of each culture grown on PDA plates and incubated at 27^{o}C for 10 days. The whole growth of each strain including mycelium and spores were used as fungal inocula.

Composting substrates

Paddy straw (P.S), cattle manure (CM) and farm yard manure (FYM) used as N and P additives was obtained from Agronomy Division, Indian Agricultural Research Institute (IARI), New Delhi. However, poultry manure (PM) was procured from one of the poultry farms located in peri-urban area of New Delhi, India.

Ten kg P.S, chopped to a size of 6-8 cm was filled in plastic bin of 80 litre capacity and referred as T1 (Table 1). The dried CM, FYM and PM (each added separately) was thoroughly mixed with shredded P.S @ 15 % (w/w) as per schedule given in Table 1. The initial moisture content of all the composting mixtures was maintained at 65-70 % by adding water as per the requirement. The bins were covered with lids and mixture allowed to decompose. After 25 days of initial composting, when the temperature in the bins subsided to ~37^{o}C , the composting mixture in T6, T7 and T8 bins were inoculated with mixed fungal broth (obtained after mixing the growth of each strain separately with 200 ml sterilized water). T2, T3 and T4 served as their respective controls. The composting mixture was aerated by manual turning at regular fortnight interval. A moisture content of 60 % was maintained during thermophilic phase and at 50 % afterwards. The composting period lasted for 120 days. The samples were drawn from different locations of the each bin. A total of ~ 1 kg sample drawn was divided into two parts, one was preserved in cold room for microbial biomass P, while the other was air dried ground and passed through 2 mm sieve for subsequent analysis.

Chemical evaluation of compost and germination index

The pH measurements were performed on aqueous suspension of fresh compost samples (1:5 w/v sample to water ratio) using digital pH meter. The organic matter (OM) content of compost samples was determined by combustion of oven dried samples at 450°C for 24 h. The total organic carbon (C_T) was calculated according to the equation C_T % = 0.51 x OM % + 0.48 [17]. Total nitrogen of oven dried samples was determined by Kjeldahl's method [10]. Effect of compost maturity on seedling emergence was determined by measuring germination index (GI) using mustard seeds. On the basis of percentage of seed germination, GI % was calculated [26].

Estimation of total P, microbial biomass P and P fractions in compost

Total P (TP) in different compost samples was estimated by digestion with tri acid mixture [10]. Microbial biomass P (M_{BP}) was measured by fumigation- extraction method[11] as per equation given below.

$$\text{Microbial biomass P} = Ep/k_{EP}$$

Ep = (P extracted from fumigated –P extracted from non-fumigated compost), k_{EP} = 0.40 [1].

For P fractionation, the different compost samples equivalent to 0.5 g (on oven dry basis) were extracted with 30 ml each of water (1: 60), 0.5 M $NaHCO_3$, (pH 8.5), 0.1 M NaOH and 1.0 M HCl. After shaking for 16 h at 175 rev min^{-1} with each extractant, the suspensions were centrifuged and filtered. A portion of $NaHCO_3$ and NaOH extracts was analyzed for inorganic P [9]. Another portion of $NaHCO_3$ and NaOH extracts was digested with acidified ammonium per-sulphate and analyzed for total P. The organic P (Po) in $NaHCO_3$ and NaOH extract was calculated by subtracting the inorganic P from total P of respective extracts. The P concentration in all the extracts was determined spectro-photometrically [16]. The residual P in compost samples was calculated by subtracting the sum of all P fractions from the total P contents of the compost sample.

Statistical analysis

Data were reported as the means of three replicates and analyzed using ANOVA. Correlation coefficient between different P fractions was determined using Pearson coefficient tool [7].

Results and discussion

Enzyme profile of phytate mineralizing fungi

A.niger (F1) was the highest producer of both CM Case (145.22 IU ml^{-1}) and phytase (103.3 EU ml^{-1}), whereas *A. flavus* (F2) produced high amount of acid phosphatase (830 µ mol pNP ml^{-1} h^{-1}), and phytase activity (Fig. 1). *T. harzianum* exuded high quantity of cellulase (Fig 1). As none of the fungal strain could produce all the tested enzymes in optimum amount, a fungal consor-

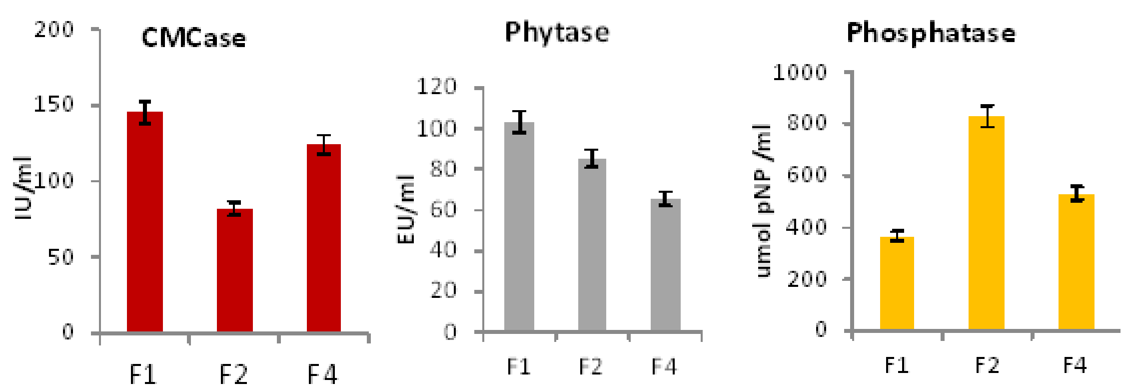

Fig. 1. Enzyme profile of phytate mineralizing fungi (F1- *A.niger*, F2- *A.flavus*, F4- *T.harzianum*), CMCase-carboxy methyl cellualse activity, IU-International unit, EU-enzyme unit, pNP-p-nitrophenol

tium was developed by mixing them in 1:1:1 ratio for inoculating the composting substrate mixture. Besides, phytate mineralization, *A. niger* was also the potential inorganic phosphate solubilizer (data not shown)

Composting substrates

Paddy straw, a ligno-cellulosic cereal residue had an initial C: N ratio as high as 78:1, (Table 1). A blend of P.S. with PM (C:N ratio 7:1) at 15 % (w/w) resulted in the desirable C : N ratio (50:1) to initiate microbial decomposition by the indigenous and exogenous population (Table 1). The amendment of P.S with CM and FYM was also confined to 15 % (w/w) to minimize the load of exogenous microbial population, but this percentage of manure supplementation resulted in an initial C: N ratio of composting mixture to 71:1 and 72:1 respectively (Table 1). Both CM and PM had N: P ratio of 1: 0.85 and 1: 0.93 whereas FYM had N: P ratio of 2:1 (data not shown). The low N: P ratio of manures will certainly result in high concentration of P in soil, if their direct land application is based on crop's N requirement.

Evaluation of compost maturity parameters

The C : N ratio, is one of the important indicator of compost maturity. Both T6 and T8 recorded the lowest C: N ratio of 18.6 and 18.4 respectively. A decrease in C:N ratio during composting may be attributed to transformation of organic carbon into carbon dioxide and the corresponding increase in N %. A C: N ratio below 20 is an indicative of acceptable maturity. The composts with high C: N ratio on application to soil result in nitrogen immobilization and too low C: N ratios (<10:1) of compost indicate its instability [19]. In the present investigation, all the inoculated composts showed a C: N ratio ranging from 18.4 to 19.4:1. However, T1 compost recorded the highest C : N ratio of 32:1 compared to manure amended and fungal inoculated counterparts. Nitrogen supplementation of paddy straw and subsequent fungal inoculation enhanced the mineralization of composting substrates in agreement with the reports of Pandey *et al.*[20].

The germination index (GI) for different composts ranged from 77 to 215 % (Table 1), a value far above the threshold limits of 60 %. Both C: N ratio and GI are important maturity parameters but could not be correlated. T8 compost had the lowest C: N ratio of 18.4:1 and GI of 144 % whereas T6 and T7 compost had C: N 18.6: 1 and 19.4:1 but had GI of 113 and 215 % respectively.

The pH values for different manure amended P.S composts were alkaline (8.53 to 8.62, Table 1). The high pH values may be due to high calcium content present in livestock manure and mineralization of organic N to NH_4-N [21]. Fungal inoculated series recorded the reduced pH values and lowest being for T7 (Table 1). Low pH values in FYM amended compost (T7) may be due to low content of NH_4-N.

Table 1. Schedule and selected properties of composting substrate mixture before and after decomposition

Treatments	Substrates	Before decomposition Inoculation	Before decomposition C:N ratio	After 120 d decomposition C:N ratio	GI %	pH
T1	P.S	No	78:1	32.92	94	8.68
T2	P.S + CM	No	71.1:1	20.33	131	8.53
T3	P.S +FYM	No	72:1	22.54	176	8.62
T4	P.S + PM	No	50:1	20.21	77	8.60
T6	P.S + CM	Yes	71.1:1	18.60	113	7.78
T7	P.S + FYM	Yes	72:1	19.40	215	7.60
T8	P.S + PM	Yes	50:1	18.40	141	7.78

GI: germination index

Total P, microbial biomass P and P fractions in compost

Total P (TP) content of compost gives an idea of how much P is added to soil through its land application. The highest TP content of 1.0 % was recorded in T8, attributed to the initial high P concentration (2.5 %) in poultry manure. The T6 and T7 composts recorded the TP value of 0.77 and 0.67 % respectively, an increase of 42.5 % and 43.5 % respectively over their respective un-inoculated controls (Fig. 2). The increase in TP content due to inoculation was in agreement with the findings of Goyal and Sindhu [8], who reported higher total P concentration in fungal inoculated and cattle dung amended P.S compost. The improved OM mineralization in the inoculated series resulted in high reduction of biomass. Carbon as CO_2, hydrogen as H_2O and nitrogen as NH_3 are lost with the exit gas, but P is retained and increases due to concentration effect [14].

Microbial biomass P (M_{BP}) represents the organic and inorganic form of P present in microbial cell and is an important source of P for plants as it can turn over rapidly to labile P, following land application of organic wastes [4]. M_{BP} content in different composts varied with the type of manure used as co-composting partner of P.S (Fig. 2). The variation in M_{BP} values may be attributed to the difference in the nutrient composition of the supplemented substrates as well as on the availability of P for microbial assimilation (Fig. 2). The M_{BP} content of T1 was 16.07 % of the total P (Fig 2). The supplementation of P.S with animal manure reduced the percentage of total P stored as M_{BP} in T2, T3 and T4 to 13.2, 7.34 and 6.33 % respectively compared with T1. A M_{BP} content of 11.08 % of the total extracted P in final compost prepared by P.S and pig manure has been reported by Dui-en *et al.*[2]. The fungal inoculation in T6, and T8 treatments reduced percentage of total P recovered as M_{BP} to 6.08 % and 5.70 % respectively. The low M_{BP} values in inoculated treatments showed that fungal inoculation improved the availability of P in composted substrates with net result of P mineralization than immobilization.

Sequential extraction of total P with different extracting solutions gives an idea of labile, less labile and non-labile or stable P in developed composts. The water soluble P is the most labile P fraction. The amount of P recovered from different composts (T1-T8) using water as an extract-

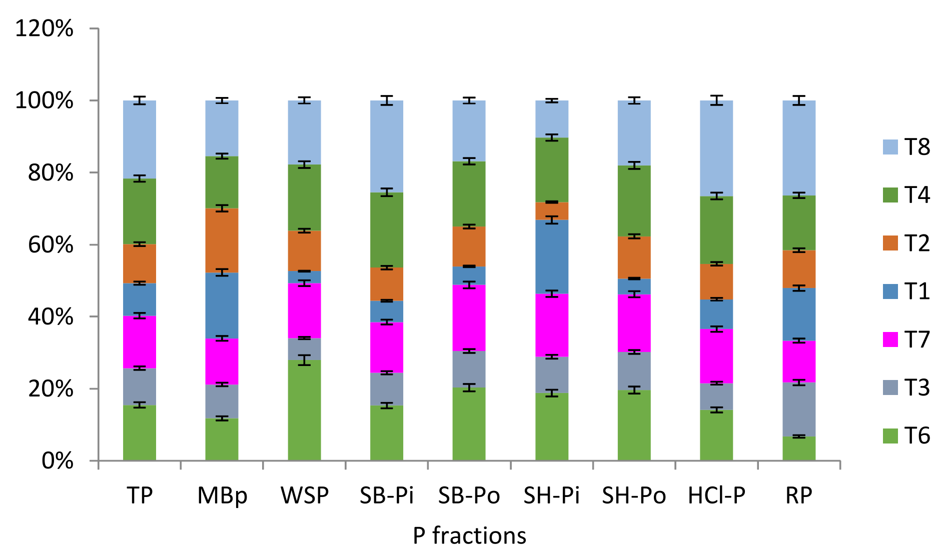

Fig. 2. Percentage content of P fractions in different composts (TP-total P, M_{BP}- microbial biomass P, WSP-water soluble P, SB-Pi -sodium bicarbonate extractable inorganic P, SB-Po -sodium bicarbonate extractable organic P, SH-Pi-sodium hydroxide extractable inorganic P, SH-Po- sodium hydroxide extractable organic P, HCl- hydrocholoc acid extractable P, RP-residual P)

ant varied from 3.45 % -17.05 % of total P, the highest being in T6 (Fig. 2). Cattle manure has 73 % of total P as inorganic fraction (data not shown). Production of organic acids during OM decomposition must have contributed towards release of higher P in solution.

Sodium bicarbonate extractable inorganic P (Pi) fraction (Fig 3a) with respect to their total P content was lowest for T1 (16.31 %) and highest for T8, followed by T4, T6 and T7 composts (28.59 %, 29.37 %, 24.74 % and 24.03 % respectively). Poultry manure had high content of inorganic P. Moreover, inorganic P is more readily soluble than organic P which gets bound to minerals. These results were higher than the range (3-22 %) reported by Khan and Joergensen [13] for biogenic compost. Dui-en *et al.*[2] reported a sodium bicarbonate extractable Pi fraction of 27.45 % for pig manure amended rice straw compost. This affirms the role of fungal inoculation in improving the plant available Pi content of final compost. The variation in labile Pi value in different composts may be due to substrate composition.

Labile organic P (Po) represents weakly absorbed but available form of P in soil. Mineralization of organic matter in the crop/animal residue release orthophosphate ions in the solution. The recovered sodium bicarbonate extractable organic P content (Po) with respect to their total P content was highest in T6 and T7 (15.61 % and 15.09 % respectively) and lowest in T1 (6.66 % Fig. 3a). These values were much higher than reported by Dui-en *et al.*[2], who reported a labile Po content of 1.29 % in mature pig manure amended rice straw compost. In the present study, inoculation with phytate mineralizing fungal consortium resulted in 2 fold increases in organically bound P, compared with uninoculated control. The total concentration of $NaHCO_3$ extractable Pi +Po in the T2, T3 and T4 amounted to 33.54, 34.03 and 40.24 % respectively and improved by 20.42 % and 14.72 % respectively in T6 and T7 respectively compared with T2 and T3.

Sodium hydroxide extractable inorganic P (Pi) fraction was highest in T1 and lowest in T2, accounting for 8.19 and 1.71 % respectively of the total P content (Fig. 3b). The content of sodium hydroxide extractable Po was higher than Pi in

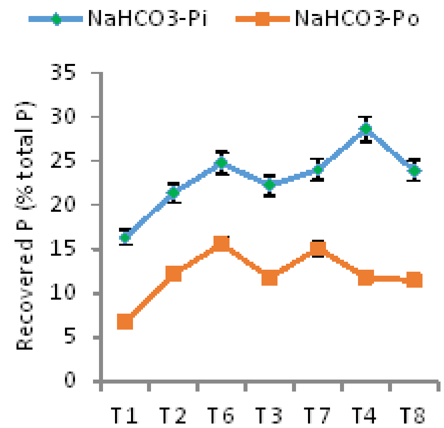

Fig. 3a. Sodium bicarbonate extractable organic and inorganic P fractions of different composts

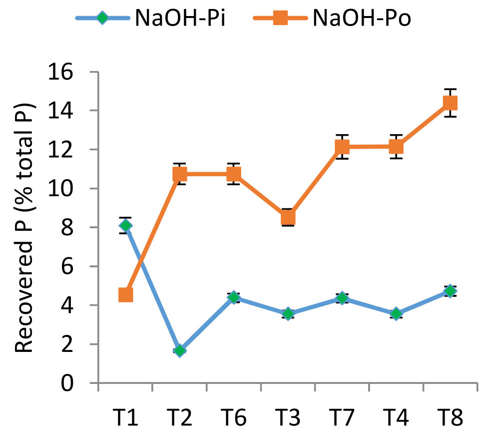

Fig. 3b. Sodium hydroxide extractable organic and inorganic P fractions of different composts

all the manure amended composts. The maximum value was recorded in T6 followed by T7 and T4 statistically at par with each other. Sodium hydroxide extractable Pi fraction is considered as poorly soluble in soil solution. This fraction consists of iron and aluminium bound P as well as the P present as calcium and magnesium phosphate. The combined values for sodium hydroxide extractable P (Pi +Po) ranged from 12.61 % (T1) to 16.45 % (T6) of the total P (Fig. 3b), were in agreement with 15.98 % reported by Dui-en *et al.*[2].

The content of most stable fraction of P extracted with HCl ranged from 8.51 % to 14.39 % (Fig. 4). The low level of HCl-P indicated that straw-manure compost had higher percentage of P in the labile form than in stable form. The amount of P which could not be exchanged within

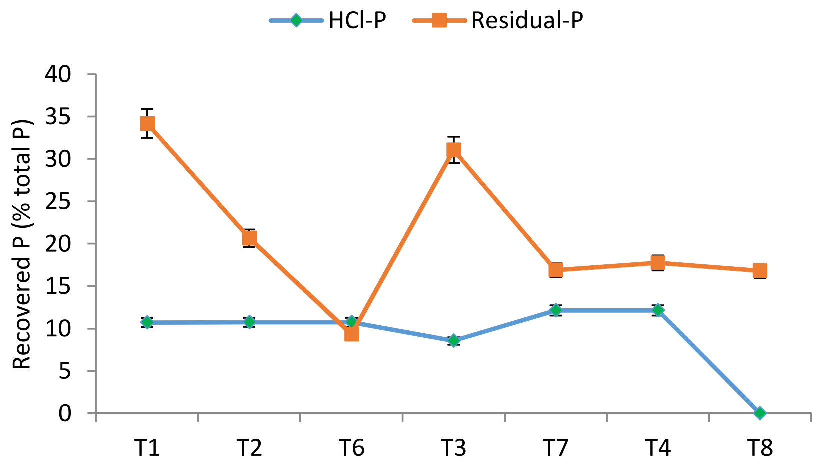

Fig. 4. Acid extractable and residual P fractions in manure amended paddy straw composts

4 months of decomposition was slowly or non-exchangeable P and referred as residual P (RP) and must be bound to calcium in the form of apatite or octa-calcium phosphates. The RP content was lowest in T6, T7 and T8 (9.29 % 16.88 % and 16.74 % of the total P respectively, indicating improved P releasing efficiency of inoculated composts. The inoculated fungal consortium was competent to mineralize and solubilise the organic and inorganic P respectively present in composting substrates. The RP could not be exchanged within 4 months of decomposition and from an agronomic point of view this fraction of compost-P will probably have a low availability to plants in neutral and alkaline soils.

Relationship among different phosphorus pools

Correlation data in Table (2) showed that most of the phosphorus fractions were positively correlated with each other. However, a strong positive correlation between bicarbonate extractable Pi and sodium hydroxide extractable Po was observed with correlation value of 0.917. A positive correlation between water soluble P and bicarbonate extractable Po and hydroxide extractable Po and water soluble P was also recorded. However, M_{BP} was negatively related to all other P fractions extracted with sodium bicarbonate, sodium hydroxide and hydrochloric acid.

Bio-augmentation of animal manures and P.S

Table 2. Correlation coefficient among different phosphorus fractions

P	† MBP	WSp	SB- Pi	SB -Po	SH- Pi	SH -Po	HCl-P
† MBp	1						
WSp	-0.0326	1					
SB-Pi	-0.314	0.762	1				
SB-Po	-0.0473	0.917	0.867	1			
SH-Pi	-0.0184	0.284	0.322	0.260	1		
SH- Po	-0.0452	0.718	0.925	0.973	0.165	1	
HCl-P	-0.0109	0.732	0.954	0.841	0.442	0.857	1

† MBP: Microbial biomass P
WSP: Water soluble P
SB-Pi: Sodium bicarbonate- extractable inorganic P
SB-Po: Sodium bicarbonate- extractable organic P, SH-Pi-Sodium hydroxide- extractable inorganic P
SH-Po: Sodium hydroxide- extractable organic P
1.0 M HCl: Extractable P

mixture with phytate mineralizing fungi was effective in developing organic fertilizer with high P availability. The efficiency of fungal inoculation in improving the availability of P was more pronounced when CM or FYM were used as co-composting substrates compared to poultry manure. Cattle manure and FYM amended P.S compost recorded 57.4 % and 49.06 % of the total P in the labile fraction an increase of 14 and ~ 10 % over their respective un-inoculated counterparts. With increased concerns over phosphorus pollution in the areas of intensive livestock and availability of organic phosphorus in phytate form; phytases have immense potential in environmental applications.

Acknowledgement:

Author expresses her sincere gratitude to Head, Division of Microbiology for providing necessary facilities during the course of investigation.

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