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ORIGINAL PAPER

Chemical and biological properties of wheat soil in response to paddy straw incorporation and its biodegradation by fungal inoculants

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Abstract A field experiment was conducted to evaluate the relative contribution of organic fertilizers (paddy straw, microbial inoculants and vermicompost) and inorganic fertilizers (urea and superphosphate) in improving pH, C, N, humus, microbial biomass, dehydrogenase, phosphatase, cellulase, β-glucosidase and xylanase activities of soil under wheat crop. Vermicompost fertilization resulted in highest microbial biomass, available phosphorus, and nitrogen content of wheat soil. It was also found effective in minimizing the alkalinity of soil compared to other treatments as indicated by pH change. However incorporation of paddy straw in conjunction with $N_{60}P_{60}$ and *T. reesei* inoculation resulted in maximum dehydrogenase, alkaline phosphatase and highest humus content of soil. Mixed inoculation of *A. awamori* and *T. reesei* did not prove effective in improving the soil biochemical properties in comparison to single inoculation of *T. reesei*. Results showed that in situ incorporation of paddy straw in combination with $N_{60}P_{60}$ and *T. reesei* inoculation can be used as an effective measure for valuable disposal of paddy straw and to improve the soil health by reducing mineral fertilization.

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Keywords *Aspergillus awamori* · Enzymes · Paddy straw · *Trichoderma reesei* · Wheat · Vermicompost

Introduction

Soil is one of the most precious natural resources of earth and to maintain its health is the moral responsibility of mankind. However, the urge for producing more food, feed and fuel is causing an irreparable damage to its environment. In addition, excessive mineral fertilization and modern cultivation practices are adding to the deterioration of soil fertility status. Environmental and soil concern have prompted the agriculture researchers to look for improved management strategies. The utilization of organic wastes as soil amendment may hold a good promise for improving the soil health, crop productivity and reduce the waste disposal problem. Paddy straw is one such waste whose huge production needs some valuable disposal solution. Though, a portion of it is being recommended for compost production, but still a major portion of it is being burnt resulting in the harmful environmental implication through global addition of CO_2. Therefore, its possible use as direct incorporation into farm soil and subsequent effect on soil properties must be explored. However, the high C:N ratio and presence of polymers such as cellulose and lignin in paddy straw may act as natural barrier for its biological degradation. In such cases, microbial interaction of specific microorganisms (cellulolytic and lignolytic) with soil and crop residue may be considered as an appropriate strategy for effective decomposition of added substrate. Fungi belonging to genus *Aspergillus, Trichoderma, Phanerochaete* and *Coprinus* are known to decompose paddy straw, wheat straw (Gaind and Mathur 2001) and horticultural wastes (Elorrieta et al. 2002), whereas *Pleurotus sajor caju, P. platypus and P. citrinopileatus* are known to colonize coir fibre, cotton stalks and sorghum stover (Ragunathan and Swaminathan 2003). These fungi may be specific for each substrate and can be used as an effective tool for in situ degradation of crop residues. They produce enzymes that can degrade cellulose, hemicellulose and lignin (Gaind et al. 2006b). Incorporation of organic residues influences soil enzyme activities. Enzyme assay, soil respiration and CO_2 evolution are the characteristics which respond more quickly to soil management practices compared to organic matter status (Brookes 1995; Gaind and Mathur 2001). Soil microbial biomass, xylanase and urease activity have been improved with the addition of FYM (Kandler et al. 1999; Ladd et al. 2004), whereas amendment with nitrogen rich substrates, crushed cotton gin compost improved the soil microbial community and oxidative enzymes as dehydrogenase, alkaline phosphatase, urease and β glucosidase (Gallo et al. 2004; Tejada et al. 2006). Addition of palletized poultry manure, green waste composts and straw based composts have been reported to improve CO_2–C evolution and nitrogen mineralization rates of soil (Flavel and Murphy 2006). As enzymes depend upon on variety of other factors such as pH, microbial biomass, carbon and nitrogen content of amendment, therefore their assay along with chemical parameters is essential for the complete evaluation and recommendation of any additive. Present study was undertaken with the twin objectives (i) to evaluate the effectiveness of cellulolytic fungi *Aspergillus awamori* and *Trichoderma reesei* in degrading the paddy straw incorporated into soil (ii) the subsequent effect of organic matter on chemical and biological properties of soil under wheat crop.

Material and methods

Substrates

The field experiment was conducted in a Typic haplustepts at experimental farm of Indian Agricultural Research Institute (IARI). The alluvial soil of experimental site was sandy loam in texture with pH 7.9. The mean maximum and minimum temperature from December to March (wheat season) were 20°C and 5°C, respectively. Paddy straw and vermicompost used as organic amendments were obtained from Agronomy and Entomology Division, IARI, New Delhi, respectively. Vermicompost was prepared using chopped wheat straw, cow dung and soil in the ratio of 6:3:1 (w/w). *Eisenia foetida* was inoculated at 15 cm depth of pits @ 10 adult earthworms kg^{-1} of waste after 30 days of decomposition. Compost was ready within 90 days. Paddy straw was incorporated in the field 15 days prior to sowing and allowed to decompose after sprinkling culture of cellulolytic fungi.

The analytical characterization of soil, paddy straw and vermicompost used as amendments are given in Table 1. There is wide variation in all the parameters studied starting with pH to C/N ratio. Soil was found to be in alkaline range, whereas both paddy straw and vermicompost tended to have pH near neutrality. The neutral pH of vermicompost may be due to production of CO_2 and organic acids during microbial metabolism (Albanell et al. 1988).

Preparation of inoculum

Phosphate dissolving and cellulolytic culture of *Aspergillus awamori* (F18) was obtained from

Table 1 Characteristics of various amendments used

Parameters	Soil	Paddy straw	Vermicompost
pH	7.92	7.2	6.8
Carbon %	0.33	44.82	23.0
Nitrogen %	0.033	0.49	1.95
C/N	10:1	91.5:1	11.79
Phosphorus %	0.065	0.10	3.87

culture collection section of Microbiology Division, IARI and *Trichoderma reesei* MTCC164 was procured from Microbial Type Culture Collection, Institute of Microbial Technology, Chandigarh. Both the cultures were maintained on Potato Dextrose agar slants. A spore suspension was prepared by inoculating a loopful of each culture into 500 ml sterilized broth of 2% malt extract separately and incubated at 30°C for 7–10 days under stationary conditions. Both the cultures were homogenized separately in a blender, before application to the field soil.

The field consisted of 5 × 8 sq m plots of seven different treatments arranged randomly in rows. The treatments were (i) $N_{120}P_{60}$, (ii) Paddy straw (P.S) @ 3 t ha^{-1}, (iii) P.S + $N_{60}P_{60}$, (iv) P.S + $N_{60}P_{60}$ + *Aspergillus awamori*, (v) P.S + $N_{60}P_{60}$ + *Trichoderma reesei*, (vi) P.S + $N_{60}P_{60}$ + *Aspergillus awamori* + *Trichoderma reesei*, (vii) Vermicompost @ 3 t ha^{-1}. All the treatments were replicated thrice. The seeds of known cultivar of wheat (var. HD-2687) were sown @ 80 kg ha^{-1}. In treatment (vi) equal quantity of fungal inoculum consisting of *A. awamori* and *T. reesei* was mixed and applied at 350 g wet biomass t^{-1} paddy straw. The mineral fertilization consisting of urea and super phosphate were applied to provide nitrogen and phosphorus as per dose given in treatment schedule. The plots were irrigated as and when required.

The soil was sampled manually at 0–15 cm depth using soil sampler at monthly interval. Three sub samples taken from different locations of the same plot were mixed thoroughly. Moist samples were stored in refrigerator at 8°C for enzyme assay. A portion of soil samples drawn at maturity of crop were oven dried, ground and passed through 2 mm sieve and used for determination of chemical parameters.

Soil pH was determined in 1:2.5 soil : water extract after shaking the solution for 30 min. Total nitrogen was estimated by Kjeldahl's method (Jackson 1967). Total organic carbon content was measured by dichromate oxidation method and subsequent titration with ferrous ammonium sulphate (Walkley and Black 1934). Available phosphorus was estimated following the method of Olsen et al (1954). Microbial biomass of soil was determined by fumigation extraction method (Vance et al. 1987). Total humus content (humic acid, fulvic acid and humin) was extracted by shaking the soil sample with alkaline sodium pyrophosphate (a mixture of 0.1 M NaOH and 0.1 M sodium pyrophosphate) in the ratio of 1:5 for 1–2 h on shaker. The contents were allowed to stand overnight and centrifuged at 10,000 rpm for 10 min. The dark brown colored solution was dialyzed in the dialyzing tubes under running water for 24 h. The total amount of humus present in the solution was estimated by gravimetric method after drying the solution at 50°C in a water bath (Konnonova 1966). The levels of different enzymatic activities, viz., dehydrogenase, alkaline phosphatase, cellulase, β-glucosidase and xylanase were assayed at monthly interval.

Enzyme assay

Estimation of dehydrogenase

Air dried soil (6 g) was treated with 3% triphenyl tetrazolium chloride (TTC) in distilled water for 24 h at 28°C in darkness. The triphenylformazone (TPF) formed was extracted with 20 ml methanol by shaking vigorously for 1 min and filtered through whatman 42 filter paper. TPF was measured spectrophotometrically at 485 nm following the method of Casida et al. (1964).

Alkaline phosphatase

Four milliliter of modified universal buffer (pH 11.0) and 1 ml *p*-nitrophenyl phosphate disodium (0.025 M) was added to 1 g soil and incubated at 37°C for 60 min. It was followed by the addition of 1 ml 0.5 M $CaCl_2$ and 4 ml 0.5 M NaOH. Mixture was centrifuged at 4000*g* for 5 min. *p*-nitrophenol was determined spectrophotometrically at 400 nm and phosphatase activity expressed as μmoles of PNP released g^{-1} h^{-1} with reference to standard curve of *p*-nitrophenol (Tabatabai and Bremner 1969).

Estimation of cellulase, β-glucosidase and xylanase

Enzyme was extracted with citrate buffer of pH 7.0. A known sample (0.5 ml) of enzyme filtrate was incubated with respective substrates (filter paper strips/cellobiose/xylan) and volume was made up to 1 ml with 0.05 M citrate buffer of pH 4.8. All the tubes were incubated at 50°C for 1 h. for cellulase and 30 min for *β*-glucosidase and xylanase. Reducing sugars liberated by action of enzyme was estimated by adding 3 ml di-nitro-salicylic acid and keeping in boiling water bath (Wood and Bhat 1988; Bailey et al. 1992). Enzyme concentration was represented as international unit (IU/g). One international unit is defined as μmol of product produced ml^{-1} min^{-1}.

Statistical analysis

All the results are means of three replicates. Data were subjected to an analysis of variance (ANOVA) using least significance difference test and comparing the difference between specific treatments (Panse and Sukhatme 1978).

Results and discussion

Changes in soil chemical properties

As decline in long-term soil fertility is the result of partial or complete removal of above ground biomass, paddy straw was incorporated into soil to provide readily available nutrients, as well as to minimize the loss of biomass. A decrease in pH was observed in all the inoculated treatments. However, it was statistically significant only in *T. reesei*, mixed inoculum and vermicompost treated soil as compared to control. The pH drop may be attributed to the effect of inoculum on the rate of organic matter degradation, leading to larger amounts of ammonium that could lower the soil pH as a consequence of nitrification. The drop in pH of vermicompost amended soil was largest and contrary to the reports of Maheshwarappa et al. (1999) who reported increase in pH of soil in which vermicompost was incorporated.

Soil organic carbon and nitrogen were found to be improved significantly in both paddy straw + $N_{60}P_{60}$ and vermicompost amended treatments (Table 2). Inorganic fertilizer in particular can affect the microbe-mediated breakdown of fresh plant residues because microbial activity is often limited by nitrogen. Therefore, use of inorganic nitrogen application when organic residue of high C/N ratio was used might have either minimized the N immobilization or speed up the microbial decay (Jenkinson et al. 1985).

Significantly high N% was recorded in vermicompost-amended soil. It may be due to high initial N% of vermicompost. A substantial increase in N% in vermicompost-amended soil used for pepper plantation has also been reported by Arancon et al. (2003). Nitrogen percent was significantly low in $N_{120}P_{60}$ treated unamended soil compared to P.S. + $N_{60}P_{60}$ and vermicompost treated soil. It had been observed that in case of

Table 2 Chemical parameters of soil at crop maturity as affected by organic and inorganic amendments

Treatments	pH	Carbon %	Nitrogen %	Humus %	Microbial biomass μg g^{-1}
Control[a]	7.69	0.41	0.033	0.60	106.09
$N_{120}P_{60}$	7.75	0.45	0.045	0.64	248.67
Paddy straw	7.58	0.47	0.036	0.64	145.13
Paddy straw +$N_{60}P_{60}$	7.71	0.48	0.071	0.70	144.24
Paddy straw + $N_{60}P_{60}$ + *A. Awamori*	7.63	0.45	0.044	0.80	118.12
Paddy straw +$N_{60}P_{60}$ + *T. reesei*	7.50	0.45	0.034	0.86	223.01
Paddy straw +$N_{60}P_{60}$ + *A. Aw* + *T. reesei*	7.57	0.46	0.059	0.81	178.76
Vermicompost	7.47	0.46	0.082	0.77	254.87
LSD (*P* = 0.05)	0.11	0.01	0.022	0.06	23.79

Paddy straw and vermicompost applied @ 3 t ha^{-1}

[a] Soil without fertilizers/organic amendments

complete fertilizer application, loss of nitrogen generally occurs to the tune of 33% and in farmyard manure (FYM) application it was 7% (Wolf and Snyder 2003). Effect of mixed fungal inoculants in improving nitrogen % was significantly higher compared to single inoculation.

Humus content was significantly higher in vermicompost amended and fungal inoculated soils as compared to control. Though, the increase was highest in Paddy straw + $N_{60}P_{60}$ + *T. reesei* treated soil (0.86%) but the results among fungus treated soils were statistically at par with each other (mixed culture 0.81% and single inoculation of *A. awamori* 0.80%). The positive effect of *T. reesei* on humus content of wheat soil was in agreeement with the findings of Gaind and Mathur (2001) who also reported statistically significant humus content in soil treated with paddy/wheat straw + *T. reesei* under rice–wheat cropping system. This showed that organic fertilizer being rich source of nutrients improved the soil fertility status. Comparatively lower humus content was recorded in vermicompost-amended treatment (0.77%). It may be due to the stabilized nature of the product applied (Hadas et al. 1996).

Application of vermicompost @ 3 t ha^{-1} resulted in microbial biomass (254.87 µg g^{-1}) though higher than the recommended dose of inorganic fertilizer (248.67 µg g^{-1}) but statistically at par with each other. Higher microbial biomass in wheat soils amended with vermicompost compared to that had received only the inorganic fertilizers was in agreement with the findings of Arancon et al. (2003). They also reported increased microbial biomass in strawberries planted soils treated with vermicompost. It may be due to the fact the vermicompost itself is the rich source of diverse microbial population. Their application to soil can add diversity and activity of indigenous soil microbial population. It was followed by *T. reesei* inoculation (223.01 µg g^{-1}).

Available phosphorus showed an increasing trend as the crop matured (Table 3). Highest available P was recorded in vermicompost-amended treatment followed by $N_{120}P_{60}$ treatment. Though not much variation in availability of phosphorus was recorded between 1st and 2nd month samples but a sharp increase was recorded in 3rd month.

Table 3 Available phosphorus (µg g^{-1}) at crop maturity in sol amended with different cellulolytic fungi

Treatments	Sampling interval in months		
	1	2	3
Control[a]	19.72	18.93	19.05
$N_{120}P_{60}$	49.67	45.90	116.86
Paddystraw	24.84	30.08	80.16
Paddystraw + $N_{60}P_{60}$	31.18	37.25	107.10
Paddystraw + $N_{60}P_{60}$ + *A. Awamori*	31.92	38.48	75.06
Paddystraw + $N_{60}P_{60}$ + *T. reesei*	31.78	40.78	97.35
Paddystraw +$N_{60}P_{60}$ + *A. Aw* + *T. reesei*	31.05	51.63	108.96
Vermicompost	33.83	55.86	121.11
LSD (P = 0.05)	4.33	7.87	4.02

[a] Soil without fertilizers/organic amendments

The dehydrogenase activity was highest in the first month of crop growth. Paddy straw + $N_{60}P_{60}$ + *T. reesei* treated soil resulted in highest activity followed by Paddy straw + $N_{60}P_{60}$ + *A. Awamori* (Fig. 1). The incorporation of substrate capable of activating autochthonous biomass might have resulted in increased microbial activity. However, the activity did not vary significantly between Paddy straw + $N_{60}P_{60}$ and Paddy straw + $N_{60}P_{60}$ + *A. awamori* in the 1st month of soil sampling. The dehydrogenase activity showed a downward trend with the maturity of the crop. It was in agreement with the previous findings of Gaind et al. (2006a).

Alkaline phosphatase activity varied with the different treatment applied to soil and was found to be maximum in 1st month sampling. The incorporation of paddy straw with half the dose of nitrogen and inoculum of *T. reesei* produced a significant increase in alkaline phosphatase activity (Fig. 2). It was directly related with the quantity of microbial biomass contained in the added material and on the subsequent activity produced by them. Added organic substrate might have resisted to initial inhibition if any. Vermicompost-treated soil though showed the highest available phosphorus but did not show higher phosphatase activity. It may be due to the

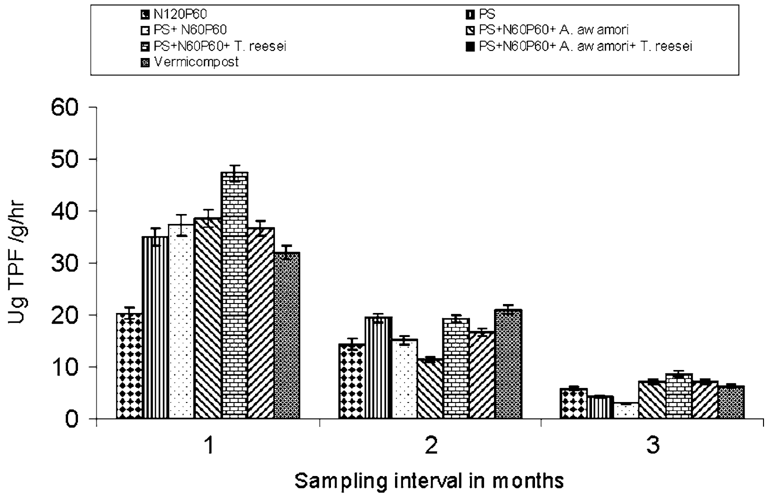


Fig. 1 Dehydrogenase activity in wheat soil as influenced by paddy straw and fungal inoculants

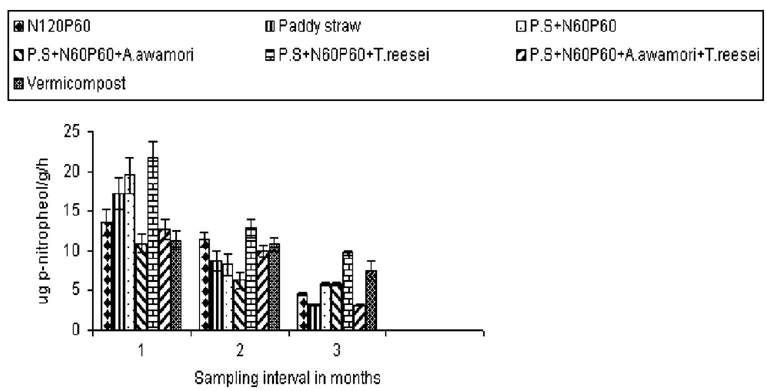


Fig. 2 Alkaline phosphatase activity in wheat soil as influenced by paddy straw and fungal inoculants

presence of certain heavy metals in the manure (Pascual et al. 2002). Generally, phosphatase enzyme is activated in soil when there is low P availability. Phosphatase can be inhibited not only by heavy metals as Cu and Zn but also by inorganic phosphate which produces feed back inhibition of this enzyme (Nannipierri et al. 1979). Enzyme assay showed higher activity in *T. reesei* inoculated soil compared to other treatments till the crop matured. An inverse relation between available phosphorus and alkaline phosphatase activity was recorded, in agreement with the findings of Gaind and Lata (2004), and Nannipieri (1990). Nannipieri reported that increase in soluble phosphorus inhibits/represses the extracellular phosphatase synthesis.

Cellulase, β-glucosidase and xylanase showed increasing trend towards the maturity of crop in agreement with the findings of Albiach et al. (2000) and Gaind et al. (2005). Cellulase was highest in soil treated with vermicompost up to 2nd month sampling followed by *T. reesei* inoculated treatment (Fig. 3). However, in the 3rd month paddy straw incorporated soil showed the maximum values for these hydrolytic enzymes. This may be a consequence of higher degradation of paddy straw after 4–8 weeks of its incorporation in soil. Cellobiase activity was also maximum in *T. reesei* treated soil (Fig. 4) followed by paddy straw incorporated soil. Xylanase was found to be highest in *A. awamori* inoculated soil, though at par with the recommended dose of inorganic fertilizer (Fig. 5).

The positive effect of single inoculation of *T. reesei* on different parameters of soil may be due to the production of hydrophobin. This protein is reported to be involved in mycelium attachment to surfaces, alteration of biotic or

Fig. 3 Cellulase activity in wheat soil as affected by paddy straw and fungal inoculants

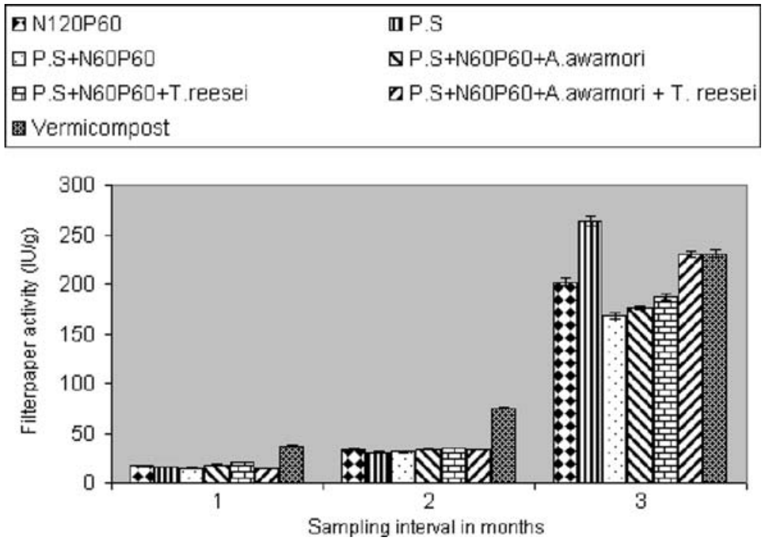


Fig. 4 *β*-glucosidase activity in wheat soil as affected by paddy straw and fungal inoculants

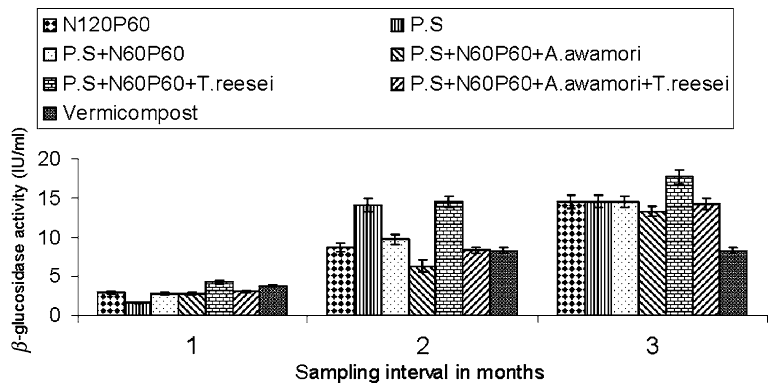
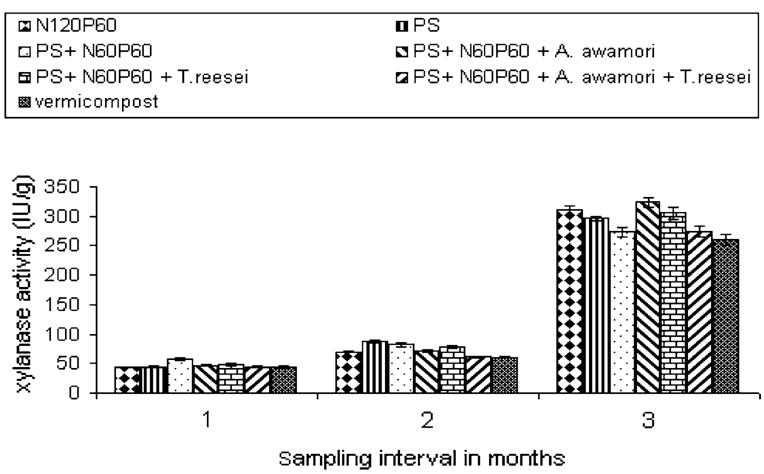


Fig. 5 Xylanase activity in wheat soil as affected by paddy straw and fungal inoculants



abiotic surface properties and lowering of water tension (Sanna et al. 2001). Due to these properties, the fungi may play a strong functional role in soil aggregation and change in soil structure (Ritz and Young 2004), which may affect microbial activity.

Conclusions

Incorporation of paddy straw in soil in conjunction with cellulolytic fungi *Trichoderma reesei* may be used as an effective measure for improving soil health in terms of soil biochemical properties as shown by the assay of different enzyme activities. It may also contribute towards the carbon and humus content of organic matter starved soil. Long-term experiments in the same field may reflect the sustainability of this practice. Application of crop residues will result in triple benefit in terms of effective and valuable utilization of waste, improvement in soil quality as well as economic gain due to low input of chemical fertilizers.

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