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Explore of ligno-cellulolytic microbial consortia on paddy straw decomposition in vertisol

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Abstract
The present investigation is an account of soil quality parameters as influenced by paddy straw decomposition and its subsequent inoculation with microbial consortia. Such information may prove useful for fast decomposition of paddy straw and improve soil carbon content and it may provide an option to combat crop residue burning because time difference between rice harvest and wheat sowing is only 15-30 days, not sufficient for decomposition and eventually clean field. Moreover residue removal from field for off-situ composting is a labour intensive process. So there is a need to find out an alternative way to address this issue. The study revealed that amongst treatments there was no significant difference observed in terms of pH and EC of the soil. However, the decomposition of paddy straw along with *Aspergillus spp. + Bacillus spp.+ Streptomyces spp*. (T_8) resulted in significant increase in organic carbon contents of the soil from 0.63 % to 0.68%. It is apparent from result that integration of straw with consortia resulted significantly the maximum content of available and potassium content in soil as compared to the treatment which receive single consortium of fungal, bacterial or actinomycetes. The phosphorus content of soil was not increased significantly with the use of paddy straw + *Aspergillus spp.+ Bacillus spp.+ Streptomyces spp*. consortia. The Easily Extractable and Total Glomalin Related Soil Protein after 60 days of residue decomposition, in moist soils were not affected by straw decomposition with microbial consortia. Addition of straw along with *Aspergillus spp. + Bacillus spp. + Streptomyces spp*. consortia increased CO_2 production as compared to the control.

Keywords: Ligno-cellulose, paddy straw, glomalin protein, soil respiration, *Aspergillus spp., Bacillus spp., Streptomyces spp*

Introduction
Soil is one of the most precious natural resources of earth and to maintain its health is the moral responsibility of mankind. (Gaind and Nain, 2007) [11]. Rice–wheat cropping system is one of the world's largest agricultural production systems, covering an area of about 26 million hectares (Mha) spread over the Indo Gangetic Plains (IGP) in South Asia and China (Sarkar 2015, Choudhary *et al.* 2016) [9]. In India there are 500-550 million tones (Mt) of crop residues are produces annually. (Mo A, 2012) [19] and about 90% of area are concentrated in the IGP (Janaiah and Hossain, 2003) [16]. Annually, a large amount of straw is accumulated as by-products from rice cultivation. With the introduction of combine harvesters, more than 75% of the rice area is harvested mechanically in north-western parts of the IGPs (Bisen and Rahangdale, 2017) [4]. Burning of rice straw is common practices in north-west India causing nutrient losses and produces serious air pollutants affecting human health. To avoid straw burning, adoption of modern crop residue management techniques should assist for achieving sustainability. Paddy straw is one such waste whose huge production needs some valuable disposal solution, which is one of the sources of organic matter, affects the soil fertility and when straw was incorporated with farmyard manure in the field, the organic N fractions was also increased (Gaur, 1984) [12] However, the recycling of organic matter may provide a source of plant nutrients and also of carbon to sustain the level of soil organic matter. In this regard, the microbial activity is closely related to the soil organic matter content which is positively affected by organic matter amendments such as the application of crop residues. The activities of soil microorganisms play a major role in nutrient recycling and organic matter decomposition (Biederbeck *et al.* 1984; Collins *et al*. 1992) [3, 8]. Long-term studies of the residue recycling have indicated improvements in physical, chemical and biological health of soil.

In addition of excessive mineral fertilizers and modern cultivation practices are adding to the deterioration of soil fertility status. The utilization of organic wastes as soil amendment may hold a good promise for improving the soil health and reduce the waste disposal problem.

Keeping this view in mind the harmful effects of *in-situ* burning of paddy straw, as well as the convenience of farmers, an economical and environment friendly straw management approaches should be adopted for effective utilization of paddy straw. According to rice straw contains high amounts of cellulose (36%), hemicelluloses (24%) and lignin (15%) and paddy straw is a potential food source for microorganisms. Inoculation of lignocellulolytic microorganisms is an effective technology to accelerate the degradation potential of lignocellulose in agricultural waste and make the paddy straw composting process economically viable. Among microbial agents of decomposition, fungi and bacteria are the most important group and they colonize very quickly and play an important role in the biodegradation of the lignocellulosic organic waste. Several fungal and bacterial species such as Rhizopus oryzae, Aspergillus oryzae, Aspergillus fumigates, Aspergillus terreus, Paecilomyces fusisporous, Micromonospora, Bacillus sp., Trichonympha, Clostridium and their consortium has been reported in literature (Sinegani *et al.*, 2005; Goyal and Sindhu., 2011; Abdulla, 2007; Borah *et al*., 2016) [27, 13, 1, 6]. Kausar *et al.*, (2011) [17] stated that the lignocellulolytic actinobacteria such as Micromonospora carbonacea was able to degrade cellulose, hemicelluloses and carbon significantly over the control. Microbial lignin and cellulose utilization is responsible for one of the largest material flow in the biosphere therefore the aim of the study to explore of ligno-cellulolytic microbial consortia on paddy straw decomposition in vertisol.

Material and methods

Substrates

The pot experiment was conducted in a vertisol at screen house of Indian Institute of Soil Science (IISS), Bhopal. The vertisol was clayey in texture with pH 7.5. The mean maximum and minimum temperature from July to October were 30.6 ^{0}C and 29.9 ^{0}C, respectively. Paddy straw used as organic amendments was obtained from farmer's field, Sukhisewania tehsil, Bhopal. Straw was chopped and prepared for pot experiment. The analytical characterization of soil and paddy straw 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 and paddy straw was tended to have pH near neutrality.

Table 1: Characteristics of various amendments used

Parameters	Soil	Paddy straw
pH	7.59	7.1
Carbon %	0.62	40.78
Nitrogen %	0.037	0.52
C/N	16.75	78.42

Preparation of inoculums

Aspergillus spp., Bacillus spp. and Streptomyces spp. was obtained from the sample collected from waste dumping area, Bhanpura, Bhopal. Bacteria, fungi and actinomycetes were isolated from the soil sample collected from the Bhanpura sewage dumping area of Bhopal. In this ways, cellulose and lignin degrading microbes were isolated and screened from these two positive samples and identified as 4 species of *bacillus,* 4 species of *Aspergillus and* 4 species of *Streptomyces* and total 12 species was identified.

Experimental Design

The experiment was laid out with completely randomized design (CRD) with three replications comprising of 8 treatments. In a pot experiment, paddy straw and microbial inoculants were applied. The treatments were designed as follows, T_l, control (Soil, 5kg) ; T_2, soil (5kg) + paddy straw (7.6g) + Fungal consortia (4 species of *Aspergillus*); T_3, soil (5kg) + paddy straw (7.6g) + Bacterial consortia (4 species of *Bacillus*) ; T_4, soil (5kg) + paddy straw (7.6g) + Actinomycetes consortia (4 species of *Streptomyces*) ; T_5, soil (5kg) + paddy straw (7.6g) + Fungal + Bacterial consortia (4 species of *Aspergillus* + 4 species of *Bacillus*); T_6, soil (5kg) + paddy straw (7.6g) + Bacteria + Actinomycetes consortia (4 species of *Bacillus* + 4 species of *Streptomyces*); T_7, soil (5kg) + paddy straw (7.6g) + Fungal + Actinomycetes consortia (4 species of *Aspergillus* + 4 species of *Streptomyces*) and T_8, soil (5kg) + paddy straw (7.6g) + Fungal + Bacteria + Actinomycetes consortia (4 species of *Aspergillus* + 4 species of *Bacillus* + 4 species of *Streptomyces*).

Soil Sampling and analysis

Soil samples were taken manually, two times during the experiment at 30 and 60 days after decomposition, to assess the effects of paddy straw decomposition with microbial consortia on different soil parameters. Three sub samples taken from three replications. Moist samples were stored in refrigerator, for biological study and some portion of soil sample was oven dried and ground to pass through 2 mm sieve for chemical study of the soil. These soil samples were examined for changes in chemical and biological properties of soil in response to organic addition. Soil pH and EC was determined in 1:2.5 soil:water extract after shaking the solution for 30 min. Total organic carbon content was measured by dichromate oxidation method and subsequent titration with ferrous ammonium sulphate (Walkley and Black 1934) [30]. Available nitrogen, phosphorus and potassium was estimated following the method of alkaline permanganate method (Subbiah and Asija., 1956) [29], Olsen *et al.*, (1954) [23] and Hanway and Heidel., (1952) [15]. The soil respiration was determined by chloroform-fumigation- incubation method as described by Anderson, 1982 [2]. Glomalin extractions from soil were carried out as described by Wright and Upadahyaya (1998) [32]. The replicate containing 1 g of soil samples of 1-2 mm aggregates were extracted with 8 mL of extractant. The easily extractable glomalin (EEG) fraction was extracted with 20 mmol (Mm) sodium citrate (pH 7.0) at 121 ^{0}C for 30 min. Samples were centrifuged at 10000 x g for 5 min immediately after extraction, and the supernatant containing the extracted protein was removed for analysis. The total glomalin (TG) fraction was extracted with 50 M mol sodium citrate (pH 8.0) at 121 ^{0}C for 60 min, and then immediately centrifuged at 10000 x g for 5 min. After each cycle, the supernatant was removed and sodium citrate was replenished for the extraction of glomalin again until the supernatant showed no red-brown color, which is typical for glomalin. The supernatant was stored for analysis. Both fractions were analyzed using the Bradford protein assay, which utilizes an acidic solution of Coomassie brilliant blue G-250 dye to bind to amino acid residues of protein. Bovine serum albumin was used to prepare as the standard for the assay. Measure the absorbance of each sample at 595 nm using a UV-visible Spectrophotometer or ELISA.

Results and Discussion

The measure of soil pH is an important parameter which helps in identification of chemical nature of the soil (Kelly *et al*., 2009) [18]. The data recorded for soil reaction (pH) are presented in table 1 revealed that there were no significantly changes in soil pH of the various treatments over control in the pot culture soil after 30 and 60 days. The highest and lowest soil pH was 7.54 and 7.50 respectively. pH measures the hydrogen ion concentration in the soil which indicate the acidic and alkaline nature of the soil. pH of the samples was found to be near neutrality. Electrical conductivity (dS/m), as the measure of current carrying capacity and was not much affected by different treatments. it gives a clear idea of the soluble salts present in the soil. It plays a major role in the salinity of soils. Even though, soil conductivity is influenced by many factors, high conductivities are usually associated with clay-rich soil and low conductivities are associated with sandy and gravelly soils. (Pattani *et al*., 2015) [24]. The highest electrical conductivity (EC) was i. e. 0.45 dSm^{-1}. There was no increase or decrease in soil EC after 60 days of decomposition.

Soil organic carbon was found to be improved significantly in paddy straw + Fungal + Bacteria + Actinomycetes consortia amended treatments. (table 1). The percentage of organic carbon ranged from 0.63 to 0.68. However, maximum was in T_8 (Soil + paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp*.) and minimum in T_1 (control). (fig.1). It is apparent from result that integration of straw with consortia resulted in significantly higher organic carbon content in soil as compared to T_1. Further, Moharana *et al., (*2012) [20] reported that the decomposition of straw with microbes into the soil increased carbon content because various microbes produce different organic acids during decomposition. After 60 days, the maximum content of available N (218.46 kg ha^{-1}) was recorded in T_8 (paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces* spp.) treatment and lowest content (192 kg ha^{-1}) of available N was associated with control treatment. (Table 2). These results are in line with the findings of Goyal *et al* (2009) [14], they reported that the nitrogen mineralization potential of soil also increased with the additions of rice straw compost. Prasad and Sinha (1995) [25] studied the effect of incorporation of rice and wheat residues soaked in 2 % urea solution after chopping (2-cm size) and inoculating with cellulytic culture (Aspergillus spp.) hasten the decomposition process. According to Rahman *et al*., (2014) [26] and Manzoni *et al*., (2008) [21], soil organic matter is known as revolving nutrient fund that supplies mainly carbon, nitrogen, phosphorus and sulfur. Therefore, decline in soil carbon generally decreases crop productivity. Degradation of crop residues and organic wastes play a major role in global carbon and nitrogen cycling. The available phosphorus of soil not increased significantly with the use of soil + paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* It is also a second most limiting factor often affecting plant growth, which exists in the soil in both organic and inorganic forms (Pattani *et al*., 2015) [24]. It is apparent from result that integration of straw with consortia not significantly affects available phosphorus content in soil but resulted in higher phosphorus content in soil as compared to control. Significant difference in available potassium content in soil was observed. Treatment T_8 recorded maximum amount (265.49 kg ha^{-1}) of available K and lowest value (243.38 kg ha^{-1}) was associated with control treatment. The addition of straw in combination with microbial consortia (*Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.)* had significant effect on potassium content of the soil. Rice straw contains about 1.5% potassium on dry weight basis (IRRI, 1984). So incorporation of rice straw in combination with microbial consortia added K to the soil. Wei *et al*. (2015) [31] and Dolan *et al.* (2006) [10] reported that regular incorporation of organic materials (crop residues) had significant effects on chemical properties of soil and increases the potassium content in soil.

The Easily Extractable Glomalin Related Soil Protein (EEGRSP) after 60 days of residue decomposition, in moist soils was ranged from 30.44 to 31.52 µg g^{-1}soil (Figure 2). EEGRSP were not affected by straw decomposition with different microbial inoculation. The highest EEGRSP protein was under treatment receiving of paddy straw with *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* (T_8) i.e. 31.52µg g^{-1}soil than the other control. Similar effect was also observed in case of Total Glomalin Related Soil Protein (TGRSP) after 60 days of residue decomposition was not significant with the application of microbial inoculation for paddy straw decomposition. TGRSP were not affected by different microbial inoculation. The highest TGRSP protein was under treatment receiving of paddy straw with *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* (T_8) i.e. 207.48 µg g^{-1}soil over other treatments (Figure 3). The lowest TGRSP protein was under control (T_1) i.e. 206.23µg g^{-1} soil. These glomalin protein is produced by arbuscular mychorryzal fungus (AMF). AMF and their product glomalin related soil protein (GRSP) play a decisive role in the soil aggregation, affecting the carbon (C) dynamics in agro ecosystems. (Singh *et al.*, 2010) [28].

The total CO_2 production among the different treatments was found statistically significant (Figure 4). Addition of straw increased CO_2 production as compared to the control treatments and increased ranged from 11.80 to 17.21 mg CO_2-C 100 g^{-1} soil day^{-1}. All inoculated straw treatments increased CO_2 evolution than the control (T_1). Among the treatments, T_8 (paddy straw + *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp*.) were significantly higher than others in term of total CO_2 production, the differences in total CO_2 produced in pots inoculated with T_5, T_6 and T_7 were not significant, indicating that application of the product had no significant effects on amount of CO_2 released from the decomposing paddy straw in this experiment. Bardgett & Saggar, (1994) [5] observed that the CO_2 has been used as a stress indicator and indicated the microbial efficiency, which is a measure of the energy required to maintain the metabolic activity in relation to the energy required to synthesize biomass. The result was agreement with the finding of Chen *et al*., (2007) [7] increase in soil respiration flux is associated with the activities of straw degrading microbes. The highest total CO_2 observed in *Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.* amended paddy straw may be due to ability of the microorganisms present in this product to establish successfully following inoculation as compared to control. Ogunniyi *et al*., (2013) [22] reported that the application of bacteria, filamentous fungus and *Saccharomycetes* inoculated straw had significant effect on total CO_2 emission during the incubation period (61 days).

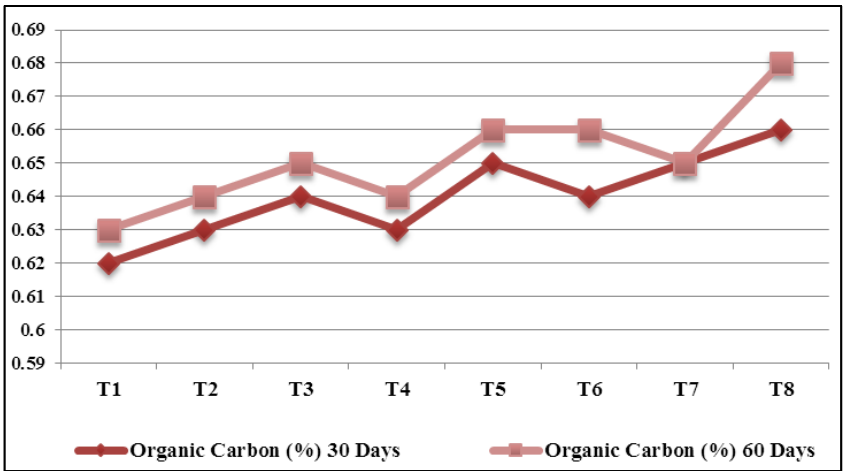

Fig 1: Effect of decomposition of paddy straw with lingo-cellulolytic microbes on Organic Carbon (%)

Table 1: Effect of decomposition of paddy straw with ligno-cellulolytic microbes on pH, Electrical Conductivity (dSm^{-1}) and Organic Carbon (%).

Tr. No.	pH		Electrical Conductivity (dSm^{-1})		Organic Carbon (%)	
	Days		Days		Days	
	30	60	30	60	30	60
T1	7.51	7.54	0.43	0.45	0.62	0.63
T2	7.52	7.52	0.43	0.43	0.63	0.64
T3	7.54	7.52	0.42	0.43	0.64	0.65
T4	7.55	7.53	0.43	0.43	0.63	0.64
T5	7.54	7.54	0.41	0.44	0.65	0.66
T6	7.55	7.50	0.42	0.43	0.64	0.66
T7	7.57	7.52	0.43	0.44	0.65	0.65
T8	7.54	7.52	0.44	0.43	0.66	0.68
SE(m)±	0.042	0.029	0.015	0.014	0.005	0.006
CD(P=0.05)	NS	NS	NS	NS	0.016	0.020

Table 2: Effect of decomposition of paddy straw with ligno-cellulolytic microbes on Available N, P and K (Kg/ha).

Tr. No.	Available N (Kg/ha)		Available P (Kg/ha)		Available K (Kg/ha)	
	Days		Days		Days	
	30	60	30	60	30	60
T1	192.98	192.10	15.25	14.83	241.05	243.38
T2	194.65	201.25	15.53	15.77	243.76	246.85
T3	195.22	202.95	15.84	16.10	243.73	247.52
T4	194.07	199.31	15.67	15.58	242.48	247.08
T5	197.60	210.31	16.02	16.26	246.48	249.01
T6	196.74	208.49	16.38	16.45	243.90	253.02
T7	197.16	212.93	16.44	16.71	244.02	250.92
T8	198.86	218.46	16.57	16.75	247.46	265.49
SE(m)±	1.400	0.229	0.31	0.613	1.583	1.337
CD(P=0.05)	NS	0.693	NS	NS	NS	4.042

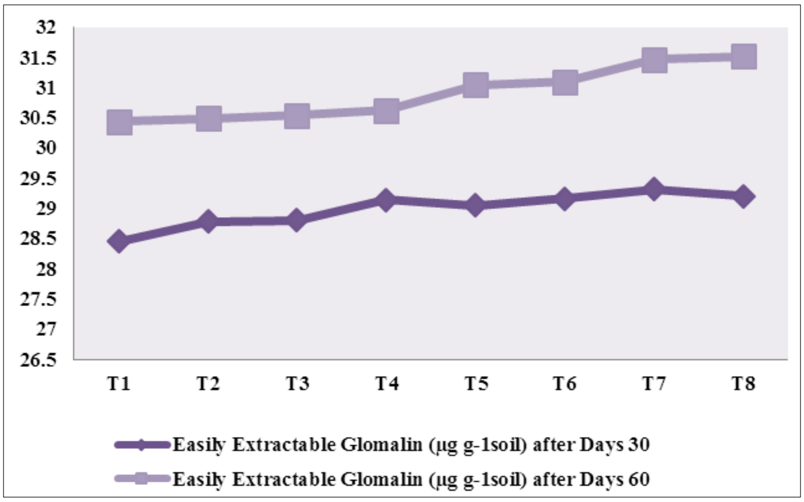

Fig 2: Effect of decomposition of paddy straw with ligno-cellulolytic microbes on Easily Extractable Glomlin Protein (µg g^{-1} soil)

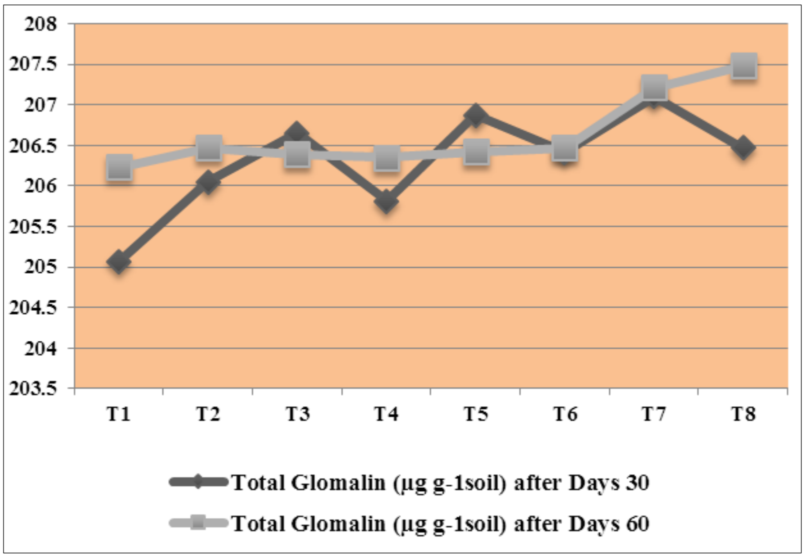

Fig 3: Effect of decomposition of paddy straw with ligno-cellulolytic microbes on Total Glomlin Protein (µg g^{-1}soil)

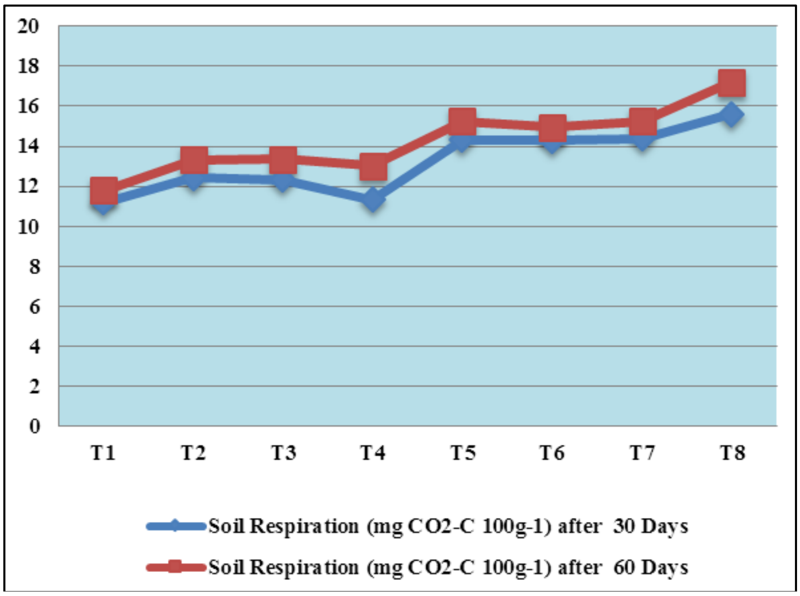

Fig. 4: Effect of decomposition of paddy straw with ligno-cellulolytic microbes on Soil Respiration.

Conclusion

It is concluded that application of paddy straw with microbial consortia (*Aspergillus spp.* + *Bacillus spp.* + *Streptomyces spp.*) was found best among all the treatments. Availability of soil nutrients particularly N, K and organic carbon also increased in treatment where straw was applied with microbial consortia compared to control. Among the biological properties, CO_2 emission increased significantly in T_8 treatment. Incorporation of paddy straw in soil in conjunction with ligno-cellulolytic microbes *Aspergillus spp.*, *Bacillus spp.* and *Streptomyces spp.* may be used as an effective measure for improving soil health. Pot culture composting study showed that it could play important role in the large scale composting of paddy straw.

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