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Efficacy of Lignocellulolytic Fungi on the Biodegradation of Paddy Straw

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ABSTRACT: Paddy straw is one of the most abundant agricultural by-products in Tamil Nadu, India. It contains high amounts of cellulose and lignin. The fungal strains were isolated from naturally decomposing sites of paddy straw. Eight fungal strains were isolated and later these eight fungi were used for finding out their potential for the degradation of lignin and cellulose content of paddy straw. Among eight fungi tested, three fungal strains showed lignocellulolytic activities. Hence, these three fungal strains were used for the decomposition of paddy straw both individually as well as in various combinations. Inoculation of these lignocellulolytic fungi in paddy straw accelerated the process of paddy straw decomposition when compared to control. Significant reduction in lignin and cellulose content were observed in paddy straw inoculated with mixed culture of *R. oryzae* + *A. oryzae* + *A. fumigatus* compared to other experiments. Mixed culture of three fungal strains reduced C:N ratio to 10:1 compared to 70:1 in paddy straw mixed with soil. A significant increase was also observed in macro nutrients of the compost harvested from E_8 experimental trays. It is evident from the results that the mixed culture of all the three lignocellulolytic fungi may be used for the degradation of paddy straw. Hence we conclude that combination of three lignocellulolytic fungi *viz.,R. oryzae, A. oryzae* and *A. fumigatus* can be recommended for the degradation of paddy straw which would result in production of good quality compost containing higher amounts of total nitrogen (1.55±0.03%), total potassium (1.57±0.01%) and total phosphorus (1.48±0.17%) content.

Key words: *Rhizopus oryzae, Aspergillus oryzae, Aspergillus fumigates* and macro nutrients

INTRODUCTION

Rice, *Oryza sativa*, is the most important food crop in the world after wheat, with more than 90% currently grown in Asia. It is grown in an area 154 million hectares (ha), yielding 636 million tons of paddy that generates huge amount of rice straw as by-product (USDA, 2008). It is not recycled in the soil due to its bulky volume, slow degradation rate, harbouring of pathogens and weed problems (Devevre and Horwath, 2000), as well as poor yield caused by short-term negative effect of nitrogen immobilization (Buresh and Sayre, 2007). Hence, the farmers usually dispose huge amount of straw by *in-situ* burning. This results on one hand in the renewable organic source waste going unutilized by not getting converted into a valuable product *i.e.*, compost and on the other hand leads to emission of green house gases (Badrinath *et al.,* 2006). It also contributes to emission of harmful air pollutants, including polycyclic aromatic hydrocarbons (PAHs) (Korenaga *et al.,* 2001), poly-chlorinated dibenzodioxins (PCDDs) and poly-chlorinated dibenzofurans (PCDFs) referred to as dioxins (Gullett and Touati, 2003; Lin *et al.,* 2007) which have significant toxicological properties and notably potential carcinogens that can cause severe impact on human health.

Keeping in mind the harmful effects of open field-burning of paddy straw, as well as the convenience of farmers, an economical, environment friendly and low labour intensive strategy should be adopted for effective utilization of paddy straw. Paddy straw is a potential food source for microorganisms and application of paddy straw compost into soil has long-term impact on nutrients recycling and maintaining soil fertility (Gaind *et al.,* 2006), increasing microbial density , biomass and enzyme activity (Hayano *et al.,* 1995). Inoculation with lignocellulolytic microbes might be an effective alternative to *in-situ* burning (Kumar *et al.,* 2008) to make the paddy straw composting process economically viable in a short period of time. The compost serves as an excellent source of nutrient in organic farming and mitigates the ill-effects due to usage of chemical fertilizers.

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Among microbial agents of decomposition, fungi are the most important group and they colonize very quickly on the solid substrates. They play an important role in the biodegradation of the lignocellulosic organic wastes (Hudson, 1972). Lignocellulolytic activity of several fungal species such as *Fusarium sp.* (Phutela and Sahni, 2012), *Aspergillusterreus* (Sinegani *et al.,* 2005), *Paecilomycesfusisporous* (Goyal and Sindhu, 2011), *Micromonospora* (Abdulla, 2007), *Coriolusversicolor* (Phutela *et al.,* 2011), has been reported in literature. In the present study, an attempt was made to isolate lignocellulolytic fungal strains from the native soil of paddy straw and the same was used for finding out their potential for the degradation of lignin and cellulose content of paddy straw under *in vitro* conditions, individually and to degrade the paddy straw by using the isolated lignocellulolytic fungal strains in various combinations, for the production of compost. The quantity of lignin, cellulose content reduction and the chemical nutrients of the compost were estimated, presented and discussed in this article.

MATERIALS & METHODS

1g of soil at the depth of 10cm was aseptically collected in a sterile polythene bag and the soil samples were stored at 4ºC for further use. The collected samples were mixed and from that mixture, dilutions of 10^{-4}, 10^{-5} and 10^{-6} were plated out in potato dextrose agar for the culture and isolation of fungi. Potato dextrose agar plates were incubated at 27° C for 3-5 days. After incubation, single colony of fungal strain was transferred aseptically onto potato dextrose media to obtain their pure culture.

The remaining soil samples were stored at 4ºC for further use. The lignolytic and cellulolytic activity of the fungi was determined by following the method of Thormann *et al.* (2002) and Hart *et al.* (2002) respectively. Identification of isolated and selected fungi was done by observing colony morphology, fungal staining, size, shape and arrangement of conidiophores (Carmichael *et al.,* 1980; Domsch and Games, 1993; Barnatt and Hunter, 1998). 10 mL molasses was taken in a conical flask and 90 mL of distilled water was added and mixed well. To this, 1 mL of pure culture of *R. oryzae, A. oryzae, A. fumigatus*obtainedindividually and the combination these three fungal strains (*i.e., R. oryzae + A. oryzae*; *R. oryzae + A. fumigatus*; *A. oryzae + A. fumigatus*; *R. oryzae + A. oryzae + A. fumigatus*) were added separately and mixed with 400mL of jaggery solution (500g of jaggery + 400mL of water). This preparation was mixed well and maintained for 7 days and used as an inoculum for the decomposition of paddy straw. The paddy straw was collected from Edumalai village, Tiruchirappalli District and chopped into small pieces. Twenty four plastic trays of size 45×15×30cm were obtained and each one was filled with 2kg of chopped paddy straw. Of these, nine trays were utilized for inoculating *R.oryzae* (three trays), *A. oryzae* (three trays) and *A. fumigates* (three trays), individually (separately). Twelve trays were utilized for inoculating a combination of these three fungal strains (*R. oryzae + A. oryzae (*in three trays); *R.oryzae + A. fumigatus* (in three trays); *A. oryzae + A. Fumigates* (in three trays) and *R.oryzae + A. oryzae + A. fumigatus* (in three trays). The remaining three trays were maintained as control (*i.e.*, paddy straw mixed with soil) in order to make comparison with the compost produced by three chosen fungal strains. These trays were kept undisturbed in shade. Watering was done regularly twice a day in order to maintain the optimum temperature and moisture content of the medium during the entire composting period. At the end of composting the samples were collected and analysed. The details of experimental setups are provided in Table 3.

The chief chemical constituents of paddy straw such as cellulose, hemicelluloses and lignin were estimated both in the raw waste and compost by following the method suggested by Updegraff (1969), Chang and Hudson (1967) and Bhat and Narayan (2003), respectively. The Organic Carbon, Total Nitrogen, Total Phosphorus and Total Potassium were estimated by Walkely Black, Micro kjeldhal, Spectrophotometric and Flame photometric methods (Tandon (2005), respectively. C:N ratio was calculated by dividing the percentage of organic carbon with percentage of total nitrogen (Anon, 2006). The total number of colony forming units of bacteria, fungi and actinomycetes present in the compost samples were estimated by serial dilution method (Allen, 1953). Nutrient Agar for bacteria, Potato Dextrose Agar for fungi and Soil Extract Agar for actinomycetes were used as suggested by Tandon (2005). Two analysis of variance (ANOVA) was performed by using the SPSS version 16.0 software. The objective of the statistical analysis was to determine any significant differences among the parameters analysed in different treatments.

RESULTS & DISCUSSION

The chemical constituents of raw paddy straw had 36.83 ± 0.75% of cellulose, 23.83 ± 1.47% of hemicelluloses and 15 ± 1.26% of lignin (Table 1). Eight different fungal strains were isolated from the soil and assigned with a number. The results of the cellulolytic activity of the eight fungal species isolated from the soil are provided in Fig.s. 1 & 3. Five fungal strains *viz.*, PSF_2, PSF_3, PSF_4, PSF_5 and PSF_7 showed cellulolytic activity. On the other hand, PSF_1, PSF_6 and PSF_8 did not show any zone of clearance (Fig. 1). Cellulose and

Table 1. The chemical constituents of raw paddy straw. Each value represents the mean (Mean ±S.D.) of three estimates

S. No.	Parameters	%
1	Cellulose	36.83 ± 0.75
2	Hemicellulose	23.83 ± 1.47
3	Lignin	15 ± 1.26
4	Organic Carbon	42.53 ± 0.19
5	Total Nitrogen	0.5 ± 0.004
6	Total Phosphorus	0.02 ± 0.004
7	Total Potassium	0.76 ± 0.04
8	C:N ratio	85:1

Table 2. Morphological characteristics and identification of fungi isolated from soil

Colour of aerial hyphae	Colour of septate hyphae	Nature of hyphae	Shape	Presence of special structure	Sporangiophore or conidiophore	Characteristics of spore head	Identified fungal strains
Gray black	Black	septate	Oval	Absence of foot cell	Long erect	Smooth round	*Rhizopus oryzae*
Brown	Brown	Septum/ nucleate	Oval greenish	Presence of foot cell	Long erect non-septate	Multinucleate	*Aspergillus oryzae*
Bluish green	Brown	Septate	Oval greenish	Presence of foot cell	Long erect non septate	Multinucleate green vesicles	*Aspergillus fumigatus*

Table 3. Experimental design adopted in the present study

S. No	Particulars	Experiment Name							
		E_1	E_2	E_3	E_4	E_5	E_6	E_7	E_8
1	Total weight of paddy straw taken in each tray (g)	2000	2000	2000	2000	2000	2000	2000	2000
2	Total quantity of fungal inoculum taken for each tray (ml)	-	500	500	500	500	500	500	500
3	Mean number of days taken for degradation of paddy straw	120	68	74	78	51	58	62	46
4	Mean weight of compost obtained in each tray (g)	750	1050	1120	1250	1310	1430	1550	1700
5	Mean percentage of degradation in each tray	38	53	56	63	66	72	78	85

Each experiment (E_1 to E_8) were done in triplicates

carboxymethylcellulose are closely related substances and therefore evidence for carboxymethylcellulose degradation can be taken for cellulose degradation (Miller, 1959). Carboxyme-thylcellulose degradation was shown by the formation of a clear yellow zone around the fungal colony. The results of the lignolytic activity of the eight fungal species isolated from the soil are given in Fig.s. 2 & 3. Three fungal isolates, *i.e.*, PSF_2 (1.8 ± 0.02 cm), PSF_4 (1.4 ± 0.03 cm) and PSF_7 (1.9 ± 0.01 cm) showed a zone of clearance. The other five fungal strains such as , PSF_1, PSF_3, PSF_5, PSF_6 and PSF_8 did not show any zone of clearance. Lignin and tannic acid are closely related substances and therefore evidence for tannic acid degradation can be taken for lignin degradation (Garcia *et al.*, 1995). Tannic acid was shown by the formation of a brown zone around the fungal colony. Out of eight fungal species, 3 fungi showed positive reaction for both cellulolytic and lignolytic activity. These three lignocellulolytic fungi were further used for paddy straw degradation. Based on the lignocellulolytic activity, three fungal strains were selected and identified as *R. oryzae, A. oryzae* and *A. fumigatus* (Table 2 & Fig. 4). The mean number of days required for degradation of paddy straw by the chosen fungal strains both individually and in various combinations was 68 (*R. oryzae*), 74 (*A. oryzae*), 78 (*A. fumigatus*), 51 (*R. oryzae + A. oryzae*), 58 (*R. oryzae + A. fumigatus*), 62 (*A. oryzae + A. fumigatus*), 46 (*R. oryzae + A. oryzae + A. fumigatus*), 120 (control) (Table 4). The average weight of compost obtained after composting of paddy straw were in the order 1700g (E_8) >1550g (E_7) >1430g (E_6) >1310g (E_5) >1250g (E_4) >1120g (E_3) >1050g (E_2) >750g (E_1).The mean percent conversion of compost was in the order 85% (E_8) >78% (E_7) >75% (E_6) >66% (E_5) >63% (E_4) >56% (E_3) >53% (E_2) >38% (E_1). Significant variations were observed in cellulose, hemicellulose and lignin content of compost obtained from all the experimental trays. Maximum cellulose and lignin degradation (76% of cellulose and 94% of lignin decrease than raw paddy straw) was observed in paddy straw inoculated with mixed culture of *R. Oryzae, A. oryzae* and *A. fumigatus*

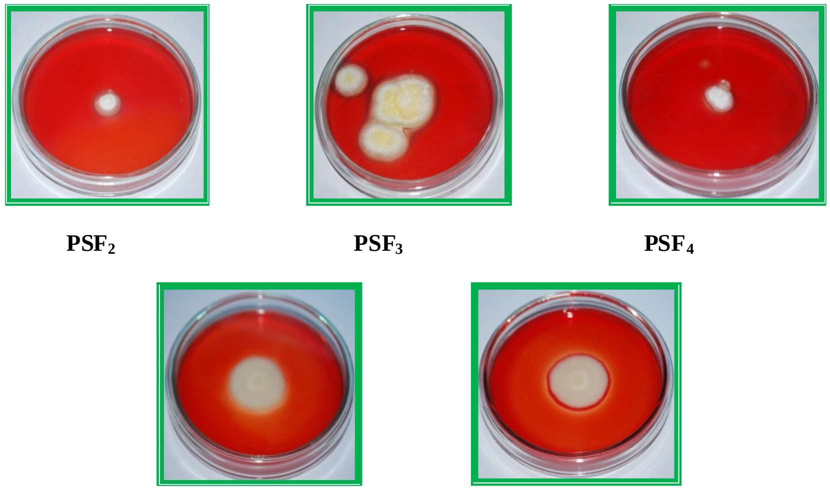
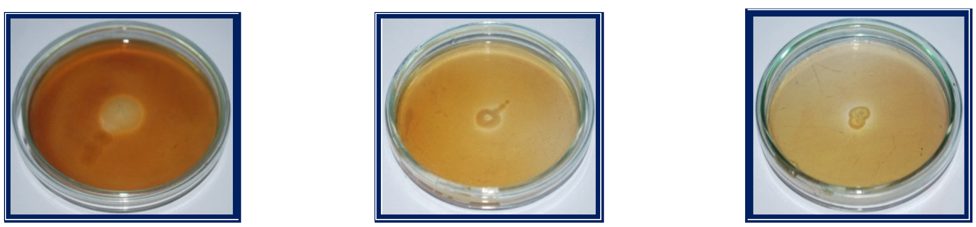


Fig. 1. Cellulolytic activity of the fungal strains isolated from native soil of the paddy straw



PSF$_2$ PSF$_4$ PSF$_7$

Fig. 2. Lignolytic activity of the fungal strains isolated from native soil of the paddy straw

Table 4. Degradation of paddy straw by using three different lignocellulolytic fungal strains both individually and in various combinations

S. No.	Experiment Name	Combination of fungal strains
1	E_1	Paddy straw + Soil
2	E_2	Paddy straw + *Rhizopus oryzae*
3	E_3	Paddy straw + *Aspergillus oryzae*
4	E_4	Paddy straw + *Aspergillus fumigatus*
5	E_5	Paddy straw + *R. oryzae* + *A. oryzae*
6	E_6	Paddy straw +*R. oryzae* + *A. fumigatus*
7	E_7	Paddy straw +*A. oryzae* + *A. fumigatus*
8	E_8	Paddy straw +*R. oryzae* + *A. oryzae* + *A. fumigatus*

For treatment combinations see Table 3.
Each experiment (E_1 to E_8) was conducted in triplicates.

when compared to other combinations of fungal strains used (Table 5). Zafar*et al.* (1980) observed that cellulose content of paddy straw treated with *Pleurotussajorcaju* decreased from 45.0 to 17.8%. Jalc*et al.* (1998) showed that initial decrease in hemi-cellulose content might be the result of breakdown or hydrolysis of hemi-celluloses into fermentable sugars. Similar results on composting of kitchen waste and municipal solid waste inoculated with effective microorganisms have been reported by Tamilarasi (2006). A significant reduction in Organic Carbon (0.70% to 62%) was recorded in compost harvested from all the experimental trays as compared to raw paddy straw. Maximum reduction (62%) was observed in paddy straw inoculated with mixed culture of lignocellulolytic fungal strains (Table 6). This study is in accordance with previous work (Shweta *et al.,* 2010; Tumuhairwe *et al.,* 2009 and Benitz*et al.*,1999). Phutela and Sahni (2011), while studying degradation of paddy straw with *Fusarium sp*. reported that the decreased organic carbon in the

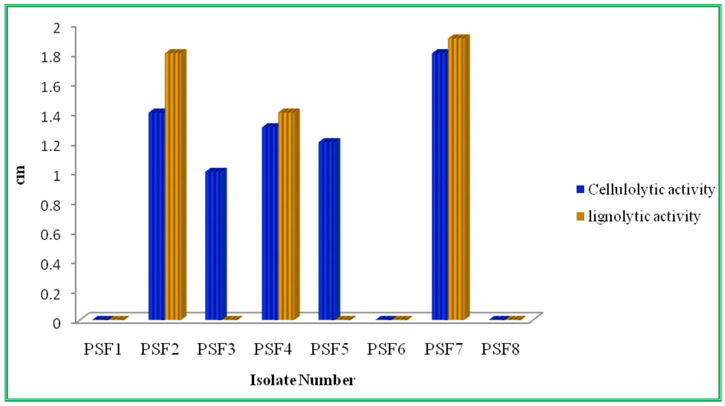
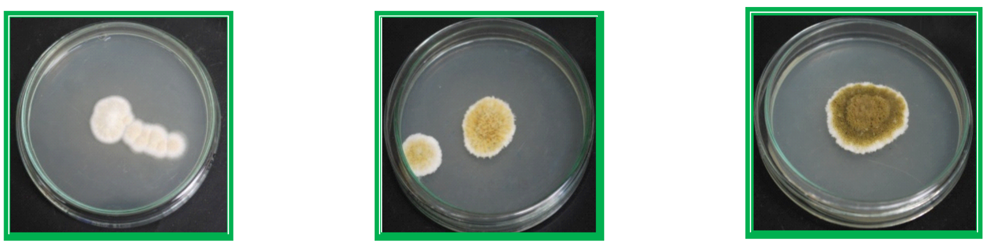


Fig. 3. The cellulolytic and lignolytic activities of the isolated fungal strains



Rhizopus oryzae *Aspergillus oryzae* *Aspergillus fumigatus*

Fig. 4. The morphology of the identified fungal strains

Table 5. Magnitude of chief chemical constituents (%) of paddy straw after decomposition. Each value represents the mean (Mean ±S.D.) of three observations

S.No.	Experiment Name	Chemical constituents					
		Cellulose	Decrease in % when compared to Raw Paddy Straw	Hemicellulose	Decrease in % when compared to Raw Paddy Straw	Lignin	Decrease in % when compared to Raw Paddy Straw
1	E_1	35.33±0.57	4.07	21.83±0.76	8.39	14.83±1.04	1.13
2	E_2	30.66±1.52*	16.74	20.20±0.3*	15.23	11.43±0.40	23.8
3	E_3	30.33±0.57	21.43	14.66±0.57*	38.48	7.2±0.26	52
4	E_4	27.33±0.51**	34.76	13±0.01**	45.44	6.3±0.28**	58
5	E_5	24.33±0.57	33.93	12.16±0.28*	48.97	6±0.01***	60
6	E_6	21.00±0.01*	42.98	11.33±0.51**	52.45	5.2±0.02	65.33
7	E_7	17.66±0.6**	52.04	8.00±0.01	66.42	2.3±0.43	84.66
8	E_8	8.66±0.61***	76.48	2.16±0.28***	90.93	0.93±0.15***	93.8

For treatment combinations see Table 3.
*, ** and *** indicates statistically significant at $p<0.05$, $p<0.01$, $p<0.001$.

final product. Vuorinen and Saharinen (1997) and Adani*et al.*(1997) reported that approximately 11-27% of the organic carbon is lost during initial stage of active composting and about 62-66% during the whole composting time. Diaz *et al.* (1996) opined that during composting, C is a source of energy for microorganisms to build cells. The total nitrogen content of the compost harvested from all the experimental trays showed significant increase when compared to raw paddy straw and control as per the ANOVA analysis (Table 6). The increase in total nitrogen content was in the order: $E_8 > E_7 > E_6 > E_5 > E_4 > E_3 > E_2 > E_1$.The increase in the total nitrogen content might be due to process of nitrification by the microorganisms (Zhu, 2007). The results observed in this study are consistent with many previous works (Viel*et al.*, 1987; Bishop and Godfrey, 1983; Bernal *et al.*, 1998; Goyal *et al.*, 2005 and Polprasert, 1996). In contrast to this finding, decreased total nitrogen was reported by Tognetti*et al.*, (1998). A significant increase in Total Phosphorus was observed

Table 6. Extent of chemical nutrients (%) of end product *i.e.*, compost of different combinations of experiments. Each value represents the mean (Mean ±S.D.) of three observations.

S.No.	Experiment Name	Chemical Nutrients				
		Organic carbon	Total Nitrogen	Total Phosphorus	Total Potassium	C:N ratio
1	E_1	40.23±0.25	0.57±0.01	0.08±0.005	0.77±0.005	70:1
2	E_2	35.07±0.12	1.15±0.02	0.13±0.01	0.88±0.005	30:1
3	E_3	31.07±0.06**	1.25±0.01	0.24±0.005**	0.95±0.005	24:1
4	E_4	30.10±0.01*	1.34±0.02**	0.75±0.21**	1.14±0.03**	22:1
5	E_5	28.07±0.64	1.39±0.02	1.25±0.01***	1.26±0.01*	20:1
6	E_6	25.09±0.08***	1.41±0.01*	1.38±0.01	1.34±0.005**	18:1
7	E_7	21.14±0.15***	1.48±0.02***	1.41±0.01**	1.42±0.01	14:1
8	E_8	16.07±0.12***	1.55±0.03***	1.48±0.17***	1.57±0.01***	10:1

For treatment combinations see Table 3.

*, ** and *** indicates statistically significant at $p<0.05$, $p<0.01$, $p<0.001$.

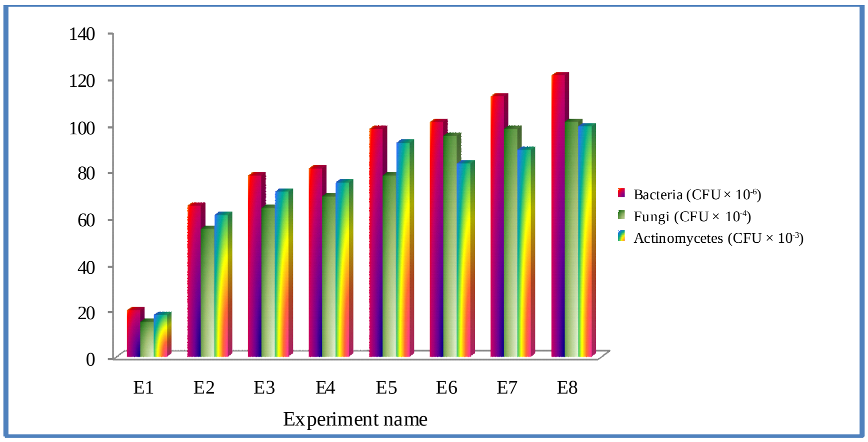


Fig. 5. Density of microbes (CFU) in compost harvsted from different experimental trays

in all the experimental trays when compared to control and raw paddy straw. The maximum increase was observed in paddy straw inoculated with the combination of three lignocellulolytic fungal strains *i.e.*,*R. oryzae* + *A. oryzae* + *A. fumigatus* (Table 6). Similar pattern of total P enhancement is well documented in the available literature (Abdulla, 2006; Anbuselvi and Jeyanthi, 2009; Vourinen and Saharinen, 1997; Sommer, 2001; Iyengar and Bhave, 2005; Tai and He, 2007. In contrast, Tumuhairwe*et al.,* (1998) reported the loss of total P during composting process and that was possibly due to the leaching of P. Total potassium was significantly higher in the end products than raw paddy straw as per the ANOVA analysis (Table 6). Total Potassium plays an important role in plant growth where its function is to increase the elongation of the root, control ion balance, improve protein synthesis, encourage enzyme reaction and improve the photosynthesis process and food development (Sabiani*et al.*, 2004). A significant decrease in C:N ratio was registered in the compost harvested from all the experimental trays when compared to raw paddy straw. The decrease in C:N ratio was in the order: $E_8 > E_7 > E_6 > E_5 > E_4 > E_3 > E_2 > E_1$ (Table 6). Suthar (2007a, b) and Garg*et al*. (2006) reported a similar result of reduced C:N ratio in decomposed materials. Composting of wheat straw by inoculation of fungi along with application of 1% urea resulted in an end product with C:N ratio of 10:7 within three months in pits (Gaind*et al*. 2005). Similarly, Brito*et al*. (2008) also observed a decline in the C:N ratio at the end of composting from 36% to 14% by the addition of solid fraction of cattle slurry. Compost production from sugarcane organic waste was performed by using *Trichoderma* fungi with different levels of urea and pH and it was observed that C:N ratio reduced below 30% in 5 weeks and below 20% in 10 weeks after composting (Torkashvand*et al.*, 2008). A C:N ratio less than or equal to 20 is considered a satisfactory value for maturity when the initial value of composting substrates is between 25 and 30 (Goyal*et al.*, 2005). In the present study, in general, an increase in the number of colonies of bacteria, fungi and actinomycetes were observed in all the experimental trays when compared to control. The maximum increase was noticed in the compost harvested from the experimental trays inoculated with the combination of three lignocellulolytic fungi *viz., R. oryzae, A. oryzae* and *A. fumigatus*. The increase in the number of colonies of bacteria, fungi and actinomycetes were in the order: $E_8 > E_7 > E_6 > E_5 > E_4 > E_3 > E_2 > E_1$ (Fig. 5).

CONCLUSIONS

Inoculation of lignocellulolytic microorganisms is deemed to be a useful strategy to accelerate the degradation potential of lignocellulose in agricultural waste. It is evident from the results that the presence of the lignocellulolytic fungi in the agro-waste facilitated to degrade lignin and cellulose contents in higher quantities than those determined in the non-inoculated. The results indicated that mixed culture of three lignocellulolytic fungal strains *viz., R. oryzae, A. oryzae* and *A. fumigatus* was found to be more efficient in degrading greater amount of lignin and cellulose in paddy straw. Macro and micro nutrients were observed at the desired levels in the compost harvested from the experimental trays inoculated with the mixed culture of these three fungal strains. Hence it is concluded that the mixed culture of *R. oryzae, A. oryzae* and *A. fumigatus* can be recommended for the degradation of paddy straw and for the production of quality compost with higher amount of macronutrients.

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