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Synergistic Effect of Fungal Consortia and C/N Ratio Variation on Rice Straw Degradation

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ABSTRACT

In this study, the efficiency of fungal consortia was evaluated on the degradation of rice straw by varying the initial carbon/nitrogen ratio of the compost piles. Consortia of three potent cellulose-degrading fungal strains: *Aspergillus fumigatus, Aspergillus terreus* and *Aspergillus flavus* were used as an inoculant to degrade rice straw in a 90-day composting process. The carbon/nitrogen ratio of the composting piles was varied by varying the proportion of bedding material in different treatments. The composts thus achieved were subjected to physic-chemical analysis and phytotoxicity assay using *Solanum lycopersicum* as test seeds. After 90 days of composting, compost from treatment 4 with initial carbon/nitrogen ratio 34 depicted maximum stability by achieving a final carbon/nitrogen ratio of 16.25. Compost from treatment 4 showed the highest Germination Index (%) followed by treatment 3 and treatment 2 as 94.32%, 88.88% and 79%, respectively on the growth of *Solanum lycopersicum* seeds. Results concluded that fungal consortia derived agro-waste compost with an initial carbon/nitrogen ratio 34 depicted the earliest maturity which is suggestive of its suitability for agricultural application.

INTRODUCTION

Crop residue management is currently a global challenge. The quantity of agricultural waste generated throughout the world is enormous. Also, the problem of pollution associated with the conventional waste disposal methods demands exploration of alternative and environment-friendly methods of dealing with agro-wastes. The demand for food has been consistently increasing with the increasing population and has, therefore, created a need for upgrading of agricultural by-products. Disposal of such a huge quantity of lignocellulosic wastes is a big challenge to our environment. Wood and crop residue burning for energy leads to a release of toxic gases that cause environmental pollution (Tuomela et al. 2000). The accumulation of agro-waste raises health, safety, environmental, and aesthetic concern. Therefore, safe disposal techniques need to be explored. Crop residue management is currently a global challenge. The Ministry of New and Renewable Energy (MNRE) has reported that about 500 Mt of agro-wastes are produced every year wherein cereals are found to produce most of the residues (352 Mt). Fibres (66 Mt), oilseeds (29 Mt), legumes (13 Mt) and sugarcane (12 Mt) are also produced subsequently. 70% of agro-waste is contributed by rice, wheat, maize and millets collectively whereas 34% is generated from rice crop alone (Jain et al. 2014).

Rice (*Oryza sativa L.*) is a major crop, grown worldwide producing a large amount of rice straw. Rice straw is a stringy, lingo-cellulosic rice crop residue that remains after the harvest. It is not considered a good substrate owing to its complex lingo-cellulosic composition that renders it hard to degrade. Wastes obtained from plants, like rice straw, are unmanageable due to their lignin content which interferes with biodegradation (Van Soest 2006). Rice straw constitutes a considerable amount of silica which exhibit various repercussions related to animal health problems and grants this crop residue a gruff appearance thereby weakening its suitability for animal intake due to its reduced digestibility. The direct incorporation of straw in the soil is coupled with problems like immobilization of plant nutrients especially nitrogen and reduces the germination of crop seeds. It can hamper seedbed preparation and add to disease and weed problems. Therefore, at present, there are limited options for rice straw because of its pitiable quality for forage, bioconversion, and engineering applications. Rice straw is usually not integrated in the crop field due to its protracted rate of decomposition and susceptibility to pest invasion (Nigam & Pandey 2009).

Farmers generally opt for open field burning to get rid of this crop residue thereby contributing to greenhouse emissions into the atmosphere. A significant amount of rice straw is burned on-farm to make way for the succeeding wheat crop. Stubble burning results in air pollution which has dire consequences on the ecosystem due to the deteriorating soil quality, soil erosion, intensification of air pollutants and greenhouse gases (Gadde et al. 2009). 80% of rice straw is burnt in Punjab, Himachal Pradesh and Haryana while 50% is burnt in Karnataka and 25% in the state of Uttar Pradesh (Gupta et al. 2003). This *in situ* burning of rice straw can be attributed to the modern method of harvesting done with the aid of combine harvesters. To tackle this issue, composting has emerged as an eco-friendly and economic treatment technology and as an effective approach to achieve enhanced and sustainable output from agriculture (Sarkar & Chourasia 2017). It aids in the reduction of waste volume, weight, moisture content, odour, pathogens and spread of diseases, thereby, increasing potential nutrients in the compost rendering it suitable for agricultural applications (Gautam et al. 2011). It is seen as a key process where the final product is safer to use and microbes such as fungi have proved to be beneficial in accelerating the degradation of lignocellulosic waste. Compost properties differ significantly depending on the composting feedstock and composting procedures. Carbon and nitrogen are imperative elements essential for microbial breakdown (Ain et al. 2017). Carbon provides energy and structural support and constitutes half of the cell biomass in microbes whereas nitrogen being a chief constituent of enzymes and nucleic acids helps in growth and performance of cells. It is important to consider the carbon and nitrogen (C/N) ratio of the compost components to establish the optimal amounts of carbon and nitrogen. Narrowing of C/N ratio of agro-wastes by using urea as a supplement has been in practice earlier (Gaind & Gaur 2000). But developments in agriculture and an increasing inclination towards organic farming has catered to the use of organic wastes rich in nitrogen. Chicken litter or poultry manure can be considered as one such alternative (Ogunwande et al. 2008). It is also recognized that when the rice straw is combined with poultry manure, it reduces the C/N ratio of rice straw and even impedes the loss of nitrogen thereby developing an end-product which is equipped to provide required nourishment to the plants (Gaind et al. 2010).

Naturally, very few microorganisms are potent enough to break the intricate structure of lignin during composting. Fungi, owing to their potential to conceive prolific spores and filamentous morphology, are quite effective in degrading lingo-cellulosic crop residues. Also, fungi are the most influential and dominant groups present in soil which impact the structure and functioning of the ecosystem and therefore play a major role in many ecological services. Fungi produce an array of hydrolytic enzymes and hence subsist in nature in saprophytic mode and are more effective than bacteria in acidic soils and decomposing cellulose rooted in lignin (Goyal et al. 2011). They can stand a wide range of pH as compared to bacteria and hence are more preferred than bacteria for the decomposition of complex organic wastes. Composting of agro residues with high C/N ratio can be efficiently achieved by inoculating cellulolytic fungi such as *Aspergillus sp* (Ashraf et al. 2007). Also, these fungi have been reported to slash down the duration of the composting process by one month.

Though microbes amended composting has been studied earlier but rice straw composting aided with fungal inoculation has less explored literature. In the present research, the synergistic effect of fungal consortia on the degradation of rice straw by varying initial C/N ratio was studied and the compost thus achieved were tested for their phytotoxic effects on *Solanum lycopersicum* (tomato) seeds.

MATERIALS AND METHODS

For composting, paddy straw was obtained after the harvest of rice from the rice field of Kanheli village, Rohtak city (Haryana). It was then pre-treated by soaking in 0.1% urea solution and stacked along with green leaves and poultry droppings to make composting piles. Pre-treatment of rice straw with urea helped in delignification of rice straw and made it susceptible to degradation by cellulase enzyme. The proportion of green leaves and poultry droppings (Fig. 1) was varied to achieve the desired C/N ratios in the composting piles.

Three cellulolytic fungal strains cultured at Department of Environmental Science, MDU, Rohtak were used as inoculants for composting purpose. Fungal spore suspension was prepared as per the method described by Samsudin et al. (2013). 5-day old culture slants were washed with 0.9% NaCl saline solution and shaken rapidly for one minute. Haemocytometer was used to count fungal spores and the number of spores was adjusted to contain approximately 10^7 spores/mL of each fungal isolate. A broth inoculum containing 10^7 spores/mL of each isolate was used for inoculation.

A 90-day experiment was conducted to degrade rice straw. Bins were set in triplicate with circular holes of 100 mm diameter to provide aeration to the compost material. Control bin was also set in which no inoculants were added. Prepared compost treatments have been described in Table 1.

Table 1: Various compost treatments prepared for the study.

Treatment	Initial C/N ratio	Composition
Treatment 1	Control	No inoculants added
Treatment 2	30:1	Rice straw = 2000 g, Green Leaves = 1000 g, Poultry droppings = 1000 g + Fungal Inoculants
Treatment 3	26:1	Rice straw = 2000g, Green Leaves = 1000 g, Poultry droppings = 1300 g + Fungal Inoculants
Treatment 4	34:1	Rice straw = 2000 g, Green Leaves = 1000 g, Poultry droppings = 750 g + Fungal Inoculants

Fig.1 (A-D): Setting of compost bins.

Fig. 2: Compost piles.

The compost bins were kept inverted to form heaps (Fig. 2). The components of the compost bins were turned at regular intervals to provide aeration (Jusoh et al. 2013). Compost samples were taken at 30, 60 and 90-days intervals and were subjected to physic-chemical analysis as per standard protocols mentioned in Table 2. The mature compost obtained after 90 days was also subjected to phytotoxicity analysis to check the effectiveness of compost on the growth of test seeds *Solanum lycopersicum*. For this, seeds of *Solanum lycopersicum* were disinfected by dipping in 7% alcohol for about 3 minutes. Later it was left in a suspension of 0.001 $HgCl_2$ for about 2 minutes where it was stirred intermittently. It was repeatedly washed with distilled water to ensure that no toxins are present. 10 mL volume of compost extract was poured on filter paper spread in a Petri plate. 20 sterilized seeds of *Solanum lycopersicum* were then set on filter paper and Petri plates were carefully sealed using tape to reduce the loss of water. They were then incubated for 72 hours at room temperature. All tests were carried out in triplicate. The percentage of root elongation, seed germination and germination index (GI) was deduced using equations 1 to 3 (Zucconi et al. 1981).

$$\text{Seed germination (\%)} = \frac{\text{Number of seeds germinated in compost extract}}{\text{Number of seeds germinated in control}} \times 100 \qquad \text{...(1)}$$

$$\text{Root elongation (\%)} = \frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100 \quad ...(2)$$

$$\text{Root elongation (\%)} = \frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100 \quad ...(3)$$

RESULTS AND DISCUSSION

After 90 days of composting, the compost obtained had a uniform and granular texture, was relatively dry, earthy, and dark brown (Fig. 3). It looked crumbly and had a pleasant aroma, like freshly turned earth. Similar results were obtained by Pandharipande et al. (2004) who stated that finished compost must be black or dark brown in colour, granular and spongy with normal smell.

Results of the physic-chemical analysis of compost samples have been served in Table 3. pH alteration is crucial to plant health. Results reveal that all treatments were recorded with an alkaline pH to some extent in the early stages of decomposition. With due course of composting, pH values show signs of gradual decrease which might be due to the production of organic acids and loss of ammonia to the atmosphere. pH in all treatments stabilized at the end of 90 days with pH 7.5, 7.1, 7.2 and 6.7 for treatments 1, 2, 3 and 4 respectively (Fig. 4). Electrical Conductivity (EC) showed an increasing trend in all treatments during composting (Fig. 5). Treatment 4 (C/N 34) showed maximum electrical conductivity (3.6 dS/m) followed by treatment 3 (3.4 dS/m) and treatment 2 (3.1 dS/m). The increase in EC can be attributed to an increase of potassium (K^+) and other ions during composting and also due to the release of inorganic salts during decomposition (Bernal et al. 2009). EC was found to be higher in treatment 4 which might be due to the release of soluble salts (Huang et al. 2004, Wang et al. 2004). Total organic carbon (TOC) depicts a decreasing trend (Fig. 6) with value of treatment 4 (18.2%) > treatment 2 (18%) > treatment 3 (17.4%) after 90 days of composting. This might be due to the loss of carbon in the form of CO_2 as decomposition proceeds. This is in accordance with results indicated by Getahun et al. (2012). Also, the maximum value for Total Nitrogen content was observed in treatment 4 (1.12%) followed by treatment 3 (0.94%) and treatment 2 (0.91%) at 90 days of composting (Fig. 7).

After 90 days of composting, treatment 4 with initial C/N ratio 34 depicted maximum stability by achieving a final C/N of 16.25 (Fig. 8) which shows that the compost from treatment 4 has attained earliest maturity and is suitable for agricultural application (Owis et al. 2016). On the contrary, a high C/N in compost obtained from control treatment depicts that carbon has been left unused in the compost mixture (Dobermann & Fairhurst 2002). This was supported by the fact that in treatment 4, the compost showed highest Germination Index (%) followed by treatment 3 and treatment 2 as 94.32%, 88.88% and 79%, respectively on the growth of *Solanum lycopersicum* seeds (Fig. 9 & Fig. 10) which implies that for most treatments phytotoxicity has been eliminated. Similar results were observed by Azim et al. (2014) and Abdelhamid et al. (2004). It can therefore be inferred from the study that rice straw can be efficiently degraded by lowering its initial C/N ratio to 34 using poultry droppings as an amendment. Similar results were recorded by Kausar et al. (2014).

Table 2: Methods used to detect parameters in compost samples.

Parameters	Methods used
pH	pH meter
Electrical conductivity	Conductivity meter
Total organic carbon	Walkley and Black method (Khiari et al. 2005)
Total nitrogen	Kjeldahl method (Katyal et al. 1987, Bremner 1996)

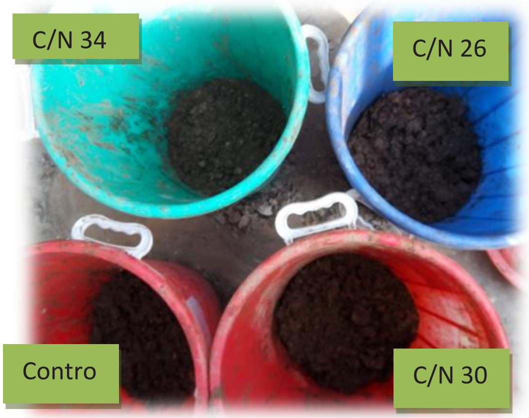


Fig. 3: Compost after 90 days of composting.

Table 3: Physico-chemical analysis of composts during the 90-day study.

Parameters	Initial C/N Ratio	30 days of composting	60 days of composting	90 days of composting
pH	C/N 34	7.8 ± 0.02	7.1 ± 0.17	6.7 ± 0.26
	C/N 30	8.1 ± 0.13	7.9 ± 0.16	7.1 ± 0.29
	C/N 26	7.9 ± 0.32	7.6 ± 0.11	7.2 ± 0.16
	Control	8.3 ± 0.11	7.5 ± 0.31	7.5 ± 0.26
Total Organic Carbon (%)	C/N 34	21 ± 0.25	19.7 ± 0.19	18.2 ± 0.09
	C/N 30	20.2 ± 0.18	19 ± 0.27	18 ± 0.27
	C/N 26	20 ± 0.22	18.9 ± 0.05	17.4 ± 0.16
	Control	21 ± 0.15	20.3 ± 0.01	19.8 ± 0.22
Total Nitrogen (%)	C/N 34	0.65 ± 0.06	0.78 ± 0.16	1.12 ± 0.07
	C/N 30	0.79 ± 0.37	0.88 ± 0.25	0.91 ± 0.13
	C/N 26	0.78 ± 0.23	0.86 ± 0.18	0.94 ± 0.27
	Control	0.71 ± 0.19	0.73 ± 0.16	0.73 ± 0.21
Electrical Conductivity (dS/m)	C/N 34	2.7 ± 0.04	3.1 ± 0.07	3.6 ± 0.15
	C/N 30	2.3 ± 0.12	2.7 ± 0.16	3.1 ± 0.19
	C/N 26	2.4 ± 0.26	2.9 ± 0.11	3.4 ± 0.34
	Control	2.3 ± 0.08	2.5 ± 0.13	2.8 ± 0.28
C/N ratio	C/N 34	32.3 ± 0.05	24.8 ± 0.18	16.25 ± 0.17
	C/N 30	25.5 ± 0.14	21.5 ± 0.04	19.7 ± 0.04
	C/N 26	25.6 ± 0.12	21.9 ± 0.25	18.5 ± 0.16
	Control	29.5 ± 0.18	27.8 ± 0.07	27.1 ± 0.11

(n = 3; Mean ± SD)

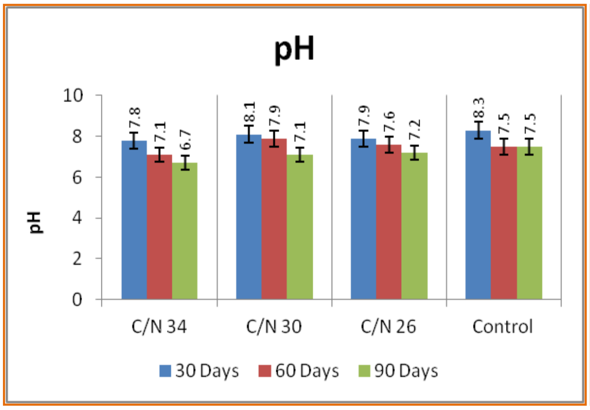


Fig. 4: pH values during composting.

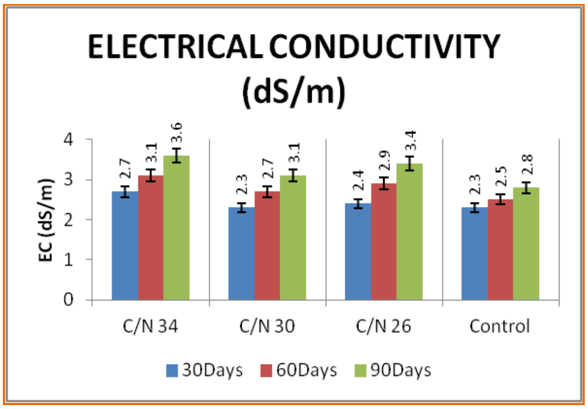


Fig. 5: Electrical conductivity (dS/m) of compost.

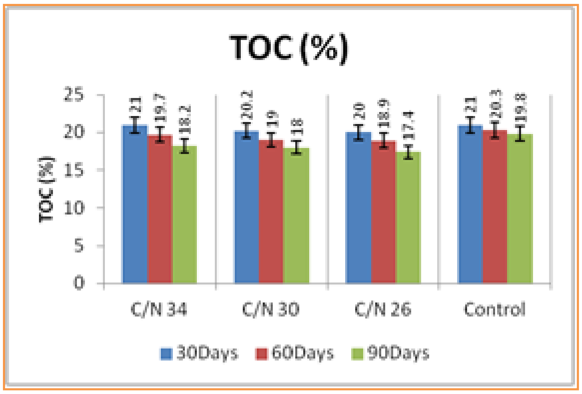


Fig. 6: Total organic carbon in compost samples.

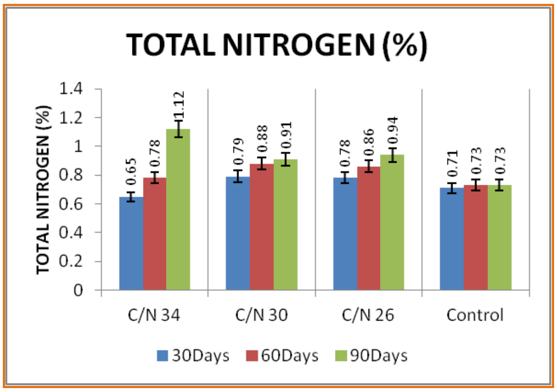


Fig. 7: Total nitrogen content in compost samples.

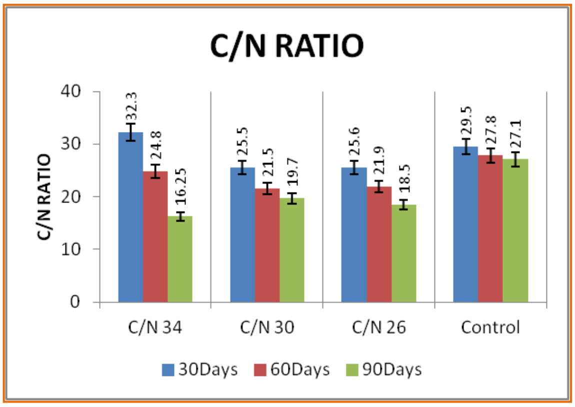


Fig. 8: C/N ratio of compost samples.

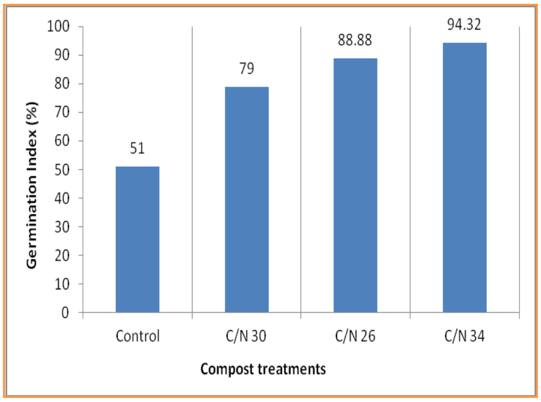
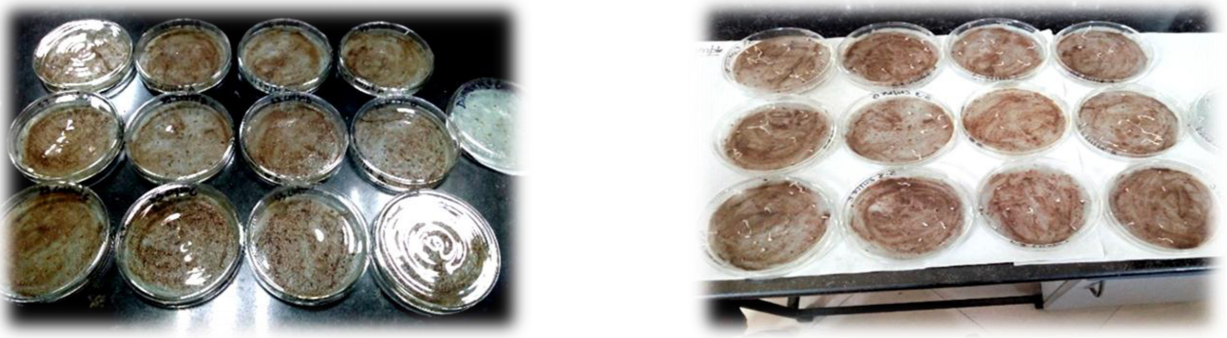


Fig. 9: Germination index (%) of final composts at the end of 90 days of composting.



(a) Seeds before Incubation

(b) Seeds after 72 hours of incubation

Fig.10 (a-b): Germination Index of mature compost.

Table 4: ANOVA: Two-factor without replication.

Source of variation	SS	df	MS	F	P-value	F crit
Rows	5237.736173	13	402.9027826	1.987737096	1.02707E-28	1.78052833
Columns	21.83360536	3	7.277868452	2.595262191	0.046159652	2.845067805
Error	109.3673196	39	2.804290247			
Total	5368.937098	55				

*null hypothesis is rejected and significant difference exists between the treatments

Statistical Analysis

To deduce the variables involved in inferring the compost quality, through physic-chemical characteristics and their relationships with each other, Two-way ANOVA (Table 4) and Pearson correlation coefficient (Table 5) were applied using SPSS 23.0 (0.05 levels).

CONCLUSION

The consortia of *Aspergillus fumigatus, Aspergillus terreus and Aspergillus flavus* efficiently degraded rice straw, thereby reducing the decomposition time. The final values of C/N observed in treatments 2, 3 and 4 are considered as acceptable levels for compost maturity. It is evident from the study

Table 5: Correlation between physico-chemical parameters of compost samples during 90 days of composting.

	pH 30	pH 60	pH 90	TOC 30	TOC 60	TOC 90	TN 30	TN 60	TN 90	EC 30	EC 60	EC 90	C/N 30	C/N 60	C/N 90
pH 30	1.000														
pH 60	0.489*	1.000													
pH 90	0.853*	0.511*	1.000												
TOC 30	0.185	-0.719	-0.105	1.000											
TOC 60	0.487*	-0.520	0.281	0.923*	1.000										
TOC 90	0.770*	-0.172	0.536*	0.760*	0.931*	1.000									
TN 30	0.246	0.935*	0.442*	-0.904	-0.713	-0.425	1.000								
TN 60	-0.349	0.645*	-0.220	-0.920	-0.980	-0.853	0.776*	1.000							
TN 90	-0.946	-0.521	-0.976	-0.012	-0.378	-0.654	-0.378	0.282	1.000						
EC 30	-0.814	-0.866	-0.866	0.418*	0.087	-0.266	-0.759	-0.208	0.876*	1.000					
EC 60	-0.990	-0.586	-0.899	-0.049	-0.374	-0.680	-0.374	0.239	0.970*	0.886*	1.000				
EC 90	-0.999	-0.526	-0.872	-0.136	-0.447	-0.739	-0.294	0.309	0.957*	0.842*	0.995*	1.000			
C/N 30	-0.202	-0.914	-0.423	0.924*	0.737*	0.462*	-0.998	-0.790	0.346	0.731*	0.333	0.250	1.000		
C/N 60	0.482*	-0.527	0.340	0.887*	0.991*	0.917*	-0.686	-0.989	-0.413	0.066	-0.379	-0.445	0.705*	1.000	
C/N 90	0.947*	0.255	0.891*	0.328	0.651*	0.857*	0.066	-0.563	-0.948	-0.691	-0.927	-0.942	-0.032	0.675*	1.000

* Values greater than p-value of 0.355 are significant

that composts attained maturity in 90 days and possessed a good amount of nutrients and organic matter. Moreover, no signs of phytotoxicity were detected in the final composts, except in control treatment. This study illustrates that supplementing rice straw with poultry droppings helped enhance the quantity of organic compounds in the final product. The study also indicates that reducing the initial C/N of rice straw to 34 by combining it with poultry droppings and inoculating with *Aspergillus* strains produced good quality mature compost as compared to the treatment done without using any inoculants. It can, therefore, be concluded that rice straw can be efficiently degraded by reducing its initial C/N ratio to 34 by amending it with poultry droppings and using a consortium of *Aspergillus fumigatus, Aspergillus terreus and Aspergillus flavus* as an inoculant. The compost thus formed can be deemed suitable for agricultural applications.

Thus we need to resort to composting rather than burning rice straw. Composting of crop residues is a promising avenue to create employment for people and it will also help to deal with the problem of increasing air pollution caused due to stubble burning.

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