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Comparative Analysis of Compost Quality Produced from Fungal Consortia and Rice Straw by Varying C/N Ratio and its Effect on Germination of *Vigna radiata*

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ABSTRACT

Composting is considered to be one of the best methods for handling organic waste. It is a natural process and takes months to give quality mature compost. Characteristics of initial wastes and process conditions are the compost maturity deciding factors. Some biological inoculants, e.g., bacteria and fungi, can reduce the compost time and improve its quality. This research is based on the hypothesis that using fungus consortia on rice straw will boost the activities of microbes and, as a result, the rate of composting. The hypothesis was tested by preparing compost using rice straw residue with and without the applications of fungal consortia. Fungal consortia of *Aspergillus flavus, Aspergillus fumigatus,* and *Aspergillus terreus* cellulose-degrading strains along with Pusa-1121 rice variety were used for the study. Different C/N ratios were achieved by varying rice straw, green leaves, poultry droppings, and fungal inoculant proportions. During various stages of composting, changes in total nitrogen, organic carbon, C/N ratios, and other parameters were calculated. The germination index of Mung beans (*Vigna radiata*) was used to measure the quality of the completed compost extract. Statistical analysis with the help of a two-tailed independent t-test at the confidence level of 95% was applied to determine the statistical difference between the treatments and control. It has been found that the Seed Germination index of treatment C/N 30 was 91.5% and that of C/N 26 was 79.1% which were significantly ($p<0.01$) different from the 54% GI of control.

INTRODUCTION

Rice is an important crop farmed all over the world, second after maize, with an annual yield of roughly 800 million metric tonnes. India ranks second in the production of rice after China (Veena & Pandey 2011). More rice production will also lead to the generation of a large amount of rice straw. Although rice fulfills the world's food requirement, mishandling its straw waste will lead to different environmental problems. In India, Punjab, Himachal Pradesh, and Haryana burn 80 percent of rice straw, while Karnataka burns 50 percent and Uttar Pradesh burns 25 percent (Gupta et al. 2003). Many toxic chemicals are produced when the rice straw is burned, including carbon dioxide, carbon monoxide, methane, and nitrogen oxides (Gupta et al. 2004a, 2004b). According to a study, open-field rice straw burning contributes 0.05, 0.18, and 0.56 percent of Green House Gas emissions in India, Thailand, and the Philippines, respectively (Gadde et al. 2009). Another problem associated with Rice straw burning is the loss of nutrients in the soil. The solution to these issues rests in using rice straw as a source, as crop leftovers are good suppliers of plant nutrients and vital components for the agricultural ecosystem's stability (Ghosh et al. 2004). As a result, the use of organic matter as mineral fertilizers is critical for long-term agricultural growth. Crop leftovers, animal shed wastes, rural and urban wastes, vegetable market wastes, and forest and industrialized wastes, all can be used as organic waste sources. India produces more than 3,000 million tonnes of organic waste annually (Gupta et al. 1998, Sharholy et al. 2008). Soil fertility and productivity get enhanced by recycling organic waste by composting in agriculture and using them as fertilizers (Tandon 1995, Chukwuka & Omotayo 2008, Ansari 2011).

When compared to other cereal straws, rice straw has a distinct chemical composition. On a dry weight basis, rice straw typically comprises lignin (10-15 percent), silica (75 percent), cellulose (40-50 percent), and hemicelluloses (9-12 percent) (Knauf & Moniruzzaman 2004). Consequently, rice straw waste is rich in silica and lignin, with a C/N ratio of roughly 80:1, making it difficult to decompose (Van Soest 2006, Kumar et al. 2008). Microorganisms are the bio-agents that degrade the waste matrix's cellulose and lignin components. These native microflorae are likely to produce higher enzymatic levels, which will speed up the composting process. However, only a few microbes efficiently use cellulose as a substrate (Kumar et al. 2010). A study by Cao et al. (2013) found that fungal consortia of *Aspergillus fumigatus* degraded cellulose before other strains. Management of rice residue by directly incorporating straw into the soil has several drawbacks, including the immobilization of plant nutrients, notably nitrogen, and reducing subsequent crop germination (Nigam & Pandey 2009). As a result, farmers resort to in situ burning of crop wastes that are strewn around the field and impossible to collect (Jacobs et al. 1997, Reinhardt et al. 2001). However, governments are increasingly advising against burning crop leftovers since it could result in a significant economic loss. On the other hand, composting rice straw is emerging as a safe alternative option that results in the reusability of the nutrients contained within the residue (Banger et al. 1989, Gaind et al. 2008, Sarkar & Chourasia 2017, Kumar & Singh 2021). Although a small amount of GHG is released during composting, the substrate with a low C/N ratio produces more NH_3 and CH_4 (Jiang et al. 2011). Carbon and nitrogen are essential for microbial breakdown. Therefore, the C/N ratio must be high at the start of the composting process for the process to get quickly (Ain et al. 2017). If the C/N ratio of paddy straw is too high, an exogenous nitrogen source must be added to lower the C/N ratio. As poultry droppings are high in nitrogen, mixing paddy straw with poultry droppings may be a suitable option for lowering the C/N ratio of paddy straw (Ashraf et al. 2007, Devi et al. 2010).

A study by Voběrková et al. (2017) revealed that the application of white-rot fungi and the fungal consortium had enhanced the degradation of organic waste which was indicated by a change in the C/N ratio, EC, pH, and higher Germination index. In the same manner, Wang & Ai (2016) reported the acceleration in the degradation process and formation of humic-like material, high composting efficiency, and degree of humification with the use of microbial activities on wheat bran. Heidarzadeh et al. (2019) found that the use of the fungal consortium of *Aspergillus* had reduced the C/N ratio and process time.

MATERIALS AND METHODS

Rice straw was collected from the nearby village Madina, part of Rohtak district (Haryana), India. Pusa-1121 variety of rice was used in this study. Rice straw was cut into small pieces and delingnify with 0.1% urea as the process makes it prone to degradation by cellulose enzymes. Rice straw was stacked in three bins for composting to achieve different C/N ratios by varying the ingredients (Rice Straw, Green leaves, Poultry droppings, and Fungal inoculant) proportions (Fig. 1 and Fig. 2). Composting was prepared by degrading rice straw using fungi (*Aspergillus sp.*) as inoculants, as shown in Table 1. Aspergillus species of fungal were provided by the laboratory at Maharshi Dayanand University, Rohtak.

Analysis of Physico-Chemical Parameters of Compost

Compost bins were set up inverted to form heaps and for aeration, they were turned regularly. Compost samples were collected at an interval of 30 days, 60 days, and 90 days (Fig. 3). These samples were analyzed for the physico-chemical parameters through standard prescribed methods mentioned in Table 2.

Seed Germination Index

Compost effectiveness was analyzed by subjecting the compost to the growth of seeds of Mung (*Vigna radiata*). First, seeds of *Vigna radiata* were dipped in 7% alcohol for about 3 min for disinfection purposes and then stirred intermittently for about 2 min in a suspension of 0.001 $HgCl_2$. Next, the toxins of the seeds were removed by washing them properly with distilled water. After that, 10 mL of compost extract was put onto filter paper that had been spread on Petri plates. Following that, ten *Vigna radiata* seeds were sterilized and placed on filter paper. To prevent moisture loss, Petri plates were carefully taped and then incubated at room temperature for 72 h.

Control C/N 26 C/N 30

Fig. 1: Compost bins set up with different C/N ratios.

The percentage of seed germination, root elongation, and percentage of germination index (GI) was calculated as follows.

Calculation:

Seed germination (%) =

$$\frac{\text{No. of seeds germinated in compost extract}}{\text{No. of seed germinated in control}} \times 100$$

Fig. 2: Compost Piles of different C/N ratios.

Table 1: Treatments prepared for the compost:

Treatment	Initial C/N ratio	Rice Straw [g]	Green leaves [g]	Poultry dropping [g]	Fungal inoculant included
Treatment 1	26/1	500	1000	500	yes
Treatment 2	30/1	500	1000	500	yes
Treatment 3	Control	500	1000	500	No

30 Days Compost Sample 60 Days Compost Sample 90 Days Compost Sample

Fig. 3: Compost samples during processing.

Root elongation (%) =

$$\frac{\text{Mean root length in compost extract}}{\text{Mean root length in control}} \times 100$$

Germination Index =

$$\frac{\text{Seed germination}(\%) \times \text{Root elongation}(\%)}{100}$$

RESULTS AND DISCUSSION

Rice straw was subjected to different physico-chemical parameters before processing for compost, as shown in Table 3. As a result, the moisture content of the straw was 11.7% and the C/N ratio calculated was 75.73:1. As the C/N ratio was high, poultry dropping was added to rice straw to decrease the ratio.

Changes of Different Physico-Chemical Parameters of Compost from Control to Treatments

Compost samples were obtained at 30, 60, and 90 days, and physico-chemical analysis was performed. Fig. 4 depicts the changes in the parameters.

Due to the creation of ammonia, pH values typically range from weakly acidic to neutral or weakly alkaline, i.e., within the range 4.5-8.1. In general, the values are directly related to the activity of microorganisms involved in the composting process (Neklyudov et al. 2006). In all the sets, there was a trend of increasing pH with due course of composting. The ammonification process and biodegradation of short-chain fatty acids might be the reason for the rise in pH. Researchers recorded a similar increase in pH throughout the degradation period during composting of rice straw (Kaur & Katyal 2021). A significant ($p<0.05$) change was observed in pH value from control to treatments, as shown in Table 4. Moisture content showed a decreasing trend with composting time. Electrical conductivity increased in all the sets, as reported by other researchers (Barapatre et al. 2020). There was a significant difference ($p<0.05$) between EC of treatments C/N30 (2.55±0.06) and C/N26 (2.02±0.09) w.r.t control (1.84±0.04). Electrical conductivity measures soluble salts in compost (Kumari et al. 2020) and regulates microbial activities (Shrivastava & Kumar 2015). The increase in EC could be due to an increase in phosphorous, calcium, sodium, potassium, and other ions concentration during composting (Bernal et al. 2009). The total organic carbon showed a trend of decreasing throughout the composting process. This might be due to the loss of carbon in the form of CO_2. The result complied with (Getahun et al. 2012). Total organic carbon of 90 days of compost showed a significant difference between control (19.19%) to first treatment (18.01%) and second treatment (18.02%). E4/E6 is the ratio of absorbance at 465 nm and 665 nm using a spectrophotometer. E4/E6 ratio is used for the humification index. With time, the ratio of E4/E6 has increased. It shows more production of humic and fulvic acid due to the degradation of organic material. Humic substances, the major component of soil organic matter in the compost, could increase shoot biomass via hormonal effects on root elongation and plant development (López-Bucio et al. 2003). Nitrogen, magnesium, Phosphorous, Calcium, Sodium, and potassium showed an increasing trend with the composting process. Treatments of all of these parameters were significantly ($P<0.05$) different from their control after 90 days of compost.

The C/N ratio is an essential metric for determining compost maturity and stability (Sharma & Garg 2018,

Table 2: Analytical techniques used for the evaluation of physico-chemical parameters of compost.

Parameters	Instrumental methods
pH	pH meter
Moisture content [%]	Gravimetric method
Electrical conductivity [$mS.cm^{-1}$]	Conductivity meter
Total organic carbon [%]	Walkley and Black method (Walkley & Black 1934)
Total potassium [%]	Flame photometer
Total phosphorus [%]	Olsen method (Olsen et al.1954)
Total nitrogen [%]	Kjeldahl method (Katyal et al.1987, Bremner 1996)
E4/E6	Spectrophotometer
Total sodium [%]	Flame photometer
Total calcium [%]	EDTA method (Tucker & Kurtz-et 1961)
Total magnesium [%]	EDTA method (Tucker & Kurtz-et 1961)

Table 3: Physico-chemical Analysis of rice straw.

Parameters	Result
Electrical Conductivity [$mS.cm^{-1}$]	5.37
Moisture content [%]	11.7
Total organic carbon [%]	46.2
Total Phosphorus [%]	0.25
Total Potassium [%]	1.26
Total Nitrogen [%]	0.61
C:N ratio	75.73:1

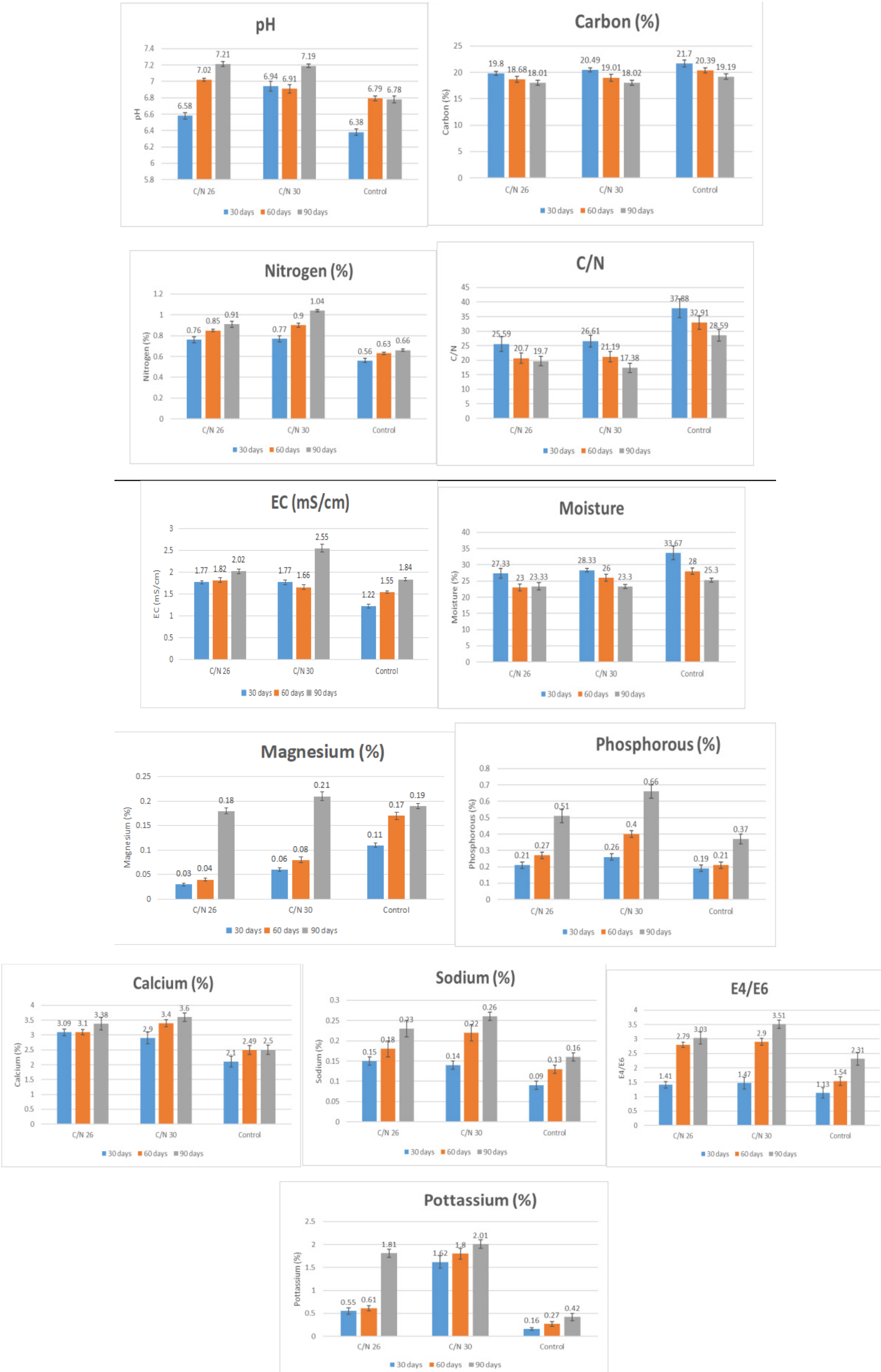
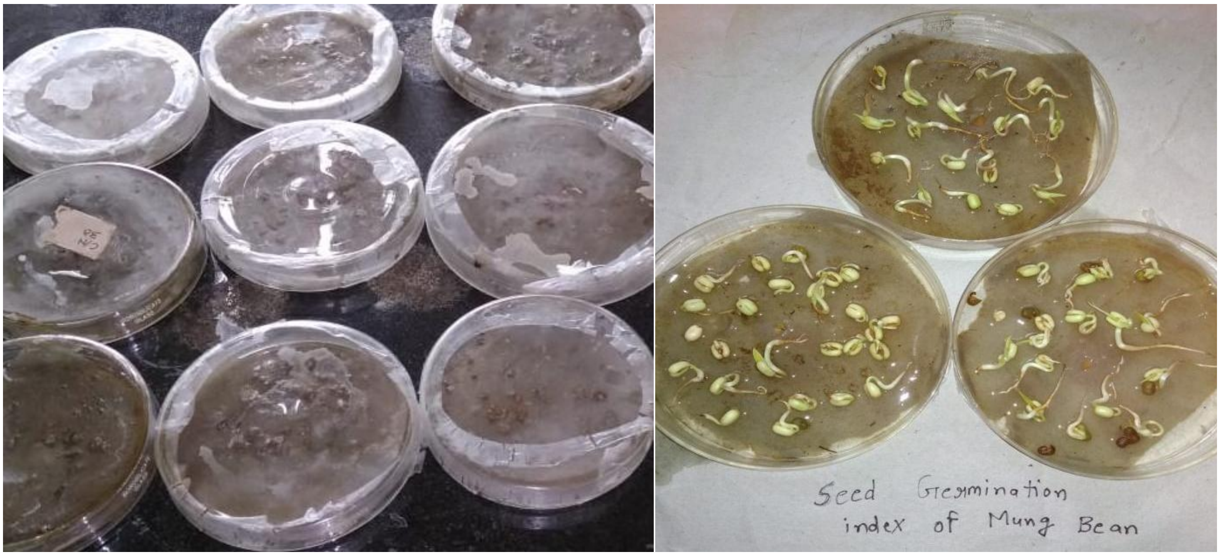


Fig. 4: Changes in the physico-chemical parameters during composting.



(A) Seed before incubation (B) Seed after 72 hours of incubation

Fig. 5: (A-B).Seed germination of Mung (*Vigna radiata*).

Nozhevnikova et al. 2019). As carbon decreases and nitrogen increases, a decreasing trend was noted in all the treatments during composting, as also reported by (Chukwujindu & Dolin 2006). Initial ratio of C:N in C/N26 treatment was 25.59, which dropped to 19.07:1. C/N30 treatment had an initial C:N ratio of 26.61:1, which dropped to 17.38:1, while in the case of control, it dropped from 37.88:1 to 28.59:1. These results are in agreement with the finding of previous researchers (Barapatre et al. 2020). There was a significant ($p<0.05$) difference in the C:N ratio between treatments and control of 90 days of compost.

Seed Germination Index of Mung Bean

Treatment two with an initial C/N ratio of 30 achieved a minimum C:N ratio of 17.38. The result showed that compost from treatment two had achieved maximum maturity and was suitable for applying in the agricultural field (Owis et al. 2016). A high C/N value in control, on the other hand, indicates that a significant amount of carbon has been left unused in compost (Dobermann & Fairhurst 2002). This is backed up by the fact that the Germination index (91.5%) of treatment two was the highest, followed by treatment one

Table 4: Changes in Physico-chemical parameters of Treatments from control.

Group	pH	Phosphorus [%]	Magnesium [%]	EC [mS.cm^{-1}]	Carbon [%]	C/N	Calcium [%]	E 4 / E6	Potassium [%]	Moisture (%)	Nitrogen (%)
Control	6.78	0.37	0.12	1.04	19.19	28.59	2.50	2.31	0.42	25.33	0.66
C/N 26	7.21*	0.51*	0.18*	2.02*	18.01*	19.7*	3.38*	3.03*	1.81*	23.33*	0.91*
C/N 30	7.19*	0.66*	0.21*	2.55*	18.02*	17.38*	3.60*	3.51*	2.01*	23.33*	1.04*

* Significant at $P<0.05$, Two-tailed independent t-test

Table 5: Seed germination index of Mung bean (*Vigna radiata*).

Treatments	Seed Germination [%]	Root Elongation [%]	Seed Germination Index [%]
Control	82.76	65.3	54
C/N 26	95.72**	82.72**	79.1**
C/N 30	98.96**	92.5**	91.5**

* Significant at $P<0.01$, two-tailed independent t-test

(79.1%) and control (54%) on the growth of Mung seeds. The same outcomes were reported by (Abdel-Hamid et al. 2004, Azim et al. 2014). Furthermore, the seed germination (%), root germination (%) and the seed germination index (%) of control were found statistically different (P<0.05) from treatment one and treatment two, as indicated in Fig. 5 and Table 5.

CONCLUSION

Agricultural waste management is great concern throughout the world. The conversion of agricultural waste into compost will reduce pollution and when this compost is applied in the field will result in a high yield of crops and vegetables. Then the second aspect is reducing the time of composting without compromising the quality of compost. This study focused on both points. In this study, *Aspergillus Fumigatus, Aspergillus terreus*, and *Aspergillus flavus* consortia efficiently decomposed the rice straw, thus lowering decomposition time. This study concluded that compost matured in 90 days and contained a good level of nutrients. According to the study, supplementing rice straw with poultry droppings improves the organic matter content of the final compost. However, in all of the treatments, the final C/N ratio was below the permissible limit of compost maturity. Out of all the treatments, C/N 30 achieved the lowest C/N ratio, followed by C/N 26. Thus using an initial C/N ratio of 30 is recommended by this study. The Germination index of Mung bean verified the maturity of the compost prepared from the initial C/N ratio of 30 as this treatment showed the highest Germination index, which was significantly different from the Germination index of control.

ABBREVIATIONS

EC: Electrical Conductivity
C/N: Carbon/Nitrogen
GHG: Green House Gas
GI: Germination Index

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