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Bio degradation of organic wastes by *Pleurotus eous* mushroom

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Abstract
Composting is an important strategy for the management of organic wastes to get quality end product with better microbial community. Various fungi and bacteria including mushrooms were selected for degradation of agro wastes. The studies were conducted on the following aspects *viz.,* collection and utilization of locally available agricultural residues as substrates, addition of inorganic and organic additives to substrates along with *Pleurotus eous* (APK1) mushroom for decomposition. The substrates like sugarcane trash, sugarcane bagasse, groundnut shell, lawn grass and coir waste and additives such as urea, gypsum, calcium carbonate, calcium hydroxide, groundnut oil cake, gingelly oil cake and neem oil cake were used in this study. The components *viz*., carbon, nitrogen and crude fibre with each additive were analysed after incubation period. Sugarcane trash with urea decomposed highly with minimum C: N ratio. Crude fibre content was less in groundnut shell with urea.

Keywords: *Pleurotus eous*, agricultural residues (substrates), organic additives, inorganic additives, carbon, nitrogen and crude fibre

Introduction
Crop residues are the noneconomic plant parts that are left in the field after harvest. The harvested refuses include straws, stubble, stover and hulms of different crops. Crop remains are also from thrashing sheds or that are discarded during crop processing. This includes wastes like groundnut shell, oil cakes, rice husks and cobs of maize, sorghum and cumbu. India's total amount of agro-industrial residues reaches 600 million tonnes, they were crop based residues (generated in field) and processing based residues (generated during wood and industrial processing) (Mande, 2005) [4]. The greatest potential as a biomass resource appears to be from the field residues of sorghum, maize, soyabean, cotton, sugarcane etc. these residues need composting before being used as manure. Compost provides a stable organic matter that enhance the soil quality and crop production. Compost application improved the soil properties by creating suitable conditions for root development and consequently promoting higher yield and higher quality of crops. Composting is brought about by a variety of organisms like bacteria, fungi and actinomycetes. Fungi grow in nature on all sorts of vegetable wastes and they play an important part in maintaining the natural cycle. Among the fungi mushrooms are capable of breaking down organic material than other microorganisms. Composting provides a medium favourable for the mushroom spawn to predominate. With this idea the research work was carried out by using *Pleurotus eous* (APK 1) as biodegrading agent for decomposition of agricultural wastes (substrates) along with organic and inorganic additives for the estimation of carbon, nitrogen and crude fibre.

Materials and Methods
Preparation of mother spawn and bed spawn: spores of *Pleurotus eous* (APK 1) were collected directly from the fruiting bodies and inoculated on Potato Dextrose Agar (PDA) medium. The inoculated Petri plates were incubated at 15 ºC for three days. The mycelium of *P. eous* appeared on the Petri plates were used as inoculum. Half cooked sorghum grains were mixed with the calcium carbonate ($CaCO_3$) @ 20g/ kg. Calcium carbonate was added to absorbs excess moisture and neutralize the pH. The prepared grains were filled in to polypropylene bags and sterilized at 15 lbs pressure for one hour. The mycelium of *P. eous* appeared in the Petri plate was inoculated into sterilized cooled spawn bags. Mouth of the bags were close with non-absorbent cotton plug and then incubated at 22 – 24 ºC in a dark place. The mycelium completely spread through the grains in about two weeks from this fully grown mother spawn bags, bed spawns were prepared by inoculating few grains with mycelium of *P. eous* to sterilized cooled sorghum grains in polypropylene bags and incubated as described earlier. Bed spawn bags were used for decomposition.

Substrates used for decomposition

The following substrates were collected from Adhiparasakthi Agricultural College campus and in and around G.B. Nagar, Kalavai.

1. Sugarcane trash
2. Sugarcane bagasse
3. Groundnut shell
4. Coir waste and
5. Lawn grass (*Zoisia tenuifolia*)

Additives used for decomposition

1. Urea
2. Gypsum
3. Calcium carbonate
4. Calcium hydroxide
5. Gingelly oilcake
6. Groundnut oilcake
7. Neem oilcake

Urea and gypsum purchased from fertilizer shop, calcium carbonate and calcium hydroxide from scientific companies and oil cakes were also obtained from local market of Kalavai.

Preparation of beds for decomposition

The substrates and additives were added layer by layer in polythene bags (60 x30 cm) with 40 per cent moisture content.

In each bag one kg of substrates was added in five layers along with 2g of additive per layer. *Pleurotus eous* added in each layer at the rate of 10g and the bags were tied and incubated at room temperature. Few holes were made in bags to provide aeration. Three replications were maintained for each substrate with each additive. Samples are taken from each bag on 75 days after incubation. Dried the substrates and powdered and then used for estimation of C: N ratio and crude fibre.

Treatment details for each bag

1. *Sugarcane trash*

Sugarcane trash + *Pleurotus eous* spawn + urea
Sugarcane trash + *Pleurotus eous* spawn + gypsum
Sugarcane trash + *Pleurotus eous* spawn + calcium carbonate
Sugarcane trash + *Pleurotus eous* spawn + calcium hydroxide
Sugarcane trash + *Pleurotus eous* spawn + ground nut oil cake
Sugarcane trash + *Pleurotus eous* spawn + gingelly oil cake
Sugarcane trash + *Pleurotus eous* spawn + neem oil cake

2. *Sugarcane bagasse*

Sugarcane bagasse + *Pleurotus eous* spawn + urea
Sugarcane bagasse + *Pleurotus eous* spawn + gypsum
Sugarcane bagasse + *Pleurotus eous* spawn + calcium carbonate
Sugarcane bagasse + *Pleurotus eous* spawn + calcium hydroxide
Sugarcane bagasse + *Pleurotus eous* spawn + ground nut oil cake
Sugarcane bagasse + *Pleurotus eous* spawn + gingelly oil cake
Sugarcane bagas se+ *Pleurotus eous* spawn + neem oil cake

3. *Groundnut shell*

Groundnut shell+ *Pleurotus eous spawn* + urea
Groundnut shell + *Pleurotus eous spawn* + gypsum
Groundnut shell + *Pleurotus eous spawn* + calcium carbonate
Groundnut shell + *Pleurotus eous spawn* + calcium hydroxide
Groundnut shell + *Pleurotus eous spawn* + ground nut oil cake
Groundnut shell + *Pleurotus eous spawn* + gingelly oil cake
Groundnut shell + *Pleurotus eous spawn* + neem oil cake

4. *Coir waste*

Coir waste + *Pleurotus eous* spawn + urea
Coir waste + *Pleurotus eous* spawn + gypsum
Coir waste + *Pleurotus eous* spawn + calcium carbonate
Coir waste + *Pleurotus eous* spawn + calcium hydroxide
Coir waste + *Pleurotus eous* spawn + ground nut oil cake
Coir waste + *Pleurotus eous* spawn + gingelly oil cake
Coir waste + *Pleurotus eous* spawn + neem oil cake

5. *Lawn grass*

Lawn grass + *Pleurotus eous* spawn + urea
Lawn grass + *Pleurotus eous* spawn + gypsum
Lawn grass + *Pleurotus eous* spawn + calcium carbonate
Lawn grass + *Pleurotus eous* spawn + calcium hydroxide
Lawn grass + *Pleurotus eous* spawn + ground nut oil cake
Lawn grass + *Pleurotus eous* spawn + gingelly oil cake
Lawn grass + *Pleurotus eous* spawn + neem oil cake

Carbon estimation (Walkley and Black, 1934) [7].

The following reagents *viz*., 1 N potassium dichromate, concentrated sulphuric acid, 85 per cent orthophosphoric acid, phenylamine indicator and 0.5 N ferrous ammonium sulphate were used for carbon estimation.

Organic carbon content of the sample was estimated as per the method described by Walkley and Black (1934) [7]. Hundred mg of the sample was added with 20 ml of 1 N potassium dichromate and 20 ml of concentrated sulphuric acid. The content was digested for 30 min. a blank was also run simultaneously. Then 250 ml of distilled water followed by 1 ml of 85 per cent ortho phosphoric acid and 0.5 ml of diphenylamine indicator were added. The content of the flask was back titrated with 0.5 N ferrous ammonium sulphate solution. The titration was continued until the appearance of a brilliant green colour, indicating the end point. The organic content was expressed in percentage using the formula.

Calculation

$$\text{Organic carbon (\%)} = \frac{\text{(BV-SV) x 10 x 0.003 x 100}}{\text{(BV x W)}}$$

Where

BV, is blank value
SV, sample value and
W, weight of the sample

Nitrogen estimation (Amma,1989)

The following reagents *viz*., concentrated sulphuric acid, Salt mixture of potassium sulphate (25g), copper sulphate (5g) and metallic selenium (0.5g), boric acid 2 per cent containing bromocresol green methyl red indicator, sodium hydroxide 40 per cent solution, 0.1 N sodium carbonate and 0.002 N hydrochloric acid.

Digestion

Transferred 250 mg of dried plant sample in to 100 ml Kjeldhal flask and added with 200 mg of salt mixture

(digestion mixture), 10 g of potassium sulphate + 1 g copper sulphate (10:1). Then added 3 ml of concentrated sulphuric acid (let the content react for I hour). Transferred the Kjeldhal flask to digestion unit. Transferred the heat briskly for subsequent digestion and continued the heating until the digest become clear (colourless digestion mixture will be obtained). Cooled the Kjeldhal flask.

Distillation

Transferred the whole plant digest material to distillation assembly. Rinsed the Kjeldhal flask thrice with 5 ml of distilled water. Added 10 ml of 40 per cent sodium hydroxide. Heated the flask filled with redistilled water to boil and the boiling steam was passed in to distillation flask. Kept a 100 ml beaker containing 20 ml of 2 per cent boric acid with double indicator below the delivery end of the condenser in the distillation set. Distilled the content and collected the liberated ammonia in boric acid. Continued the distillation until release free of ammonia about 30 ml distillate was collected (tested by litmus paper near the out let of the condenser which was turn blue as ammonia was present). Titrated the ammonia collected boric acid with N/10 sulphuric acid. End point was appearance of wine red colour.

Calculation

$$\text{Percentage of N in plant sample} = \frac{(S - B) \times N \times ME \times 100}{W}$$

Where

S, is ml of standard acid used in the titration of the sample distillate

B, is the ml of standard acid used in the titration of the blank distillate

N, is the normality of the acid used in the titration of the distillate

ME, is the milligram equivalent weight of the nitrogen *i.e.* 0.014

W, is the mass of plant sample taken in gram for the preparation of the sample digest.

Estimation of crude fibre

Procedure

Extracted 2g of ground material with ether or petroleum ether to remove fat (initial boiling temperature 35 – 38 ºC and final temperature 52 ºC) if fat content is below 1 per cent extraction may be omitted. After extraction with ether boiled 2g of dried material with 200ml of sulphuric for 30min. with bumping chips. Filtered through muslin cloth and washed with boiling water until washing were no longer acidic. Boiled with 200ml of sodium hydroxide solution for 30min. Filtered through muslin cloth again and washed with 25ml of boiling 1.25 per cent sulphuric acid, three portions 50 ml of water and 25 ml of alcohol. Removed the residue and transferred to ashing dish (pre weighed dish w_1). Dried the residue for 2 hours at 130 ± 2 ºC. Cooled the dish in a desicator and weighed (w_2). Ignited for 30 min. at 600 ± 15 ºC. Cooled in a desiccator and reweighed (w_3).

Calculation

Percentage of crude fibre in ground sample

$$\text{Percentage of crude fibre in ground sample} = \frac{\text{Loss in weight on ignition } (w2 - w1) - (w3 - w1)}{\text{Weight of the sample}} \times 100$$

Statistical analysis

Data of the experiments were analysed by Factorial Completely Randamized Block Design (CRD) using data entry module for Ag Res Statistical Software© 1994 Pascal International Software Solutions, version 3.01 for data entry and version 7.01 for analysis.

Results and Discussion

Organic manures are highly regarded as good source of material to maintain soil health and increasing soil organic carbon content. Various agro-wastes are converted in to organic manure by composting in which biodegradation with microorganisms especially mushrooms are highly used. Species of oyster mushrooms are able to degrade cellulose, lignin and hemicellulose through their enzyme production. In this study, the oyster mushroom, *Pleurotus eous* (APK 1) was used to degrade various agro-wastes (substrates). Dried and powdered samples of substrates were used from each bag for the estimation of C: N ratio and crude fibre.

Estimation of C: N ratio on biodegradation of various agro-wastes by *Pleurotus eous* mushroom

Experiment was conducted to study C: N ratio of agro- wastes with additives on biodegradation by *P. eous* and results are summarized in the Table 1.

Carbon: Nitrogen ratio was estimated in all substrates with each additives on 75 days after incubation. Among the substrates sugarcane trash with urea additive was decomposed much with C: N ratio of 23. 08: 1 followed by lawn grass 25.10: 1 and sugar cane bagasse 25.90: 1.

Table 1: Estimation of C:N ratio on biodegradation of various agro-wastes by *Pleurotus eous* mushroom

S. No.	Additives	Carbon: Nitrogen ratio (%)				
		Substrates				
		Sugarcane trash	Sugarcane bagasse	Groundnut shell	Lawn grass	Coir waste
1.	Urea	23.80: 1a	25.90: 1 c	29.20: 1 f	25.10: 1 b	32.30: 1 h
2.	Gypsum	27.40: 1d	28.50: 1 e	32.20: 1 h	29.20: 1 f	36.40: 1 k
3.	Calcium carbonate	30.30: 1 g	32.32: 1 h	36.13: 1 j	33.20: 1 i	40.20: 1 n
4.	Calcium hydroxide	33.26: 1 i	36. 47: 1 k	39.30: 1 m	38.20: 1 l	44.31: 1 p
5.	Groundnut oilcake	39.40: 1 m	40.12: 1 n	44.20: 1 p	44.35: 1 p	49.26: 1 t
6.	Gingelly oil cake	42.67: 1 o	46.70: 1 r	48.12: 1 s	50.41: 1 v	55.53: 1 w
7.	Neem oil cake	46.32: 1 q	51.20: 1 v	56.45: 1 x	57.21: 1 y	61.23: 1 z
8.	Control	83.30: 1 €	68.90: 1 {	75.28: 1 |	78.10: 1 }	81.08: 1~
		S	T	ST	**Note:** { > | >} > ~ > €	
SED		0.02962	0.03746	0.08377		
CD (0.05)		0.05894	0.07456	0.16672		

On biodegradation, coir waste showed minimum ratio of C: N that indicates the reduced bio-efficiency. Within the additives urea was effective in degradation of all substrates followed by gypsum. All other chemical and natural additives showed less degradation.

This result was coincided with the report of Pandey *et al*. (2012) [5]. They reported that sugar bagasse treated with urea and hot water along with *Pleurotus citrinopileatus* decreased the carbon content and increased the nitrogen content at different stages of growth. It showed the activities of cellulolytic, hemi cellulolytic and lignolytic enzymes produced by *Pleurotus citrinopileatus.* Theradimani *et al*. (2018) [6] studied that the inoculation of coir waste with six *Pleurotus* spp., antagonists and biofertilizers reduced the organic carbon content. Maximum reduction in organic carbon content of coconut coir pith (37.53 per cent) and maximum increase in total nitrogen (318.5 per cent) was brought about by the inoculation of *Pleurotus djamor*. In this experiment, the C:N ratio also reduced in coconut coir pith as 32.30:1 by *Pleurotus eous* after 75 days of incubation as compared to 81.08: 1 in control. Comparison of all substrates, sugarcane trash with urea additive was decomposed much by *P. eous.*

Estimation of crude fibre on biodegradation of various agro-wastes by *Pleurotus eous* mushroom

Experiment was conducted to study crude fibre of agro-wastes with additives on biodegradation by *P. eous* and results are summarized in the Table 2.

Crude fibre was an insoluble residue that remains after decomposition of plant materials. In this experiment biodegradation with *P.eous* revealed the content of crude fibre after 75 days of incubation (decomposition) in various substrates with additives. It indirectly showed the decomposition percentage of substrates with the influence of both inorganic and organic additives. Among the substrates used, groundnut shell with urea showed the minimum crude fibre content of 1.60 per cent followed by groundnut shell with gypsum as 1.80 per cent. Within the substrates groundnut shell with all additives showed less crude fibre followed by sugarcane trash and lawn grass. Maximum crude fibre was obtained from coir waste and sugarcane bagasse. Oil cakes of groundnut, gingelly and neem showed more fibre in all substrates due to less decomposition.

The result was corroborated with the report of El-Mandany (1997) [3] as crude fibre content decreased from 33.64 and 32.29 per cent for urea treated rice straw. Adenipekun and Dada (2013) [`1] also reported that crude fibre decreased significantly in cotton waste and cocoa husk from 5.88 to 5.31 per cent and from 39.88 to 34.95 per cent respectively whereas it was increased in rice straw from 18.42 to 28.08 per cent after 60 days of incubation with *Pleurotus pulmonarius*. In this experiment also groundnut shell with urea followed by groundnut shell with gypsum showed the minimum crude fibre content. Higher content of crude fibre was obtained from coir waste and sugarcane bagasse. Oil cakes of groundnut, gingelly and neem showed more fibre in all substrates due to less decomposition.

Table 2: Estimation of crude fibre on biodegradation of various agro-wastes by *Pleurotus eous* mushroom

S. No.	Additives	Crude fibre (%)				
		Substrates				
		Sugarcane trash	Sugarcane bagasse	Groundnut shell	Lawn grass	Coir waste
1.	Urea	20.12 i	23.20 l	1.60 a	21.32 j	22.51 k
2.	Gypsum	22.35 k	25.60 p	1.80 b	23.50 m	24.53 o
3.	Calcium carbonate	24.33 n	27.25 r	2.10 c	26.20 q	27.21 r
4.	Calcium hydroxide	25.43 p	29.45 v	2.50 d	28.80 t	29.40 u
5.	Groundnut oilcake	28.45 s	32.61 {	3.21 e	30.33 v	32.63 z
6.	Gingelly oil cake	30.37 v	34.50 ~	4.16 f	31.60 x	34.05}
7.	Neem oil cake	31.23 w	36.28 □	4.79 g	32.65 y	35.30 □
8.	Control	33.50 |	38.17 □	7.50 h	34.10 }	36.24 €
	S	T	ST	Note: { >l >} > ~ > □ > €		
SED	0.02877	0.03639	0.08136			
CD (0.05)	0.05725	0.07241	0.16192			

Conclusion

Pleurotus eous decomposed all the substrates used in the study in which sugarcane trash with urea was highly decomposed with minimum C: N ratio. Crude fibre content was less in groundnut shell with urea.

Reference

1. Adenipekun CO, Dada OJ. Biodegradation of three Agricultural Wastes by a white rot fungus *Pleurotus pulmonarius* (Fries) Quetlet. Nature and Science. 2013; 11(2):19-25.
2. Amma T. Nitrogen estimation in plant sample. Nitrogen determination by micro-kjeldhal digestion and distillation method, 1989, 261-264.
3. El-Mandany HA. Nutritional using of treated rice straw in goat rations. M.Sc. Thesis. Fac. Of Agric., Zagazig University, 1997.
4. Mande S. Biomass gasifier-based power plants: potential, problems and research needs for decentralized rural electrification. In: Lal B, Reddy MRVP (eds) Wealth from Waste: Trends and Technologies, New Delhi, 2005.
5. Pandey A, Soccol CR, Mitchell D. New developments in solid state fermentation: I- Bioprocesses and products. Process. Biochem. 2012; 35:1153-1169.
6. Theradimani M, Thangeshwari S, Parthasarathy S. Biological decomposition of coconut coir pith waste. Pl.Dis. Res. 2018; 33(2):142-147.
7. Walkley A, Black CA. An examination of Degtjareff method by determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science. 1934; 37:29-38.