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Changes of Cellular Content in Agro Wastes by *Pleurotus eous*

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Abstract

Bio-compost is the viable process that converting the organic substance to beneficial product such as bio fertilizers and other soil conditioner. Composting become an increasingly important strategy for the treatment of organic wastes to get quality end product with microbial community. Fungi and bacteria degraded the agro-wastes/ by-products in which mushroom fungus *Pleurotus* is successfully utilized for biodegradation of ligno-cellulosic residues with high efficiency. In this experiment locally available agricultural residues as substrates viz., sugar cane trash, sugar cane bagasse, ground nut 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 along with *Pleurotus eous* (APK 1) mushroom for decomposition. The components viz., cellulose, crude protein and crude fibre content from each substrate with various additives were analyzed after incubation period. Groundnut shell showed high protein content with less crude fibre.

***Key words*:** Cellulose, Crude protein, Crude fibre: Inorganic and organic additives, *Pleurotus eous* mushroom, Substrates (agricultural residues)

The global agro industry facing a serious crisis due to the steady build up of biological resistance to chemical pesticides and chemical fertilizers. Improper use of chemical fertilizers in soil leads to altering the physical and chemical properties of soil and pollutes the biosphere. Composting was the most suitable technique for transforming organic wastes into usable agricultural amendments.

Composting is a method of degrading solid waste by different means. Among them, the organic component of the solid waste is biologically degraded under controlled condition with microorganisms to supply plant nutrients without any detrimental effects on the environment and to crop to which applied. Compost is a rich source of organic matter and as a valuable soil amendment for centuries. Use of compost is an effective way to increase healthy plant production helps to save money, reduce the use of chemical fertilizers and conserve natural resources. Improved the aeration and encouraged the growth of beneficial microorganisms and earthworms. Compost application supresses certain soil borne diseases, parasitic nematodes and reduce crop losses. Variety of agricultural crops and their residues in our country form the potential renewable resources. Several methods have been adopted for biodegradation of agrowastes, in which solid state fermentation of straw through mushroom cultivation is prominent one and also act as an eco-friendly method of solid waste management. Agricultural wastes are rich in lignin cellulosic components which are difficult to breakdown, but can effectively be done mushroom cultivation. They are very nutritious products that can be generated from lingo cellulosic waste materials. The bioconversion of agricultural wastes into a value-added product is a good mean of their use. The property of edible mushroom fungi to convert complex organic compounds into simpler one's is used to transform the useless agricultural wastes into valuable products. (Suganthi and Krishnakumari, 2018).

Bio compost is the viable process that means converting this organic substance to beneficial product such as biofertilizers and other soil conditioner. Composting became an increasingly important strategy for the treatment of organic wastes to get quality end product, with better microbial community. Based on this the research work was carried out at Adhiparasakthi Agricultural College, G.B. Nagar, Kalavai, Tamil Nadu, India by using oyster mushroom *Pleurotus eous* (APK 1) as biodegrading agent for decomposition of agricultural wastes (substrates) with organic and inorganic additives and estimated the cellulose and crude protein content during decomposition.

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 ° C 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° C 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 oil cake
6. Ground nut oil cake
7. Neem oil cake

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 cellulose and crude protein.

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 bagasse + *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

Cellulose estimation (Updegroff, 1969)

Cellulose content was estimated with the following reagents and procedure.

Acetic acid- Nitric acid reagent: Mixed 150 ml of 80 per cent acetic acid and 15 ml of concentrated nitric acid.

Anthrone reagent: Dissolved 200 mg anthrone in 100 ml of ice- cold 95 per cent sulphuric acid. Prepared fresh and chilled for 2 hours before use.

Sulphuric acid (67 per cent): 67 ml of sulphuric acid dissolved in 100 ml of water.

Sample of substrate (1g) was added with acetic/ nitric acid reagent in a test tube and mixed in a vortex mixture. Placed the tube in a water bath at 100° C for 30 min. Cooled and then centrifuged the contents for 15-20 min. Discarded the supernatant and washed the residue with distilled water. Sulphuric acid 67 per cent (10 ml) was added and allowed for one hour. Diluted 1ml of the above solution to 100 ml. Anthrone reagent 10 ml was added to 1 ml of diluted above solution and mixed well. Heated the tubes in boiling water bath for 10 min. and then cooled. Measured the colour at 630 nm and compared with blank. Drew the standard graph by using series of known quantity of cellulose and calculated the amount of cellulose in the sample.

Protein Estimation (AOAC, 1990)

Protein content was estimated with the following reagents and procedure.

Digestion mixture: Copper sulphate and Potassium sulphate were mixed in the ratio of 1: 9
Boric acid solution: 4.0 per cent
Sodium hydroxide: 40.0 per cent
Standard sulphuric acid: 0.1 per cent

Weighed sample was digested with concentrated sulphuric acid (25 ml) and along with digestion mixture (5g) in Kjeldhal digestion flask. The contents were cooled and transferred to 250 ml volumetric flask. The volume was made up to the mark with distilled water and mixed. Measured aliquot

(5 ml) was taken in a distillation flask followed by 40.0 per cent sodium hydroxide and ammonium borate was collected through a condenser in a flask containing 10 ml of 4.0 per cent boric acid solution. The distillate was titrated with 0.1 N sulphuric acid. A blank sample was also run along with the sample.

$$\text{Nitrogen (\%)} = \frac{\text{Titre value x 0.00014 x volume made}}{\text{Aliquot x Weight of sample}} \times 100$$

Crude protein = Nitrogen % X 5.95

Estimation of crude fibre

Procedure

Two gram of ground material was extracted with ether or petroleum ether to remove fat (initial boiling temperature 35 – 38 ° C and final temperature 52 ° C) 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 ° C. Cooled the dish in a desicator and weighed (w_2). Ignited for 30 min. at 600 ± 15 ° C. Cooled in a desiccator and reweighed (w_3).

Calculation

Percentage of crude fibre in ground sample

$$\text{Nitrogen (\%)} = \frac{\text{Loss in weight on ignition } (w2 - w1) - (w3 - w1)}{\text{Weight of sample}} \times 100$$

Statistical analysis

Data of the experiments were analysed by Factorial Completely Randomized 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

Agricultural wastes are rich in various types of nutrients and their disposal is difficult to manage as excess of nutrients in them can cause leaching is left in the field, as a compost. Mostly they are disposed by means of incineration which causes pollution. Agricultural waste management method is cost effective and contribute less in environment pollution. It is rich in lignin cellulosic components which are difficult to break down, but can effectively done by mushroom. The property of edible mushroom fungi to convert complex organic compounds into simpler one's is used to transform the useless agricultural waste into valuable product.

In this study, *Pleurotus eous* (APK 1) oyster mushroom was used to degrade various agro-wastes (substrates) *viz*., sugarcane trash, sugarcane bagasse, groundnut shell, coir waste and lawn grass (*Zoisia tenuifolia*) with all additives for each substrate such as urea, gypsum, calcium carbonate, calcium hydroxide, gingelly oil cake, ground nut oil cake and neem oil cake. Dried and powdered samples of substrates were used from each bag for the estimation of cellulose and crude protein after 75 days of incubation.

Cellulose content on biodegradation of agro wastes by P. eous

Experiment was conducted to study cellulose content of agro wastes with additives on biodegradation by *P. eous* (Table 1). Cellulose is a major component in many of the farm wastes which is degraded by microbes in soil. In this study the fungus *P. eous* (oyster mushroom) was used for biodegradation of agrowastes with inorganic and organic additives. Coir waste with urea showed minimum cellulose content of 16.30 mg due to the utilization of cellulose by *P. eous* followed by coir waste with gypsum as 18.43 mg. Among the substrates, cellulose in sugar cane trash and sugar cane bagasse was utilized as minimum of 45.30 mg and 42.53 mg respectively in control. Whereas the addition of urea with these substrates broke down the cellulose and made available to *P. eous*.

Decrease in cellulose content of substrates treated with additives and *P. eous* coincidence with the report of Natarajan (1992) and found that with microbial composting, the lignin, cellulose, organic carbon and C: N ratio were reduced in coir waste. Mugilan and Elango (2012) also reported the same as coir waste inoculated with *Pleurotus sajor-caju* reduced lignin and cellulose content gradually up to 60 days of incubation. Study of Theradimani *et al*. (2018) revealed that the maximum reduction of cellulose in composted coir pith (63.13 per cent) was observed after inoculation with *Pleurotus djamor*. Report of Clauz (2004) also revealed the same result of reduction of cellulose and lignin contents in composted coir pith by *P. sajor-caju* enriched with urea. It increased the total nitrogen and other nutrients after a period of 30 days incubation.

Table 1 Estimation of cellulose on biodegradation of agro-wastes by *Pleurotus eous*

S.No.	Additives	Cellulose (mg)				
		Substrates				
		Sugarcane trash	Sugarcane bagasse	Groundnut shell	Lawn grass	Coir waste
1.	Urea	21.50 e	18.78 c	23.40 f	30.10 n	16.30 a
2.	Gypsum	24.70 h	21.53 c	26.31 k	31.30 o	18.43 b
3.	Calcium carbonate	26.10 j	25.10 i	29.20 m	32.30 q	29.15 m
4.	Calcium hydroxide	27.10 l	29.10 m	31.60 p	33.60 s	21.32 d
5.	Groundnut oilcake	31.50 p	34.50 t	35.30 u	36.20 v	23.50 f
6.	Gingelly oil cake	33.20 r	37.30 x	36.80 w	37.65 x	24.43 g
7.	Neem oil cake	35.43 v	39.61 {	37.20 x	38.22 z	26.12 j
8.	Control	45.30 €	42.53 ~	40.30 |	40.70}	27.20 l
		S	T	ST	Note: { >|>} > ~ > €	
		SED	0.02887	0.03652	0.08165	
		CD (0.05)	0.05745	0.07267	0.16250	

Obodai *et al.* (2003) statement also revealed that cellulose rich organic substance has been reported to be good substrate for cultivation of mushrooms. Substrate with high lignin and phenolic content decreased the activity of cellulose,

but less lignin would enhance enzyme activity and thus ensure higher yield of mushroom. The fast degradation of lignin and slow depletion of cellulose and hemicellulose during mycelial growth and slow degradation of lignin and fast depletion of cellulose and hemicellulose during fruit body formation revealed the differential requirement of the fungus *Pleurotus djamor* during different phase of its growth. Same pattern of biodegradation of lignocellulosic wastes by various species of *Pleurotus* have been reported (Singh *et al*., 2002: 2007; 2011). These observations suggested that the cellulose and hemicellulose serve as energy source for the formation of fruit bodies. Poonam and Deepak (2013) found that there is positive correlation of cellulose: lignin with mycelia growth and high yield in *Pleurotus ostreatus*.

Table 2 Estimation of crude protein on biodegradation of agro-wastes by *Pleurotus eous*

S.No.	Additives	Crude protein (%)				
		Substrates				
		Sugarcane trash	Sugarcane bagasse	Groundnut shell	Lawn grass	Coir waste
1.	Urea	4.20 pq	4.11 opq	5.05 t	3.85 klmn	3.63 p
2.	Gypsum	4.18 pq	3.97 no	4.94 t	3.61 hi	3.62 efg
3.	Calcium carbonate	3.97 no	3.89 lmn	4.86 t	3.42 gh	3.26 efg
4.	Calcium hydroxide	3.82 jklmn	3.73 Ikm	4.62 s	3.36 fg	3.15 de
5.	Groundnut oilcake	3.70 Ikl	3.67 Ik	4.53 rs	3.25 efg	2.97 cd
6.	Gingelly oil cake	3.42 gh	3.42 efg	4.34 dqr	3.13 e	2.83 d
7.	Neem oil cake	3.35 efg	3.24 efg	4.10 dop	3.00 c	2.70 b
8.	Control	3.20 ef	3.15 de	3.90 mn	2.90 c	2.32 a
		S	T	ST		
		SED	0.03638	0.04602	0.10291	
		CD (0.05)	0.07241	0.09159	0.20480	

Crude protein content on biodegradation of agro wastes by Pleurotus eous

Experiment was conducted to study crude protein of agro wastes with additives on biodegradation by *P. eous* (Table 2). Crude protein in various substrates was assessed by the estimation of nitrogen and multiplied with factor value. At the time of growth of mycelium, *Pleurotus* utilizes the cellulose and hemicellulose as carbon source available in plant wastes and increased the nitrogen content. In this study also *P. eous* used for decomposition of various substrates with additives increased the crude protein indirectly by increasing content of nitrogen through their enzymatic reaction. Among the substrates groundnut shell with urea showed maximum of 5.05 per cent followed by groundnut shell with gypsum as 4.94 per cent crude protein. Sugarcane trash was next to groundnut shell and least was coir waste.

The results are in accordance with the findings of Zhang *et al*. (1995), under solid-state fermentation, the crude protein contents were increased from 24.10 to 32.30 per cent and from 28.40 to 36.70 per cent for *Pleurotus ostreatus* and *Lentinus edodes* spent compost media, respectively. The crude fibre contents of the composts were significantly decreased, and the *in vitro* digestibility of the crude protein was as high as 70 per cent; the total and essential amino acid contents made up 73.30 and 37.10 per cent of the crude protein, respectively. Therefore, mushroom substrate is a potential source of nitrogen for poultry and animals. According to El-Madany (1997) as rice straw treated with urea increased the crude protein content from 3.42 to 10.47 per cent as against 3.42 – 8.09 per cent in untreated rice straw. Large number of microorganisms such as yeasts, bacteria and fungi were used to improve its performances. Among these, filamentous fungi, especially basidiomycetes, are the preferred choice for enzyme production and protein enrichment and have been widely employed (Pandey *et al*., 2000).

Abdel-Aziz and Ismail (2001) found that the biological treatments of some agricultural by-products became essential to degrade lignocellulosic materials and improved its crude protein content. Some microorganisms, including cellulose enzymes from anaerobic bacteria and white rot fungi (*Pleurotus ostreatus*) degraded lignin in the cell walls. Crude protein content was increased to 80 per cent in treated rice straw and 30 per cent in the treated wheat straw with the oyster mushroom (Kakkar and Dhanda, 1998). Crude protein increased significantly throughout the incubation period (60 days) from 6.65 to 14.82 per cent in rice straw with the use of *P. pulmonarius* (Adenipekun and Dada, 2013). There was a significant difference between the four fibrous materials, processed bagasse, raw bagasse, wheat straw, and barley straw, in the case of the content of crude protein (CP), the amount of CP content of treated sugarcane bagasse was significantly increased (Kermani *et al*., 2019). The *in vitro* organic matter digestibility (IVOMD) was significantly increased by processing with fungi, a large part of the raw fibre, and insoluble compounds were degraded by the fungus enzymatic process and converted into soluble materials. According to result, due to the reduction in the dry weight, neutral detergent fibre (NDF), and acidic detergent fibre (ADF), the degradable and water-soluble portion increased (Kermani *et al*., 2019).

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

Experiment was conducted to assess crude fibre of agro-wastes with additives on biodegradation by *P. eous* and results are summarized in the (Table 3).

Crude fibre was an insoluble residue that remains after decomposition of plant materials. In this experiment *P.eous* mushroom was used for biodegradation. It revealed the content of crude fibre after 75 days of incubation (decomposition) in various substrates added with additives. Decomposition percentage of substrates with the influence of both inorganic and organic additives were observed by estimated the crude fibre content. Among the substrates used, groundnut shell with urea showed the minimum crude fibre content of 1.80 per cent followed by groundnut shell with gypsum as 1.95 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 reports of El-Mandany (1997) and Adenipekun and Dada (2013) as crude fibre content decreased from 33.64 and 32.29 per cent for urea treated rice straw and 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, the minimum crude fibre content was obtained in groundnut shell with urea followed by groundnut shell with gypsum. Coir waste and sugarcane bagasse showed higher content of crude fibre. Oil cakes of groundnut, gingelly and neem showed more fibre in all substrates due to less decomposition.

Table 3 Estimation of crude fibre on biodegradation of agro-wastes by *Pleurotus eous* mushroom

S.No.	Additives	Crude fibre (%) Substrates				
		Sugarcane trash	Sugarcane bagasse	Groundnut shell	Lawn grass	Coir waste
1.	Urea	20.22 i	23.30 l	1.80 a	21.42 j	22.61 k
2.	Gypsum	22.45 k	25.70 p	1.95 b	23.60 m	24.63 o
3.	Calcium carbonate	24.53 n	27.35 r	2.20 c	26.30 q	27.31 r
4.	Calcium hydroxide	25.53 p	29.55 v	2.60 d	28.90 t	29.50 u
5.	Groundnut oilcake	28.55 s	32.71 {	3.31 e	30.43 v	32.73 z
6.	Gingelly oil cake	30.47 v	34.60 ~	4.26 f	31.70 x	34.15 }
7.	Neem oil cake	31.33 w	36.38 □	4.89 g	32.85 y	35.40 □
8.	Control	33.60 |	38.27 □	7.60 h	34.20 }	36.34 €
		S	T	ST	Note: { >l >} > ~ > □ > €	
		SED	0.02877	0.03639	0.08136	
		CD (0.05)	0.05725	0.07241	0.16192	

CONCLUSION

Utilization of cellulose by *Pleurotus eous* was higher in coir waste with urea. Addition of urea with these substances broke down the cellulose and made available to *Pleurotus eous* growth. Maximum cellulose utilization was occurred in coir waste. Crude protein was higher and crude fibre content was in less in groundnut shell with all additives. Higher crude protein content with less crude fibre was due to decomposition of groundnut shell by *Pleurotus* mushroom along with additives.

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