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RECYCLING OF LIGNO-CELLULOSIC WASTE MATERIALS THROUGH OYSTER MUSHROOM CULTIVATION FOR SUSTAINABLE FOOD PRODUCTION

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ABSTRACT

Various lingo-cellulosic waste materials were tested for their suitability in cultivation of oyster mushroom (*Pleurotus florida*) under the agro-ecological condition of lateritic zone of West Bengal. Paddy straw was found to be most appropriate substrate for cultivation of *Pleurotus florida which exhibited* 96 % biological efficiency followed by wheat straw (89.20 %) and maize leaf and stalk (81.5%). Wheat straw has taken minimum time (14 days) for completing the spawn run followed by paper waste (15 days), paddy straw (16 days), banana pseudo-stem and leaf (17 days). Biological efficiency of oyster mushroom was increased further up to 12.6 % by mixing of 5 % rice bran to paddy straw substrate (107.52 %). Five percent maize flour supplementation gave higher average weight of sporophore (5.469 g.) in comparison to other supplements. The present findings deals with the techniques for enhancing the biological efficiency of oyster mushroom which will catch the attention of the poor farmers towards mushroom cultivation and also help them for generating extra income.

KEY WORDS
Pleurotus florida
Biological efficiency
Supplementation
Oyster mushroom cultivation

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INTRODUCTION

Mushrooms have been used by human being since ancient times and they are closely related to the history of mankind.Mushroom growing is a recycling process done indoors, which does not require arable land or fertile soil, and the potent cash crop can be grown by utilizing farm/forest and industrial wastes.Besides being a fast spinning cash crop,it is also an ideal health food capable to fight malnutrition in general and protein-deficiency in particular (Verma, 2014). Oyster mushroom (*Pleurotus* spp.) is an efficient lignin degrading mushroom and can grow well on different types of ligno-cellulosic materials including agricultural and forest waste. Mushroom is the choicest food of nutritionists because of its hypolipidemic, hypocholesterolemic, hypoglycaemic and antitumor properties. Mushroom contains 20-30% protein which is higher than vegetables and fruits and is of high quality.Oyster mushroom modulate the immune system, inhibit tumour growth and inflammation, have hypo-glycaemic and antithrombotic activities, lower blood lipid concentrations, prevent high blood pressure and atherosclerosis and have antimicrobial and other activities (Gunde-Cinerman, 1999). The popularity of oyster mushroom has been increasing due to its ease of cultivation, high yield potential and high nutritional value (Banik and Nandi, 2004 and Gregori *et al.*, 2007). It also helps in environmental protection by removing the toxicity produces by the agro wastes (Fan *et al.*, 2000a; Fan *et al.*, 2000b and Murthy and Manonmani, 2008). Oyster mushroom has the ability to breakdown directly the cellulose and lignin bearing materials without chemical or biological preparation (Chang and Miles, 1991).Therefore, a broad variety of lignocellulosic wastes can be utilized and recycled by solid state fermentation (SSF). Only using 25% of the yearly volume of burned cereal straws the world could harvest of 317 million metric tonnes of fresh mushroom per year (Chang and Miles, 1989). India produces about 600 million tonnes of agricultural waste per annum and a major part of it is left out to decompose naturally or burnt *in situ*. The per capita consumption of mushroom in India is about 90 g, which is very less as compared to 1.49 kg in USA and in China 1.16 kg.FAO recognizes mushroom as a protein-rich food for the poor people in underdeveloped countries.West Bengal is well known rice belt in India, which has tremendous potential and scope for oyster mushroom cultivation due to the easy availability of basic substrate (paddy straw).But, the total production of oyster mushroom and its productivity in West Bengal are still very meager in comparison to other states of the country.A wide range of diverse cellulosic substrates were used for cultivation of *P. sajor-caju* and other *Pleurotus* spp. by different workers (Jandaik and Kapoor, 1974; Pani and Patra,1997 and Mane *et al.*, 2007). These wastes can be used by

simple moistening or untreated, pasteurized, fermented or sterilized for cultivation oyster mushroom (Poppe and Hofte, 1995). Supplements or additives are normally used in the substrate of oyster and other mushrooms to enhance the yield of the mushroom or increase the bio-efficiency (Philippoussis *et al.*, 2001; Sharma *et al.*, 2013 and Neelam *et al.*, 2014). A high concentration of nitrogen encourages mycelial growth and decreases the formation of fruiting bodies in the Basidiomycetes (Chang and Miles, 1989). Considering the tremendous nutritive values of edible mushrooms, their bioconversion ability and also the poor performance of the state in terms of mushroom cultivation an attempt was made to identify a suitable substrate of oyster mushroom fromthe locally available agro-waste materials and also to increase the biological efficiency of oyster mushroom by mixing of various supplements to the best substrate.

MATERIALS AND METHODS

Isolation of pure culture

Pure culture of *Pleurotus florida* was prepared from fresh fruiting body by tissue culture technique (Jonathan *et al.*, 2009). Healthy sporophores were selected and small pieces from the junction of pileus and stipe of sporophore were cut and surface sterilized with 0.1% mercuric chloride solution for 30 seconds and transferred to malt extract agar slants aseptically, which were incubated at 25ºC ± 1ºC for 4-5 days. Small bit of fungal mycelium seen around the sporophore piece in the slant is transferred to another culture tube and incubated at 25ºC ± 1ºC for 3-4 days and thus pure culture was obtained.

Preparation of master culture or stock culture

For preparation of master culture healthy wheat grains were soaked in tap water over night and next day it was boiled for about 10-15 minutes till they become soft but do not rupture. These grains were put on wire netting to remove the excess water, air dried and mixed with calcium carbonate and calcium sulphate at the ratio of 1:2 by wet weight basis. Grains were filled in 250 mL conical flasks and plugged with non absorbent cotton before autoclaved at 20 lbs P.S.I. for 2 hours. The sterilized grains inoculated aseptically with pure culture of *Pleurotus florida* and incubated for 7 days at 25±1ºC.

Preparation of spawn

Healthy wheat grains were dipped in water, boiled, air dried and calcium carbonate and calcium sulphate 1:2 ratio (wet weight basis)were mixed in similar way. These grains were filled in small polypropylene bags (25cm.x 15cm.). In each bag 200 gm grains were filled which were autoclaved at 20 lbs P.S.I. pressure for 2 hours. The sterilized bags were inoculated with master culture and incubated at 25±1ºC for 15 days. Fresh spawns were prepared separately for each experiment.

Preparation of beds

One year old fresh paddy straw free from any noticeable contaminants was used as substrate for cultivation. For preparation of mushroom bed, the substrates were cut into small pieces (1 to1.5 inches) and the straw was treated chemically by dipping the substrate into a solution containing 75 ppm Bavistin (carbendazim 50 WP) and 500 ppm formalin for 16-18 hours (Vijay and Sohi, 1987). After complete decantation of water the straw was allowed for air drying by spreading the substrate on a cemented floor. Layering method of spawning at the rate of 4% by wet weight basis was followed (Kang *et al.*, 2004). The spawned substrate was filled in polypropylene bags (45x30 cm). The mouths of the bags were tied with nylon string and perforations were made in the lower portion in the bags. A unit of 2 kg of dry straw was used for each treatment, which was equally distributed in four bags representing each as a replication. The moisture content of the straw at the time of spawning was kept around 72-75%. The filled bags were incubated in a dark room at a temperature ranging between 24-30ºC where 90% Relative Humidity was maintained till the spawn run was complete.When the straw is fully covered with milky white mycelium in the bag, it is regarded as complete spawn run, then the bags were cut open and compacted mass of aggregated straw called, as "bed" was ready for cropping. The beds were hanged by nylon string at a distance of 60 cm. Harvesting was done when the small primordial converted into a full grown sporophore.

Evaluation of different agricultural waste substrates for *Pleurotus florida*

Different agricultural waste materials such as paddy straw, wheat straw, rice husk, sugarcane bagasses, maize leaf and stalk, banana pseudo-stem and leaf, water hyacinth and vegetable waste of potato, brinjal and cucurbits, available easily in this region were tested for their suitability in cultivation of oyster mushroom (*Pleurotus florida*). All the substrates were chopped into 1.5"-2" size and treated chemically with 75 ppm bavistin and 500 ppm. A unit of 500 g of substrate was taken for each bed and four replications were maintained for each treatment

Evaluation of different suppliments for increasing the biological efficiency of oyster mushroom (*Pleurotus florida*)

Various supplements viz. rice bran, wheat bran, gram dal flour, maize flour, mustard cake, chicken manure, nitrogen fixing bacteria (*Azotobactor* sp.) @ 2% and 5%, and saw dust @ 5 and 10% (dry weight basis) were mixed with paddy straw substrate to know their effects on biological efficiency of *Pleurotus florida*. Paddy straw without any supplement was served as control. All the supplements were sterilized by autoclaving at 15 lb psi for 15 min. A unit of 2 kg of dry straw was used for each treatment, which was equally distributed in four bags representing each as a replication. The moisture content of the straw at the time of spawning was kept around 72-75%.

RESULTS

Evaluation of different agricultural waste substrates

Results indicated the suitability of all the substrates tested for the cultivation of *Pleurotus florida* under the agro-ecological condition of undulating red and lateritic belt of West Bengal. Maximum biological efficiency (94.85%) was obtained from paddy straw followed by wheat straw (87.40%) and maize leaf and stalk (79.5 %), which differs significantly from other substrates (Fig. 3.1). Banana pseudo-stem and leaf was however respondedmoderatelyin terms of yield (66.25%B.E.) in

Table 1: Effects of various substrates on the biological efficiency of *Pleurotus florida*

Sl..No	Treatment	Average yield (g.)	Biological Efficiency %	Time required for spawn run(days)	Average number of spropro-phores	Average weight of sporophore (g.)	Remarks
1	Paddy Straw	474.25	94.85	16	75	6.321	
2	Wheat Straw	437.00	87.4	14	56	7.753	
3	Rice Husk	248.50	49.7	15	42	5.987	
4	Maize Leaf & Stalk	397.50	79.5	18	61	6.523	
5	Water Hyacinth	331.25	55.7	21	53	6.256	
6	Banana Pseudo-stem & Leaf	278.50	66.25	17	46	6.012	
7	Sugarcane Bagassae	218.00	43.6	24	37	5.82	MA,II
8	Vegetable Waste	167.00	33.4	26	32	5.145	MA,II,NI
	C.D. at 5% level	16.651	3.330	2.189	6.182	0.740	
	Sem ±	5.705	1.1409	0.750	2.118	0.253	

MA = Mould Attack, II = Insect Infestation , NI = Nematode Infestation

Table 2: Effect of various supplements on the biological efficiency of *Pleurotus florida*

Sl.No	Treatment	Average yield (fresh weight of mushroom(g)	Biological Efficiency (%)	Time required for complete spawn run (days)	Average number of sporophore	Average weight of sporophore (g)	Remarks
1	Rice bran 2%	495.5	99.10	15	71	6.952	
2	Rice bran 5%	524.25	104.85	14	74	7.012	
3	Wheat bran 2%	485.5	97.10	14	68	7.114	
4	Wheat bran 5%	509.5	101.90	13	69	7.354	
5	Saw dust 5%	477.25	95.45	15	71	6.765	
6	Saw dust 10%	494.5	98.90	14	73	6.81	
7	Gram dal flour 2%	501.75	100.35	18	72	6.95	
8	Gram dal flour 5%	465	93.00	22	68	6.865	
9	Maize flour 2%	494.25	98.85	18	70	7.11	
10	Maize flour 5%	512.25	102.45	16	69	7.469	
11	Nitrogen fixing bacteria 2%	486.25	97.25	18	63	7.675	
12	Nitrogen fixing bacteria 5%	475.5	95.10	19	72	6.621	
13	Mustard cake 2%	437.25	87.45	22	79	5.564	MA,II
14	Mustard cake 5%	345.5	69.10	26	61	5.66	MA,II
15	Chicken manure 2%	421.75	84.35	23	82	5.125	MA,II,
16	Chicken manure 5%	306.75	61.35	25	60	5.1	MA,II ,NA
17	Control(Paddy Straw)	469	93.80	16	71	6.42	
	C.D. at 5% level	19.106	3.820	1.90	4.22	0.285	
	Sem ±	6.729	1.345	0.671	1.489	0.100	

MA = Mould Attack, II = Insect Infestation , NI = Nematode Infestation

comparison to water hyacinth (55.70%) and rice husk (49.70%). Sugarcane bagasses and vegetable waste were found to be less suitable in this region and gave 43.60% and 33.40 % biological efficiency respectively.The total spawn run periods were found to be varied with the substrates. Wheat straw has taken minimum time (14 days) for completing thespawn run followed by rice husk (15 days), paddy straw (16 days), banana pseudo-stem and leaf (17 days).Sugarcane bagassesand vegetablewaste took maximum period 24 and 26 day respectively for completing the spawn run. The effect of various agricultural waste substrates on the average weight of sporophore was also recorded. Bigger size of sporophores were recovered from wheat straw (7.753g) followed by maize stalk and leaf (6.523g), which was significantly superior than other substrates. Average weight of sporophore was found to be minimum (5.145g) in the beds of vegetable waste substrate.

Evaluation of different supplements

It has been observed from the Table 2 that, majority of the supplements i.e. rice bran, wheat bran, maize flour, saw dust, nitrogen fixing bacteria were contributed positively towards the yield of oyster mushroom at both 2 and 5 % dose. While, others *i.e.* chicken manure and mustard cake contributed negatively and were given less yield than control. Highest biological efficiency (104.85%) was obtained from the substrate supplemented with 5% rice bran followed by 5% maize flour (102.45%) and wheat bran (101.95%) supplemented substrates which differ significantly over control. Some supplements *i.e.* gram dal flour and nitrogen fixing bacteria were found suitable only at lower dose (2%) and increased the biological efficiency of *Pleurotus florida*, 100.35 % and 97.25% respectively but both of the supplements were found less effective at higher dose (5 %) and showed 93.00 % and 95.10 % biological efficiency respectively. Chicken manure and mustard cake contributed negatively towards the yield of oyster mushroom at both dosages and produced fewer mushrooms. Minimum biological efficiency 61.35 % was obtained from the substrate supplemented with 5 % chicken manure (Fig. 3.2). Wheat bran supplementation @ 5% to the substrate has taken minimum number of days (13) for completing the spawn run followed by 14 days (wheat bran

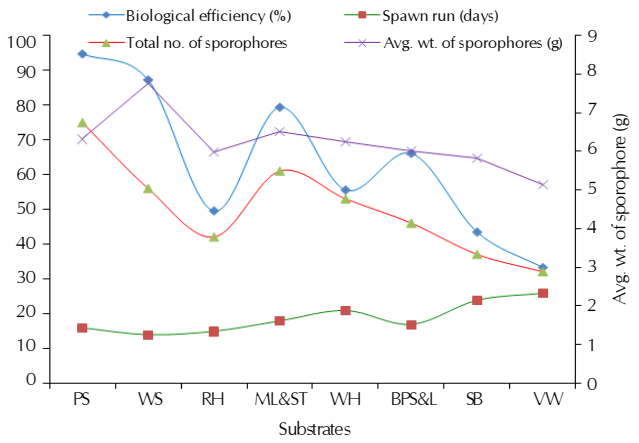

Figure 3.1: Effect of substrate on B.E. and other parameters

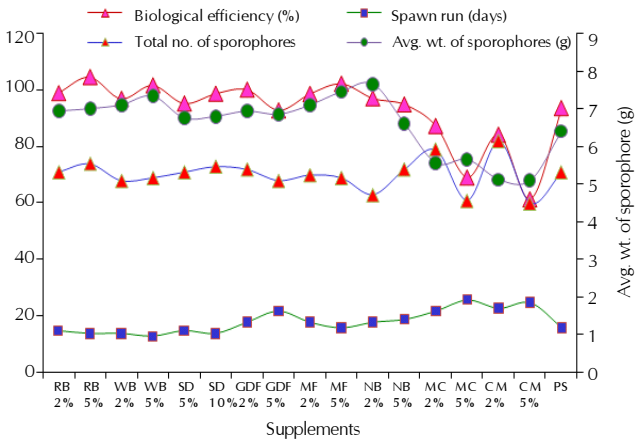

Figure 3.2: Effect of different supplements on B.E. and other parameters

2%, rice bran 5 % and 10 % saw dust) and 15 days (rice bran 2 % and saw dust 5 %), which were 1 to 3 days earlier than paddy straw (control). Most of the supplements except chicken manure and mustard cake have contributed appreciably on the size of the sporophore. Maximum size of the sporophore was obtained from 5 % maize flour supplemented substrate (7.469 gm.) followed by (7.354 gm) from 5 % wheat bran, (7.114gm) from 2 % wheat bran and (7.110 gm) form 2% maize flour supplemented substrate which were significantly superior thancontrol.

DISCUSSION

Paddy straw contains 41% cellulose, 13% lignin, 0.8% total nitrogen, 0.25% phosphorus penta-oxide (P_2O_5), 0.3% potassium oxide (K_2O), 6% Silicon oxide (SiO_2) and has a carbon-nitrogen ratio of 58:1 (Hetley *et al.*, 1960). Appropriate nutrients of fresh paddy straw and compactness of the mushroom beds facilitates the mycelium growth of *P. florida* under the environmental conditions of lateritic belt of West Bengal which may be contributed for higher yield and biological efficiency in paddy straw substrate. Bano *et al.* (1979) observed similar result with *Pleurotus flabellatus*. Higher incidence of competitor moulds *i.e Aspergillus niger*, *Coprinus* spp., *Penicillium* spp, *Trichoderma* spp, *Rhizopus* spp , insects infestation *i.e.* Phorids fly, larva of Sciarids fly,and presence of myceliophagous nematodes *i.e. Aphelenchoides* and *Ditylenchus myceliophagus* were probably the main reasons for lower yield and biological efficiency in vegetable wasteand sugarcane bagasse substrates. The results of sugarcane bagassefor cultivation of *P. florida* are corroborating with the findings of Martinez - Carrera (1989), Biswas *et al.* (1997) and Moda *et al.* (2005). Oyster mushroom has the ability of lignin degradation INair, 1980) and wheat straw contains more cellulose (48%) and lignin (20%) in comparison to paddy straw (Hetlay *et al.*, 1960) which gave faster spawn run in wheat straw substrate (13 days for complete spawn run) and bigger size of sporophores 7.753g in comparison to paddy straw substrate (16 days) and 6.321g. Cellulose, hemicelluloses and lignin were the source of carbon to the oyster mushroom. Nitrogen of the cereal straw was released by the action of fungal enzymes (Adejoye *et al.*, 2006 and Upadhyay *et al.*, 2002). Supplementation of nitrogenous materials to the substrates favors the absorption of nitrogen rich nutrient for the fungal biomass which probably the reason for increased biological efficiency in 5% rice bran and maize bran supplemented substrates. Upadhyay *et al.* (2002) reported higher yield of oyster mushroom with rice bran supplementation in wheat straw substrate.

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