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Technical Report

Cultivation Of The Oyster Mushroom (*Pleurotus ostreatus*) On Cellulosic Residues from Rice Straw

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Abstract

The use of cellulosic residues and additives in Ghana to improve the biological efficiency (BE) and nutrient content of the oyster mushroom *Pleurotus ostreatus* strain EM-1 has been an area of continuous study in Ghana. The effect of varying substrate formulations of rice straw with rice husk and bran on the weekly mycelia growth rate, spawn run period, yield and biological efficiency (BE) of *P. ostreatus* strain EM-1 were studied. Based on the results obtained, Treatment A {Rice straw (97.5%) with rice husk (2%) and calcium carbonate (0.5%)} recorded the highest mean mycelial growth rate (10.15 cm) in the first week and also recorded the lowest mycelia growth rate (5.30 cm) in the third week. There were significant differences ($P<0.05$) in the mycelia growth rates for the 1st week among the treatments. All the treatments containing rice straw: ranging from 43.75% to 97.5% took 28 days to be fully colonised compared to the control which took 35 days. The suitability of the rice straw and rice husk treatments in descending order are combinations of rice straw (50%) and rice husk (50%); rice straw only (100%) and rice straw (87.5%) and rice husk (2%) and sawdust only, having biological efficiencies of 154.29, 124.91, 107.47 and 75.08 respectively with the rice straw and rice husk showing a 37% increase over the control. All the treatments showed significant differences in biological efficiencies at $P\leq0.05$. Based on this increase in the BE of *Pleurotus ostreatus* strain EM-1, rice straw and rice husk at 1:1 ratio can be used as an alternate substrate for producing more mushrooms in rice growing areas.

Key words: *Pleurotus ostreatus,* oyster mushroom, substrate formulation, rice straw, sawdust, biological efficiency

Introduction

Oyster mushrooms (*Pleurotus* species), the third largest commercially produced mushroom in the world, are found growing naturally on rotten woody material (Phillips, 2006). *Pleurotus ostreatus* one of the species of the oyster mushroom are widespread in many temperate and subtropical forests throughout the world, although it is absent from the Pacific of Northwest of North America, being replaced by *P. pulmonarius* and *P. populinus* (Trudell and Ammirati, 2009). *In vivo* research has shown that consumption of oyster mushrooms lowers cholesterol levels (Rop *et al.,* 2009) because they naturally contain lovastatin (Gunde-Cimerman and Cimerman, 1995).

In Ghana, *P. ostreatus* is the most cultivated mushroom with its production standing at 120 tons a year with an annual increase of 2.7% (Obodai *et al.*, 2009). It is grown on composted sawdust of *Triplochiton scleroxylon* locally known as 'wawa' supplemented with rice bran and lime (Obodai *et al.*, 2002). However, due to the current concerns about deforestation and the unavailability of sawdust in some regions of Ghana, it makes it imperative that other sources of substrates and additives be utilized for its cultivation.

Mushrooms can be grown on a great variety of substrates. The choice of substrates depends on availability and cost. Previous research has shown great potential for using ligno-cellulosic wastes of nature as raw material for its production (Poppe, 2000).

Rice straw, husk and bran by-products of rice are some of these wastes that can be used in mushroom production. Yearly production of rice straw is about 537 million tons worldwide and rice husk accounts for 23% of total paddy weight (Shashirekha et al., 2005). In Ghana, rice is usually cultivated in the Northern and Volta regions. The straw is usually disposed of by open-field burning and soil incorporation, which are associated with environmental pollution problems. Finding ways of using the rice straw for mushroom cultivation will help curb this problem, while generating income and providing nutritious and/or medicinal food, especially when there is a world food crisis (Narh *et al.*, 2010).

The objective of this study was to investigate the influence of varying rice husk and bran as additives on rice straw with its resultant effect on mycelia growth rate, spawn run period, its yield and biological efficiency of *P. ostreatus* (Jacq.ex.Fr.) Kummer.

Materials and Methods

Mushroom culture

A culture of *P. ostreatus* (Jacq.ex.Fr.) Kummer strain EM -1 originally from Mauritius and maintained on Potato Dextrose Agar slants was used to prepare sorghum grain spawn as described by Oei, 1991.

Substrate preparations:

i) Rice straw preparation

Rice straw obtained from Dorwenya- Ghana, was chopped into 4cm length and steeped overnight in a plastic basin. This was covered with a wooden board to enable anaerobic fermentation of the straw. The steeped rice straw was then removed from the water and the excess water allowed to drain out by spreading the substrate on a mat for 30 mins. The moisture content was determined by using a hot oven (Gallenkamp oven, 300 plus series, England) at 107°C.

ii) Rice husk preparation

Rice husk obtained from Dorwenya – Ghana, was steeped overnight in a plastic basin and was covered with a wooden board to enable anaerobic fermentation of the husk. The steeped rice husk was then removed from the water and the excess water allowed to drain by spreading the substrate on a mat for 30 mins. The moisture content was determined by performing the squeeze test (Buswell, 1984) for accurate formulation of the substrate.

iii) Sawdust substrate preparation

Sawdust was prepared by using the outdoor single-phase solid waste fermentation in accordance with Obodai *et. al,* (2002).

Bagging and spawning

The treatments used in this study are as stated in Table 1. Sawdust supplemented with rice bran and calcium carbonate served as the control. The mixtures were bagged, sterilized and inoculated with 5 g of spawn and incubated till total spawn run. The mean radial growth rate per week, the spawn run period (the number of days from inoculation to complete colonization of the compost bag by the mycelium), the mycelia density and the number of days taken for the appearance of pinheads were recorded. There were five replicates for each treatment.

Table 1: List of treatments and their acronyms

Acronyms	Treatments
A	Rice straw (97.5%) with rice husk (2%) and calcium carbonate (0.5%)
B	Rice straw (87.5%) with rice bran (12%) and calcium carbonate (0.5%)
C	Rice straw (85.5) with rice husk (2%), rice bran (12%) and calcium carbonate (0.5%)
D	Rice straw (86%) with rice husk (2%) and rice bran (12%)
E	Sawdust (87.5%), rice bran (12%) and calcium carbonate(0.5%)
F	Rice straw (43.75%) with rice husk (43.75%), rice bran (12%) and calcium carbonate (0.5%)

Cropping

After spawn run the bags were transferred and packed horizontally, in stacks, onto horizontal racks inside a cropping house. The mushrooms were harvested as described by Obodai *et al.,* (2002). The biological efficiency was determined as a percentage of the weight of fresh mushrooms to the dry weight of substrate at spawning as described by Mueller *et al.,* (1985).

Analysis

Moisture and pH determinations

Moisture content of the sterilized substrates were determined by drying 5 g of each substrate at 107°C overnight in a hot oven (Gallenkamp oven, 300 plus series, England). The acidity of the substrates was also measured using and Alpha 500 model laboratory pH meter. There were five replicates in all analysis.

Experimental Design

A 2×2 experimental design was employed in this experiment. The principal factors were two substrates: rice straw and sawdust of *Triplochiton scleroxylon*, and the process treatment were: compost and substrate formulation. The cultivation of *P. ostreatus* strain EM1 on composted sawdust of *T. scleroxylon* with additives was the control experiment.

Data Analysis

The mycelia growth rates, total number of fruit bodies, total yields and biological efficiencies were subjected to a one-way analysis of variance and their means separated by the Duncan's multiple range tests (SPSS 16.0 for windows).

Results and Discussions

Moisture content and pH readings

Moisture contents and mean pH readings are presented in Table 2. The pH readings at bagging of the treatments at 25°C ranged from 6.15 to 6.75, these were not significantly different ($P>0.05$) from each other and were all within the optimum range of 6.0-8.0 (Stamets, 2000). The effect of rice husk or rice bran did not have any influence on the pH value recorded for treatment A and B. This was however not the case to the slightly higher acidic value recorded for the addition of $CaCO_3$ in treatment D in comparison to C (Table 2). No significant values ($P>0.05$) were recorded for the moisture content of the various treatments at bagging (75-79 %) but these values were lower than the required relative humidity of 85-95% (Stamets, 2000).

Table 2: Mean pH at 25°C and moisture content of substrates at bagging

Treatment	pH at 25°C	Moisture content (%)
A	6.23[a]	79[b]
B	6.23[a]	79[b]
C	6.23[a]	79[b]
D	6.75[a]	79[b]
E	6.15[a]	75[b]
F	6.23[a]	79[b]

Values in the same column followed by a common letter do not differ significantly (P>0.05)

Mycelial growth rate

The results of the mycelia growth rate on rice straw using rice husk or bran or both as additives and composted sawdust of *Triplochiton scleroxylon* are presented in Table 3. In general, the rice husk and rice bran within the rice straw and the sawdust accelerated spawn run and therefore increased the mycelia growth rate (Table 3). The spawn run periods recorded in this study (28-35 days), conform to the periods of 4- 5 weeks as stated by Oei (1996) for *P. ostreatus* cultivated on sawdust supplemented with rice bran. However, Obodai *et al* (2003) obtained 21 and 33 days as spawn run periods (total colonization period) for the same strain of *P. ostreatus* cultivated on fresh and composted 'wawa' sawdust supplemented with rice bran and calcium carbonate at the rates used in this study. Based on the results obtained from Table 3, Treatment A recorded the highest mean mycelial growth rate (10.15 cm) in the first week and also recorded the lowest mycelia growth rate (5.30 cm) in the third week. There were significant differences (P<0.05) in the mycelia growth rates for the 1st week.

All the treatments containing rice straw (A, B, C, D and F) took 28 days to be fully colonised compared to the control which took 35 days. This can be attributed to the physical nature and high porosity the rice straw is, as compared to the sawdust which is compact (Salmones *et al* (1999); Frimpong - Manso *et al* (2010).

Mycelial density

Treatments B and D were not uniformly white even though the mycelia totally grew through the bag but the mycelium density was thick and dense for treatments A, C, E, and F and this is in accordance with Thomas *et al* (1998) who reported that the yield of the mushroom is directly related to the spread of mycelium into the substrate.

Table 3: Mycelial growth rate of *P. ostreatus* on different pretreatments of rice straw using rice husk and bran as additives

Pretreatments	**1st week**	**2nd week**	**3rd week**	**Spawn run period (days)**	**[1]Surface mycelia density**
A	10.15±1.58[b]	5.92±0.42	5.30±1.48	28	++++
B	8.10±0.41[ab]	5.95±0.15	6.75±0.86	28	+++
C	7.45±0.24[a]	6.42±0.23	7.40±0.25	28	++++
D	7.62±0.43[a]	5.77±0.61	6.20±0.96	28	+++
E	6.62±0.11[a]	5.92±0.24	6.37±0.21	35	++++
F	8.20±0.14[ab]	6.95±0.32	8.72±0.33	28	++++

Values in the same column followed by a common letter do not differ significantly (P>0.05)

[1]Degree of mycelial density when mycelia fully colonize the substrate

+++ Mycelium totally grows through the bag and is uniformly white

++ Mycelium totally grows through the bag but not uniformly white

\+ Poor patchy growth

Physical characteristics for first flush

The physical characteristics of the first flush of mushroom fruit bodies are presented in Table 4. Varying differences were observed in the cap and stipe weights, the cap diameter, and the stipe circumference (Table 4). Fruit bodies with treatment A had the highest cap weight (26.0 g), whilst fruit bodies with treatment B had the lowest (2.5 g), also, fruit bodies from treatment F recorded the highest stipe weight, cap diameter and stipe circumference with values: 8.0 g, 12.0g and 5.2 g respectively whilst treatment D recorded the lowest stipe weight and circumference with values: 0.5 g, and 1.8 g respectively and treatment B recorded the lowest cap diameter of 5.0 g (Table 4). Royse *et al* (2004) and Mamiro and Royse (2008) have attributed the difference in mushroom size to type of substrate, spawn rate, type and level of supplements and type of mushroom species and strain.

Number of fruitbodies

The fruit body of treatment A and E become apparent only 7 days after opening the bags in the cropping house as compared to 6 days for treatments B, C and D, this was not the same for treatment F which become apparent in four days. Treatments A, B, C and D recorded three flushes each within the period. Whilst E and F recorded four flushes each within the period. In general, the number of fruit bodies per flush recorded decreased from flush to flush (Table 5) indication that the nature and the amount of nitrogen available in the substrate after each flush influence the degree of cellulose degradation which in turn affects the yield (Zadrazil and Brunnert, 1980). The number of fruit bodies ranged from 12 in the first flush to 8 by the 3rd flush.

The number of fruit bodies per bag in ascending order was 15, 18, 23, 23, 27 and 28 for treatment D, A, C, E, F and B respectively (Table 4). Treatment B had the highest number of fruit bodies. However, Treatments B, F, E and C had a significantly higher number of fruit bodies as compared to treatment A and D. An average of 31 fruit bodies have been recorded by Frimpong-Manso *et al.* (2010) for the same strain of oyster mushroom cultivated on composted *T. scleroxylon* sawdust supplemented with rice husk at varying concentrations. Also, Shah *et al.* (2004) have recorded 7-22 fruit bodies for *P. ostreatus* cultivated on wheat straw, sawdust and leaves singly and in combination.

Table 4: Physical characteristics of fruit bodies for first flush of mushrooms

Treatments	Cap weight (g)	Stipe weight (g)	Cap diameter (cm)	Stipe circumference (cm)
A	3.5-26	1.0-4.0	6.0-10.5	2.4-5.1
B	2.5-20.0	1.0-3.5	5.0-10.4	2.1-4.8
C	7.5-12.5	2.5-5.5	7.6-9.5	3.2-4.8
D	5.0-12.0	0.5-4.5	6.4-9.0	1.8-3.8
E	10.5-25.0	2.5-4.5	7.8-11.7	3.0-4.8
F	6.5-23.5	3.0-8.0	6.3-12.0	2.7-5.2

Table 5. Mean number of fruit bodies per flush

Treatments	Period of bag opening to first flush (days)	First	Second	Third	Fourth	Total number of fruit bodies
A	7	8	7	3	0	18^{ab}
B	6	12	11	6	1	28^{d}
C	6	10	7	6	0	23^{bc}
D	6	8	7	0	0	15^{a}
E	7	10	6	4	3	23^{bc}
F	4	9	7	7	4	27^{cd}

Values in the same column followed by a common letter do not differ significantly (P>0.05)

Mean yield and biological efficiencies of mushrooms

With the exception of treatment F (rice straw and rice husk in a 1:1 ratio) which had its highest yield in the second flush (68.83g) all other treatments had the highest yield in the first flush (Table 6). Among the various treatments, treatment F gave the highest yields with the 2nd and 3rd flushes showing significantly higher ($P<0.05$) yields as compared to all other treatments (Table 6).

There was reduction in yield from flush to flush (Table 6). This trend agrees with results obtained by other researchers (Obodai *et al.* 2003; Tisdale *et al.* 2006; Mshandete and Cuff, 2008) and demonstrates that the trend of steadily reducing mean yield per flush remains unchanged in spite of mushroom species or strain, the substrate (straw and sawdust) , and the treatment. This reduction in yield has been attributed to nutrient depletion in the substrate being directly proportional to fruit bodies harvested in each flush (Stamets and Chilton, 1983).

The biological efficiencies varied significantly ($P<0.05$) among the treatments (Table 6). The highest biological efficiency obtained in this study was 154.29 % for treatment F showing a 37% increase over the control. This is significantly higher ($P<0.05$) than the biological efficiencies obtained for all the other treatments. Treatment D showed the least biological efficiency of 42. 86%. It was observed that biological efficiency is enhanced significantly when rice straw and rice husk is used in the same ratio. Rice straw and rice husk has been identified as rich in cellulose (Datta and Chakravarty, 2001; Obodai *et al.,* 2003) and are therefore used for the cultivation of *Volvariella volvacea* and *P. ostreatus* (Mahmoud and El-Kattan, 1989) and these are available in large quantities. Earlier studies conducted have shown that rice straw only is the best alternate substrate for the cultivation of *P. ostreatus floridarus* strain and also a combination of rice straw and composted sawdust of *Triplochiton scleroxylon* in a 1:1 ratio on wet basis is the best alternate substrate for both *P. ostreatus* strain EM1 and *P. pulmonarius* strain PPO (Narh *et al* 2010 unpublished). Studies have also shown that pre-treatment and substrate formulation can improve yield of *Pleurotus ostreatus* strain EM-1 when chopped rice straw and sawdust of *T. scleroxylon* are used as substrates (Obodai *et al.* 2010). Frimpong-Manso *et al.* 2010, indicated that rice husk can be used as an additive in rice growing areas for production of mushrooms with sawdust as substrate since it showed an 11% increase in the biological efficiency over the control at 2% supplementation

with subsequent increases in some proximate analysis and mineral contents such as calcium and phosphorus.

Conclusion

Pre-treatments and substrate formulation can improve yield of *Pleurotus ostreatus* strain EM-1 when chopped rice straw is used as substrate in combination with rice husk in a 1:1 ratio. The biological efficiency is enhanced significantly with a 37% increase over the control.

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Table 6. Total yield and Biological efficiency of *P. ostreatus* on different pretreatments of rice straw using rice husk and bran as additives

Pretreatments	Yield/ Flush (g)				Total yield (g)	Biological Efficiency (%)
	First	Second	Third	Fourth		
A	38.33	22.17	18.33	0.00	78.83^{b}	75.08^{b}
B	55.83	41.33	34.00	0.00	131.16^{de}	124.91^{de}
C	50.67	37.17	25.00	0.00	112.84^{c}	107.47^{c}
D	24.17	20.83	0.00	0.00	45.00^{a}	42.86^{a}
E	45.57	32.89	26.53	15.25	120.24^{cd}	96.19^{cd}
F	33.00	68.83	40.50	19.67	162.00^{e}	154.29^{e}

Values in the same column followed by a common letter do not differ significantly (P>0.05)

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