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EFFECT OF SUBSTRATE FORMULATION OF RICE STRAW (*ORYZA SATIVA*) AND SAWDUST (*TRIPLOCHITON SCLEROXYLON*) ON THE CULTIVATION OF *PLEUROTUS OSTREATUS* (JACQ. EX. FR.) KUMMER

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Abstract: The use of agricultural by-products and additives to improve the biological efficiency and nutrient content of the oyster mushroom *Pleurotus ostreatus* (Jacq. ex. Fr.) Kummer has been an area of continuous research in Ghana. The effect of varying pre-treatments and substrate formulations of rice straw and sawdust of T*riplochiton scleroxylon* on the yield and biological efficiency of *Pleurotus ostreatus* strain EM-1 was studied. The suitability of the substrates for cultivation of *P. ostreatus* strain EM-1 in descending order is rice straw with composted sawdust with additives, composted sawdust with additives, rice straw only, rice straw with fresh sawdust with additives and fresh sawdust with additives, having biological efficiencies (BEs) of 76.07%, 65.23%, 56.33%, 46.55% and 43.50% respectively. All the treatments showed significant differences in BEs at $P<0.05$. The combined substrates generally gave significantly higher BEs than the single substrates. Composted sawdust gave significantly higher BE than fresh sawdust ($P<0.05$). Substrate combinations increase the yield of *P. ostreatus* strain EM-1 when rice straw and sawdust of *T. scleroxylon* are used. Also composting increases the BE of sawdust of *T. scleroxylon* when used to cultivate *P. ostreatus* strain EM-1.

***Key words*:** *Pleurotus ostreatus*, Oyster mushrooms, Composting, Substrate formulation, Rice straw, Sawdust, Biological efficiency.

INTRODUCTION

The oyster mushrooms (*Pleurotus* spp.), is a common primary decomposer of wood and agricultural residues (Zadrazil and Kurtzman, 1982). These mushrooms can naturally be found in tropical and subtropical rainforests, and can be artificially cultivated (Maziero et. al., 1992). Previous research has shown great potential for using some lignocellulosic substances as raw material for the production of *Pleurotus ostreatus* (Zadrazil, 1987; Gapinski and Ziombra, 1988). A wide array of enzymes such as laccases, peroxidases, glucosidases, cellulases, hemicellulases and xylanases (Ortega et. al., 1992; Hatakka, 1994; Datta and Chakravarty, 2001; and Lo et. al., 2001) produced by mushroom mycelia are capable of utilizing complex organic compounds which occur in organic matter residues (Tisdale et. al., 2006; Mane et. al., 2007; Olfati and Peyvast, 2008). Thus, the enzymes enable mycelia of various mushrooms including *Pleurotus* spp. to colonize a wide array of lignocellulosic substances (Miles and Chang, 1997; Chang, 1999). However, availability and cost of the substrates for *Pleurotus* spp. cultivation are major determining factors in the type of substrates and substrate formulations used in countries and even regions (Balazs, 1995; Croan, 1999; Labuschagne et. al., 2000; Cohen et. al., 2002).

Pre-treatment of lignocellulosic substances vary widely. They include mechanical, physical, physicochemical, chemical and biological pre-treatments (Mtui, 2009). All pre-treatments of these substances are aimed at altering or removing structural and

compositional impediments to hydrolysis and subsequent degradation processes in order to enhance digestibility, improve the rate of enzyme hydrolysis and increase yields of intended products (Mosier et al., 2005; Hendriks and Zeeman, 2009).

Some of the lignocellulosic substances found in Ghana include sawdust of various woods, maize stover, corncobs, wheat husks, and rice straw. Rice straw is becoming increasingly abundant due to an increase in production of the grain in the country with more emphasis being placed on improving the agricultural sector in recent times. Finding highly efficient ways of using this bioresource in the country, such as for mushroom cultivation, is very essential to avoid the environmental pollution problems associated with improper waste disposal methods, while providing a source of nutritious food and income.

This paper reports on varying pre-treatments and substrate formulations of rice straw (*Oryza sativa)* and 'wawa' sawdust (*Triplochiton scleroxylon*) as substrates for the cultivation of *Pleurotus ostreatus* (Jacq ex fr) Kummer strain EM-1 under Ghanaian conditions.

MATERIALS AND METHODS

Culture maintenance and spawn preparation

Pleurotus ostreatus strain EM1 originally obtained from Mauritius was maintained alternately on Potatoe Dextrose Agar (PDA) and Malt Extract Agar (MEA) (OXOID Ltd., Basingstoke Hampshire, England). Spawn of this mushroom was prepared using grains of sorghum and millet, obtained from the Nima Market in Accra, combined in a 3:1 (w/w) ratio. Both the cultures and the spawn were incubated at 26-28ºC and 60-65% RH in an incubator (Tuttlingten WTC Binder, Germany).

Substrate preparation

Rice straw preparation

The rice straw was manually chopped into 4cm length and steeped overnight in a plastic basin, which was covered to encourage anaerobic fermentation of the straw. The soaked straw was then drained and rinsed three times with tap water. The straw was squeezed and spread on a rectangular metallic strainer for 30mins, to allow excess water to drain out, such that a moisture content of 65-70% was attained. The moisture content was determined by performing the squeeze test (Buswell, 1984).

Composting of sawdust

Sawdust compost was prepared in accordance to Obodai et. al. (2000).

Bagging and spawning

The treatments used were: rice straw only (RS), fresh sawdust with additives only (FS_A), composted sawdust with additives only (CS_A), rice straw with fresh sawdust with additives ($RSFS_A$) in a ratio of 1:1 (w/w), and rice straw with composted sawdust with additives ($RSCS_A$) also in a l:1 ratio (w/w) as shown in Table 1. The composted sawdust with additives only (CS_A) was the control. Twelve percent of rice bran and 0.5% of Calcium carbonate ($CaCO_3$) were added on dry weight basis and thoroughly mixed with the substrates, serving as the additives. To the treatments containing sawdust, water was added while mixing such that a moisture content of 60-70% was attained. There were five replicates for each treatment.

The bags were steam sterilized for 3hrs, cooled to room temperature and inoculated with 5g spawn of *P. ostreatus* strain EM1. These were then incubated at 28±2ºC for 31 to 34 days in a semi-sterile room (Auetrugal, 1984). The spawn run period (the number of days from inoculation to complete colonization of the substrate by the mycelia) was recorded.

Table 1: List of treatments and their acronyms

Treatment	Acronym
Rice straw only	RS
Fresh sawdust with additives only	FS_A
Composted sawdust with additives only	CS_A
Rice straw with fresh sawdust with additives (1:1w/w)	$RSFS_A$
Rice straw with composted sawdust with additives (1:1w/w)	$RSCS_A$

Cropping

The bags were transferred into the cropping house with the environmental conditions: light, moisture, air exchange and temperature altered to induce fruiting (Stamets, 2000). The parameters recorded were; days till primordia formation, days from bag opening to first flush, interval between flushes, flush number within 2 months of cropping and number of fruit bodies per bag. Also, mushroom size determined as

total weight of fresh mushroom harvested per bag over the total number of mushrooms harvested per bag, crop period (sum of incubation and fruiting periods) and biological efficiency (BE) determined as a percentage of the weight of fresh mushrooms (g) to the dry weight of substrate (g) at spawning according to Royse et. al. (2004) was recorded.

pH and moisture determinations

The acidity of the sterilized substrates was measured using a pHM92 Lab pH meter (MeterLab^{TM}, Radiometer Analytical A/S, Copenhagen, Denmark). Moisture content of the sterilized substrates was determined using a hot oven (Gallenkamp oven, 300plus series, England) at 107ºC. All weight measurements were done by the use of a Digital Computing Scale (Hana Electronics Company Limited, Korea).

Statistical Analysis

Values presented are means of data obtained. However, the flush number presented is the modal flush number obtained for each treatment, whereas the biological efficiency was presented as the mean±standard error (SE). Data analysis was conducted by the separation of means by Fischer's Least Significant Difference (LSD) at a 95% level of probability

Experimental Design

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

RESULTS AND DISCUSSIONS

pH and Moisture Content of Substrates at Bagging

The pH and moisture content of the substrates at bagging ranged from 6.94-7.69 and 60-80% respectively (results not shown). These values were not significantly different ($P<0.05$) among the treatments. Both the pH and the moisture content for all the substrates were generally within the optimum range of 6.0-8.0 and 60-75% respectively (Stamets, 2000) for *P. ostreatus* cultivation.

Spawn running, primordia and fruit body formation

The spawn run period, days till primordial formation and the days from bag opening to first flush were not significantly different among the treatments (Table 2). The spawn run periods recorded in this study (30-34days) (Table 2), conform to the period of 4-5weeks (ie 28-35days) 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 colonisation period) for the same strain of *P. ostreatus* cultivated on fresh and composted 'wawa' sawdust supplemented with rice bran and calcium oxide at the rates used in this study.

The difference between the days till primordia formation and the days from bag opening to first flush (Table 2) indicates that it takes 2-3days for a fruit body of *P. ostreatus* to mature from the primordial to the matured stage. This is in accordance with Oei (1996).

Interval between flushes, flush number and total fruiting and crop periods

The interval between flushes ranged from 12-19 days with the modal mean interval between flushes being 14 days (Table 2). This is partly in agreement with Stamets (2000) who has stated the interval between flushes for *P. ostreatus* to be 7-14 days. However, the highest mean interval between flushes of 19 days obtained in this study is notably higher than the maximum (14 days) stated by Stamets (2000).

The modal flush number for the treatments ranged from 2-5 over the 2 months of cropping (Table 2). This result corresponds with the 2-6 flushes recorded by Mandeel et al. (2005) when *P. ostreatus* was cultivated using various lignocellulosic wastes (paper, cardboard, fibre and sawdust). Rice straw only (RS) had the least flush number of 2. Rice straw with composted sawdust with additives ($RSCS_A$) showed a higher flush number of 5 flushes while the control (i.e. composted sawdust with additives only) yielded 4 flushes within the study period.

The fruiting periods obtained for the various treatments were in ascending order, 28, 44, 46, 47 and 50 days for RS, FS_A, $RSCS_A$, $RSFS_A$ and CS_A respectively (Table 2). The fruiting period is influenced by the interval between flushes and the flush number. The crop periods obtained in this study ranged from 75-82 days and were not significantly different among the treatments.

Table 2: Days for completion of spawn running, pinhead and fruit body formation and crop period of *P. ostreatus* strain EM-1 grown on various treatments.

Treatment	[a] Spawn run period (days)	Days till primodia formation (days)	Days from bag opening to first flush (days)	[b] Interval between flushes (days)	[c] Flush number	[d] Total fruiting period (days)	[e]Crop period (days)
RS	nd	7	9	14	2	28	nd
FS_A	31	6	8	16	3	44	75
CS_A	32	3	6	14	4	50	82
$RSFS_A$	30	6	8	19	3	47	77
$RSCS_A$	34	5	7	12	5	46	80

nd: not determined
a: days taken until complete colonization of substrate by mycelium
b: number of days between successive flushes
c: number of flushes recorded during the 2 months of cropping
d: number of days from the day of bag opening to the last flush
e: number of days from bag inoculation to last flush (sum of the spawn run and total fruiting periods).

Yield per flush

Among the various substrates, rice straw with composted sawdust and additives ($RSCS_A$) gave the highest yields with the 1st, 2nd and 3rd flushes showing significantly higher yields as compared to all the other substrates (Fig. 1). Although rice straw only (RS) showed comparable yield with the other substrates in the 1st flush, the treatment gave significantly lower yields ($P>0.05$) in the subsequent flushes. However, fresh sawdust with additives only (FS_A) and composted sawdust with additives only (CS_A) showed similar yield with CS_A generally giving slightly higher yields (Fig. 1). With the exception of $RSCS_A$ which gave high yields even after the 4th flush, there was a drastic decline in yield among the other treatments. This indicates that it would be economically proper to discard the bags produced with all the treatments with the exception of the $RSCS_A$ bags after the 3rd flush.

This fact is also evident in Fig. 2, which shows a cumulative percentage yield of 81% across the treatments from the first three flushes as compared to a cumulative percentage yield of 19% from the last three flushes. Though these values are lower than the 93-99% cumulative percentage yield for the first three flushes obtained by Mshandete and Cuff (2008), both results indicate that, the highest economically viable flush number an oyster mushroom cultivator should expect from a compost bag should be 4 flushes. Any higher flush number would mean a waste of space, other resources and a condition, which would encourage proliferation of pests and diseases.

There is a reduction in yield from flush to flush (Fig. 2). 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/strain, the substrate (straw or sawdust) and the treatment (whether fresh, composted or combined). 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). Tsang et al. (1987) have also demonstrated a reduction in cellulose, hemicellulose, lignin and other nutrient contents of wheat straw with the cultivation of four *Pleurotus* spp. including *P. ostreatus*. Some other forms of substrate modifications in terms of pH, moisture content, texture of substrate etc could also contribute to this phenomenon. These parameters would be investigated in further studies.

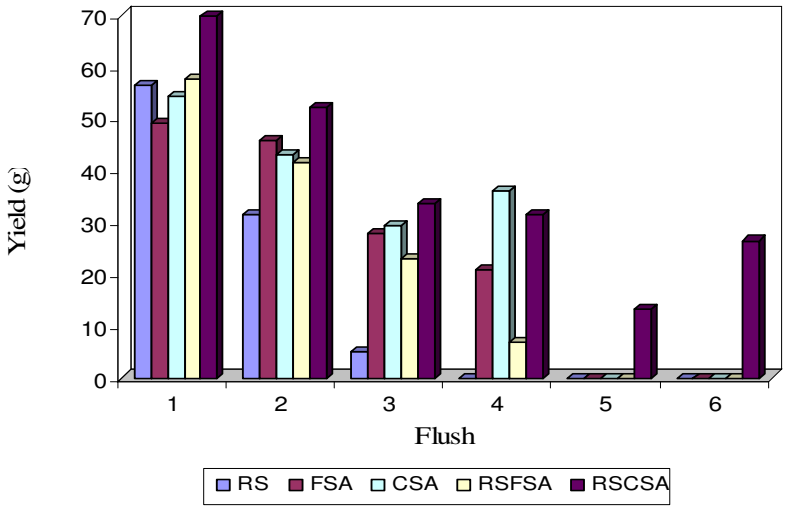

Fig. 1: Mean yield per substrate per flush

RS: Rice straw only
FSA: Fresh sawdust with additives only
CSA: Composted sawdust with additives only
RSFSA: Rice straw with fresh sawdust with additives in a ratio of 1:1 (w/w)
RSCSA: Rice straw with composted sawdust with additives in a ratio of l:1 (w/w)

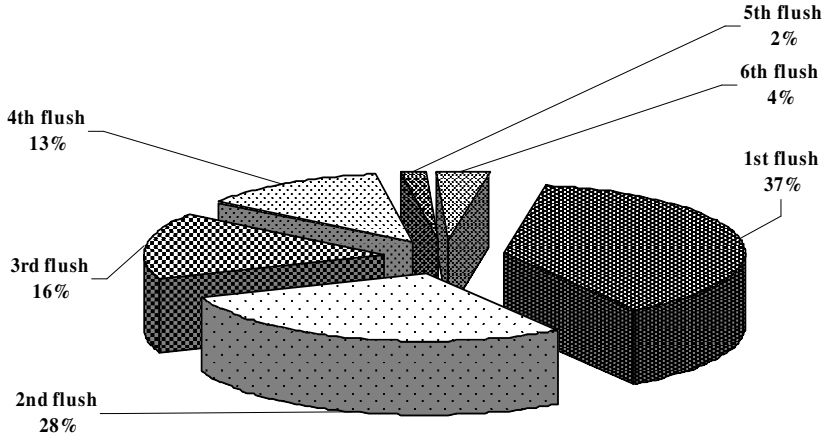

Fig. 2: Percentage yield per flush of fresh fruit bodies across the treatments

Number of fruit bodies, mushroom size, and biological efficiency

The number of fruit bodies per bag in ascending order was 16, 20, 31 and 35 for RS, FS_A and $RSFS_A$, CS_A and $RSCS_A$ respectively (Table 3). $RSCS_A$ and CS_A had a significantly higher ($P<0.05$) number of fruit bodies as compared to the RS, FS_A and $RSFS_A$. 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.

The mushroom size ranged from 5.3-5.9g/fruit body and was not significantly different for the fruit bodies harvested from the various treatments (Table 3). Royse et al. (2004) and Mamiro and Royse (2008) have attributed the differences in mushroom size to type of substrate, spawn rate, type and level of supplements and type of mushroom species and strains. The insignificant difference ($P>0.05$) in mushroom size among the fruit bodies obtained from the various treatments in this study (Table 3) indicates that mushroom size can probably be influenced by the genes of the mushroom species/strains cultivated in addition to other factors during the cropping period such as light intensity and C/N ratio (Stamets, 2000).

Table 3: Number of fruit bodies, mushroom size and biological efficiency of *P. ostreatus* strain EM-1 grown on various treatments.

Treatment	Number of fruit bodies per bag	Mushroom size	Biological efficiency (%)
RS	16	5.7	56.33±4.74c
FS_A	20	5.9	43.50±2.49a
CS_A	31	5.3	65.23±4.45d
$RSFS_A$	20	5.6	46.55±2.65b
$RSCS_A$	35	5.5	76.07±2.66e

Values in a column followed by a different letter are significantly different at 95% level of probability according to Fisher's Least Significant Difference (LSD). n=5

Conversely, the biological efficiencies (BEs) varied, generally significantly ($P<0.05$), among the treatments (Table 3). The highest BE obtained in this study was 76.07% for $RSCS_A$ (Table 3). This is significantly higher than the BEs obtained for all the other treatments. Fresh sawdust with additives only (FS_A) showed the least BE of 43.50%.

However, the significantly higher BE ($P<0.05$) of 65.23% for CS_A than the BE of 43.50% for FS_A (Table 3) is not in agreement with the observation of Mshandete and Cuff (2008) which states that non-composted substrates are more productive substrates in

terms of bioefficiency as far as *Pleurotus* species are concerned. This is also evident in the observation that even when combined with fermented rice straw, fresh sawdust showed significantly lower BE as compared to the composted sawdust (Table 3). This disparity could be associated with the proximate and nutrient compositions, pH, as well as the texture of the sawdust, used in this study, and that of the sisal decortications, used in the study by Mshandete and Cuff (2008), before and after composting of the lignocellulosic substances.

It was observed that the sawdust (fresh or composted) with additives showed significantly higher BE when combined with rice straw in a 1:1(w/w) ratio than when used singly (Table 3). The fresh or composted sawdust had smaller particle size than the chopped rice straw and therefore had fewer air spaces than the rice straw after compaction. The rice straw in the combined substrates therefore, compensated for this and hence, air exchange in the combined substrates was enhanced. Good air exchange is good for fruit body development of mushroom (Stamets, 2000). The rice straw could also have modified the nutrient content of the combined substrate to suit the requirements of the mushrooms (Obodai et al., 2003).

CONCLUSION

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. The biological efficiency is enhanced significantly when the sawdust is composted before usage.

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