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ORIGINAL PAPER

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Comparative study on the growth and yield of *Pleurotus ostreatus* mushroom on different lignocellulosic by-products

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Abstract Eight lignocellulosic by-products were evaluated as substrates for cultivation of the oyster mushroom, *Pleurotus ostreatus* (*Jacq*. ex. fr) Kummer. The yields of mushroom on the different substrates were 183.1, 151.8, 111.5, 87.8, 49.5, 23.3, 13.0 and 0.0 g for composted sawdust of *Triplochiton scleroxylon*, rice straw, banana leaves, maize stover, corn husk, rice husk, fresh sawdust, and elephant grass, respectively. The biological efficiency (BE) followed the same pattern and ranged from 61.0% for composted sawdust to 0.0% for elephant grass. The yield of mushroom was positively correlated to cellulose ($r^2 = 0.6$), lignin ($r^2 = 0.7$) and fibre ($r^2 = 0.7$) contents of the substrates. Based on the yield and BE of the substrates tested, rice straw appeared to be the best alternate substrate for growing oyster mushrooms.

Keywords *Pleurotus ostreatus* mushroom · Comparative · Lignocellulosic by-product · Yield

Introduction

Oyster mushrooms (*Pleurotus* species), the third largest commercially produced mushroom in the world [3], are found growing naturally on rotten wood material. The growing increase in consumption of oyster mushroom is largely due to its taste, medicinal and nutritional properties [4]. *Pleurotus ostreatus*, one of the most-produced species, is cultivated mainly on sawdust. The unavailability of sawdust and the fact that felling of trees in most regions of the world is prohibited makes it imperative that other sources of substrates be utilised for its cultivation. In the tropics and sub-tropics, large volumes of unused lignocellulosic by-products can be found. These by-products are left to rot in the field or are disposed off through burning. Cultivation of mushrooms on these by-products may be one of the solutions to transforming these inedible wastes into accepted edible biomass of high market value. The spent substrates from mushroom cultivation can also potentially be used as an animal feed supplement, possibly providing additional animal feed resources [11]. *Pleurotus* species, a widely accepted mushroom cultivated in Ghana, degrades and grows directly on these lignocellulosic by-products [7]. Although, large volumes of by-products are available in Ghana, their use as substrate for mushroom cultivation has not been fully exploited. This paper reports on the comparative utilisation of eight lignocellulosic by-products as substrates on the growth rate and yield of *P. ostreatus* (*Jacq*. ex .fr) Kummer using the plastic bag method [6]. This method is more reliable, in that it produces better and more stable yields, than the traditional commercial method of cultivation.

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Materials and methods

Substrate preparation and spawning

P. ostreatus (*Jacq*. ex.fr) Kummer strain EM-1, originally obtained from Mauritius, was maintained on malt extract agar slants and spawn was prepared on sorghum grains [12]. Both cultures and spawn were incubated in the laboratory at 26–28°C and 60–65% relative humidity (RH).

Freshly milled sawdust of *Triplochiton scleroxylon*, moisture content 30% (w/w) (88 parts), was thoroughly mixed with 11.5 parts rice bran and 0.5 part of calcium oxide. Water was sprinkled on the mixture until its moisture content was about 70% (w/w). The mixture was piled up into a pyramidal heap and allowed to ferment for 28 days. It was turned every fourth day to ensure proper aeration. Aliquots of composted sawdust (1 kg) were put into 33×18 cm heat-resistant, 0.1 μm polypropylene bags [2]. Corn husk, banana leaves, elephant grass, rice straw and maize stover were chopped into 4 cm lengths and soaked in water overnight in basins. Excess water was drained and the substrates dried in the sun for 2 h. Fresh uncomposted sawdust and the substrates were also bagged in heat-resistant polypropylene bags. Each bag was closed with a plastic neck, steam-sterilised for 2.5 h

and inoculated with 5 g sorghum spawn (the substrates were subjected to these different treatments to ensure maximum yields). The bags were then incubated at 26–28°C and 60–65% RH for 20–34 days in a well-ventilated, semi-dark room. The mean radial growth per week and the spawn run period to total colonisation (i.e. the number of days from inoculation to complete colonisation of the compost bag by the mycelium) were recorded.

Cropping

After completion of the spawn run, the bags were transferred onto horizontal racks in a cropping house – a wooden-frame structure covered with woven mats. The bags were then opened and the mats were watered twice a day to increase the humidity and induce fruit body formation. The interior of the house reached 26–28°C and 90–95% RH. The number of days until the first appearance of the mushroom was recorded. The biological efficiency (BE), i.e. the weight of fresh mushrooms as a percentage of the dry weight of the substrate was determined [5]. The experiment was replicated five times for each by-product substrate.

Chemical analysis

Proximate analyses were carried out on the substrates on which the mycelium grew. The substrates were powdered and analysed for various constituents: crude protein and fibre, cellulose, hemicellulose, lignin and ash [1]. Moisture content was determined by drying 5 g of each substrate at 107°C overnight. Acidity (pH) was measured using an Alpha 500 model laboratory pH/mV meter. For each analysis, there were four replicates.

Data were submitted to a one-way analysis of variance. The total yield of mushroom per substrate was separated by Duncan's multiple range tests at $\alpha = 0.05$. Correlation analyses were carried out in order to determine the relation of each chemical constituent with the total yield of mushroom pooled from all the substrates. All statistical analyses were performed using SPSS 10 for Windows [9].

Results

The highest mean radial growth of the mycelium was recorded on rice husk, followed by fresh sawdust (Table 1). The density of the mycelium was comparatively poor on corn, rice husks and fresh sawdust. That of composted sawdust, banana leaves, maize stover and rice straw was uniform and white. The mycelium of the fungus totally colonised the substrates within a period of 34 days of spawn run. There was, however, no growth in the case of elephant grass. The primordia started appearing 4–6 days after the bags had been transferred to the cropping house and opened. This varied from substrate to substrate. Data on the quantity of sporophores harvested in different flushes are presented in Table 2. Composted sawdust, corn husk, banana leaves and rice

Table 1 Comparison of weekly mycelial growth of *Pleurotus ostreatus* strain EM-1 on different substrates

Substrate	Surface mycelial density[a]	Total colonisation period (days)	Diameter (cm) of mycelia growth/week				Mean	Days from bag opening to primordia formation
			1	2	3	4		
Fresh sawdust	+	21	5.2	5.1	5.0		5.1 ± 0.82	5
Composted sawdust	+ + +	33	3.0	4.2	6.1	5.5	4.70 ± 0.64	4
Rice husk	+	15	5.8	5.5	c[b]	c	5.65 ± 0.31	4
Corn husk	+	27	4.3	4.2	4.2	4.1	4.20 ± 0.50	5
Banana leaves	+ + +	34	4.3	5.1	4.5	4.8	4.68 ± 0.43	4
Maize stover	+ + +	30	4.2	4.7	4.5	4.1	4.38 ± 0.91	5
Rice straw	+ + +	28	4.1	4.3	4.4	4.0	4.20 ± 1.01	6
Elephant grass	No mycelia growth							

[a]Degree of mycelial density when the mycelia fully colonises the substrate: + poor running growth, + + mycelium grows throughout the whole bag but is not uniformly white, + + + mycelium grows throughout the whole bag and is uniformly white
[b]Complete colonisation

Table 2 Cumulative mushroom yields and biological efficiency (*BE*) on different by-products

Substrate	Fresh weight of mushrooms by flushes (g)				BE (%)
	First	Second	Third	Total fresh weight (g)	
Fresh sawdust	13.0 ± 0.32	nf[a]	nf	13.0 ± 0.32	4.30
Composted sawdust	83.6 ± 0.41	79.2 ± 0.61	20.4 ± 0.41	183.12 ± 1.23	61.04
Rice husk	23.3 ± 0.51	nf	nf	23.30 ± 0.91	7.76
Corn husk	25.2 ± 0.72	14.2 ± 0.57	10.1 ± 0.51	49.50 ± 0.79	16.50
Banana leaves	58.2 ± 0.81	43.1 ± 0.92	10.2 ± 0.62	111.46 ± 0.81	37.15
Maize stover	50.7 ± 1.21	37.1 ± 0.41	nf	87.80 ± 0.21	29.26
Rice straw	50.2 ± 1.30	49.2 ± 0.32	12.2 ± 0.71	151.8 ± 0.44	50.64
Elephant grass	nf				

[a]No flushes recorded

Table 3 Proximate and chemical composition of some of the lignocellulosic materials used

	Composted sawdust	Banana leaves	Maize stover	Rice straw
Moisture	58.44 ± 0.04	67.62 ± 0.32	69.30 ± 0.11	69.02 ± 0.81
Nitrogen	0.16 ± 0.09	0.94 ± 0.51	0.76 ± 0.32	0.91 ± 0.11
Cellulose	46.47 ± 0.14	29.47 ± 0.03	39.04 ± 0.05	38.42 ± 0.32
Hemicellulose	8.82 ± 0.25	21.83 ± 0.06	25.27 ± 1.21	28.57 ± 0.01
Lignin	31.68 ± 0.51	15.74 ± 0.11	6.15 ± 0.31	6.73 ± 0.21
Crude fibre	63.28 ± 0.19	32.72 ± 0.51	32.35 ± 0.32	28.78 ± 0.41
Ash	15.22 ± 0.03	14.17 ± 0.04	8.65 ± 0.62	8.37 ± 0.53
pH of medium	7.60 ± 0.05	6.92 ± 0.02	7.02 ± 0.31	7.37 ± 0.24

Table 4 Correlation between mushroom yield and constituents of the substrates

Constituents of substrates	Mushroom yield
Lignin	0.70**
Cellulose	0.64**
Fibre	0.71**
Hemicellulose	−0.62*
Organic matter	−0.44

**Significant at 1%, *significant at 5%

straw each recorded three flushes, with maize stover recording two flushes. Fresh sawdust and rice husk recorded only one flush each. The first flush of crop gave a maximum yield of 83.56 g on composted sawdust followed by 58.2 g on banana leaves. More than 70% of the yield was obtained in the first two flushes in all the by-product substrates tested.

During the 8 weeks of cropping, the highest total weight of mushrooms harvested on 1 kg wet substrate was recorded on composted sawdust (183.12 g). This was followed by rice straw with a total weight of 151.8 g. Analysis of mushroom yield revealed significant differences ($P < 0.05$) between substrates. Composted sawdust and rice straw were superior to all the other substrates. BE of mushroom production varied in different substrates. The maximum BE of 61.04% was recorded with composted sawdust followed by rice straw, at 50.64%.

Data on the chemical composition of the by-products tested for mushroom production are presented in Table 3. The by-products differed significantly ($P < 0.05$) in the concentration of constituents such as lignin, cellulose, and nitrogen. Composted sawdust had higher cellulose and lignin contents compared to the other by-products while banana leaves had the highest nitrogen content of all the by-products tested. Correlation studies between the constituents of the substrates and mushroom yield (Table 4) revealed a significant ($P < 0.05$) positive relationship with cellulose content ($r^2 = 0.64$), lignin content ($r^2 = 0.70$) and fibre content ($r^2 = 0.71$). However, sporophore production was negatively related to hemicellulose ($r^2 = -0.62$) and organic matter ($r^2 = -0.44$).

Discussion

Rice husk gave the fastest mycelial growth rate; however, this did not correspond with yield, indicating that mycelial growth and yield of mushrooms have different requirements [6]. Also, this substrate is very susceptible to drying, which affected sporophore formation [8]. Given the physical nature and high porosity of the rice husk, and also the fact that it dries up very fast, it will be advisable to use it as an additive to sawdust for use as mushroom substrate. In an experiment in which 2% (w/w) of rice husk was added to composted sawdust, there was an 11% increase in mushroom yield (J. Cleland-Okine et al. unpublished).

Among the different lignocellulosic by-products tested as substrates for the cultivation of *P. ostreatus*, composted sawdust and rice straw were found to best support growth of the fungus, with the mycelium fully colonising the substrates at 33 and 28 days, respectively. The mycelium density was very thick and dense in the two substrates. In an experiment to evaluate the use of lignocellulosic biomass from coconut palm as substrate for cultivation of *Pleurotus sajor-caju*, [10] the authors reported that the yield of the mushroom is directly related to the spread of the mycelium into the substrate. In the present study, the quantity of mushrooms harvested was significantly ($P < 0.05$) greater in composted sawdust than in any of the other substrates. The superiority of the two substrates was also evidenced in their BE, with composted sawdust showing 61.04%, and rice straw 50.64%. Variable ranges of BE have been reported when different lignocellulosic by-products were used as substrates. When *P. ostreatus* was grown on fermented coffee pulp, a BE value of 132% was obtained, while 37.7% was recorded on *P. sajor-caju* grown on leaf-sheath of arecanut palm.

Pleurotus spp. are reported to be efficient colonisers and degraders of lignocelluloses [7]. The fungus accomplishes enzymatic degradation of the lignocellulosic portion of substrates by using enzymes such as endoglucanase, β-glucosidase, xylase, laminarinase, laccase and polyphenol oxidase that are involved in the degradation of lignocellulose [8]. The positive relationship obtained in the present study between mushroom yield and cellulose ($r^2 = 0.64$) and lignin ($r^2 = 0.70$) contents revealed that these components are an important factor for fruit body formation. Cellulose-rich organic materials were reported to be good substrates for the cultivation of mushrooms. Cellulase production was positively correlated with yield of sporophores. Experiments carried out by Xiujin et al. [11], revealed that during fruitbody formation of *P. ostreatus* on cotton

seed hulls there is a significant decrease in cellulose content after the flushing of mushrooms, indicating that more cellulose is used during fruiting. There was no mycelial growth and thus no yield when elephant grass was used. This does not compare favourably with the results of Zhanzi and Zhanhua [13] who recorded high yields of mushroom. Further treatments, such as composting the substrates for varying periods, can improve yield.

The selection of substrate for cultivation of mushroom is largely determined by the abundance and cost of the substrate. The most widely used substrate for the cultivation of oyster mushroom in Ghana is sawdust, but its shortage or unavailability in some areas makes it imperative to find alternative sources. Thus, rice straw, which gave relatively good yields, could be an alternative substrate for mushroom cultivation in rice-growing areas.

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