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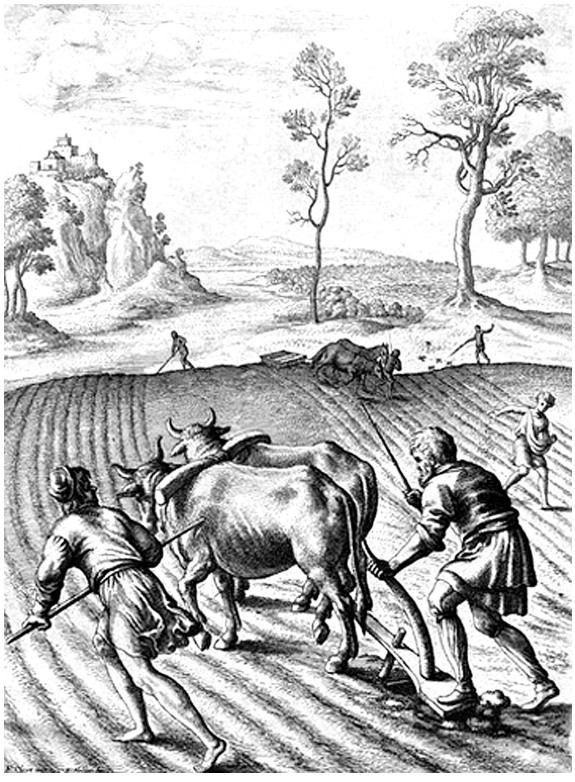

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Liginocellulosic Materials as a Possible Substrate for *Pleurotus ostreatus* (Fr.) Kummer Cultivation

Sopit Vetayasuporn
Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand

Abstract: Four lignocellulosic substrates (sawdust, peat of coconut husk, narrow leaf cattails and bagasse) were used for *Pleurotus ostreatus* cultivation and 3-6 flushes were obtained from these substrates. A bagasse substrate accelerated the mushroom growing processes. The mycelial completed colonization, primordium initiation and fruiting body formation were found within 28, 40 and 44 days, respectively. The sawdust (control) gave the maximum mushroom yield (536.85 g per 1 kg substrate) and this yield was significantly different to those found from bagasse (360.84 g), peat of coconut husk (278.78 g) and narrow leaf cattails (112.10 g) at a confidence level of 95%. Even the mushroom yield obtained from bagasse was lower than sawdust but in term of biological efficiency (BE) value the result achieved from bagasse (103.56%) was slightly higher than sawdust (95.02%). Low BE values were revealed in both peat of coconut husk (56.76%) and narrow leaf cattails (44.67%) and these values were significantly different and two times less than those found in bagasse and sawdust. Therefore, when BE value was taking into account the lignocellulosic substrate likes bagasse has shown great potential for use as a raw material instead of sawdust since this substrate provides an economically acceptable production alternative for *P. ostreatus* cultivation.

Key words: Lignocellulosic, biological efficiency, primordium initiation, *Pleurotus ostreatus*

INTRODUCTION

Pleurotus ostreatus (oyster mushroom; OM) is an edible basidiomycete which widely grow in Thailand. OM has high protein content and has many other constituents, such as Vitamin B1 and B2 and low calory levels. This makes it very popular for consumption among people who are dieting (Bano and Rajarathnam, 1988). *P. ostreatus* is in class Basidiomycetes, subclass Hollobasidiomycetidae, order Agricals. This white rot fungi can decompose and grow well in many lignocellulosic materials. The component of lignocellulosic substrates include mostly cellulose and hemicellulose. Digestion of cellulose produces glucose and cellobiose, while digestion of hemicellulose produces mostly xylose and other sugars, such as galactose, mannose, arabinose, pyranose, plus glucoronic acid and galacturonic acid as secondary products (Albersheim, 1976; Clarke, 1997; Keller, 1993). Since many sugars are released which are converted into sources of carbon when lignocellulosic substrates are digested. Therefore, lignocellulosic substrates are likely be used as substrate for *P. ostreatus* cultivation.

In Thailand, OM s. are cultivated on sawdust, but this raw material is relatively high cost and not available in many places. Moreover, OM cultivation substrate has a problem with contamination of pure mushroom culture from foreign micro-organisms that cause effects on the growth of mycelia, which grow at sub-optimum levels and give low yields. Up to 50% of *P. ostreatus* substrates was contaminated by green fungi in the farmer's mushroom house. Therefore, searching for other more cost effective substrates that are readily available to nearby mushroom farms and reduce the contamination of OM cultivation substrates are the major work of this study. Peat of coconut husk, narrow leaf cattails and baggasse are lignocellulosic substrates which are low-value wastes and widely found in Thailand. Therefore, these lignocellulosic substrates were selected for use as a substrate for OM cultivation. This experiment has the objective of studying the production and growth of *P. ostreatus* when peat of coconut husk, narrow leaf cattails, baggasse and sawdust (control) will be mixed with Effective micro-organisms (EM; pH 3.49), dissolved in water at concentrations of 15%. EM are used to reduce the contamination since one of the main micro-organism found in EM are the group of lactic acid bacteria which are able to produce lactic acid during their growth phase (Higa, 2001). Lactic acid may help to destroy the impure micro-organisms in the OM substrate.

MATERIALS AND METHODS

All the mushroom growing processes were carried out between April to August 2006 in the farmer's mushroom house at Mahasarakham province in the northeast of Thailand. The temperature, relative humidity and ventilation were not controlled.

Preparation of substrate of OM: The composition of the substrate for OM cultivation was as following: lignocellulosic materials (1000 kg); soft rice bran (80 kg); pumice (10 kg); lime (10 kg); gypsum (2 kg) and soaked with Effective Microorganiosms (EM) dissolved in water at concentrations of 15% until suitable of moisture content is gained. The substrate types of lignocellulosic materials were prepared as follows:

- 100% sawdust+15% EM (control substrate)
- 100% Peat of coconut husk+15% EM
- 100% Narrow leaf cattails (2 cm long)+15% EM
- 100% Bagasse+15% EM

Each substrate was mixed thoroughly together and left to stand for 7 days in the shade to allow the EM to breakdown the culture medium. After 7 days, each substrate was put in cylindrical plastic bag without adjusting the moisture content. Cotton wool was used to block the entrance to the OM blocks and then they were tightly sealed with paper before the bags were sterilised. One thousand bags of each substrate were used in this study.

Method of OM cultivation: The sterilized OM culture blocks were spawned with pure OM culture using a sterile method. The room was acclimatized at room temperature until the mycelia were widespread. They were then moved to the farmer mushroom house, the block entrance was opened up; the sorghum seeds were pulled out and left for the large mycelia to develop into OM s. In laying out the cultivation blocks in the mushroom house, the bags were arranged in a Randomized Complete Block Design (RCBD).

Method of irrigation: Each culture medium block was irrigated using tap water with irrigation being done every morning and evening.

Method of data concerning and harvesting OM: Spawn running data and the percentage contamination of the OM substrates were recorded. Moreover, primordium initiation, fruiting body formation and initial moisture content of each substrate before put in cylindrical plastic bag were determined. Bunches/clusters of OM flowers were harvested by pulling them off from the block and weighted. Harvests were started 1-2 weeks after the first primordial emerged. Harvesting was done until full OM culture medium comsumption. At the end of the harvesting period, yield and % BE (Biological efficiency) were calculated. BE is the ratio of kg of fresh mushroom weight per kg dry substrate and counted as a percentage.

Method of Analysis: Analyses was performed to find the percentage contamination of the OM substrates; compare the rate of growth of the mycelia; pin head and fruiting body formation; compare the mean weights and % BE and then data groups were analyzed using SPSS for windows 10.0. Treatment means were compared using Ducan's multiple range test.

RESULTS

Initial moisture content and contamination of the OM substrates: The substrates initial moisture content obtained in this study were ranged between 43-74% and the narrow leaf cattails showed the highest percent of moisture content (74.91%; Table 1). Narrow leaf cattails showed the highest percentage of contamination (15 %) compared to the control (sawdust; 1%) while 7 and 4% were found in peat of coconut husk and bagasse respectively, as shown in Table 1. A contamination of the OM substrates occurred during the spawn running. The OM substrates became green or black in colour when they were infected by the green and black fungi. The experiment found that, growth of spawn running, primordium initiation and fruiting body formation can be occurred in the OM substrates which contaminated with black fungi but these OM substrates are classified as contamination. Therefore, the mushrooms yields obtained from black fungi contaminated substrates were excluded from the analysis data.

Growth of spawn running, primordium initiation and fruiting body formation: The results found that the growth of the *P. ostreatus* mycelia in bagasse and peat of coconut husk were 28 and 29 days respectively, which was quicker than sawdust (34 days) and narrow leaf cattails (40 days; Table 2). The primordium initiation was formed within 2-3 weeks after spawn running. In this study, the bagasse substrate showed the highest mushroom growing processes. The period of spawn running (28 days), primordium initation (40 days) and fruiting body formation (44 days) however these mushroom growing periods were insignificantly different to those found in sawdust alone at a confidence level of 95%.

Comparison of the yield of OM (g/kg of wet substrate), number of flushes and % BE: It was found that the cultivation substrate sawdust gave the highest mushroom yield (536.85 g) and it was significantly different to those

Table 1: Initial moisture content and percentage of *P. ostreatus* contamination

Substrate type	Initial moisture content (%)	Contamination (%)
100% Sawdust+15% EM (Control)	43.50	1
100% Peat of coconut husk+15% EM	50.89	7
100% Narrow leaf cattails+15% EM	74.91	15
100% Bagasse+15% EM	65.16	4

Table 2: Time periods of spawn running, primordium initiation and fruiting body formation

Substrate type	Spawn running (days)	Primordium initiation (days)	Fruiting body formation (days)
100% Sawdust+15% EM (Control)	34.00±4.61^{ab}	56.85±13.76^{NS}	62.55±15.47^{NS}
100% Peat of coconut husk+15%EM	29.00±2.50^{a}	43.99±12.43	49.70±9.42
100% Narrow leaf cattails+15% EM	40.00±2.19^{b}	66.30±13.75	71.00±13.25
100% Bagasse+15% EM	28.00±3.05^{a}	40.25±15.75	44.65±15.40

Mean±SD in each column with different superscripts indicate statisticant differences ($p<0.05$), NS = Not Significantly different

Table 3: Comparison of the number of flushes, % Biological efficiency and yield of *P. ostreatus*

Substrate type	No. of flushes	Biological efficiency (%)	Yield of OM (g kg^{-1} wet substrate)
100% Sawdust+15% EM (Control)	6	95.02±6.01^{a}	536.85±12.93^{a}
100% Peat of coconut husk+15%EM	5	56.76±3.30^{b}	278.78±6.63^{c}
100% Narrow leaf cattails+15% EM	3	44.67±4.10^{b}	112.10±5.94^{d}
100% Bagasse+15% EM	5	103.56±6.35^{a}	360.84±9.02^{b}

Mean±SD in each column with different superscripts indicate statisticant differences ($p<0.05$)

found from bagasse (360.84 g), peat of coconut husk (278.78 g) and narrow leaf cattails (112.10 g) at a confidence level of 95% (as shown in Table 3). Three to six mushroom flushes were obtained from the cultivation substrates and higher mushroom flush was found in sawdust (6 flushes) than bagasse (5 flushes), peat of coconut husk (5 flushes) and narrow leaf cattails (3 flushes). In all cultivation substrates, the percentages of biological efficiency were ranged between 44-103% (Table 3). The percentages of biological efficiency obtained from peat of coconut husk (56.76%) and narrow leaf cattails (44.67%) were significant differences to those found in bagasse (103.56%) and sawdust (95.02%) at a confidence level of 95%. Even the highest percentage of biological efficiency was obtained from bagasse but this percentage was insignificant differences at a confidence level of 95% to those found in control (sawdust).

DISCUSSION

High risk of contamination and differences in fruiting body formation will be obtained in different substrates. The sawdust (control) gave the maximum mushroom yield (536.85 g per 1 kg substrate) and this yield was significantly different to those found from bagasse (360.84 g), at a confidence level of 95%. This may have been caused by the *P. ostreatus* culture showed high efficient in utilizing different sugars that are released from the digestion of sawdust. Controlling the initial moisture of *P. ostreatus* substrate is known to present difficulties, since humidity varies between lots and even within a single lot (Lopez *et al*., 1996; Contreras *et al*., 2004). Even the mushroom yield obtained from bagasse was lower than sawdust but in term BE values the result achieved from bagasse (103.56%) was slightly higher than sawdust (95.02%). This may resulted by initial moisture of substrate since it is probable that with better moisture control, higher BE could be obtained. Moreover, good control of initial moisture content of substrate can limit contamination of pure mushroom culture from foreign microorganisms. Thus, the narrow leaf cattails substrate showed the lowest BE (44.67%) and maximum contamination (15%) might be a result from its structure and high in initial moisture content (74.91%).

For small farmers, cost effective production of *P. ostreatus* depends on the availability and cost of substrates. In Thailand, bagasse is one of the cellulolytic materials that is readily available and more cost effective than sawdust. Based on this study, a short period for complete mycelia colonization, primordium initiation and fruiting body formation was found in bagasse substrate. These results can be explained by the remaining sucrose and other sugars in bagasse are available and suitable for the growth of *P. ostreatus* culture. The source of carbon that the mushroom culture uses for growth is mostly carbohydrate. Therefore, *P. ostreatus* may be easily digested and uses the retaining sugars in bagasse which is a reservoir of carbon directly for growth of spawn and colonization during the generative stage. In final conclusion, it is clearly indicated that the availability, shorter production process time and reduced cost show bagasse to be a cost effective alternative substrate to sawdust in the production of mushrooms.

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