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Bagasse as a Possible Substrate for *Pleurotus ostreatus* (Fr.) Kummer Cultivation for the Local Mushroom Farms in the Northeast of Thailand

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Abstract: A substrate combination of sawdust and bagasse were used for *Pleurotus ostreatus* cultivation, and 6-9 flushes were obtained from these substrates. A substrate combination of 50% bagasse +50% sawdust accelerated the mushroom growing processes. The mycelial completed colonization, primordium initiation and fruiting body formation were found within 22, 27 and 32 days, respectively. Even the 100% sawdust +15% EM solution gave the maximum mushroom yield (536.85 g per 1,000 g substrate) but this yield was insignificantly different to those found from 100% sawdust substrate + tap water (control; 508.98 g), the substrate combination of 75% bagasse +25% sawdust (524.28 g) and 50% bagasse +50% sawdust (494.05 g) at a confidence level of 95%. However, 107.61 and 106.89% of the Biological efficiency values were revealed in the substrate combination of 75% bagasse +25% sawdust and 50% bagasse +50% sawdust, respectively. The substrate combination of sawdust and bagasse has shown great potential for use as a raw material since this mixed substrate provides an economically acceptable production alternative for *P. ostreatus* cultivation.

Key words: Effective microorganisms, contamination, irrigation, *Pleurotus ostreatus*

INTRODUCTION

The Oyster Mushroom (OM) is a mushroom that Thai people know very well. It is becoming increasingly popular for consumption, because it is clean and white, has high nutritional value, has a delicious flower and the flesh is not chewy like other types of mushroom. The OM also has high protein levels, second only to beans and it 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).

In Thailand, OMs are cultivated on sawdust, but this raw material is relatively high cost and not available in many places. Therefore, searching for other more cost effective substrates that are readily available to nearby mushroom farms is the major work of this study. Many cane and sugar industries are located in the northeast of Thailand. Many by-products such as bagasse, filter mud and molasses are obtained from the sugar extraction process. Bagasse is the residual cane fibre which remains after the sugar juice has been separated and its composition consists of moisture 46-52%, fiber 43-52% and soluble solids 2-6%. Presently, bagasse is used as a fuel source for steam, electricity generation and production of paper.

In OM cultivation there are problems with the breakdown of raw materials used in mushroom culture and 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. Hence, in this study, effective micro-organisms (EM; pH 3.49) are used to help in mushroom cultivation because the main micro-organism found in EM are the group of bacteria that produce lactic acid, yeasts, photosynthetic bacteria, actinomycetes and fungi. Because EM are able to reduce the time taken to breakdown the waste products of animals, plants and agricultural residues and are able to produce lactic acid during their growth phase (Higa, 2001), it is therefore EM can be used to breakdown materials used in OM cultivation into minor components. This should have an effect on OM pure culture being able to utilize those products of material breakdown, resulting in better growth, or EM may be able to check and destroy the impure micro-organisms in the pure OM culture.

For the local farmers in the northeast of Thailand, bagasse is one of the cellulolytic materials that is readily available and more cost effective than sawdust. Therefore, bagasse was selected for use as a substrate for OM cultivation. This experiment has the objective of studying the production and growth of *P. ostreatus* when bagasse

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has been used alone or supplemented with saw dust. Furthermore, these substrate combinations will be mixed with EM, dissolved in water at concentrations of 15%, left for 7 days in the shade to allow the EM to break down the OM substrate before autoclaving to sterilize it, and then inoculated with pure *P. ostreatus* culture.

MATERIALS AND METHODS

All the mushroom growing processes were carried out 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 O.M. cultivation was as following: cellulolytic materials (1000 kg); soft rice bran (80 kg); pumice (10 kg); lime (10 kg); gypsum (2 kg) and soaked with Effective Microorganisms (EM) dissolved in water at concentrations of 15 until 60% of moisture content is gained. The substrate types and combination of cellulolytic materials between bagasse and sawdust (W/W) were prepared as follows: a) 100% sawdust, b) 100% bagasse, c) 25% bagasse +75% sawdust, d) 50% bagasse +50% sawdust and e) 75% bagasse +25% sawdust. 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. For the standard control substrate (f) 100% sawdust was mixed with ordinary tap water and left to stand for 7 days. 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 sterilized. 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 OMs. 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 consumption. 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 analyses: 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 % Biological efficiency 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 substrate which contained bagasse had a percentage of contamination (3-5%) higher than sawdust alone (0-1%), as shown in Table 1. A contamination of the OM substrates occurred during the spawn running, and it was found that the OM substrates became green or dark green in colour, with different characteristics from the normal white colour of *P. ostreatus* mycelia.

Growth of spawn running, primordium initiation and fruiting body formation: The experiment found that the growth of the *P. ostreatus* mycelia in substrates which contained bagasse ranged between 22-28 days, which was quicker than control (34 days). The most rapid spawn running was 22 days, which was found on the substrate combination between 50% bagasse +50% sawdust

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

Substrate type	Initial moisture content (%)	Contamination (%)
100% Sawdust + 15% EM	43.50	1
100% Bagasse + 15% EM	65.16	4
25% Bagasse + 75% Sawdust +15% EM	47.87	5
50% Bagasse + 50% Sawdust +15% EM	53.78	4
75% Bagasse + 25% Sawdust +15% EM	51.28	3
100% Sawdust + Tap water (Control)	52.15	0

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

	Spawn running (days)	Primordium initiation (days)	Fruiting body formation (days)
Substrate type	$\bar{X}$ ±SD	$\bar{X}$ ±SD	$\bar{X}$ ±SD
100% Sawdust + 15% EM	34.00±4.61[a]	56.85±13.76[a]	62.55±15.47[a]
100% Bagasse + 15% EM	28.00±3.05[ab]	40.25±15.75[ab]	44.65±15.40[ab]
25% Bagasse + 75% Sawdust + 15% EM	28.00±3.75[ab]	47.85±13.45[ab]	52.30±13.28[ab]
50% Bagasse + 50% Sawdust + 15% EM	22.00±5.15[b]	27.10±11.77[b]	32.40±13.07[b]
75% Bagasse + 25% Sawdust + 15% EM	28.00±4.05[ab]	39.80±12.84[ab]	44.10±13.04[ab]
100% Sawdust + Tap water (Control)	34.00±4.62[a]	49.90±15.23[ab]	53.85±15.19[ab]

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

Table 3: Comparison of the number of flushes, % Biological efficiency and yield of *Pleurotus Ostreatus*

		Biological efficiency (%)	Yield of OM (g kg^{-1} wet substrate)
Substrate type	No. of flushes	$\bar{X}$ ±SD	$\bar{X}$ ±SD
100% Sawdust + 15% EM	6	95.02±6.01[ab]	536.85±12.93[a]
100% Bagasse + 15% EM	5	103.56±6.35[a]	360.84±9.02[c]
25% Bagasse + 75% Sawdust + 15% EM	6	88.10±3.28[ab]	459.24±6.46[b]
50% Bagasse + 50% Sawdust + 15% EM	9	106.89±2.22[a]	494.05±3.41[ab]
75% Bagasse + 25% Sawdust + 15% EM	8	107.61±4.24[a]	524.28±6.89[ab]
100% Sawdust + Tap water (Control)	5	106.37±10.30[a]	508.98±14.61[ab]

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

(Table 2). The primordium initiation was formed within 1-2 weeks after spawn running. In this study, the substrate combination of 50% bagasse +50% sawdust accelerated the mushroom growing processes. The period of spawn running (22 days), primordium initiation (27 days) and fruiting body formation (32 days) were significantly different to those found in 100% sawdust alone at a confidence level of 95%.

Comparison of the yield of OM (g kg^{-1} of wet substrate), number of flushes and % BE: From the experiment it was found that the cultivation substrate of 100% sawdust +15% EM solution gave the highest mushroom yield (536.85 g) when compared to bagasse alone (360.84 g). However, this yield was insignificantly different to those found from the control (508.98 g), the substrate combination between 75% bagasse +25% sawdust (524.28 g) and 50% bagasse +50% sawdust (494.05 g) at a confidence level of 95% (Table 3). Six to nine mushroom flushes were obtained from the mixed cultivation substrate between sawdust and bagasse which were higher than those found from sawdust and bagasse alone. In all cultivation substrates, the percentages of biological efficiency showed insignificant differences at a confidence level of 95% and these values were ranged between 88-107%. Even the highest percentage of biological efficiency was obtained from the substrate combination between 75% bagasse +25% sawdust (107%) but this percentage was similar to those found in control (100% Sawdust + Tap water; 106%).

DISCUSSION

By using EM to breakdown the OM substrate for seven days before sterilization of the mushroom substrate, it was found that the sawdust substrate mixed with EM did not show growth of mycelia, primordium initiation and fruiting body formation to be any faster than those cultures without EM (control). Furthermore, the rate of substrate contamination and wastage of the OM substrate was similar to the control. Even the effective microorganisms contain the groups of micro-organisms that are able to digest organic substances, such as *Tricoderma* sp., *Aspergillus* sp., *Pennicillium* sp., *Streptomyces* sp., *Micromonospora* sp., *Streptosporangium* sp. and *Nocardia* sp. These micro-organisms are able to produce enzymes in the cellulytic enzyme group, such as hemicellulase and cellulase, which can digest rubber tree sawdust and soft rice bran which are the main raw materials used in the OM substrate. The components of sawdust and rice bran 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). When EM digests the OM substrate, different sugars are released which are converted into sources of carbon thus the *P. ostreatus* culture should be easily utilized and show rapid growth of mycelia, primordium initiation and fruiting body formation. However, these results did not concur with the previous controlled experiments at the controlled mushroom house of the Biotechnology Department at Mahasarakham University, in which the use of EM solution to digest the sawdust substrate showed good growth of mycelia and increased production of mushroom yields (Vetayasuporn, 2004). The contradictory results obtained in this study might be caused by the uncontrolled conditions of the processes that took place

(environmental, temperature, relative humidity and air ventilation, light and the presence of many various microorganisms) in the farmer's mushroom house. Since the environmental conditions can not be controlled, the EM may be unable to grow and produce cellulolytic enzymes properly, or the dominant presence of microorganisms nearby the farmer's mushroom house may have had a greater effect in the compost process than the EM.

The substrate ingredient which contained bagasse showed a high risk of bacterial contamination. This could be ascribed to the fact that the remaining sucrose and other sugars in bagasse are available and suitable for the growth of micro-organisms which are responsible for contaminating the OM substrate. However, a short period for complete mycelia colonization, primordium initiation and fruiting body formation was found in the substrate ingredient that contained bagasse. These results can be explained by the *P. ostreatus* culture that can be easily digested and uses the remaining sucrose and other sugars directly for growth. The source of carbon that the mushroom culture uses for growth is mostly carbohydrate, by utilization of hexose molecules, such as glucose, sucrose and lactose. Hexose is converted into mostly glucose-6-phsophate or fructose-6-phosphate before being digested through the process of glycolysis and the tricarboxylic acid cycle (TCA) using energy from carbon dioxide and water (Nielsen, 1996).

In the mixture substrate, 75% of the bagasse combination increased both yield and %BE when compared to the control. The 75% bagasse +25% sawdust combination has the highest % BE (107.61) followed by 50% bagasse +50% sawdust (106.89) and control (106.37). The high % BE that was found in those mixed substrates may be caused by bagasse, which is a reservoir of carbon that the *P. ostreatus* culture easily utilizes during the growth of spawn and colonization of substrates during the generative stage. The ability of *Pleurotus* sp. To grow on agricultural wastes depends on the ligninolytic and other adaptive enzymes of their fungal life cycles (Jennings and Lysek, 1999). Rapid growth and colonization of mycelia may increase the mushroom yield and number of flushes during the fructification stage. 100% sawdust substrate +15% EM solution gave the maximum mushroom yield (536.85 g per 1,000 g substrate). This may have been caused by the *P. ostreatus* culture that can easily utilize different sugars that are produced from the digestion of sawdust. However, this yield was insignificantly different to those found from 100% sawdust substrate + tap water (control; 508.98 g), the substrate combination of 75% bagasse +25% sawdust (524.28 g) and 50% bagasse +50% sawdust (494.05 g) at a confidence level of 95%.

The small farmers cost effective production of *P. ostreatus* depends on the availability and cost of substrates. In the northeast of Thailand, bagasse is one of the cellulolytic materials that is readily available and more cost effective than sawdust. Based on this study, yield and %BE of mushrooms obtained from bagasse were similar to those found from sawdust. Bagasse can be used independently or combined with sawdust for oyster mushroom cultivation. However, rapid days of spawn running, primordium initiation and fruiting body formation were observed in substrates which contained bagasse. In 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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