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The Feasibility of Using Coconut Residue as a Substrate for Oyster Mushroom Cultivation

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Abstract: The effects of two different substrates, sawdust and coconut residue and mixture ratios on oyster mushroom cultivation were determined. The mycelium growth was unable to completely colonized the coconut residue substrates. Percentage of mycelium colonized on the substrate was reduced when percentage of coconut residue supplemented in cultivation substrate was increased. Even the mycelium growth was not totally colonized the coconut residue substrates but the mass of whitish mycelium was thick, dense and comparatively compact when compared to sawdust (control). A substrate combination of 25% coconut residue + 75% sawdust accelerated the mushroom growing processes and gave the highest in both mushroom yield (559.67 g) and percentage Biological efficiency (BE; 109.80%). Even the substrate combination of 25% coconut residue + 75% sawdust gave the maximum mushroom yield but this yield was insignificantly different to those found from 100% sawdust (536.85 g). Least yield (278.78 g) and low BE (56.76%) were revealed from coconut residue alone and these values were significantly different to those found in all cultivation substrates at a confidence level of 95%. For oyster mushroom (*Pleurotus ostreatus*) cultivation, it is clearly indicated that the by-product waste such as coconut residue can be used as a supplementation material with sawdust and the appropriate ratio added should not be exceeded 25%.

Key words: Biological efficiency, supplementation, mycelium, *Pleurotus ostreatus*

INTRODUCTION

Coconut palm (*Cocos nucifera* L.) is a member of the kingdom Plantae, division Magnoliophyta, class Liliopsida, order Arecales, family Arecaceae and it is widely growing in tropical countries. The mature dehusked coconut possesses of 50% wet meat or kernel, 33% shell and 17% water (http://www.aphorticulture.com). The chemical composition of fresh coconut meat comprises approximately 3.33% protein, 33.49% total fat, 15.23% carbohydrate, 3% total fiber, 3.23% total sugar and small amount of minerals and vitamins (www.nutritionanalyser.com). In Thailand, flesh of coconut kernel is a raw material for producing coconut milk. For the local market, fresh coconut kernel is finely grated and hand-squeezed or expeller pressed to produce coconut milk. The coconut residue obtained after the extraction of milk are inedible by-product and small part of them are being utilized as fertilizer or feed for cows. Hence, large quantity of coconut residue by-product after pressing out milk are left to rot on the fields as waste material.

Pleurotus ostreatus (Oyster Mushroom; OM) is edible basidiomycete which can grow naturally on rotten lignocellulosic materials. OM has high nutritional properties, rich in mineral contents and medicinal properties (Bano and Rajarathnam, 1988). Moreover, oyster mushroom has short life cycle, can grow well in many agricultural wastes and less demand on resources and technology (Wang *et al*., 2001; Yildiz *et al*., 2002; Kalmis and Sargin, 2003). Therefore, cultivation of oyster mushroom becomes attractive and commercially produced in the world. Many agricultural by-products, such as plant fibers (Mandeel *et al*., 2005), coconut palm leafstalk (Thomas *et al*., 1998) and sugarcane residue (Singh, 1998; Vetayasuporn, 2006), are being used as substrates for oyster mushroom cultivation. In Thailand, oyster mushroom is cultivated mainly on sawdust. The unavailability and relatively high cost of sawdust makes it imperative that other sources of substrates be utilised for oyster mushroom cultivation.

Since coconut residue obtained after the extraction of milk is by-product waste which are rich in nutrition and are available everywhere without cost. Therefore cultivation of oyster mushroom on this by-products may be one of the solutions to transforming this inedible waste into accepted edible biomass of high market value. This present study aims at the cultivation of oyster mushroom from inedible coconut residue after pressing out milk. The coconut residue after pressing out milk are left to dry by sun light before used. The present observation was undertaken with a view to see the feasibility of utilizing coconut residue as potential substrate for the oyster mushroom cultivation. Contamination, growth and yields of oyster mushroom were determined when coconut residue has been used alone or supplemented with sawdust and compare their growth to the control substrate (sawdust alone).

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 coconut residue by-product: Fresh coconut kernel is finely grated and steeped in hot water. The soaked pieces are hand-squeezed or expeller pressed through cheesecloth to produce coconut milk. This process may be repeated once or twice to produce lighter coconut milk. The coconut residues obtained after the extraction of milk were sun-dried and used as oyster mushroom cultivation substrate.

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

- 25% coconut residue+75% sawdust+15% EM
- 50% coconut residue+50% sawdust+15% EM
- 75% coconut residue+25% sawdust+15% EM
- 100% coconut residue+15% EM
- 100% sawdust + 15% EM (control substrate)

Each substrate was mixed thoroughly together and left to stand for 7 days (composed) 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 hundred bags of each substrate were used in this study.

Method of OM cultivation: The sterilized OM culture bags 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 bag and weighted. Harvests were started 1-2 week 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 analysis: Analyses was performed to find the percentage contamination of the OM substrates; compare the rate of growth of the mycelia per day; compare (%) mycelium colonized on substrate; 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 not much different and ranged between 43-58%. However, the contamination on cultivation substrates have manifested variable levels. Coconut residue cultivation substrates showed higher percentage of contamination compared to sawdust (control). In this study, 100% coconut residue substrate showed the highest percent of contamination (15%) whereas contamination on sawdust was quite low (1%) than other cultivation substrates (Table 1).

Growth of spawn running, primordium initiation and fruiting body formation: The results found that the distances of spawn running of *P. ostreatus* on substrates

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

Substrate type	Initial moisture content (%)	Contamination (%)
25% coconut residue + 75% sawdust	49.03	2
50% coconut residue + 50% sawdust	51.60	9
75% coconut residue + 25% sawdust	58.72	2
100% coconut residue	50.89	15
100% Sawdust (control)	43.50	1

which contained coconut residue were less than control (sawdust). The mycelium growth was unable to completely colonized the coconut residue substrates. Percentage of mycelium colonized on the substrate was reduced when the ratio of coconut residue in cultivation substrate was increased. Sawdust was found to be a good support growth of oyster mushroom, with the mycelium fully colonizing (100%) the substrate at 34 days. However, hyphal growth on sawdust was less profuse than all coconut residue substrates. The percentage of mycelium colonized on coconut residue substrates was 86.11% followed by 64.44, 46.15 and 42.86%, the corresponding substrates being 25% coconut residue +75% sawdust, 50% coconut residue +50% sawdust, 75% coconut residue +25% sawdust and 100% coconut residue, respectively (Table 2). Even the mycelium was not totally colonized the coconut residue substrates but the mass of whitish mycelium was thick, dense and comparatively compact when compared to sawdust.

In this experiment, the lowest and fastest average length of mycelium growth were 0.26 and 0.53 cm day^{-1} which were found in 100% coconut residue and 25% coconut residue +75% sawdust, respectively (Table 2). However, the average length of mycelium growth in 25% coconut residue +75% sawdust was quite similar to those found in control (sawdust; 0.50 cm day^{-1}). In trial of coconut residue substrates, the average length of mycelium growth per days was decreased when increased the ratio of coconut residue. Spawn running on these substrates was stopped after 28-29 days from *P. ostreatus* inoculation and their primordial began appearing approximately 10-14 days after the cylindrical plastic bags were opened. The result obtained in this study found that primordium initiation and fruiting body formation periods in coconut residue substrates were approximately 15 and 25 days faster than control, respectively.

Comparison of the yield of OM (g kg^{-1} of wet substrate), number of flushes and %BE: Five to six mushroom flushes were obtained from the cultivation substrates. In all cultivation substrates, the percentages of biological efficiency were ranged between 56-109% (Table 3). The greatest %BE (109.80%) was seen in the substrate where the yield was the highest (559.67 g). Mushroom yields are proportionally correlated with %BE, the least %BE (56.76%) was found in the substrate where the yield was the lowest (278.78 g). In this work, %BE and mushroom yields obtained from 25% coconut residue +75% sawdust (109.80%) were insignificantly different to those found from control (95.02%) at a confidence level of 95%. However, % BE obtained from coconut residue alone (56.76%) was significant differences to those found in all cultivation substrates at a confidence level of 95% and its mushroom yield (278.78 g) was little more than one-third different from others. The highest mushroom yield was obtained from 25% coconut residue +75% sawdust followed by sawdust (control), 75% coconut residue + 25% sawdust, 50% coconut residue +50% sawdust, 100% coconut residue, the corresponding mushroom yield being 559.67, 536.85, 443.94, 433.98 and 278.78 g, respectively.

Table 2: Average length of mycelium growth (cm day^{-1}), percentage of mycelium colonized on substrate and days from primordium initiation to fruiting body formation

Substrate type	Average length of mycelium growth (cm day^{-1})	Percentage of mycelium colonized on substrate	Days from primordium initiation to fruiting body formation
25% coconut residue +75% sawdust	0.53	86.11	5
50% coconut residue +50% sawdust	0.41	64.44	4
75% coconut residue +25% sawdust	0.31	46.15	6
100% coconut residue	0.26	42.86	6
100% Sawdust (control)	0.50	100.00	6

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

Substrate type	No. of flushes	(%) Biological efficiency	Yield of OM (g kg^{-1} wet substrate)
25% coconut residue +75% sawdust	5	109.80±18.48^{a}	559.67±38.45^{a}
50% coconut residue +50% sawdust	6	89.67±5.28^{b}	433.98±8.51^{b}
75% coconut residue +25% sawdust	6	107.53±3.44^{a}	443.94±4.72^{b}
100% coconut residue	5	56.76±3.31^{c}	278.78±12.93^{c}
100% Sawdust (control)	6	95.02±6.01^{ab}	536.85±12.93^{a}

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

DISCUSSION

Oyster mushrooms are able to colonize various types of lignocellulosic materials but the mushroom yields and %BE are manifested variable and different.

These variations are caused from rate of spawn running, percentage colonization of mycelium and nutrients added on substrate. Experiments have revealed that, mushroom yields were decreased with increasing the ratio of coconut residue into sawdust and the most suitable supplemented ratio for coconut residue was 25%. Moreover, the spawn running on the substrate formulated with coconut residue was stopped after 28-29 days from *P. ostreatus* inoculation and the mycelium extension was unable to completely colonized in all coconut residue substrates. Since coconut meat contains approximately 33.49% of total fat therefore high concentration of oil was increased when large quantity of coconut residues were applied in mushroom cultivation substrate. Hence, high concentration of oil presented in coconut residue substrate may also be factor affecting the mushroom yield and spawn running. The results from this experiment confirm those of Zervakis *et al.* (2001), who found that supplemented oak sawdust and olive press-cake were poor substrates for mycelium extension of *Pleurotus* species. And also, report of cultivation *Pleurotus sajor-caju* on coconut palm substrate showed that yield of the mushroom is directly related to the spread of the mycelium into the substrate (Thomas *et al.*, 1998).

Ginterova and Janotkova (1981) reported that plant oil was found to stimulate the formation of biomass in *P. ostreatus* from Florida and *P. cornucopiae*. Adding growth limiting mineral and nutrients can increased the mycelial growth rate and the degradation of polysaccharide compounds is associated with the fruiting stage (Curvetto *et al.*, 2002; Rajarathnam and Bano, 1988). Rapid growth and colonization of mycelia may increase the mushroom yield and number of flushes during the fructification stage. In trial of substrates formulated with coconut residue, the mass of whitish mycelium was thick, dense and comparatively compact when compared to sawdust alone. In the mixture substrate, 25% of the coconut residue combination increased both yield and % BE when compared to the control. The 25% coconut residue +75% sawdust combination has the highest % BE (109.80) and mushroom yield (559.67 g). The highest % BE and mushroom yield that was found in those mixed substrates may be caused by the most suitable ratio of coconut residue, which is a reservoir of main energy and nutritional ingredients such as carbon, nitrogen, lipid and minerals that the *P. ostreatus* culture easily utilizes during the growth of spawn and colonization of substrates during the generative stage. Increase level of nutrient available in spawn would provide more energy for mycelium growth and development. Therefore, primordium initiation and fruiting body formation of 25% coconut residue +75% sawdust combination were approximately 15 and 25 days faster than control, respectively.

Cost effective cultivation of *P. ostreatus* is depended on the availability, utilization and cost of substrate ingredients. In conclusion, it is clearly indicated that the by-product waste such as coconut residue can be used as a supplementation material with sawdust and the appropriate ratio added should not be exceeded 25%. Even fungal mycelium was unable to completely colonized in substrate combination of 75% sawdust +25% coconut residue but high yield, high %BE and less time consuming were obtained in its mushroom growing processes. Therefore, the substrate combination of 75% sawdust +25% coconut residue has shown the high potential for use as a raw material in *P. ostreatus* cultivation.

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