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Thermophile mushroom cultivation in Cambodia: Spawn production and development of a new substrate, *acacia* tree sawdust

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ABSTRACT: To minimize cultivation costs, prevent insect-pest infestation, and improve the production efficiency of thermophilic mushrooms, plant substrates obtainedfrom local areas in Cambodia were used for production of both spawn and mushrooms. In this experiment, different sawdusts different organic wastes and grain ingredients and analyzed for improvement of spawn-production efficiency. Four thermophilic mushroom species, *Pleurotus sajor-caju* (oyster mushroom, Sambok), *Ganoderma lucidum* (deer horn shaped), Auricularia auricula (ear mushroom), and Lentinula edodes (shiitake), were used to identify efficient new substrates for spawn and mushroom production. Although the mycelia in the rubber tree sawdust medium showed a slightly slower growth rate (10.9 cm/15 days) than mycelia grown in grains (11.2 cm/15 days in rice seeds), rubber tree sawdust appeared to be an adequate replacement for grain spawn substrates. Th findings indicate that rubber tree sawdust, sugarcane bagasse, and *acaciatree* sawdust supplemented with rice bran and calcium carbonate could be new alternative the substrates for . Although sugarcane bagasse and rubber tree sawdust showed similarly high biological efficiencies (BE) of 60% and 60.8%, respectively, *acacia* tree sawdust exhibited relatively a low biological efficiency of 22.4%. However, it is expected that *acacia* sawdust has potential for the mushroom cultivation when supplemented with currently used sawdust substrates in Cambodia, because of its relatively low price. The price of the sawdust (20 kg sawdust= 6500 Riel or 1.6 USD) currently used was 6.5 times higher than the price of *acacia* sawdust (201000 Riel or 0.25)USD). Therefore, utilization for *acacia* sawdust for mushroom cultivation could become feasible as it would reduce by producing costs of mushrooms in rural areas of Cambodia.

KEYWORDS: *Auricularia auricula*, *Ganoderma lucidum*, *Lentinus edodes*, *Pleurotus sajor-caju*, Sawdust spawn, Substrates

Introduction

Mushrooms have been valued throughout the world as both food and medicine for thousands of years. To begin with, mushrooms are exceptionally nutrient-rich foods and contain more than twice the amount of protein as other vegetables and 10% of the daily value for potassium and phosphorus. They are also a good source of important micro-nutrients such as selenium, ergothioneine, copper, etc. In addition to these compounds, mushrooms are rich in vitamins, cholesterol-free, fat-free (United Nations - N.U., 2007) and very low in sodium and calories. Since mushrooms also possess a large quantity of polysaccharides and triterpenes, polyphenol compounds as well as polysaccharides and triterpenes, and over 1,000 other bioactive compounds, they are good candidates as medicinal foods for patients with high blood pressure, diabetes, and obesity etc. Mushrooms also play an active role in modulating and enhancing immune system for human beings (Chang et al., 2004). Besides, mushrooms do not cause any communicable diseases, which is a major public health and socio-

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economic problems in the world.

Mushrooms are an important source of income in rural areas as well in many parts of the world. Mushroom substrates such as agricultural and industrial wastes are cheap or free of charge. The total growing period of mushrooms is approximately 60 days, which is relatively short compared to other agricultural crops. Since it is cultivable all year round without much of climate control, there is a growing commercial demand for the cultivation of edible mushrooms throughout the world(Shah et al., 2004). Currently, only oyster mushrooms and straw mushrooms are being cultivated in Cambodia; and cultivation substrates are very limited. Therefore, study was initiated to develop the cultivation method for thermophile mushrooms, such as *L. edodes*(shitake), *Ganoderma lucidum* (deer horn type), *Auricularia auricula* (ear mushroom), as well as *P. sajor-caju* (oyster sambok). Those mushrooms are easy to grow at 25°C and do not require controlled environmental condition for fruiting body formation. In the first stage, we gathered mushroom spawns of all kinds(*P. sajor-caju*(oyster sambok), *G. lucidum*(deer horn type), *A. auricula* (ear mushroom) and *L. edodes*(shitake). After selecting proper spawn media, we tried to find the most effective mushroom growing substrates (Nasir et al., 2012).

Materials and Method

Mushroom strains

Mycelial cultures were obtained from fruiting bodies of oyster and straw mushrooms via tissue culture. Small pieces of the inner tissue of fruiting bodies was removed with a sterilized scapel and placed aseptically on the Potato Dextrose Agar(PDA) plates and incubated for 7 days. The pure mycelial cultures of the mushrooms were maintained on PDA through periodical sub-culturing for every 10 days. *G. lucidum*(deer horn type) and *A. auricula*(ear mushroom) were obtained from Dept. of Mushroom Science, Korea National College of Agriculture and Fisheries. *L. edodes*(Shiitake mushroom 'Sanjo 701')was obtained from Korea Spawn Center located in Jeonju-si Jeollabuk-do.

Preparation of Media for Mushroom Mycelium

Most mushroom species incubated on PDA (Potato Dextrose Agar) Media. PDA was made with 200 g of sliced potato, 20 g of agar powder and 20 g of white sugar in a liter of water. For sub-culturing of the mushroom species, PDA slant test tubes were used.

Preparation of Spawn

Grain spawn: Wheat, rice seed and sorghum were used. The water content of the grain is around 50% after boiling and drying.

Sawdust spawn: Sawdust spawn consists of 89% sawdust, 10% rice bran, 1% CaCO3 and 65% of water.

Proper spawn media was selected through large test tube (3 cm × 20 cm) experiments: 50 g of spawn media was filled up to 13.5 cm high in a large test tube (3 cm × 20 cm) and sterilized in an autoclave for 20 minutes and mycelium (size of 1.5 cm × 1.5 cm) on a PDA petri-dish was inoculated. The growth rate was observed every four days.

Spawn production : 300 g to 500 g spawn media was placed into a High Density Poly Plastic(HDPP) and autoclaved for 40 min at 121°C. After cooling, the bags were spawned with 1/3 of mycelium in one PDA petri-dish inside the clean bench. They were, then, cultured at 24°C in a dark area for approximately 12days to 2 weeks. *P. sajor-caju* (oyster mushroom, sambok), *L. edodes* (shitake), *G. lucidum* (deer horn type) and *A. auricula* (ear mushroom) mushroom spawn were produced.

Mushroom Cultivation

Cultivation in various substrates and selection of proper substrates : To compare the growth rate, large test tube was used with various kinds of substrates, and mushrooms were cultivated.

Commonly used substrates in this experiment were a combination of 79% of various sawdust (rubber tree, acasia tree, eukalyptus tree, long-zeim tree, perdeer tree, mixed tree sawdust and Korean oak tree sawdust), 20% rice bran, 1% CaCo3 and 60% to 65% water.

To remove wood inhibitors, some sawdust was boiled for 30 minutes and washed. Proper mushroom culture media such as rubber tree sawdust, acasia sawdust and sugarcane bagasse were selected by a large test tube test. Selected substrates were put in the HDPP bags and autoclaved at 121°C, for 40 min. Cooled 1 kg sawdust bags(should be below 30°C) were spawned with 20 g to 25 g of spawn. They were placed in an incubation room for around 4 weeks during the growth period.

Oyster mushroom fruiting formation and harvest: Cultivation bags were opened when mycelium was fully grown. Depending on the mushroom strains used, they required proper temperature (around 24°C to 28°C), aeration for oxygen, at least 500 LUX of light and 90 to 95% of humidity. Around 5 to 10 days after, mushrooms were harvested.

***G. lucidum*(deer horn type) cultivation**: Deer horn mushroom spawn was made the same way as other mushrooms in wheat substrates. This spawn was also inoculated in various mushroom substrates such as rubber tree sawdust, acasia tree sawdust, sugarcane bagasse and oak tree sawdust. Inoculated bags with spawn were cultivated for about 4 weeks in the dark at 25°C, and the lid was opened after mycelium fully grew in a bag. After opening the bag around 10 days later, the fruiting body started to appear. It was grown for about 3 to 4 months.

Results and Discussion

Mycelial growth in various grains and different sawdust spawn media

The average growth was measured after carrying out the same experiment three times. In Table 1, the oyster mushroom showed a relatively fast mycelium growth on grain spawns(13.5 cm/15 days in wheat and 11.2 cm/15 days in rice seed) as compared to sawdust spawn (10.9 cm/15 days), but rubber tree sawdust showed potential as a spawn substrate.

Mushroom spawn production

In Cambodia, most mushroom cultivators use grain spawn such as rice seeds. In this experiment, wheat, sorghum and rice seeds were used. Table 2 shows that oyster mushroom spawn required 7 to 12 days to complete spawn running on wheat and rice seed. Deer horn spawn showed a relatively slow growth on rice seeds so only wheat spawn was used for deer horn spawn. Even though it is clear from the result that wheat is the most effective spawn substrate, it is costly and often unavailable in Cambodia. Therefore, rice seeds were selected for oyster sambock(*P. sajor-caju*) spawn, and wheat for deer horn(*G. lucidum*), and rubber tree sawdust for shitake mushroom 'Sanjo 701'(*L. edodes*). Shitake mushroom 'Sanjo 701' mycelium grew slowly on rubber tree sawdust; It took 4 to 6 weeks for spawn to run completely. Shitake 'sanjo 701' rubber tree spawn (Table 2) has been made and is to be used for mushroom production.

Table 1. Mycelial growth rate of *P. ostreatus* in various spawn substrates

Name of spawn media	Mycelial growth (cm, days)			**Mycelial density in test tube
	5	10	15	
Wheat	3.4	8.2	13.5	++++
Sorghum	3.3	8.5	13.5	++++
Rice seed	3.0	7.3	11.2	+++
*Rubber sawdust	2.9	6.8	10.9	+++
Eucalyptus sawdust	2.2	3.8	6.0	++
Mixed sawdust	No growth	No growth	No growth	***N/A
Oak tree sawdust (control)	2.8	6,7	11.9	+++

*Sawdust spawn media consist of 89% sawdust, 10% rice bran and 1% $CaCO_3$.
**++++ very thick , +++ good ++ thin, + very thin
*** N/A means not available

Table 2. Media used for mycelial culture of mushrooms and time for spawn production

Mushrooms	Spawn media	Time for spawn production
P. sajor-caju (oyster sambok)	Wheat or rice seeds	7 - 12 days
G. lucidum (Deer horn type)	Wheat seeds	12 - 15 days
A. auricula (Ear mushroom)	Wheat or rice seeds	7 - 12 days
L. edodes, (Shitake mushroom 'Sanjo 701')	Oak sawdust Rubber tree sawdust Pseudo-acasia tree sawdust	1 month 4 - 6 weeks 4 - 6 weeks

Oyster mushroom grain spawn is active and efficient, but it is susceptible to pest infestation, and the process for making rice seeds spawn is laborious. The average price of grain substrates is high, and the development of new spawn substrates is in demand. In order to investigate new oyster mushroom spawn substrates, oyster mushrooms were grown on rubber tree sawdust. It took 4 to 5 weeks for spawn to run completely.

The outcome was similar to Sopit's experiment (2006), in which oyster spawn on sawdust required 34 days to run completely. It was also comparable to experiments conducted by Shah et el.'s report (2004), in which the growth of oyster mushroom was completed in 2 weeks respectively. Seeing that oak tree spawn has been widely used and recognized as an efficient spawn substrate in Korea, it would be possible to assume that rubber tree sawdust can act as a suitable spawn substrate.

Oyster mushroom pinhead formation was observed for about 4 weeks after inoculation of spawn. This result is in accord with Quimio(1976 and 1978) who reported that mushroom appeared 3 to 4 weeks after spawn inoculation. Fruiting bodies appeared about 5 to 10 days after pinhead formation.

Selection of sawdust substrates for mushroom production

Nowadays, the price of substrates for mushroom cultivation have increased noticeably in Cambodia. Mushroom cultivators are in urgent need of affordable and efficient local lignocellulose wastes.

Oyster mushrooms were experimented on different locally available sawdust substrates. As can be seen in Table 3, mushroom mycelium that grew on rubber tree sawdust(10.3 cm/15 days) and acasia tree sawdust(9.6 cm/15 days) showed fast growth. As Oei, P., (2005) stated, sawdust and sugarcane bagasse are more reliable substrates for Oyster mushroom production than other agricultural waste substrates. Therefore, rubber tree sawdust, acasia tree sawdust and sugarcane bagasse were selected as mushroom production substrates. Acasia tree sawdust displayed lower biological efficiency than rubber tree sawdust, but considering that the price of rubber tree sawdust is 6,500 Riel or 1.6 USD per 20 kg and acasia tree sawdust is 1000 Riel or 0.25 USD per 20 kg, acasia sawdust is a far more economical option. If acasia tree sawdust is combined with rubber tree sawdust, it could be a high- potential substrate.

Table 3. Oyster mushroom growth in various sawdust substrates using wheat spawn.

Spawn	Substrates	5 days (cm)	10 days (cm)	15 days (cm)
Oyster mushroom wheat spawn	Rubber tree sawdust	3.3	6.8	10.3
	Acacia tree sawdust	3.0	6.6	9.6
	Longzaim sawdust	2.0	4.5	5.8
	Perdeer sawdust	1.6	2.8	4.0
	Eucalyptus sawdust	2.2	4.6	5.0
	Oak sawdust(control)	3.3	6.7	10.0

*Sawdust Substrates is a mixture of 79% Sawdust, 20% Rice Bran and 1% CaCO3

Biological Efficiency of Oyster mushroom production on selected substrates

Oyster mushroom cultivation experiment was done three times, and each time, the mushroom was harvested in three flushes for 6 weeks. The highest yield was obtained from dried rubber sawdust(BE of 60.7%). Sugarcane bagasse substrates(BE of 60.0%) produced almost the same amount of mushroom as rubber tree

Table 4. Total weight of fresh oyster mushroom harvested from different sawdust substrates and biological efficiency on various substrates

Sawdust substate	Harvested fresh oyster mushroom weight(g)				Biological efficiency(BE)	
	1st Expt.	2nd Expt.	3rd Expt.	Average weight	Dry weight of used Substrate(g)	Biological efficiency(%)
Acasia sawdust	306.0	378.0	321.0	335.0	1500	22.4
Bagasse	666.0	645.0	335.0	542.0	900	60.0
Dried rubber sawdust	589.8	664.0	580.0	607.4	1000	60.7

Table 5. Comparison of oyster mushroom harvested from grain spawn and sawdust spawn: total weight of fresh oyster mushroom and biological efficiency of each spawn

Sawdust substate	Harvested fresh oyster mushroom weight(g) on rubber tree sawdust spawn				Biological efficiency(BE)	
	1st Expt	2nd Expt	3rd Expt	Average weight	Rubber tree sawdust spawn	Grain spawn
Acasia	232.00	*NA	NA	232.00	23.2%	22.4%
Bagasse	413.16	319.77	357.06	363.30	36.3%	60.0%
Rubber	429.20	501.00	434.00	482.90	48.3%	60.7%

* N/A means not available

sawdust did.

In an experiment comparing the biological efficiency of grain spawn and sawdust spawn, grain spawn showed higher yield (60.7% on rubber tree sawdust and 60% on bagasse substrate) than rubber tree sawdust spawn.

When rubber tree sawdust spawn was inoculated on different fruiting body substrates (acasia sawdust, rubber sawdust and bagasse), rubber sawdust produced a higher yield of (BE of 48.3%) oyster mushrooms than bagasse substrates (BE of 36.3%). This indicated that when rubber tree sawdust spawn was inoculated on proper sawdust substrate for fruiting, it yielded more mushrooms. This experiment corresponded to a study done by Hami(1990), in which the highest amount of oyster mushrooms were produced on sawdust substrates. Oyster mushroom mycelial growth experiment results in table 1 and these results both imply that rubber tree sawdust could serve a purpose as a spawn substrate in Cambodia, as it is economical and easy to handle.

적 요

이 연구는, 느타리버섯과 풀버섯만 재배하던 캄보디아에서 영지버섯, 목이버섯 그리고 표고버섯 등과 같은 여러 종류의 고온성 버섯들의 종균을 생산하고, 캄보디아에서 생산되는 경제적이고 효율적인 새로운 버섯배지를 찾기 위해 시도되었다. 종균 및 버섯재배 배지로, 여러 종류의 유기물찌꺼기, 곡물, 그리고 톱밥이 사용되었다. 고무나무 톱밥배지에서 자란 균사 (10.9 cm/15일)는 곡물배지에서 자란 균사 (11.2 cm/15일)에 비해 성장이 조금 느렸지만, 고무나무 톱밥은 낮은 가격 및 취급의 용이성이 있어, 비싼 곡물종균을 대치할 수 있는 매우 적절한 배지인 것으로 나타났다.

버섯생산을 위해서, 고무나무톱밥(60.8% BE), 사탕수수박(60% BE), 그리고 아카시아나무 톱밥 등에 미강과 탄산칼슘을 혼합하여 사용하였는데, 사탕수수박과 고무나무톱밥은 매우 비슷한 높은 생물학적 효율(BE)을 보여주었고, 아카시아나무 톱밥은 비교적 낮은 22.4%의 생물학적 효율을 보여주었다. 따라서 낮은 가격에 구입할 수 있는 아카시아톱밥이 현재 사용되고 있는 높은 가격의 배지들과 혼합하여 사용된다면 매우 가능성이 높은 새로운 배지가 될 수 있을 것으로 보였다. 현재, 사용되고 있는 톱밥의 가격 (20 kg 고무나무톱밥=6500 Riel 혹은 1.6 USD)과 비교했을 때, 아카시아톱밥의 가격(20 kg=1000Riel 혹은 0.25USD)이 매우 낮아 버섯의 생산에 투자되는 비용을 크게 줄여 경제성을 높일 수 있을 것으로 사료된다.

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