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Article

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Testing agricultural wastes for the production of *Pleurotus ostreatus*

Thongklang N[1]* and Luangharn T[1]

[1]*Center of Excellence in Fungal Research, Mae Fah Luang University, Chiang Rai 57100, Thailand*

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Abstract

An edible mushroom, *Pleurotus ostreatus* (oyster mushroom) has a worldwide cultivation and is popular in Thailand where rubber sawdust is traditionally used in its cultivation. However, rubber sawdust is a relatively expensive substrate that affects the price of *P. ostreatus* production. Six different grain media were tested for spawn production. The best spawn production was in *Sorghum bicolor* (sorghum) media. Five different agricultural wastes for promoting mycelium growth for spawn production were tested. This was followed by sorghum mixed with corn cobs (16.8317 mm/day), and sorghum mixed with rice husks (11 mm/day). Agricultural wastes for *P. ostreatus* cultivation were also investigated. A comparative study using rubber sawdust, versus rubber sawdust mixed with different supplements of rice straw, rice husks or corncobs to grow *P. ostreatus* was carried out. The fruiting bodies of *P. ostreatus* were produced at room temperature and 70–80% humidity. The first primordia and fruiting bodies of *P. ostreatus* appeared on day 30 after inoculation. The optimal substrate to cultivate *P. ostreatus* is sawdust + rice husks with an average wet weight harvest of 277.50 ± 79.74 g in a 40 day production cycle.

Key words – alternative substrates – basidiomycota – optimal condition – *Pleurotaceae*

Introduction

The genus *Pleurotus* (Fries) Kummer (*Pleurotaceae*, Basidiomycota) has a worldwide distribution and comprises 14 sections and approximately 40 species (Patel et al. 2012, Index Fungorum 2016). The genus is important as it comprises several edible species which also have medicinal properties (Bonatti et al. 2004). Several *Pleurotus* species are used as dietary food due to their high fiber, protein and mineral content (Reis et al. 2012). These mushrooms produce soluble polysaccharides, phenolics, ascorbic acid, lectins and ergothioneine and in addition to their pharmaceutical properties, these have anti-tumor, anti-oxidant and anti-microbial activities (De Silva et al. 2013, Yang et al. 2013, Mishra et al. 2013, Liang et al. 2013, Deepalakshmi & Mirunalini 2014).

In Thailand 22 mushroom species are cultivated commercially and many are species of *Pleurotus* i.e, *Pleurotus giganteus*, *P. ostreatus*, and *P. eryngii* (Thawthong et al. 2014). In addition, *P. giganteus* could be domesticated and introduced (Klomklung et al. 2012). *Pleurotus ostreatus* (oyster mushroom) is known as *Hed Nanglom* in Thai. The mushroom has a high potential for cultivation as it has nutritional and medicinal properties for example anti-cancer, anti-oxidant, anti-tumor, anti-diabetic, anti-hypercholesterolic, anti-arthritic and antimicrobial properties (Bauerova et al. 2009, EI-Fakharany et al. 2010, Weng et al. 2010, Ghaly et al. 2011, Wu et al. 2011, Mirunalini et al. 2012, Vamanu 2012, De Silva et al. 2013, Patra et al. 2013, Devi et al.

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Corresponding Author: N. Thongklang – e-mail – fah_naritsada@hotmail.com

2013). The species is the second most consumed species in the world after the button mushroom (*Agaricus bisporus*) (Sánchez 2010) and is also popular in Thailand. Sorghum is commonly used in spawn media for mushroom cultivation, however there are many other substrates used instead of sorghum for example kurakkan, maize, paddy, brown rice, yellow corn, wheat, and millet (Pathmashini et al. 2008, Hoa & Wang 2015). Likewise, rubber sawdust most common substrates used for cultivation of *P. ostreatus* in Thailand. However rubber sawdust increases the price of production as the price is between 0.34−0.57 US Dollar per kilogram (our observation). There are many other substrates/ agricultural wastes can used for *P. ostreatus* production e.g. cassava peels, cotton seed hulls, coffee husks, wheat straw, barely straw, saw dust and sinar straw (Sánchez 2010, Da Silva et al. 2012, Ajayi et al. 2015, Tesfaw et al. 2015). Agricultural wastes should be explored for alternative cultivation.

The aim of the research was to cultivate *Pleurotus ostreatus* with various agricultural wastes. A comparative study using rubber sawdust, versus rubber sawdust mixed with different supplements of rice straw, rice husk and corncobs to grow *P. ostreatus* was carried out. In addition, substrates for optimal spawn media production were investigated. The results of this study are presented and their implications for industrial production are discussed.

Materials & Methods

Mushroom strains

A pure culture of *Pleurotus ostreatus* was isolated from pileus tissues using sterile forceps by plating on to Petri-dishes with potato dextrose agar (PDA) and incubated at 25 °C for 14 days. The cultures of mushrooms are deposited in Mae Fah Luang University Culture Collection (as MFLUCC 16-0765) and dried fruiting bodies are deposited in Mae Fah Luang University herbarium (MFU herbarium).

Spawn production

In this study six different grain media were obtained from local market in Thailand, *Sorghum bicolor* (sorghum), *Pennisetum glaucum* (L.) R. Br. (millet), *Vigna radiata* (L.) R. Wilczek, Steve Hurst. (mung bean), *Arachis hypogaea* Linn (peanut), *Phaseolus vulgaris* L. (black turtle beans) and *Vigna angularis* (Willd.) Ohwi & Ohashi (kidney bean). The bottles containing 50 g of grains were inoculated with five mycelial plugs of approximately 0.5 cm diam. from the mycelia colony on PDA plates. The cultures were incubated in the dark at 25 °C. The experiment was carried out in five replicates. The best grain medium, sorghum was used to mix with others agricultural substrates. The ingredients of the five substrates used to determine suitable media for promoting mycelium growth for spawn production are presented in Table 1. Each spawn medium was washed and soaked overnight, water was drained off, and boiled for 15 minutes, and left to cool for 20 minutes. Fifty grams of each spawn medium was placed in the tissue culture bottles, autoclaved at 121°C for 15 minutes and left to cool. Before mycelia of *P. ostreatus* was inoculated, the bottles were shaken to prevent clump formation and to displace the 0.5 cm diam. mycelium discs from the upper side of the bottles. Tissue culture bottles were incubated in the dark at 25 °C for 10 days. The linear mycelium length was measured every 2 days for 10 days to calculate the growth rate. All the analyses were carried out in five replicates.

Suitable agricultural wastes for growing Pleurotus ostreatus

Four different formulae were tested for cultivating *Pleurotus ostreatus*. Rubber sawdust was used as the main substrate. The sawdust was mixed (w/w) with rice straw, rice husks and corn cob agricultural waste supplements (Table 2). Rice bran, spent brewery grain, flour, pumice sulfate and calcium carbonate were also added. All substrate supplements were manually mixed with 70% moisture. The mixture (800 g) was packed into polypropylene bags then capped with a plastic ring and lid. The sawdust bags were sterilized at 121 °C for 15 minutes or at 90–100 °C for 3 hr. After the temperature cooled to 25 °C, spawn was inoculated to the sawdust bags, and appropriately 5 g

Table 1 The composition of spawn media.

Nutritional reagents	The composition of spawn media (g/bottle)				
	Sorghum	**Sawdust**	**Corn cobs**	**Rice straw**	**Rice husks**
Sorghum	50				
Sorghum+Sawdust	25	25	-	-	-
Sorghum+Corn cobs	25	-	25	-	-
Sorghum+Rice straw	25	-	-	25	-
Sorghum+Rice husks	25	-	-	-	25

Table 2 The ratio of experimental substrates (w/w) for cultivation of *Pleurotus ostreatus*.

Treatments (w/w)	Quantity/Bag (g)
Sawdust	800
Sawdust + Corn cobs (1:1)	800
Sawdust + Rice straw (1:1)	800
Sawdust + Rice husks (1:1)	800

of mushroom spawn were inoculated into the sawdust bags under aseptic conditions. The bags were incubated in the dark in the mycelial running room temperatures (25±1 °C), at 60–75% relative humidity. All analyses were carried out from four replicates. After the mycelial had completely colonized the bag substrates, they were removed from the shelves and opened at the ends. The bags were maintained at 25±1 °C, 70–80% humidity. Watering was carried out every morning and evening by using a sprayer with tap water until the fruiting bodies had fully developed.

Yield data and statistical analysis

The mycelia growth rate of *P. ostreatus* in spawn culture was determined and the data analyzed statistically in terms of variance of means by using Tukey's test with significant for $P < 0.05$. The fruiting bodies that matured were manually collected, counted and measured daily. The mushrooms were harvested and recorded for 40 days. The data set was analyzed statistically for variance of means by one-way ANOVA analysis by using Tukey's test. Differences were considered significant for $P < 0.05$.

Results

Effect of grain media for spawn production

Optimal growth occurred in the sorghum medium after incubation at 25 °C for 14 days. Sorghum mixed with different agricultural wastes was tested for promoting mycelia growth and spawn production. After 10 days of incubation, *P. ostreatus* mycelium was able to colonize all spawn media and agricultural waste. The data for mycelium growth on different spawn media were investigated and is shown in Table 3. Sorghum mixed with corn cobs had the highest growth rate (16.83 mm/day), followed by sorghum mixed with rice husks (11.07 mm/day), sorghum (8.11 mm/day), sorghum mixed with rice straw (5.27 mm/day) and sorghum mixed with sawdust (4.42 mm/day). Therefore, the results showed that the various supplemented cereal grain media can be used in order to promote mycelia growth of *P. ostreatus*.

Suitable agricultural wastes for mushroom production

The fruiting bodies of *P. ostreatus* were manually harvested and measured daily. The mushrooms were produced at room temperature in 70–80% humidity. Sawdust, sawdust + corn cobs, sawdust + rice straw and sawdust + rice husks were used for the fruiting body production of *P. ostreatus*. We found that the mushroom grew in all media. The first primordia and fruiting bodies of the *P. ostreatus* appeared on day 30 in sawdust + rice husks media after inoculation (Fig. 1). We found that the best substrate was sawdust + rice husks with an average wet weight of 277.50

Table 3 Effect of different types of spawn media on mycelia growth rates (mm/day) of *P. ostreatus*. Values with the same letter are not significantly different ($p < .05$) in the Tukey's test.

Spawn media	Mycelial growth rate
Sorghum	8.11 ± 0.29^{c}
Sorghum + Sawdust	4.42 ± 0.24^{d}
Sorghum + Corn cobs	16.83 ± 0.89^{a}
Sorghum + Rice straw	5.27 ± 0.19^{d}
Sorghum + Rice husks	11.07 ± 0.46^{b}

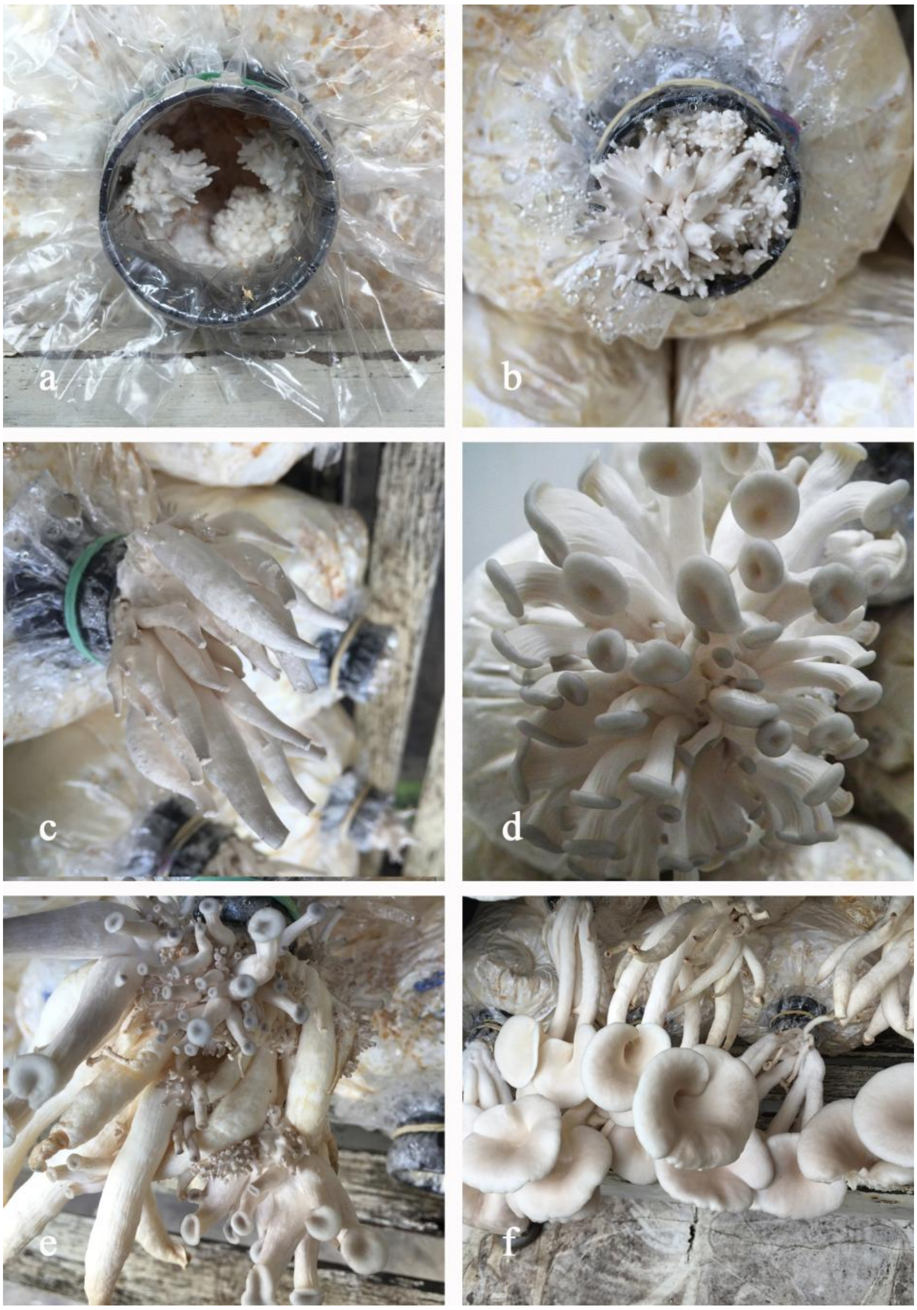

Fig. 1 – The first primordia of *P. ostreatus* appeared on day 30 on sawdust + rice husks media.

± 79.74 g of *P. ostreatus* fruiting bodies in 40 days, followed by sawdust + rice straw, sawdust + corn cobs and sawdust as the wet yields were 262.50 ± 45.73 g, 175.00 ± 64.55 g and 162.50 ± 28.72 respectively in 40 days of production cycle.

Discussion

In mushroom cultivation, sorghum grain is frequently used for spawn production and costs 0.23−0.57 US Dollar per kilogram. However, cereal grains such as red bean, black bean (0.43−0.57 USD per kilogram), mung bean (0.57−0.99 USD per kilogram), soy bean (0.85−1.14 USD per kilogram), kurakkan, maize (0.23−0.43 USD per kilogram), paddy (0.09−0.14 USD per kilogram), corn (0.43−0.57 USD per kilogram), wheat (2.55−3.97 USD per kilogram), barley (1.42−1.70 USD per kilogram) and millet (0.85−1.14 USD per kilogram) could also be used for spawn production (Pathmashini et al. 2008, Klomklung et al. 2012, Sofi et al. 2014). This study confirms that *P. ostreatus* can grow on all types of grain media and grain media mixed with agricultural wastes (i.e. corn cobs, rice husks, rice straw, sawdust). Optimal growth was found in sorghum + corn cobs spawn media after 10 days of incubation. Thus, agricultural waste can be utilized as substrates for spawn production to decrease the price of mushrooms.

Oyster mushrooms can be cultivated on several substrates including paddy straw, maize stalks/cobs, vegetable plant residues and bagasse (Alemu & Fisseha 2015). In our fruiting trials, *P. ostreatus* produced fruiting bodies in sawdust + rice husks, sawdust + rice straw, sawdust + corn cobs and sawdust, indicating that Thai agricultural and industrial waste can be used as substrates for cultivation. Sawdust + rice husks had the highest yield and fastest mycelial growth rate. Obodai et al. (2003) also reported that *P. ostreatus* grew fastest in rice husks bags. Yang et al. (2013) observed that 80% cotton seed hull mixed with 20% wheat bran produced significantly larger pilei, followed by 45% cotton seed hull + 45% rice straw + 10% wheat bran and 40% cotton seed hull + 40% wheat straw + 20% wheat bran. In our study the largest pileus diameter was produced in sawdust, followed by sawdust + corn cobs, sawdust + rice husks and sawdust + rice straw. In both studies the addition of agricultural waste was better than the traditionally used substrates, i.e. sorghum alone for spawn production, sawdust + rice husks gave better yields of fruiting bodies than sawdust alone. Therefore the use of agricultural waste has high potential in mushroom production. Thus, further research on how to increase the production yields on sawdust + rice husks substrates by changing the supplement is necessary.

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