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Recycling of spent *Pleurotus* compost for production of the *Agrocybe cylindracea*

Noonsong V[1], Puttakun N[1], Tinsirisuk M[1] and Seephueak P[1]

[1]*Division of Plant Science, Faculty of Agriculture, Rajamangala University of Technology Srivijava, Thung Yai, Nakhon Si Thammarat, 80240, Thailand.*

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Abstract

Spent *Pleurotus* mushroom compost and rubber sawdust were used as a substrate for a cultivation of *Agrocybe cylindracea*. The five mixed ratios for spawning media were entirely spent mushroom compost, spent mushroom compost and rubber sawdust (ratio 3:1, 1:1 and 1:3 respectively) and entirely rubber sawdust. The comparisons were evaluated on number of days for full colonization of the mycelia, time to first flush, number of basidiocarp and yield on 850 g in plastic bag. The average higher yield of *A. cylindracea* for the formula spent mushroom compost alone were obtained 135.63 g/bag were not significant different when they were compared with rubber sawdust only yield obtained 56.46 g/bag.

Key words – agricultural waste – mushroom – supplement

Introduction

Mushrooms are appreciated for their good taste and nutritional value. The income from edible mushrooms is an important source of revenue for farmers, especially in developing countries. Presently, in Thailand and some Asian countries, the most commonly cultivated mushrooms are oyster mushroom *(Pleurotus* spp.), ear mushroom (*Auricularia* spp.) and straw mushroom (*Volvariella* spp.) (Thawthong et al. 2014). Moreover, other types of mushroom such *Lentinula edodes*, *Lentinus* spp., *Ganoderma* spp. *Macrocybe* spp. and *Agrocybe* spp. can also be cultivated and successfully (Hanko 2001, Know & Thatithatgoon 2004). One of them, *Agrocybe cylindracea* (Brig.) Singer (black poplar mushroom) is an excellent, high quality mushroom with a pleasant aroma (Uhart et al. 2008), delicious taste, and nutritive and healthy values. *A. cylindracea* is normally grown on a wide range of lignocellulosic material the same as is *Pleurotus* spp. (Sánchez 2004). Some have reported of agricultural waste studies as substrate for *Agrocybe,* such as on orange peel and grape stalks (Nicolini et al. 1987), poplar sawdust, barley, wheat straw, reed, rice husks, sunflower, corn cobs (Philippoussis et al. 2001, Uhart et al. 2008, Isikhuemhen et al. 2009).

The substrates used in each region depend on the locally available agricultural wastes (Cohen et al. 2002, Liang et al. 2005). In Thailand, rubber sawdust is the popular basal ingredient used in the main substrate for producing the mushroom. These mushroom sawdust wastes were partially used as organic fertilizer. The mushroom compost contains high percentage of three primary nutrients (nitrogen, N; phosphorus, P or P_2O_5; potassium, K or K_2O) as a fertilizer. Thus a

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Corresponding Author: Pornsil Seephueak – e-mail – spornsil@gmail.com

substantial amount of spent substrate, containing potential nutritional components for additional mushroom production, is generated in the farm of the solid waste (Rinker et al. 2004, Jonathan et al 2011).

The spent mushroom compost could be used to cultivate other edible mushrooms such as *P. sajor-caju* after cultivation of *Lentinula edodes* (Royse 1992), *Coprinus conatus* and *Agaricus bisporus* after cultivation of *Ganoderma lucidum* or *Flamulina velutipes* (Chen 1998, Zhang et al. 2013), *Pleurotus abalonus* or *Auricularia polytricha* after cultivation of *Pleurotus pulmonarius* (Sripheuk 2007), *Pleurotus ostreatus* and *P. florida* after cultivation of oyster mushroom (Ashrafi et al. 2014). However, the production of *A. cylindracea* on spent substrates used to grow *P. pulmonarius* was not reported. This study was designed to investigate the mycelial growth, time to first flush, number of basidiocarp and yield of *A. cylindracea* on a supplemented basal medium of spent *P. pulmonarius* compost.

Materials & Methods

Culture and their maintenance

A culture of *A. cylindracea* was prepared by tissue transplanting technique in laboratory. The culture was multiplied on potato dextrose agar media (PDA) and maintained in glass tubes at 10°C for further studies.

Cultivation technique

Spawn preparation: Sorghum (*Sorghum bicolor*) grain spawn was prepared using the standard method (Siddhant & Singh 2009).

Substrate preparation: The oyster mushroom (*P. pulmonarius*) was cultivated on a rubber sawdust substrate supplemented with 20% palm oil sludge (Seephueak et al. 2014). After the final harvest the spent mushroom compost was reused as the main substrate for *A. cylindracea* cultivation. Before reusing, the spent mushroom compost was mixed with 5% rice bran, 2% $Ca(OH)_2$ and 0.2% $Mg(SO_4)$, on a dry weight basis. Four different formulae of substrates were used to grow *A. cylindracea* in plastic bags compared with the standard formula of rubber sawdust substrate (Sripheuk 2007).

Treatment 1 spent mushroom compost
Treatment 2 spent mushroom compost : rubber sawdust (3:1, w:w)
Treatment 3 spent mushroom compost : rubber sawdust (1:1, w:w)
Treatment 4 spent mushroom compost : rubber sawdust (1:3, w:w)
Treatment 5 rubber sawdust (control)

The substrates were packed in plastic bags (850 g/bag) and the openings of the bag were plugged with cotton and secured with plastic rings. The bags were pasteurized at 100 °C for 3 hours. After the substrates cooled down, they were inoculated with 15-20 grains of sorghum mother culture. The inoculated substrate bags were incubated at room temperate (28-32°C) until the substrates were fully colonized with mycelia, approximately 35 days. The bags were then exposed by opening the top of the plastic bags in a growth room for basidiocarps to fruit out.

The experimental design

The experiment was laid out in a randomized complete block design (RCBD) with 4 replications. The following data were collected; number of days for full colonization of the mycelia, time to first flush, number of basidiocarp and yield. The data were analyzed according to standard method. Means were compared using Duncan's multiple range tests. Biological efficiency was measured using the following formula:

$$\text{Biological efficiency or B.E. (\%)} = \frac{\text{Weight of fresh mushroom basidiocarps}}{\text{Weight of dry substrate}} \times 100$$

Results

Days of mycelial growth

The period for spawn to fully colonize the substrates had significant differences among treatment ($P<0.01$). The periods are shown in Table 1. The period of *A. cylindracea* colonized the substrate of rubber sawdust (control) gave the fastest mycelial growth rate which was 36.05 days followed by the spent mushroom compost mixed with rubber sawdust 1:3, 1:1 and 3:1 w/w which were 40.10, 41.03 and 41.40 days, respectively. Whereas with the use of spent mushroom compost the spawn on the substrate tended to be the slowest mycelial growth rate which was 43.65 days.

Time to the first flush of basidiocarp started 46.50-73.00 days were significant difference among treatments ($P<0.01$). Time to the first flush was according to the number of day for full colonization of mycelia. The first flush on the substrate of rubber sawdust gave the fastest at 46.50 days followed by the substrate of spent mushroom compost mixed with rubber sawdust 1:3 and 1:1 at 53.25 and 54.25 days, and had no significant difference when compared with the spent mushroom compost only substrate, which the first flush was 54.00 days. However, time of the first flush on spent mushroom compost : rubber sawdust (3:1) trended to be the slowest which was 73.00 days (Table 1).

Number of basidiocarps and yields

Number of basidiocarps on 850 g in plastic bag of 60 days harvesting are shown in Table 2 and Fig. 1. The average higher basidiocarp of *A. cylindracea* from the formula spent mushroom compost were obtained 61.00 basidiocarps/bag followed by spent mushroom compost mixed with rubber sawdust 3:1, 1:1 and 1:3 were obtained 42.29, 35.75 and 35.32 basidiocarps/bag, respectively. While, the formula rubber sawdust (control) the average lowest basidiocarps were obtained 31.25 basidiocarps/bag, no difference significant ($P<0.01$).

Average mushroom yields are shown in Table 2 and Fig. 2. The highest yield (135.63 g/bag, B.E. = 40.79%) was obtained on spent mushroom compost. Following spent mushroom compost mixed with rubber sawdust 3:1, 1:1 and 1:3 yields were obtained 111.42, 97.38 and 84.44 g/bag the biological efficiency (B.E.) were obtained 33.51, 29.28 and 25.40%, respectively. Whereas, the lowest yield obtained on rubber sawdust was 56.46 g/bag (B.E. =16.91%), no difference significant ($P<0.01$).

Table 1 Number of day for full colonization of mycelia and time to first flush of *A. cylindracea* on different ratios of spent mushroom compost : rubber sawdust substrates.

Substrate (850 g/bag)	No. of day for full colonization	Time to first flush (days)
1. spent mushroom compost	43.65±0.39 a	54.00 ±1.29 b
2. spent mushroom compost : rubber sawdust (3:1)	41.40±0.42 b	73.00 ±2.16 a
3. spent mushroom compost : rubber sawdust (1:1)	41.03±0.99 bc	54.25 ±1.25 b
4. spent mushroom compost : rubber sawdust (1:3)	40.10±1.42 c	53.25 ±1.29 b
5. rubber sawdust (control)	36.05±0.78 d	46.50 ±1.00 c

Means within a column followed by same letter are not significantly different ($P<0.01$) by DMRT.

Table 2 Number of basidiocarps and yield obtained of *A. cylindracea* on different ratios of spent mushroom compost: rubber sawdust substrate.

Substrate (850g/bag)	No. of basidiocarps	Yield (g/bag)	B.E. (%)
1. spent mushroom compost	61.00±26.84	135.63±73.29	40.79±22.04
2. spent mushroom compost : rubber sawdust (3:1)	42.29±19.11	111.42±42.37	33.51±12.74
3. spent mushroom compost : rubber sawdust (1:1)	35.75±7.73	97.38±34.02	29.28±10.23
4. spent mushroom compost : rubber sawdust (1:3)	35.32±11.70	84.44±29.56	25.40±8.89
5. rubber sawdust (control)	31.25±5.95	56.46±2.61	16.91±0.78

Remark: non- significant at $P<0.01$ probability levels.

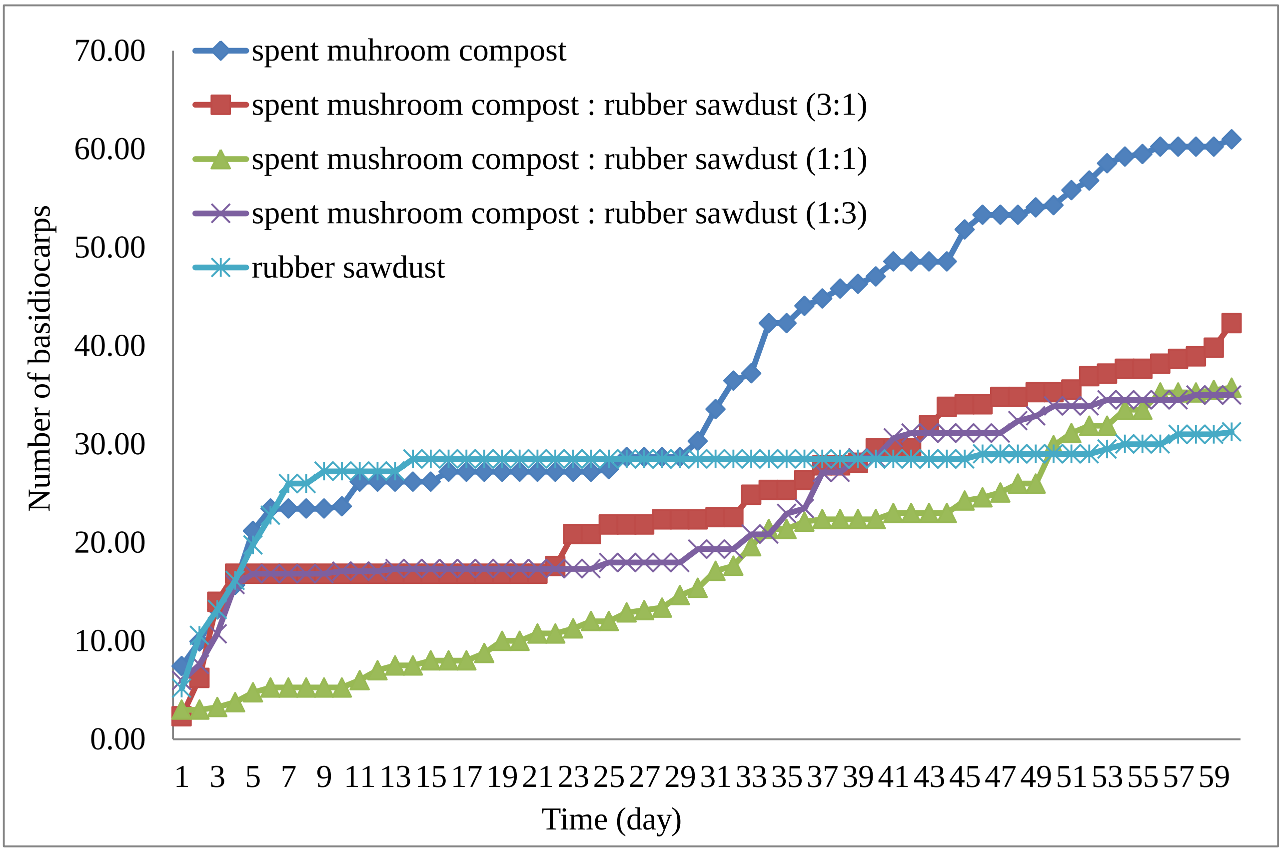

Fig. 1 – Cumulative number of basidiocarps of *A. cylindracea* on different ratios of spent mushroom compost : rubber sawdust substrate, during 60 days of harvesting.

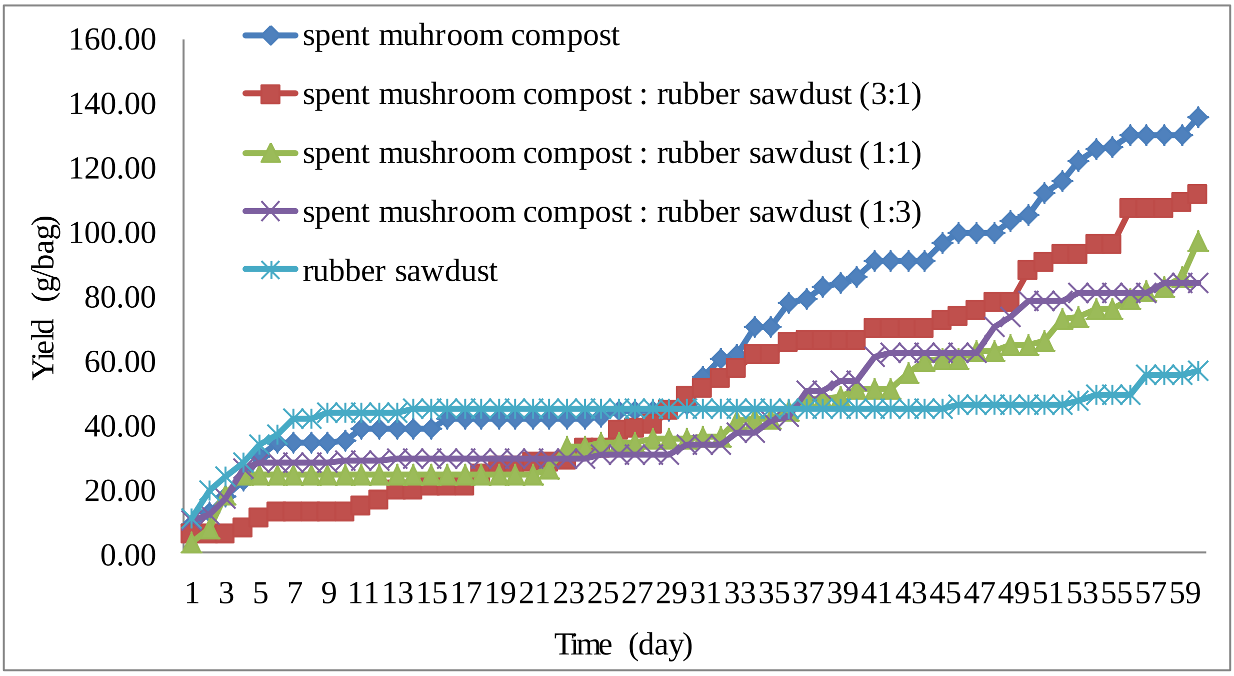

Fig. 2 – Cumulative yield of *A. cylindracea* on different ratios of spent mushroom compost : rubber sawdust substrate, during 60 days of harvesting.

Discussion

In this study, the mycelium growth rate is different depending on the substrate, according to Bilay et al (2000), Obodai et al. (2003), Liang et al. (2005) investigated growth of mycelium of mushroom and reported that the rate depended on the substrate, especially *Agrocybe* mycelium which was easy to reproduce (Jasińska et al. 2011). Stamets (2005) reported that *Agrocybe* is a common mushroom that appears on many species of trees, and the cultivation substrate is mostly chosen according to its availability. Normally, mycelial growth is a preliminary step that creates a suitable internal condition for stated fruiting bodies (Pokhrel et al. 2009). In experiments, among of the five formula substrates for the cultivation of *A. cylindracea*, the rubber sawdust (control) gave the fastest mycelial growth and time to first crop. Whereas spent mushroom compost gave the slowest mycelial growth and time to the first crop, however the yields obtained were reversed. According to Obodai et al. (2003), Liang et al. (2005) reported that period for spawn to fully colonize the substrate does not correspond with mushroom number and yield. For the results indicate that slow spawn running for *A. cylindracea* on rubber sawdust waste might be due to excessive nitrogen and fat contents from palm oil sludge (2.20% Nitrogen and 15.62% Fat) which used as a supplemented in *P. pulmonarius* spent mushroom (Seephueak et al. 2014). According to Baysal et al. (2003) reported that slow spawn running for *P. ostreatus* on paper waste maybe due to excessive nitrogen of chicken manure. Moreover, Royse & Rahler (1988), Jong (1989), Song et al. (1990), Fakoya et al. (2014) reported that high fat content in supplemented had effected to slow spawn running.

Liang et al. (2005) studied using mushroom sawdust wastes for cultivation of *P. citrinopileatus* and the results had shown that the period for spawn to fully colonize had significant difference among treatments use of sawdust of *M. indica* (control) gave the fastest mycelial growth rate which was 15.9 days, followed by use of sawdust mixed with sawdust waste of *P. sajor-caju* (2:1) mycelial growth rate was 18.1 days, whereas use of the substrate of sawdust waste of *L. edodes*, *P. sajor-caju* and sawdust mixed with sawdust waste of *L. edodes* gave the slowest mycelial growth rate which was 29.5-34.3 days. However, the mushroom yield was significantly greater in sawdust supplemented with 50% sawdust waste than in sawdust of *M. indica*, mushroom yield increased 20% and 6% than that of sawdust of *M. indica* only.

The results showed that number and yield of basidiocarp were reverted rate of mycelium growth, according to Obodai et al. (2003), Liang et al. (2005), Jasińska (2011) reported that rapidly of mycelium growth mean high yield and quality of mushroom. Philippoussis & Diamantopoulou (2000) reported that yield of *A. cylindracea* of investigated strains was different and depended on the used cultivation substrate. Zadrazil (1994) recommended that states of better mycelium growth, as well as higher yield of *Agrocybe* can be obtained by increasing inorganic nitrogen content or by addition of protein rich additives in the substrate. Chen (1998) reported that the total nitrogen content in the sawdust waste increased 10% over pre-cultivation for mushroom cultivation. According to this result all formulae with used sawdust waste yields obtained higher than rubber sawdust may be due to spent mushroom compost were supplemented with palm oil sludge which high nitrogen contents.

For the experiment confirms that the spent mushroom compost was used as ingredient substrate in the cultivation of *A. cylindracea*. Where all of them showed highest yield and biological efficiency especially in the set spent mushroom compost alone. In present communication, possibilities of reuse of spent substrate were carried out for the cultivation of various mushroom species. The spent mushroom compost could be used to recultivate other edible mushroom especially *Pleurotus* spp. (Royse 1992, Sharma & Jandaik 1992, 1994, Pardo-Giménez 2012, Ashrafi et al. 2014). Siddhant & Singh (2009) studied recycling of spent oyster mushroom as a substrate for cultivation of *P. florida* strain-P1 and *P. flabellatus* and *P. sajor-caju* strain Malaysia. The result showed that mixed fresh wheat straw with 25% spent mushroom compost showed the highest yield and biological efficiency of mushroom than control. It was obtained 345 g (B.E.= 69%), 565 g (B.E.=113%) and 525 g (B.E.=105%) for *P. sajor-caju*, *P. florida* and *P. flabellatus*, respectively.

Sharma & Jandaik (1985) reported recycling of *Pleurotus* waste for the cultivation of *P. sajor-caju* and found significant yield of *P. sajor-caju* on starch, peptone and wheat bran supplement spent mushroom substrate. Nakaya et al. (2000) reported that recycled *P. cornucopiae* waste for the cultivation of two oyster species viz., *P. cornucopiae* and *P. ostreatus*, and the results showed the high effective yield. However, Siddhant & Singh (2009), Zhang et al. (2013), Ashrafi et al. (2014) suggest that there is a good potential for reuse of spent mushroom for the cultivation of mushroom after supplementation of certain organic and inorganic materials. Liang et al. (2005) studied using mushroom sawdust wastes of *L. edodes* and *P. sajor-caju* for cultivation of *P. citrinopoleatus* the results showed that sawdust mixed with sawdust waste (2:1) obtained the highest yield were 89.6 and 79.2 g/bag.

Zadrazil (1994) suggested that during growth on substrate, mushroom releases humic acid like fractions when added to soil which increase its fertility. In addition, humic substance may affect the reproduction of mushroom. Silva et al. (2002), Campos et al. (2009) reported that growth of mushroom on spent mushroom compost it might be due to increased mineral and protein content in spent mushroom compost which is available to fungus after supplementation. Moreover, Zhao (1993) reported that straw mushroom used a little nutrient from straw, so they were added some manure to ferment again to cultivate *Coprinus* mushroom in a good yield. Beside, some growers cultivated straw mushroom *Volvariella* with spent mushroom compost of button mushroom (Oei et al. 2007).

In Taiwan, mushroom farmers used spent king oyster mushroom substrate mixed with sawdust (1:3 to 3:4) and replace parts of new sawdust to grow king oyster mushroom, oyster mushroom, shiitake (*Lentinula edodes*), almond mushroom (*Agaricus subrufescens*) and shimeji (*Hypsizigus tessellates*), the mushroom yields grown with parts of spent king oyster mushroom substrate are comparable to those grown with 100% new sawdust. Moreover, shiitake grown with one-third of spent king oyster mushroom substrate has a 20% higher yield than those grown without the spent substrate (Taiwan Agricultural Research Institute 2012).

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