International Journal of Agricultural Technology 2017 Vol. 13(6): 883-892
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Cultivation of *Lentinus squarrosulus* and *Pleurotus ostreatus* on Cassava Bagasse Based Substrates

Kupradi, C.[*], Khongla, C., Musika, S., Ranok, A., Tamaruay, K. Woraratphoka, J. and Mangkalanan, S.

Department of Applied Biology, Faculty of Sciences and Liberal Arts, Rajamangala University of Technology Isan, Nakhon Ratchasima, 30000, Thailand.

Kupradit, C., Khongla, C., Musika, S., Ranok, A., Tamaruay, K., Woraratphoka, J. and Mangkalanan, S. (2017). Cultivation of *Lentinus squarrosulus* and *Pleurotus ostreatus* on cassava bagasse based substrates. International Journal of Agricultural Technology 13(6):883-892.

Abstract Cassava is a major food crop in Thailand. A large quantity of cassava waste is produced after processing. In this work, the utilization of cassava bagasse as an alternative substrate for mushroom cultivation was performed. The mycelia growth and yield of *Lentinus squarrosulus* and *Pleurotus ostreatus* on different mixtures of sawdust and cassava bagasse were evaluated. The highest rate of mycelia growth for *L. squarrosulus* and *P. ostreatus* was observed in the control (without cassava bagasse, 100% sawdust). However, yield observed from mushroom cultivation using a mixture of cassava bagasse and sawdust as substrate was higher than that using control. The highest yield of *P. ostreatus* (2.670 kg) and *L. squarrosulus* (1.515 kg) were obtained from 20 bags of substrate containing 25% cassava bagasse after 39 days of mushroom production. The results showed that the potential use of cassava bagasse as adjunct substrate (at 25% of substrate) could boost the yield of mushroom with 2.36-3.29 increase.

Keywords: Cassava bagasse, Sawdust, Mushroom cultivation, *Lentinus squarrosulus*, *Pleurotus ostreatus*

Introduction

Mushroom belongs to the genus fungi which are classified based on edibility and shape of the fruit body. The fungal *Pleurotus* and *Lentinus* genus have been intensely studied and cultivated in many different parts of the world including Thailand. *Pleurotus ostreatus* (oyster mushroom) is an edible mushroom with excellent flavor and taste (Ibekwe *et al*., 2008).

For *Lentinus* spp. mushroom, research on *L. squarrosulus* Mont. has shown antimicrobial activity (Giri *et al*., 2012).

Studies on mushroom cultivation including different strains, different lignocellulosic substrates, different types of spawn, moisture physicochemical

[*] **Coressponding author:** Kupradit, C.; **Email:** lego7823@hotmail.com

conditions are important for the cultivation productivity of each particular mushroom (Sánchez, 2010). *L. squarrosulus* and other species of mushrooms are known to grow on a wide variety of substrates and habitats. This mushroom has been successfully cultivated on cassava peels, rice straw and *Andropogon* straw (Adesina *et al*., 2011). For *Pleurotus*, this mushroom can be cultivated using the largest variety of lignocellulosic wastes including instant coffee pulp, cotton seed hulls, cassava peels, rice straw, corncobs, crushed bagasse, water hyacinth, water lily, bean, wheat straw, oil-palm fiber, paper and cardboard. The fungal contain multilateral enzyme systems that can biodegrade nearly all types of available wastes (Amirta *et al*., 2016).

The fact that mushrooms can be cultivated on a variety of materials, especially agricultural wastes, could be of benefit in term of low cost production system. In Thailand, especially in northeastern province, a huge amount of residual cassava is generated from cassava processing industry. Industrial processing of cassava tubers is mainly done to isolate flour and starch, which generate more liquid and solid residues. Solid residues include brown peel, inner peel, unusable roots, crude bran, bran, bagasse and flour refuse, among which bagasse is the main residue. Cassava bagasse is made up fibrous root material. It contains starch that physical process is could not be extract (40-70%), poor nitrogen content (2.3%) and does not show any cyanide (Soccol and Vandenberghe, 2003). To produce mushroom in low cost systems and zero waste application, cassava bagasse should be used as substrate for *L. squarrosulus* and *P. ostreatus* cultivations. Used of cassava bagasse as an alternative substrate could also solve the pollution problem.

In this research, cassava bagasse was used as substrate mixture for *L. squarrosulus* and *P. ostreatus* production. The effects of different ratios of cassava bagasse and sawdust substrate mixture on mycelia growth and yield of mushroom were investigated.

Materials and methods

Preparation of mushroom mycelium

The pure mycelium of the *L. squarrosulus* and *P. ostreatus* were prepared from spores in a piece of fruiting body mushroom. To obtain the pure mycelium, the small piece of *L. squarrosulus* and *P. ostreatus* from fresh fruiting body were aseptically transferred to Potato Dextrose Agar (PDA). The mycelium was incubated at 30°C for 5 days. The pure mycelium culture of each *L. squarrosulus* and *P. ostreatus* were isolated by transferring the culture to the PDA using sterile cork borer. The pure mycelium of each mushroom was used for spawn preparation.

Preparation of grain spawning

Millet grains were washed and soaked for 24 h in water, and then sieved. Then, the grains were filled halfway into heat resistant bottle and sterilized at 121°C for 45 min. The sterile millet grains in the bottle were cooled down. The 15 mm mycelial discs of each pure mycelium on PDA were transferred to the sterile millet grains in the bottle using sterile cork borer. The cultivations were incubated at room temperature for 14 days.

Preparation of substrate mixtures and bagging

The substrate mixture composed of 0.02 kg $CaSO_4 \cdot 2H_2O$, 0.01 kg $CaCO_3$, 0.003 kg pumice powder, 0.002 kg Na_2SO_4, 0.05 kg rice bran, and 1 kg mixture of cassava bagasse and sawdust. The varying cassava bagasse and sawdust mixtures were prepared at different proportions as shown in Table 1. All ingredients were mixed and water was added to obtain the moisture content about 80%. One kilogram of each substrate mixture was bagged into heat resistant polypropylene bags of dimension 29×9 cm. For each treatment, 20 bags were used. The bagged substrates were then sterilized with moist heat using hot stream at temperature 95-100°C for 4 h and cooling down for 48 h.

Inoculation and cropping

The sterilized substrate bags were inoculated with a few spawn under aseptic conditions. The inoculated bags were transferred to a clean and disinfected incubation room with dark environmental condition at ambient temperature (28-32°C) for approximately 30-40 days to allow ramification of the mushroom mycelia. The length of mycelium growth of each mushroom on each substrate mixtures was recorded. For mushroom production, all full colonization of the bags were transferred to the cropping house where the substrate bags were placed on horizontal shelves. The bags were opened by a slit at the neck for cultivation of *L. squarrosulus* or removing the plastic cork of cultivation for *P. ostreatus*. The humidity in the cropping house was maintained at approximately 80-85% by watering twice a day.

Harvesting

The weights of the complete mushroom from 20 bags of each substrate mixture were recorded every 3-4 days continuously for a period of 6 weeks. Weights of each mushroom from each substrate proportion mixtures were compared.

Table 1. Substrate compositions and codes of the substrate formulas for mushroom cultivation

Substrate code number	Mixture proportion (%)	
	Sawdust	Cassava bagasse
Control	100	0
S75	75	25
S50	50	50
S25	25	75

Results and Discussion

Mycelia growth of L. squarrosulus and P. ostreatus on different substrate mixture

The results of the mycelia growth of *L. squarrosulus* and *P. ostreatus* on each proportion of sawdust and cassava bagasse substrate (Table 1) are shown in Figure 1. The average length of mycelia ramification (cm) for 30 days obtained from 20 substrate bags of each substrate mixture are summarized in Table 2. The highest rate of mycelia growth for *L. squarrosulus* (14.69 ± 0.88 cm) and *P. ostreatus* (14.16 ± 1.06 cm) were observed in control (sawdust 100%) (Table 2). Results on Table 2 indicated that using cassava bagasse as the component mixtures reduce the mycelia growth of both *L. squarrosulus* and *P. ostreatus*. The reduction of mycelia growth of the mushroom is probably because cassava bagasse is a poor nitrogen and protein sources (Pandey *et al*., 2000). Similar results were reported by (Adebayo *et al*., 2009). The researchers found that no mycelia ramification of *Pleurotus pulmonarius* (oyster mushroom) on 100% cassava peels. It might be due to the presence of little or complete lack of some vital nutrients, especially nitrogen, needed for *P. pulmonarius* growth (Adebayo *et al*., 2009). Nitrogen is an important basic nutrient for microorganisms which required for protein, nucleic acid and chitin (component of mycelia cell wall in fungi) synthesis (Adebayo *et al*., 2009). However, mycelia observed from the culture of *L. squarrosulus* and *P. ostreatus* on substrate mixture containing cassava bagasse was very dense (Fig.

1). These results revealed that carbohydrate content in cassava bagasse could support the growth of mycelia of the mushroom.

Table 2. Mycelia growth of *L. squarrosulus* and *P. ostreatus* on each substrate mixtures after cultivation for 30 days

Mushroom	Mycelia growth on each substrate mixture(cm)			
	Control	S75	S50	S25
P. ostreatus	14.16 ± 1.06	9.37 ± 1.79	9.67 ± 1.52	10.09 ± 2.30
L. squarrosulus	14.69 ± 0.88	13.48 ± 1.77	12.36 ± 1.08	10.49 ± 1.94

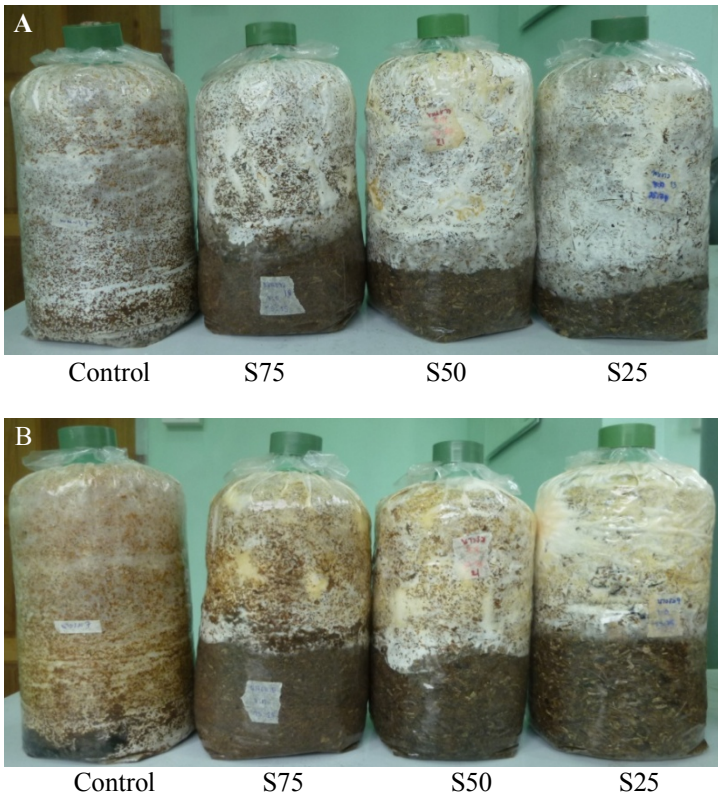

Figure 1. Mycelia growth of *L. squarrosulus* (A) and *P. ostreatus* (B) on each substrate mixtures after cultivation for 30 days

Yield of L. squarrosulus and P. ostreatus production on different substrate mixture

The fruiting body of *L. squarrosulus* and *P. ostreatus* cultured on substrate mixture containing cassava bagasse are shown in Fig. 2. The fruiting body of *L. squarrosulus* and *P. ostreatus* occurred after 8 days of mushroom production on the combine substrate containing cassava bagasse while slower was found on the control (Table 3 and 4). These results indicate that the fruiting bodies of *L. squarrosulus* and *P. ostreatus* cultured on the combined substrate comprising of cassava bagasse and sawdust were formed earlier than that on the control. Results of *P. ostreatus* and *L. squarrosulus* yields (Table 3 and 4) showed that yield of the mushroom combined substrate were higher than that using control (100% sawdust) with 1.08 to 3.29 fold increase. The highest yields (2.670 kg for *P. ostreatus* and 1.515 kg for *L. squarrosulus*) were obtained from S75 substrate mixture after 39 days of mushroom production while the control yeilds only 1.130 kg and 0.460 kg for *P. ostreatus* and *L. squarrosulus*, respectively. These results revealed that *L. squarrosulus* and *P. ostreatus* are able to utilize these cassava wastes. Mushroom are saprophytes or decomposers of organic matter. They convert waste organic matter into food. Fungal mycelium excretes extensive enzymes complexes which can directly attack and degrade the component of cellulose, hemicelluloses and lignin (Sonnenberg *et al*., 2014). This research finding agrees with the finding that cassava waste (stems and peels) can be used for the production of oyster mushroom (Amuneke *et al*., 2011). In the case of cassava peel substrate, mixture substrate containing up to 75% cassava peels has yield productions comparable to the yields obtained on the traditional sawdust based substrates (Amuneke *et al*., 2011). The proximate composition of cassava peel substrate for cultivation of *P. ostreatus* is 54-59% moisture content, 4.8% protein content and 65% carbohydrate content (Sonnenberg *et al*., 2014). This composition in cassava peel should support the mushroom production yield when used as a combination substrate. Adebayo *et al*. (2009) reported that 0.0728 kg of *Pleurotus pulmonarius* was obtained from mushroom cultivation using the substrate mixture comprising of 0.02 kg cassava peel and 0.08 kg cotton waste. These results indicated that cassava peel is the potential substrate adjunct (at 20% of the substrate) for the cultivation of oyster mushroom (Adebayo *et al*., 2009). In case of cassava bagasse, it has been used for mushroom cultivation as reported by other researchers. In 1995, the potential of the cultivation of *Lentinula edodes* (shiitake) mushrooms on cassava bagasse and sugarcane bagasse were evaluated under solid state fermentation (Beux *et al*., 1995). The fructification of mushroom in plastic bag and biological efficiency of the

process was found to be good. Both the substrates were found suitable for mushroom production, but the best results were obtained when a mixture of cassava bagasse (80%) and sugarcane bagasse (20%) was used. Data on kinetics of starch consumption and protein synthesis showed the consumption of 77.42% starch during the biotransformation process, with three times increase in protein content. For cultivation of oyster mushroom, Barbosa *et al.* (1995) also evaluated cassava bagasse and sugarcane bagasse for *Pleurotus sajor-caju* production. The research found that cassava fibrous waste was a high potential source for *Pleurotus* mushroom cultivation. The average production of fresh fruit bodies and the biological efficiency of the process are excellent when 80:20 ratio of cassava bagasse : sugar cane bagasse was used as substrate for cultivation (Barbosa *et al.*, 1995). All available data claimed cassava bagasse to be useful for mushroom cultivation when mixed with other substrates. Cassava bagasse is a fibrous residue, which contains about 50% starch on a dry weight basis. Starch is the main component determined as carbohydrate. Cassava bagasse showed good potential for mushroom cultivation (Pandey *et al*., 2000). Cassava bagasse is readily available and cheap in Thailand. In this research, application of cassava bagasse as a substrate component (25%) could increase yield of *L. squarrosulus* and *P. ostreatus* with 3.29 and 2.36 fold, respectively. Moreover, the use of agricultural waste products in producing edible mushroom would reduce not only production cost but also help recycle agro-waste which could be accumulated and cause pollution problems.

Table 3. Yields (g) of *P. ostreatus* from 20 bags of each different substrate mixture

Time of mushroom production (days)	Yield of mushroom on different substrate (kg)			
	Control	**S25**	**S50**	**S75**
8	-	0.550	0.150	0.590
16	-	0.250	0.200	0.480
19	-	0.050	0.260	0.140
21	0.370	-	0.300	0.110
23	0.100	-	0.300	0.280
27	0.280	0.090	0.090	0.210
29	0.380	0.140	0.580	0.220
32	-	0.210	0.180	0.100
35	-	-	-	0.110
36	-	-	-	0.290
39	-	0.120	0.050	0.140
Total	**1.130**	**1.410**	**2.110**	**2.670**

However, yield of mushroom can vary for different mushroom species, seasons and cultivation regions. Not only substrate mixtures but also season and size of substrate bag can affect mushroom production yield. As suggested by Ediriweera *et al*. (2015), the size of the bag should be increased to obtain a higher yield (Ediriweera *et al*., 2015). Moreover, the yield of oyster mushroom can be improved by addition of nitrogenous supplements (Adebayo *et al*., 2009).

Table 4. Yields (g) of *L. squarrosulus* from 20 bags of each different substrate mixture

Time of mushroom production (days)	Yield of mushroom on different substrate (kg)			
	Control	**S25**	**S50**	**S75**
8	-	-	0.050	0.590
16	0.100	-	-	0.090
19	0.140	-	0.150	0.140
21	0.100	-	0.120	0.180
23	-	-	0.200	0.130
27	-	-	0.210	0.160
29	-	-	-	0.105
32	-	0.120	-	0.060
35	-	-	-	0.060
36	-	-	-	-
39	0.120	0.380	-	-
Total	**0.460**	**0.500**	**0.730**	**1.515**

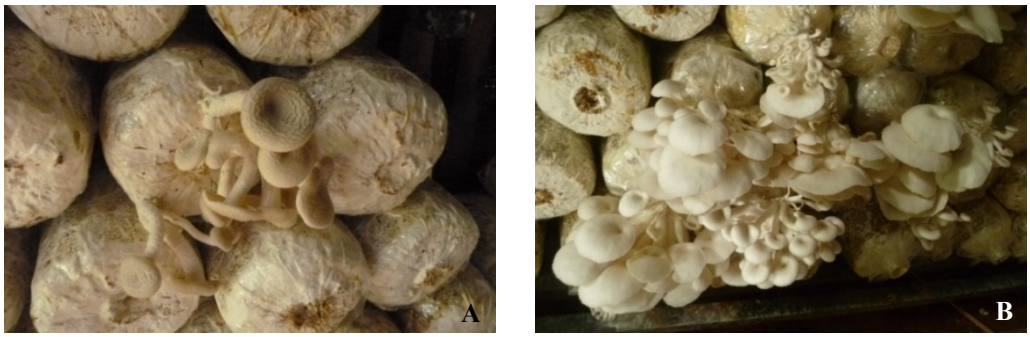

Figure 2. Representative photographs of fruiting bodies of *L. squarrosulus* (A) and *P. ostreatus* (B) grown on substrate prepared from cassava waste

In conclusion of this research, *L. squarrosulus* and *P. ostreatus* can be successfully cultivated by mixing substrates of cassava bagasse and sawdust. The highest rate of mycelia growth for *L. squarrosulus* and *P. ostreatus* was observed in the control while the highest yield of *L. squarrosulus* (1.515 kg) and *P. ostreatus* (2.670 kg) were obtained from the substrate mixture containing sawdust: cassava bagasse at ratio of 75:25 (w/w). The result indicates that

cassava bagasse can be used as substrate (25%) for higher yield in mushroom production. The agro-waste, cassava bagasse, which could be accumulate and cause pollution problems, could be recycled for mushroom cultivation. This way, the cassava bagasse would be useful in the production of a high valued nutrition food. In future work, the various nitrogen sources from agricultural wastes will be used as the supplement with cassava bagasse and sawdust for oyster mushroom production to increase the mushroom production yield.

Acknowledgement

This work was supported by research grant from the Plant Genetic Conservation Project Under the Royal Initiation of Her Royal Highness Princess Maha Chakri Sirindhorn (RSPG) and Rajamangala University of Technology Isan, Thailand. The authors thank the Faculty of Science and Liberal Art, Rajamangala University of Technology Isan for providing some chemical and instruments. Some parts of this work were performed at Conservation Husbandry Board Under Royal Initiatives Project, Nong Ra Wiang, Nakhon Ratchasima, Thailand.

References

Adebayo, G. J., Omolara, B. N. and Toyin, A. E. (2009). Evaluation of yield of oyster mushroom (*Pleurotus pulmonarius*) grown on cotton waste and cassava peel. African Journal Biotechnology 8:215-218.

Adesina, F. C., Fasidi, I. O. and Adenipekun, O. C. (2011). Cultivation and fruit body production of *Lentinus squarrosulus* Mont. (Singer) on bark and leaves of fruit trees supplemented with agricultural waste. African Journal Biotechnology 10:4608-4611.

Amirta, R., Herawati, E., Suwinarti, W. and Watanabe, T. (2016). Two-steps utilization of shorea wood waste biomass for the production of oyster mushroom and biogas-a zero waste approach. Agriculture Agricultural Science Procedia 9:202-208.

Amuneke, E. H., Dike, K. S. and Ogbulie, J. N. (2011). Cultivation of *Pleurotus ostreatus*: an edible mushroom from agro base waste products. Journal of Microbiology and Biotechnology Research 1:1-14.

Barbosa, M. C. S., Soccol, C. R., Marin, B., Todeschini, M. L., Tonial, T. and Flores, V. (1995). Prospect for production of *Pleurotus sajor-caju* from cassava fibrous waste 1st edition. In Roussos, S., Lonsane, B. K., Raimbault, M. and Viniegra-Gonzalez, G. (Eds.), Advances in Solid State Fermentation. Dordrecht: Kluwer Academic Publishers. pp. 513-525.

Beux, M. R., Soccol, C. R., Marin, B., Tonial, T. and Roussos, S. (1995). Cultivation of *Lentinula edodes* on the mixture of cassava bagasse and sugarcane bagasse. In Roussos, S., Lonsane, B. K., Raimbault, M. and Viniegra-Gonzalez, G. (Eds.), Advances in Solid State Fermentation. Dordrecht: Kluwer Academic Publishers. pp. 499-511.

Ediriweera, S. S., Wijesundera, R. L. C., Nanayakkara, C. M. and Weerasena, O. V. D. S. J. (2015). Comparative study of growth and yield of edible mushrooms, *Schizophyllum commune* Fr., *Auricularia polytricha* (Mont.) Sacc. and *Lentinus squarrosulus* Mont. On lignocellulosic substrates. Mycosphere 6:760-765.

Giri, S., Biswas, G., Pradhan, P., Mandal, S. C. and Acharya, K. (2012). Antimicrobial activities of basidiocarps of wild edible mushrooms of west Bengal, India. International Journal of PhamTech Research 4:1555-1560.

Ibekwe, V. I., Azubuike, P. I., Ezeji, E. U. and Chinakwe, E. C. (2008). Effects of nutrient sources and environmental factors on the cultivation and yield of oyster mushroom (*Pleurotus ostreatus*). Pakistan Journal of Nutrition 7:349-351.

Pandey, A., Soccol, C. R., Nigam, P., Soccol, V. T., Vandenberghe, L. P. S. and Mohan, R. (2000). Biotechnological potential of agro-industrial residues. II: cassava bagasse. Bioresource Technology 74:81-87.

Sánchez, C. (2010). Cultivation of *Pleurotus ostreatus* and other edible mushrooms. Applied Microbiology and Biotechnology 85:1321-1337.

Soccol, C. R. and Vandenberghe, L. P. S. (2003). Overview of applied solid-state fermentation in Brazil. Biochemical Engineering Journal 13:205-218.

Sonnenberg, A. S., Baars, J. J. P., Obodai, M. and Asagbra, A. (2014). Cultivation of oyster mushrooms on cassava waste. Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products (ICMBMP8), New Delhi. India. pp. 286-291.

(Received: 7 July 2017, accepted: 30 October 2017)