African Journal of Biotechnology Vol. 10(22), pp. 4608-4611, 30 May, 2011
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB10.627
ISSN 1684–5315 © 2011 Academic Journals

Full Length Research Paper

Cultivation and fruit body production of *Lentinus squarrosulus* Mont. (Singer) on bark and leaves of fruit trees supplemented with agricultural waste

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Accepted 18 March, 2011.

The mycelia growth of *Lentinus squarrosulus* culture on the leaves and bark of common fruit trees were investigated. The effect of supplementing these fruit trees with 25% each of rice bran, horse dung, poultry droppings, cow dung, fresh cassava flour and oil palm waste fiber on the mycelia growth of this fungus was also observed. All the substrates supported the growth of the fungus, while supplemented substrates gave higher mycelia growth. The best mycelia growth was recorded on the leaves of *Spondias mombin* with 8.90 cm length. Among the supplements, rice bran gave the highest mycelia growth of 10.43 cm on *S. mombin*. Fruiting of the fungus on the logs of the best two substrates commenced after 28 days of inoculation and occurred every four days. The highest yield of fruit bodies and the best biological efficiency was obtained on the log of *S. mombin*, while the leaf substrates did not fructify even after 35 days of inoculation.

Keywords: *Lentinus squarrosulus,* supplements, fruit body, biological efficiency.

INTRODUCTION

Mushrooms are cultivated world wide for their taste, nutritional attributes and potential application in industries (Sunagawa and Magae, 2005; Mata et al., 2005). They are useful for the preparation of medical concoctions and drugs (Lim et al., 2004). The importance of mushrooms in bioremediation and biodegradation has been reported by various scientists (Adenipekun and Fasidi, 2005; Estevez et al., 2005).

Lentinus squarrosulus and other species of mushrooms are known to grow on a wide variety of substrates and habitat. However the fact still remains that mushrooms show preference for a particular substratum within a habitat. Many species of *Lentinus* have been reported to live in nature on special substrates and can be cultivated on pasteurized substances (Morais et al., 2000; Philippousis et al., 2001).

In the cultivation of *Pleurotus, Auricularia* and *Lentinus edodes,* the grain mother spawns were prepared on sawdust, which were in turn, inoculated in grain straw before being used as planting spawns (Quimio et al., 1990; Stamets, 1993; Quimio, 2002). Fasidi and Kadiri (1993) found that the best mycelial growth of *Pleurotus tuber-regium* was on the straw of *Andropogon tectorum*, while cotton and peel (pericarp) wastes supported the least. They reported that the highest yield for edible sclerotia was obtained from cotton waste and rice straw, while banana leaves gave the lowest. Kadiri and Kehinde (1999) also reported that mixtures of rice straw (86%) + rice bran (10%) + C_uSO_4 (4%) produced the best planting spawn for *L. squarrosulus* with a view for paving way for possible cultivation of mushroom fruit bodies for consumption.

L. squarrosulus has been successfully cultivated on cassava peels, rice straw and *Andropogon* straw, so also on tree barks of *Spondias mombin.* It was found to have high crude fiber content (Fasidi and Kadiri, 1999). The protein content of this fungus has been reported to be double that of Irish potatoes and six times that of orange (Atikpo et al., 2008), while its essential amino acid content exceeds that of kidney beans. The fact that mushrooms can be cultivated on materials that would otherwise be considered as waste makes it

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Table 1. Mycelia growth of *L. squarrosulus* on fruit tree waste after 7 days.

Fruit tree sample	Leaf substrate (cm)	Bark substrate (cm)
A. occidentalis	6.27±0.15	5.67±0.33
C. sinensis	8.30±0.25	5.20±0.17
M. indica	8.29±0.32	5.50±0.13
P. guajava	6.50±0.00	4.30±0.28
S. mombin	8.90±0.00	5.67±0.76
T. catappa	8.28±0.15	4.27±0.25

Each value is a mean of linear growth diameter of readings ± standard error on the 7th day.

a valuable venture in self sustaining and empowered community we envision in future. The aim of this research is therefore to study the growth and fructification of *L. squarrosulus* on waste leaves, logs and bark of common fruit trees in Nigeria, and the effect of addition of agro waste supplement with these substrates on the growth and fructification of the mushroom.

MATERIALS AND METHODS

Collection of samples

The pure cultures of *L. squarrosulus* used in this work were from the Department of Botany and Microbiology, University of Ibadan. A pure and fresh culture of the fungus was obtained by regular sub culturing on potato dextrose agar (oxoid).

Substrates and their collection

The substrates used for this study were waste leaves, barks and logs of some selected fruit trees such as *Anacardium occidentalis, Citrus sinensis, Mangifera indica, Psdium guajava, Terminalia cattapa* and *Spondias mombin.* Fresh leaves that dropped from the trees of each of the fruit trees were collected from the Nigeria Horticultural Research Institute at Idi-Isin, Ibadan.

Fresh cassava flour, cow-dung, poultry droppings, horse dung, oil palm waste fiber and rice bran used as supplements were obtained from Polo Club stables, Oje market and University of Ibadan farm.

Preparation of fruit tree substrates

The fruit trees substrates were crushed into pieces after which they were prepared according to a modified method of Adenipekun and Fasidi (2003). Each of the substrates were soaked in warm sterile water for 30 min, the water was squeezed out through a muslin cloth and twenty grammes of each was weighed into clean petri dishes wrapped in aluminum foil, autoclaved at 121°C for 30 min and allowed to cool.

On cooling, the substrates were inoculated with 7-days old pure stock culture of the fungus using a sterile 5 mm cork borer and incubated at 30 ± 2°C. The linear growth diameter was measured at 2-das time intervals for a period of one week.

Effect of supplemented fruit tree waste on growth of fungus

This was done according to the modified method of Adenipekun and Fasidi (2003).The different substrates were thoroughly mixed in 3:1 ratio with the supplements. Twenty grammes of the supplemented substrate was weighed into Petri dishes, the Petri dishes were wrapped with aluminum foil and autoclaved at 121°C for 30 min. After cooling, the substrate in each dish was inoculated with a 7 day old pure culture of the fungus with a 5 mm cork borer and incubated at 30 ± 2°C. The linear growth diameter was measured after a period of seven days.

Fruit body production of *L. squarrosulus* on fruit tree logs and leaves

The dried drilled logs of each fruit trees were soaked in sterile distilled water for 24 h after which the excess water on the logs were aseptically drained for 6 h. Five grams of spawn was aseptically inoculated into each of the drilled holes of each fruit tree log. This was done in replicates and kept in sterile feed bags tied at the open end to create a dark incubation environment for 3 days at 30 ± 2°C. After which, the logs were then removed from the bags kept at the same temperature and watered everyday and observed regularly for fruit body formation.

Fruit body production of *L. squarrosulus* on some of the fruit tree leaves

This was done using a modified method of Alofe (1985). 250 g of each of the leaves substrates was measured and logs of the selected fruit tree samples were soaked in 480 ml of warm clean water for 30 min and the excess water was drained out through clean muslin cloth. Each of these substrates was replicated thrice, packed into aluminum containers and sterilized at 121°C for 30 min. On cooling to room temperature, the leaves substrates were decanted to sterile polyethylene bags. Twenty grams of spawn was inoculated in the middle of each substrate and the bags were tied at each of the open end, perforated with sterile inoculating needle, incubated in the dark at 30 ± 2°C for 12 days. They were brought out of the dark and kept in airy environment after 12 days of incubation. The substrates were watered on daily basis and the weight of the emerging mushroom was noted.

RESULTS AND DISCUSSION

Table 1 shows the result of the mycelia growth of the fungus on fruit trees substrates. The best mycelia growth on all the substrates was on *S. mombin.* There was also considerable good growth on *M. indica, C. sinensis and T. catappa* leaves. The fruit trees bark gave lower mycelia growth values as compared to the leaf

Table 2. Effect of addition of supplements with fruit tree substrates on the mycelia growth of *L. squarrosulus.*

Fruit Tree	Supplement effect on mycelia growth (cm)					
	Cowdung	Horsedung	Fresh cassava flour	Oilpalm waste fibre	Poultry droppings	Rice bran
A. occidentalis	6.85^{ab}±0.07	7.4^{ab}±0.00	7.77^{a}±0.15	97^{c}±0.38	6.43^{bc}±0.45	7.93^{a}±0.06
C. sinensis	8.70^{cd}±0.44	9.8^{ab}±0.26	9.30^{bc}±0.19	6.07^{e}±0.70	8.40^{d}±0.10	10.13^{a}±0.29
M. indica	8.73^{b}±0.23	8.96^{b}±0.55	8.0^{c}± 0.17	7.0^{d}± 0.00	7.0^{d}±0.10	9.93^{a}±0.49
P. guajava	6.70^{c}±0.44	7.03^{ab}±0.23	6.90^{b}±0.25	5.47^{d}± 0.23	7.57^{dc}±0.32	7.50^{a}±0.00
S. mombin	9.30^{b}±0.45	9.67^{b0}±0.06	8.40^{c}±0.35	7.8^{d}± 0.28	8.00^{c}±0.00	10.43^{a}±0.12
T. catappa	8.60^{dc}±0.35	9.70^{b}±0.30	9.00^{c}±0.85	7.7^{e}± 0.30	8.00^{d}±0.20	10.30^{a}±0.00

Each value is a mean of linear growth diameter of readings + standard error on the 7th day. Values followed by different letters are significantly different.

substrates. The lowest growth was recorded on the bark of *P. guajava* and leaves of *A. occidentalis*

The results of the effect of addition of supplements with fruit tree leaves on the mycelia growth of *L. squarrosulus* showed that rice bran gave the best mycelia growth as supplement, followed by horse dung and the difference was significant, while the lowest mycelia growth was recorded when oil palm fiber was used as supplement. For some of the substrates supplemented with oil palm fiber, the mycelia diameter was even lower than the one recorded on the substrates when supplements were not used (Table 2). *S. mombin* has the highest mycelia growth with all supplements. Mycelia growth was generally enhanced when supplements were used, except when oil palm fiber was supplemented with the substrates and when poultry dropping was supplemented with *M. indica, S. mombin and T. catappa* leaves.

Table 3 shows that fruit body formation of *L. squarrosulus* was initiated on the logs of *S. mombin* and *C. sinensis* after 28 days of inoculation with spawn. Flushing was noticed on the leaves of both fruit tree substrates; no fruit body was produced on these substrates but mycelia ramification was very dense on the 35th day of inoculation. The logs of the fruit trees produced fruit bodies but the logs of *S. mombin* had the highest yield of fruit bodies (12 fruit bodies).

Table 1 shows that generally, mycelia growth was higher on fruit tree leaves than on fruit tree barks. This may be due to the complexity of the tree bark as compared to the leaves. Thus, the nutrients in the fruit tree leaves were more accessible for the fungus than those in the bark. Of the fruit tree substrates used, *S. mombin* supported the best mycelia growth, this agrees with the result of Fasidi and Kadiri (1991). They attributed it to the high availability of fructose which is the main sugar source for the fungus in *S. mombin* as compared to the other substrates and fructose is the main sugar source for the fungus (Fasidi and Kadiri, 1991).

From Table 2, among the supplements used along with the substrates, rice bran supported the best mycelia growth followed by horse dung. This is also in agreement with Fasidi and Kadiri (1991). Horse dung had been reported to boost compost nutrient composition thus supporting better mushroom yield (Meire et al., 2007). Adenipekun and Fasidi (2003) also recorded best mycelia growth of *Pleurotus sajor-caju* on horse dung supplemented with cassava peels. The lowest mycelia growth was recorded on oil palm fiber and poultry droppings. The low growth on poultry droppings may be due to the presence of ammonia which has been reported to be a respiration and mycelia growth inhibitor since it would not be completely removed at the incubation temperature of 30°C (Ross and Harris, 1982; Kadiri and Kehinde, 1999; Mshandete and Cuff, 2008). Lim (1981) found that *Volvariella* can be successfully cultivated with rice bran as supplements on horse dung. Oil palm fiber may contain complex lipids which may hinder easy access of the fungus to simpler carbon sources, thus, the low mycelia growth recorded on it.

From Table 3, the fruit tree leaves were unable to support fructification of the fungus, while the logs did. Alofe (1985) was also unable to fructify *L. squarrosulus* and *Volvariella esculenta* on rice straw and horse dung supplemented banana and pawpaw leaves. Kadiri (1991) however was able to produce 12 fruit bodies in 2 flushes using *L. squarrosulus* on *Andropogon tecturum* straws supplemented with horse dung. On *S. mombin* log + rice bran supplemented spawn, flushing began on the 23rd day up to the 28th day and the high fruit body yield of this fungus in a short time is an advantage for obtaining maximum yield in a short time, thus, ultimately lowering the cost of production (Mshandete and Cuff, 2008).

Conclusion

This study shows that *L. squarrosulus* can be successfully cultivated in the laboratory on fruit tree waste, especially the leaves of *S. mombin and C. sinensis*. The fungus can successfully fructify on the logs of *S. mombin and C. sinensis*, while the spawns for cultivating the mushroom can be obtained by inoculating dry *S. mombin* leaves which are readily available and cheap on vine plantations. Production of this spawn can also be enhanced

Table 3. Mycelia growth, fructification, yield and biological efficiency of *L. squarosulus* on logs and leaves

Substrate	Days				
	15	23	28		
			Fruit body Yield (g/kg waste)	Biological efficiency	No of Fruit bodies
Control	Growing	Death			
S. mombin log + r. b. s. s.	Very good growth	Flushing begins	20.50	10.25	12
S. mombin leaves + r. b. s. s.	Very good growth	very dense ramification	-	-	-
C. sinensis log + r. b. s. s.	Very good growth	Flushing begins	11.70	5.85	4
C. sinensis leaves + r. b. s. s.	Growth	-	-	-	-

r. b. s. s.: Rice bran supplemented spawn; -: means no growth. Control consisted of fruit tree logs and leaves inoculated with fungus mycelium.

by using agricultural waste like rice bran and horse dung as supplements.

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