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Enriched cultivation of *Lentinus squarrosulus* (Mont.) Singer: A newly domesticated wild edible mushroom in the Philippines

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Abstract

Lentinus squarrosulus (Mont.) Singer is a tropical white rot macro fungus that belongs to family Polyporaceae. In order to domesticate this wild edible species, the influence of indigenous culture media, physical conditions (pH, aeration, illumination and temperature) and grains spawn materials were evaluated. Moreover, the fruiting body performance was evaluated on enriched rice straw and sawdust based substrate formulations. The mycelia grew best on potato sucrose gelatin with a pH of 6.5−7.0 cultured in sealed plates incubated in alternating light and dark condition at room temperature (32 C). Both *Sorghum bicolor* grain and crack *Zea mays* grain produced a very luxuriant mycelial growth with the shortest incubation period of 6 days at 31.5 C ± 0.55. The recorded incubation period for the fruiting spawn took 16 days to fully ramify the substrate. The highest number of fruiting bodies (9.33 ± 1.35), (18.33 ± 6.62), mean weight (90 g ± 16.67), (90 g ± 29.06) and biological efficiency (18%) were obtained in substrates supplemented with 15% rice bran and 20% rice hull respectively. The substrate supplemented with 5% rice hull registered the longest length and widest diameter of stipe with means of 43.01 mm ± 5.09 and 7.24 mm ± 0.75 correspondingly. The largest pileus (71.92 mm ± 2.69) was recorded in substrate with 20% rice hull. However no significant differences among the different treatments were noted.

Key words – basidiocarp – biological efficiency – enriched cultivation – optimum condition

Introduction

Mushrooms are natural recycler of agricultural wastes because they can convert lignocellulosic materials into protein rich healthy food (FAO 2002). Their flavor, texture and nutritional value provide an excellent food source (Mata et al. 2005). In the Philippines, edible mushrooms particularly paddy straw mushroom (*Vovariella volvacea*) and oyster mushroom (*Pleurotus* spp.) are now cultivated commercially in different parts of the country (Reyes et al. 2009b). However, aside from these mushroom species, there are a number of wild edible mushrooms which can be cultivated because of their potential use in the culinary and nutraceutical industry (Sunagawa and Magae 2005, Mata et al. 2005).

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Lentinus squarrosulus is a wild edible mushroom which is commonly found growing in the wild on decaying logs of trees during rainy season. Similar to other species of macrofungi, this mushroom can grow on a wide variety of substrates and habitats. Many species of *Lentinus* have been reported to grow in nature on special substrates and can be grown on pasteurized substrates (Morais et al. 2000, Philippousis et al. 2001). Recently, *L. squarrosulus* was found growing in Zambales, Philippines (De Leon et al. 2013c). This mushroom species has not been cultivated on a large scale for the production of fruiting bodies (Mhd Omar et al. 2011). The tough fruiting body of this mushroom is rich in proteins, sugars, lipid, amino acids, vitamin B, C, and D, and minerals (Royse et al. 1990). It has been reported that liquid fermentation of mushrooms in general, produces large amounts of uniform mycelial biomass as a source of bioactive compounds (Mhd Omar et al. 2011).

The tropical climate of the Philippines favors the growth of many wild species of macrofungi, e.g. *Schizophyllum commune* Fr. (Bulseco et al., 2005), *Coprinus comatus* (O.F.Mull.:Fr) Pers. (Reyes et al. 2009b), *Collybia reinakeana* P. Henn. (Reyes et al. 1997), *Lentinus sajor-caju* Fr. (Cuevas et al. 2009), *Lentinus tigrinus* (Bull) Fr. (Dulay et al. 2012a), including *L. squarrosulus* (De Leon et al. 2013b). Moreover, there are many agricultural and industrial wastes that can be used in mushroom cultivation. Also, many mushroom species have potential uses and if cultivated, these wild edible fungal species can also be used to generate livelihood among local communities to ensure food security (Reyes et al. 2009a). Although many mushroom species can be successfully cultivated through the use of rice straw and sawdust based substrate formulation developed by the Center for Tropical Mushroom Research and Development, Central Luzon State University, addition of supplements might substantially increase the yield per unit weight of this mushroom. Therefore, this study was undertaken to investigate the optimum condition for mycelial growth and fruiting body performance of *L. squarrosulus* on rice straw based substrate formulation supplemented with rice bran and rice hull.

Materials & Methods

Mushroom collection

Pure culture of *Lentinus squarrosulus* strain ZB12MF02 was obtained from the culture collection of the Center for Tropical Mushroom Research and Development (CTMRD), Department of Biological Sciences, College of Art and Sciences, Central Luzon State University. This culture was collected from the Aeta communities in Zambales and optimized by De Leon et al. (2012, 2013b,c). The strain was inoculated into the potato dextrose agar (PDA) plates. The culture was covered with parafilm then it was incubated at 27 C until the plates are fully ramified with the mycelia.

Study I. Nutritional Requirements for the mycelial growth of Lentinus squarrosulus

The mycelial growth performance was evaluated using different indigenous culture media: potato sucrose gelatin (PSG), rice bran decoction gelatin (RBDG), crack corn decoction gelatin (CCDG), and coconut water gelatin (CWG). In the preparation of PSG, RBDG and CCDG, one liter in separate decoctions of potato, rice bran, yellow corn grits, were added with 20 g each of shredded white gelatin bars and 10 g of sucrose. To prepare CWG, one liter of coconut water was filtered using cheese cloth, boiled and added with 20 g of shredded gelatin. The newly prepared indigenous culture media were sterilized at 121 C or 15 pounds per inch (psi) for 15 minutes. After sterilization decoctions were pour plated in sterile petri plates. Then, from the revived culture, 10 mm diameter mycelial disc was inoculated centrally on the prepared indigenous culture media. The inoculated plates were incubated at room temperature 31.5 C ± 0.55.

Study II. Physical Requirements of Mycelial Growth

pH Level

The most appropriate culture medium that was obtained in study I, were adjusted to different pH levels: 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 using 0.1 M NaOH and 0.1 M HCl. The

culture medium with different pH levels was aseptically inoculated with approximately 10 mm fungal disc to allow the ramification of mycelia.

Aeration

The lid of the Petri plates inoculated with *Lentinus squarrosulus* were sealed with parafilm to prevent entrance of air into the petri plates and the other group of inoculated petri plates was not sealed. The plates were incubated at room temperature to allow the full ramification of the mycelia.

Illumination

One group of the inoculated petri plates was expose to artificial light (fluorescent light) until fully ramified. The other group of inoculated petri plates was covered with carbon paper. While the last group which served as the control was the inoculated plates that are incubated in a normal light and dark condition. All treatments were incubated to allow the full ramification of mycelia.

Temperature

The inoculated plate was placed in different temperature condition such as 32, 15 and 40 C. The temperature was monitored using a thermometer. All the inoculated plates (in triplicates) were incubated to allow full ramification of its mycelia. The daily mycelial growth in diameter of *L. squarrosulus* was measured using a vernier calliper until full ramification in all the treatments of study I and II.

Study III. Evaluation of Different Materials for the Mother Spawn Production for Lentinus squarrosulus

Preparation, Sterilization and inoculation

This study evaluated the mycelial growth of *L. squarrosulus* on *Sorghum bicolor*, crack *Zea mays* and unmilled *Oryza sativa* seeds for the production of *L. squarrosulus* mother grain spawn.

Each grain was boiled until tender. Dried until 60% humidity was attained. The grain was dispensed in clean glass bottles. The bottled substrates were plugged with cotton and wrap with aluminum foil, then, it was sterilized at a pressure of 15 psi for 45 minutes. Once cooled, the sterilized bottled substrates were inoculated with 10 mm diameter mycelial disc of *L. squarrosulus*. This was incubated at room temperature (32 C). The spawn which is fully ramified in the shortest period of incubation time and has thickest mycelial density was the criteria used in choosing the best mother spawning materials.

Statistical analysis

All the treatments were laid out in complete randomized design under laboratory conditions. One-way analysis of variance (ANOVA) was used to determined significant differences between treatments using least significant differences (LSD) at 5% level of significance. The SAS 9.1 program were used for the analysis.

Study IV. Evaluation of fruiting body performance in enriched rice straw-sawdust based substrate formulation

Preparation of enriched substrates

Rice straw was initially composted by soaking in a water tank for three days to ferment. Then, the tank was drained with water. The rice straw was hauled from the tank, piled and covered with sacks to stimulate the growth of natural decomposers. The rice straw was composted for one week and aerated by turning the pile with a spading fork every two days interval. Finally, the composted rice straw was chopped into small pieces, and the substrates were formulated by mixing 8 parts of the composted rice straw with 2 parts of saw dust (De Leon et al. 2013a) and enriched separately with varying amount (5–25%) of rice bran (RB) and rice hull (RH). The formulated

substrates were placed on a 6 × 12 inches pp bags with three replicates and 5 experimental units per replicates. A pvc neck with cotton ball was placed at the open end of the bags, covered with aluminum foil and pasteurized in a pasteurization chamber for 5 hours at 60-80 C.

Inoculation of enriched substrata for mass production of Lentinus squarrosulus

The bagged substrates were aseptically inoculated with 40 grams of the grain spawn. The inoculated bags were incubated at room temperature to allow the spread of mycelia. Bags with fruiting initials were opened at one end to allow maturation of fruiting bodies. The opened fruiting bags were watered using a mister three times a day to prevent drying of the fruiting initials. Mature fruiting bodies were harvested, the size of the pileus and stipe were measured using a vernier calliper and then weighed to determine the biological efficiency. The biological efficiency was computed using the following formula:

$$\% \text{ Biological efficiency} = \frac{\text{weight of the fresh mushroom in a fruiting bags (g)}}{\text{weight of the substrate per bag (g)}} \times 100$$

Results and Discussion

Study I. Nutritional requirements for the mycelial growth of Lentinus squarrosulus

A culture medium is an enriched material which is often times derived from plant based sources that can promote and sustain the mycelial growth of the desired mushroom (Reyes et al. 2009a). Table 1 presents the mycelial diameter and mycelial density of *Lentinus squarrosulus* on different indigenous culture media evaluated. The largest mean mycelial diameter was observed in PSG with a mean of 92.60 mm ± 0.31 and very thick mycelial density (Fig. 2). On the other hand, RBDG recorded the smallest mycelial diameter with a mean of 88.25 mm ± 0.08 and very thick mycelial density. Statistical analysis revealed significant differences in mycelial diameter among the different indigenous culture media. The luxuriant mycelial growth observed in PSG could be due to the nutrient components of potato such as carbohydrates, proteins, fats and several minerals like calcium, potassium, phosphorus, iron and vitamins such as niacin, thiamine and riboflavin (Prokop and Albert 2008). According to Lander (1984), thiamine stimulates mycelial growth of *Cercospora arachidicola* in liquid culture. Moreover, Madunagu (1998), revealed that thiamine is required for good growth in mushrooms and that different vitamins produce different effects on mycelial growth within a certain concentration range.The result obtained in the present study is similar with the result of Valdez (2014), who reported the luxuriant and thick mycelial growth of *P. djamor* on PSG. However De Leon et al. (2013b) reported that the mycelial growth of both *L. squarrosulus* and *P. grammocephalus* was significantly highest in CWG with mean mycelial growth of 80 mm in diameter and with thick mycelial density on the 8th day of incubation. The same result was obtain by Dulay et al. (2012a) on *Lentinus tigrinus* wherein the mycelia grew best on coconut water gulaman (local crude agar) as solid medium.

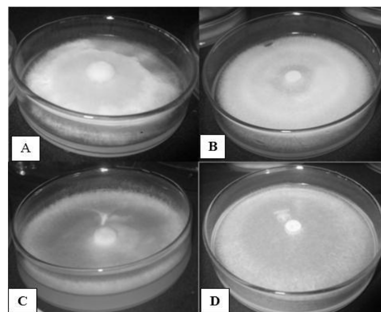

Fig. 1 – Mycelial growth of *Lentinus squarrosulus* on different indigenous culture media: **a** potato sucrose gelatin, **b** coconut water gelatin, **c** crackcorn decoction gelatin and **d** ricebran decoction gelatin after 6 days of incubation period.

Table 1 Mycelial diameter and density of *Lentinus squarrosulus* on various nutritional and physical requirements for mycelial growth

	Mycelial diameter (mm)	Mycelial density
Culture media		
PSG	92.60±0.31[a]	++++
CWG	91.34±0.27[b]	++
CCDG	90.06±0.86[c]	+
RBDG	88.25±0.08[d]	++++
pH		
5	85.07±6.33[ab]	+++
5.5	84.33±6.59[ab]	+++
6	81.95±8.14[b]	+++
6.5	92.04±3.26[a]	+++
7	92.39±0.37[a]	++++
7.5	79.52±0.55[b]	+++
8	79.45±1.40[b]	++++
Aeration		
Sealed	89.19±0.70[a]	+++
Unsealed	73.69±1.45[b]	+
Illumination		
Light	60.49±8.41c	+
Dark	81.18±1.69[b]	++
Light and Dark	93.18±0.73[a]	+++
Temperature		
15 C	10.00±0.00[b]	-
32 C	92.39±0.37[a]	+++
40 C	10.35±0.20[b]	-

Values are means ± SD. Means with the same superscript in a column are not significantly different at 5% level of significance using LSD. Note: Mycelial density were evaluated as (+) very thin, (++) thin, (+++) thick, (++++) very thick and (-) no growth

Study II. Physical requirements for the mycelial growth of Lentinus squarrosulus

pH levels

Maximizing of growth rate and biomass yield and minimizing the duration of lag phase for growth of mushroom are influenced by pH of the media as well as inoculum size and nutrient composition (Chang and Miles 2004). The mycelial growth response of *L. squarrosulus* is presented in Table 1. Among the different pH levels evaluated, pH 7.0 recorded the shortest mycelia colonization with the mean of 92.39 mm ± 0.37 however this result is statistically comparable to pH 6.5, pH 5.0 and pH 5.5. On the other hand, pH 8.0 and pH 7.5 registered the longest incubation period which is statistically comparable to pH 6.0, pH 5.0 and pH 5.5. Luxuriant and very thick growth of mycelia was observed in pH 7.0 and 8.0. This result is comparable to the result of Akinyele and Adetuyi (2005) and Dulay et al. (2012a) who found that the maximum mycelia yield was observed between pH 5.5 to 8.5 as well as and 6 to 8 in *V. volvacea* and *L. tigrinus* respectively. Also, presented by Dulay et al. (2012b) in the basidiocarp production of *L. tigrinus* that pH of 7.5 had the highest percentage germination with means of 82% and 88.67% after 7 hours and 10 hours, incubation respectively. On the other hand, Osman et al. (2009) reported that

the two strains of *Lentinus edodes* grow over a wide range of pH value, but an initial pH 7.0 favored the highest growth and the highest extract production. While the highest mycelia dry weight was recorded at pH 7.0. Jonathan and Fasidi (2003) reported appreciable growth of *Psathyrella atroumbonata* at pH 6.5 which is comparable to the observations of Anyakuorah et al. (1998) on the cultivation of *L. squarrosulus.* According to Jacob et al. (2014) the luxuriant and thickest mycelia growth was recorded in pH 8.0 for the three strains of *Pleurotus*. Most common fungi grow well over the range pH 3 to 7, although some can grow at pH 2 and below (Smith et al., 2001). Adequate pH and moisture content is essential to ensure good aeration and fast mycelial growth. Van Aarle et al. (2002) reported that the low growth of mycelium at lower pH is caused by an aversion on the substrate. While Reyes et al. (2009b) stated that the mycelium growth of *C. comatus* was cottony and suitable in pH 6.0 and 7.0.

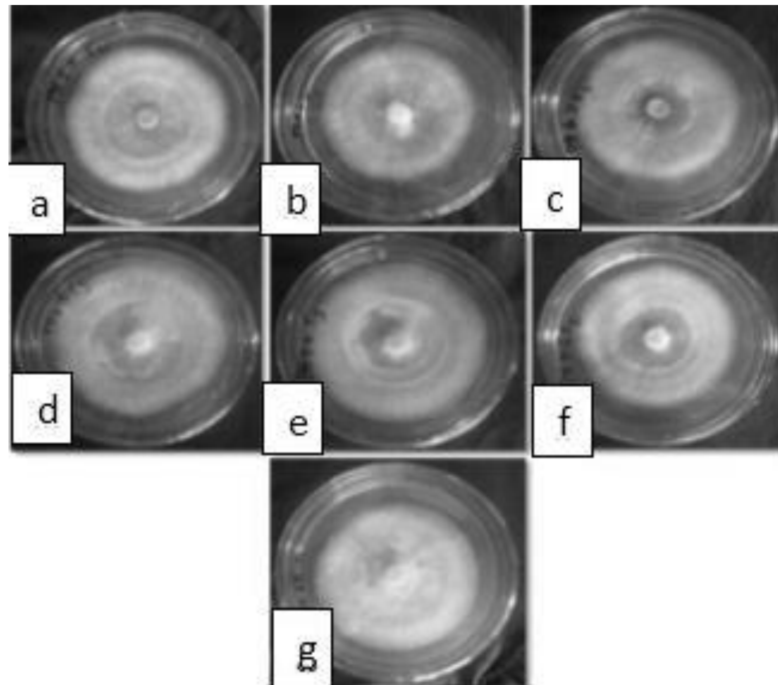

Fig. 2 – Mycelial growth of *Lentinus squarrosulus* on different pH levels: **a** pH 5.0, **b** pH 5.5, **c** pH 6.0, **d** pH 6.5, **e** pH 7.0, **f** pH 7.5 and **g** pH 8.0 after 6 days of incubation period

Aeration

The components of the air that are important to most fungi are oxygen and carbon dioxide. Aeration is one of the most important factors that affect production and activity of ligninolytic enzymes (Chang and Miles 2004). *Lentinus squarrosulus* cultured in sealed condition produced significantly larger mean diameter of 89.19 mm ± 0.70 with a thick mycelial density while those that grow in unsealed condition exhibited significantly smaller mycelial diameter with a mean of 73.69 mm ± 1.45 and very thin mycelial density after six days of incubation. The difference in the mycelial growth rate could be due to the high concentration of CO_2 on the sealed plate that stimulated the growth of mycelia. In a related study, Miguel (2012) observed luxuriant and thick mycelia growth of *P. pulmonarius* in sealed condition. Kang (2004) reported that the mycelial growth *P. ostreatus* and *P. florida* is stimulated in high CO_2 concentrations up to 28% and 22%, respectively. Contrary to this result *L. tigrinus* grew luxuriantly in neither sealed or unsealed condition (Dulay et al. 2012a).

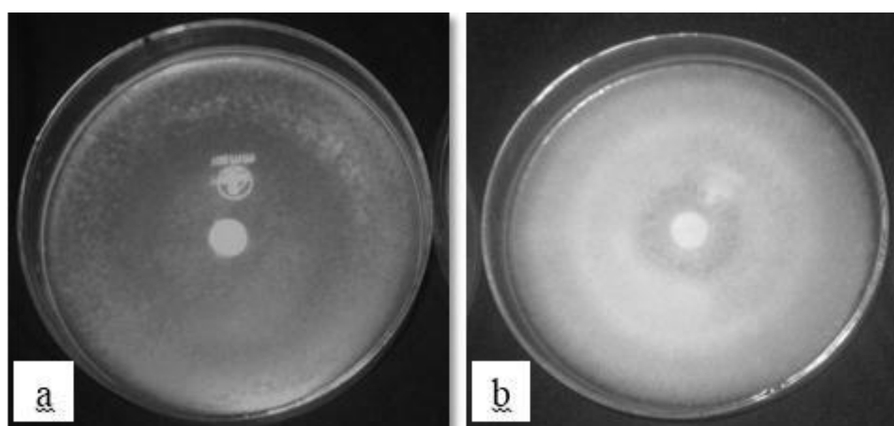

Fig. 3 – Mycelial growth of *Lentinus squarrosulus* on different aeration: **a** unsealed and **b** sealed after 6 days of incubation period

Illumination

Most cultivated fungi are exposed to alternating periods of daylight and darkness (Chang and Miles 2004). The influence of light conditions on mycelial growth of *Lentinus squarrosulus* is shown in Table 1. Among the three light conditions, cultures exposed to alternating light and dark condition significantly recorded the largest mycelial diameter with a mean of 93.18 mm ± 0.73 and thick mycelial density while those exposed to lighted condition registered significantly smallest mycelial growth with a mean of 60.49 mm ± 8.41 and very thin mycelial growth after 6 days of incubation. Chang and Miles (2004) reported that the growth of most fungi is not sensitive to light, although strong light may inhibit or even kill the organism (possibly a temperature effect). Positive response to darkness of this mushroom is comparable to the other basidiomycetes like *V. volvacea* (Reyes et al. 1998), *C. comatus* (Lopez et al. 2009), *Agaricus blazei* (Galamgam et al. 2009) and *Lentinus tigrinus* (Dulay et al. 2012a). However, this results contradicts the repot of Dulay et al. (2012b) on basidiospore germination of *Lentinus tigrinus*, wherein incubation in lighted condition (30 footcandles) significantly obtained higher percentage germination with means of 79% and 88% after 7 hours and 10 hours, respectively. Variation on the responses in illumination suggests that different basidiomycetes have varying response to light (Dulay 2011).

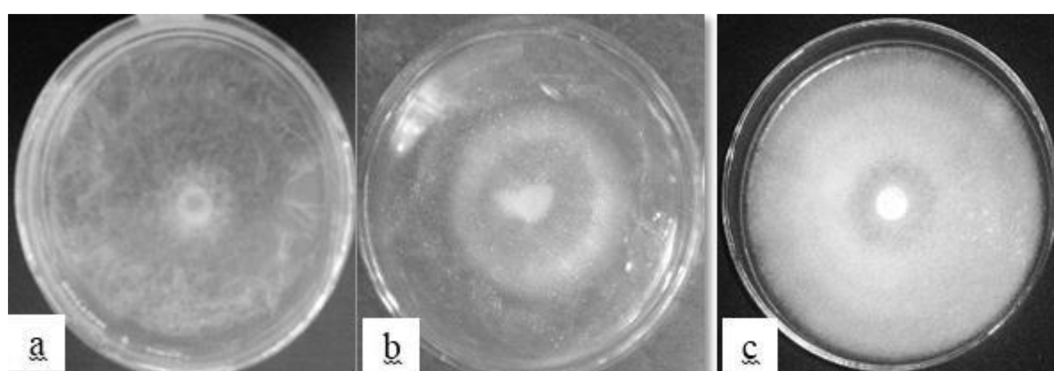

Fig.4 – Mycelial growth of *Lentinus squarrosulus* on different illumination: **a** Light (expose to artificial light), **b** Dark (cover with carbon paper) and **c** Light and dark or normal condition after 6 days of incubation period

Temperature

The temperature extremes (maximum and minimum) are of great importance in determining the survival and distribution of a fungal species in nature (Chang and Miles, 2004). Results revealed that room temperature recorded the largest mycelial growth with 92.39 mm ± 0.37 and thick mycelial density. While at 15 C and 40 C inhibited the mycelial growth of *Lentinus*

squarrosulus. The result obtained in the present study is the same with the response of the tropical mushrooms like *C. reinakeana* (Reyes et al. 1997), *S. commune* (Bulseco et al. 2005), *V. volvacea* (Reyes et al. 1998) and *Lentinus tigrinus* (Dulay et al. 2012a). However it differs to the basidiospore germination of *L. tigrinus* presented by Dulay et al. (2012b) wherin basidiospores at 23 C (air-conditioned with 48% relative humidity) recorded the highest percentage germination with means of 86% and 91.33%, after 7 hours and 10 hours, respectively and the lowest percent basidiospore germination was observed at room temperature (32 C) with means of 50% and 54% after 7 hours and 10 hours, respectively. Lin et al. (2003) reported that fungi can be classified as temperate, semi-temperate or tropical based on the optimum temperature for mycelial growth. Jayasinghe et al. (2008) disclosed that the most suitable temperature for the mycelial growth was 30 C but generally the optimal temperature range for the mycelia growth was found at 25-30 C.

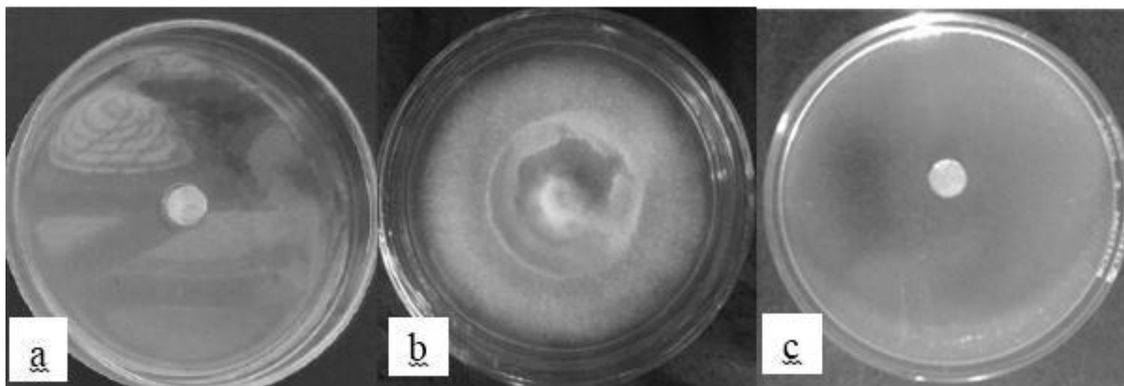

Fig. 5 – Mycelial growth of *Lentinus squarrosulus* on different temperature: **a** incubator (40 °C), **b** room temperature (32 °C) and **c** refrigerator (15 °C) after 6 days of incubation period

Study III. Granulated spawning materials

The grain spawn is the starter for the mass/bulk production of mushroom. It facilitates the rapid colonization of the mushroom substrate thus reducing the days of mycelia colonization. It is essential for mushroom production to have a good mycelia growth (Chang 2009). *Sorghum bicolor* and cracked *Zea mays* recorded the shortest incubation with a mean of 6 days, while unmilled *O. sativa* seed registered the longest period with the mean of 12 days. The efficient growth of *L. squarrosulus* on *S. bicolor* seeds and crack *Zes mays* could be attributed to their nutrient composition. According to Leder (2004), 100 g sorghum seeds contain 10.9 g protein, 3.2 g fat, 2.3 g crude fiber, 1 g ash, 329 kcal of energy, 27 mg calcium, 4.3 mg iron, 0.3 mg thiamin, 3.83 mg niacin and 0.138 mg rivoflavin. Moreover, the suitable moisture capacity of sorghum (55-60%) contributed to mycelial proliferation (Gizaw 2010).

The result of the present study is consistent with the finding of Royse (2003), who disclosed that sorghum grains provide optimum spawn growth and quality inoculum in oyster mushroom. Also, *L. squarrosulus* grown in corn grits and sorghum seeds had very thick mycelial density and the shortest incubation period of 6 days (De Leon et al. 2013b). Moreover, those grain material was also found to be the best spawning material for *Lentinus sajor-caju, Agaricus blazei, Auricularia polytricha* and *Agrocybe aegerita* (Cuevas 2009, Galamgam 2009, Yabo 2011, Marcelo 2011). Futhermore, De Leon et al. (2013a) also reported that mycelial growth of *L. tigrinus* grown in the three spawning medium did not vary considerably. All were fully ramified at 7 days of incubation and had very thick mycelial density. This result was not consistent with the data reported by Dulay et al. (2012a) on similar spawning materials. In their study, *L. tigrinus* mushroom grew better on unmilled rice seeds (with 5 days incubation period) than corn grits and sorghum seeds (with a mean of 6.7 days incubation period). Cuevas et al. (2009) also reported that the spawning of mycelia of *L. sajor-caju* was superior in sorghum seeds compared with unmilled rice. For the enrichment type, corn grits provided the fastest period of primordial initiation for *C. comatus* and *P. sajor-caju*, (Dulay et al. 2014). Stanley et al. (2011) also reported that *P. pulmonarius* grew well on white

maize, followed by red sorghum seed, millet, gunica corn, yellow corn and wheat. Ramos (2004) also confirmed that the fastest incubation and thickest mycelial density of *P. pulmonarius* on corn grits. Tinoco et al. (2001) mentioned that the larger surface area and pore of substrates support more mycelium growth rate. The thin mycelia growth observed in unmilled rice seeds could be due to the protective covering of the rice grain. Dulay et al. (2012a) explained that the hull serves as the barrier that limits the penetration of the fungal mycelia needed to acquire the nutritious part of the rice. While Oghenekaro et al. (2009) used sorghum seeds as spawning material for the cultivation of *L. squarrosulus* in Nigeria while in Cuba, wheat kernels were used as a spawning material for the cultivation of *P. ostreatus.*

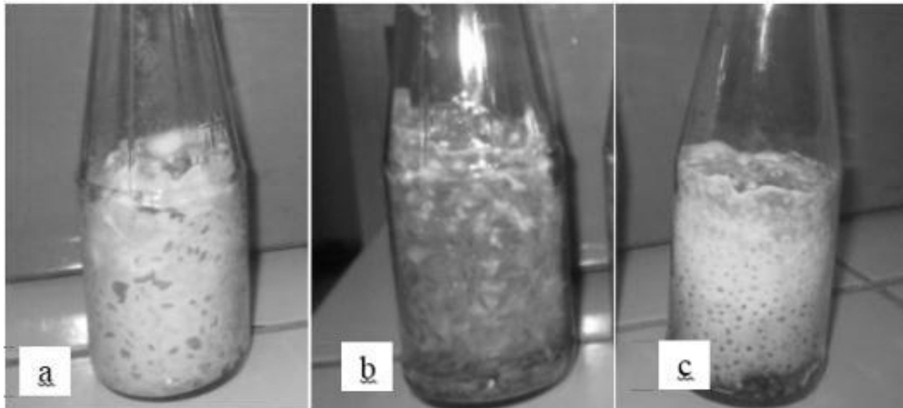

Fig. 6 – Mycelial density of *L. squarrosulus* on different grain spawning material: **a** cracked *Zea mays*, **b** unmilled *Oryza sativa* grains and **c** *Sorghum bicolor* after 6 days of incubation

Study IV. fruiting body performance in enriched rice straw-sawdust based substrate formulation

Incubation period and Pinhead formation of L. squarrosulus

Spawn running is the growth of mycelium into the compost, which usually lasts for two weeks (Royse et al. 1990). Substrate supplemented with 5% and 10% rice bran registered the shortest spawn running with a mean of 16 days (Table 2). The rest of the treatments registered a mean of 15 days to fully ramify the substrate. The one day difference in the spawn running of *L. squarrosulus* could be due to the supplementations used in the cultivation which might have affected the mycelial ramification. Okhuoya et al. (2005) in their report on the cultivation of *L. squarrosulus* on sawdust of selected tropical tree species, showed that the mushroom was able to colonize all the different substrate/supplement combinations, except the sawdust of *Celtis* sp., which totally failed to support the growth of the mushroom mycelium at the wheat bran supplementation of 20%. Time for mycelium colonization of the substrate ranged from 7.80 ± 0.49 days in *Celtis* sp., supplemented with 1% sugar, 1% CaCO3, and 1% sugar; to 17.50 ± 6.50 days in the sawdust of *C. excelsa* supplemented with 1% CaCO3, 1% sugar, and 10% wheat bran. On the other hand, Alemu (2015) reported that spawn running of *L. edodes* on sorghum based spawn took 25 days to colonize the substrate completely. In a study conducted by Dumale (2015) the difference in incubation period of *Pleurotus florida*, *P. djamor* and *P. ostreatus* is only 2 days in rice straw while 3 days in sawdust substrate, which is not very far from the result of this study. Mata et al. (2005) mentioned that factors like, temperature of the incubation room, light and humidity affect the spawn running time of mushrooms.

The formation of pinheads initially marks the start of fruiting body production. In this study, the pinhead formation of *Lentinus squarrosulus* on enriched RS and SD based substrate formulation is presented in Table 2. *Lentinus squarrosulus* grown in substrate supplemented with 5% rice hull registered the fastest pinhead formation (1 day) while the longest period to pinhead formation was noted in 15% rice bran with a mean of 4 days.

Table 2 Number of incubation of *Lentinus squarrosulus* on enriched RS and SD based substrate formulation

Treatments	Incubation period (days)	Pinhead formation (days)
5%RB	16	2
10%RB	16	3
15%RB	15	4
20%RB	15	2
25%RB	15	3
5%RH	15	1
10%RH	15	2
15%RH	15	2
20%RH	15	3
25%RH	15	3

The results obtained in the present study is faster than the 2.07 mean number of days to primordial formation of *Pleurotus djamor* cultured on rice straw and sawdust substrate (Dumale 2015) as well as the 3.29 days appearance of primordia of *Pleurotus flabellatus* cultured in mango sawdust (Islam et al. 2009). However Okhuoya et al. (2005) presented that the earliest time of primordial emergence in *Lentinus squarrosulus* was 20.60 ± 0.16 days (on sawdust of *B. nigerica* without any addition of supplements).

Size of pileus and stipe size

The diameter of pileus of *Lentinus squarrosulus* grown on substrate RS and SD enriched with RB and RH is presented in Table 3. *L. squarrosulus* cultivated in substrate supplemented with 20% rice hull registered the largest pileus diameter (71.92 mm ± 2.69) while the smallest pileus diameter was recorded in 25% rice hull supplementation with a mean of 52.82 mm ± 7.33. This result indicates that supplementation affects the size of the pileus of the mushroom as it produced larger pileus diameter compared to the mean pileus size (64.79 mm ± 18.70) reported by De Leon et al. (2013b) in un-supplemented cultivation. However the amount and type of the supplementation did not significantly affect the size of the pileus. Royse et al. (1990) reported that there are several sources of variations on mushroom size, which include characteristics of species of mushroom as well as their strains.

Table 3 Mean size of pileus, stipe length and stipe diameter, number, weight and percent biological efficiency of fruiting bodies of *Lentinus squarrosulus* on enriched RS and SD based substrate formulation

Treatment	Size of pileus	Stipe diameter	Stipe length	No. of fruiting bodies	Weight of fruiting bodies	Biological efficiency
5%RB	55.94^{ab}±7.59	5.62±0.93	34.92±2.63	5.67^{d}± 0.19	46.67 ^{d}± 4.81	9.33 ^{b}
10%RB	66.84^{ab}±5.98	6.78±1.11	37.65±4.52	5.00 ^{d}± 0.58	43.33 ^{b}± 3.47	8.67 ^{b}
15%RB	61.29^{ab}±10.70	6.26±0.72	40.98±7.87	9.33^{bc}± 1.35	90.00 ^{a}± 16.67	18.00^{a}
20%RB	57.14^{ab}±19.00	6.20±1.29	39.75±3.08	11.33^{b}± 3.37	71.67^{ab}± 15.84	14.33^{ab}
25%RB	55.26^{ab}±11.17	6.23±0.82	39.47±6.53	8.00^{bcd}±1.33	66.67^{ab}± 15.49	13.33^{ab}
5%RH	68.77^{ab}±6.63	7.24±0.75	43.01±5.09	9.67^{bc}±2.41	83.33 ^{a}± 13.57	16.67 ^{a}
10%RH	57.51^{ab}±11.01	5.40±0.23	36.27±4.30	18.33^{a}±6.62	90.00 ^{a}± 29.06	18.00 ^{a}
15%RH	62.90^{ab}±8.90	5.84±1.86	34.49±7.33	6.67^{cd}±0.51	48.33 ^{b}±6.31	9.67 ^{b}
20%RH	71.92 ^{a}± 2.69	6.87±1.05	41.98±10.39	5.67 ^{d}± 0.51	65.00^{ab}±6.67	13.00^{ab}
25%RH	52.82^{ab}±7.33	6.09±0.29	34.41±5.51	6.33^{cd}±0.38	48.33 ^{b}± 2.55	9.67 ^{b}

Values are means ± SD. Means with the same superscript in a column are not significantly different at 5% level of significance using LSD. RH= Rice Hull, RB= Rice Bran

The size of stipe of *L. squarrosulus* grown on enriched RS and SD based substrate formulation is also shown in Table 3. The largest stipe diameter and longest stipe was recorded on substrate supplemented with 5% rice hull with mean value of 7.24 mm ± 0.75 and 43.01 mm ± 5.09, respectively. However, statistical analysis revealed no significant difference in the size of stipe among different treatments. This indicates that the amount of supplementation does not affect the size of the mushroom. However supplementation has an effect as proven by this result which is much higher than the report of De Leon et al. (2013b) in unsupplemented cultivation of *L. squarrosulus* where as the longest stipe length is only 33.08 mm ± 3.53 and the widest stipe diameter is 5.94 mm ± 1.78. On the other hand, Nwanze et al. (2005) reported that various media, oil type and rate had a highly significant effect on the stipe length and diameter as well as, pileus diameter of *L. squarrosulus*.

Number of fruiting bodies and weight of fruiting bodies

In this study, the fruiting body performance of *L. squarrosulus* on enriched RS and SD based substrate formulation is also presented in Table 3.

Results showed that substrates supplemented with 10% rice hull produced the most number of fruiting bodies (with a mean of 18.33 ± 6.62). The lowest number of fruiting bodies was observed in 10% rice bran with a mean of 5.00±0.58. Statistical analysis showed significant difference in the number of fruiting bodies of the different treatments. The result of this study proved that supplementation could enhance mushroom production since it produced more fruiting bodies compared to the report of De Leon et al. (2013b) where *L. squarrosulus* produced only 14.40±12.50 fruiting bodies in un-supplemented cultivation. The values obtained in the present study are also higher than the results obtained by Rossi et al. (2002) in *L. edodes* which produced a mean of 2.73-3.45 fruiting bodies with 30% rice bran supplementation. Adesina et al. (2011) reported that cultivation of *L. squarrosulus* on bark and leaves of fruit trees supplemented with rice bran exhibited best result as supplement in the mycelial growth. Supplementation of substrates has become one of the major aspects of mushroom cultivation. This is done to increase the yield of mushroom which is in line with the finding of Zadrazil (1978) that supplements usually change the decomposition rate and the sequence of decomposition of substrate components during mushroom growth.

The weight of fruiting bodies of *L. squarrosulus* on enriched RS and SD substrate formulation is also presented in Table 3. The highest mean weight of fruiting bodies was observed in substrate supplemented with 10% rice hull and 15% rice bran with 90.00 g ± 29.06 and 90.00 g ± 16.67 respectively while the lowest mean weight was recorded in 10% rice bran with mean weight of 43.33 g ± 3.47. However, this is comparable with substrates supplemented with 5% rice hull, 20% rice bran, 25% rice bran and 20% rice hull. The results obtained in the present study is much higher than the report of De Leon et al. (2013b) wherein un-supplemented cultivation of *L. squarrosulus* yielded only 39.14 g ± 13.74 total weight of fruiting bodies.

Biological efficiency

As presented in Table 3, both treatments supplemented with 15% rice bran and 10% rice hull produced the highest biological efficiency of 18%, while 10% rice bran supplementation got the lowest value of 8.67% Although this result is also comparable with different supplementations (20% rice hull, 5% rice hull, 20% rice bran and 25% rice bran). This result proved that both supplementations have the same effect that can help to improve the biological efficiency of mushrooms. This result is higher than the biological efficiency of enriched cultivation of *L. tigrinus* which only registered 14.66%. Ayodele et al. (2007) reported a 4.27% biological efficiency of *L. squarrosulus* grown in *M. altissima* and Adesina et al. (2011), on the other hand, reported a 10.25% biological efficiency of *L. squarrosulus* cultivated in *S. mombin* supplemented with in rice bran. Likewise, the biological efficiency reported by Cuevas et al. (2009) of *L. sajor-caju* was 7.2% at 9 parts rice straw + 1 part sawdust substrate formulation. However, these results except for the findings in this paper were lower compared to the biological efficiency of *L. tigrinus* (15.93%) as reported by Dulay et

al. (2012a). Perhaps, different species and even different strains of same species exhibited differences in biological efficiency.

Moonmoon et al. (2011) reported that rice bran increased productivity of mushroom due to its components such as carbohydrates, amino acids and mineral elements. On the other hand, Frimpong-manzo et al. (2011) claimed that both rice bran and rice hull contains cellulose which is important for the growth of mushroom since they are lignocellulosic.

Based on the result of this study, supplementation is important in mushroom cultivation since it can increase the biological efficiency compared to the reported 7.83% biological efficiency of un-supplemented cultivation of *L. squarrosulus* (De Leon et al. 2013b).

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