Journal of Agricultural Technology 2013 Vol. 9(5): 1199-1214
Available online http://www.ijat-aatsea.com
ISSN 1686-9141

Enriched cultivation of three wild strains of *Lentinus tigrinus* (Bull.) Fr.using agricultural wastes

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De Leon, A.M., Reyes, R.G. and dela Cruz, Tee (2013) Enriched cultivation of three wild strains of *Lentinus tigrinus* (Bull.) Fr.using agricultural wastes. Journal of Agricultural Technology 9(5):1199-1214.

Lentinus tigrinus, a wood-decaying species of macrofungi, is utilized as food by the indigenous Aeta tribes from Botolan, Zambales. In this study, the cultivation of this wild edible species using enriched media is reported. Results showed that mycelia grew best on coconut water-gelatin medium. Unmilled rice seeds, corn grits seeds, and sorghum seeds were all suitable materials as spawning media for mycelial mass production. The best substrate formulation for the growth of *L. tigrinus* (ZB12MF03 and ZB12MF06) were 8+2 rice straw-sawdust enriched with 25g rice bran or 50g rice grits. One strain, *L. tigrinus* ZB12MF05, grew best on 8+2 rice straw-sawdust formulation enriched with 75g rice grits. This is the first report of enriched cultivation of *L. tigrinus* in the Philippines using agricultural wastes.

Keywords: rice bran, rice grits, sorghum seeds, mushroom cultivation, agricultural wastes

Introduction

Mushrooms are cultivated worldwide for their taste, nutritional attributes, and potential application in industries (Mata *et al*., 2005, Sunagawa and Magae, 2005). During the last decades, major efforts in the cultivation of edible mushrooms were focused on development of new technologies and increasing yields at large-scale (Van Griensven, 2000; Oei *et al.*, 2003). However, small-scale mushroom production represents an opportunity for farmers interested in obtaining additional sources of income. This is also a special option for farmers without much land. Mushroom production can also play an important role in managing organic farm wastes when agricultural and food processing by-products are used as growing media. These agricultural by-products, if not treated properly, can also pose a problem in disposal in the environment (Onuoha, 2007). However, mushrooms grew on almost all cellulosic

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agricultural waste materials like rice straw, banana leaves, and dried paddy straw (Reyes and Abella, 1997). Fasidi and Kadiri (1993) reported that the highest yield for *Pleurotus tuber-regium* edible sclerotia was obtained from cotton waste and rice straw. So, the cultivation of mushroom using agro-wastes can be a way of reducing environmental waste materials (Reyes and Abella, 1997). Furthermore, the used substrata can also be composted and applied directly back to the soil as organic fertilizer (Alice and Kustudia, 2004).

The bioconversion of agricultural wastes into edible mushroom fruit bodies is developing into a profitable enterprise with a lot of advantages. In the Philippines, studies on the cultivation of mushroom include those of *Collybia reinakeana* (Reyes *et al*., 2004), *Coprinus comatus* (Reyes *et al*., 2009), *Schizophyllum commune* (Garcia *et al*. 2004; Bulseco *et al*., 2005), *Volvariella volvacea* (Reyes, 2000; Reyes *et al*., 2004), *Pleurotus sajor-caju* and *Pleurotus florida* (Reyes *et al.,* 2009), and *Lentinus tigrinus* (Dulay *et al*., 2012). Efficient utilization of rice straw-based substrate for the cultivation of edible mushrooms has also been developed (Reyes *et al*., 2006; Villaceran *et al.*, 2006). Rice straw is the principal substrate for mushroom growing in the Philippines, while sawdust is one of the most readily available agro-industrial wastes that has been extensively used for the cultivation of mushrooms (Staments 2000; Joshua and Agina 2002; Kuforriji *et al.*, 2003). Although adequate production can be achieved through the use of rice straw-saw dust based formulation, additionof supplements might substantially increase the yield per unit weight. Oghenekaro *et al.* (2009) reported a 3.52g increase in the mean dry weight of fruiting bodies of *L. squarrosulus* cultivated in *Brachystegia nigeria* sawdust supplemented with 20% (w/w) wheat bran, 1% (w/w) CaCO3, and 1% (w/w) sugar. Adesina *et al.* (2011) also claimed that mycelial growth of *L. squarrosulus* was generally enhanced when supplements were used, particularly rice bran and horse dung. In this study, we evaluated the fruiting body performance of three wild strains of *Lentinus tigrinus* on rice straw-sawdust based substrata enriched with rice bran and rice grits.

Materials and methods

The cultivated mushroom

Fruiting bodies of three *L. tigrinus* strains, i.e. ZB12MF03, ZB12MF05 and ZB12MF06, were collected from Brgy. Bucao and Brgy. Bihawo in Botolan, Zambales. Fruiting bodies were scraped off from the barks of trees, wrapped in brown paper, coded, and brought to the laboratory. General description of the mushroom was as follows: *Pileus* 4-11 cm, umbilicate, white to ochraceous, covered by white scales, margin undulate. *Gills* decurrent,

crowded, white. *Stipe* eccentrical or lateral, 1.2-2 × 0.3-1.5 cm, solid, white to brown, with scales. *Spores* elliptical, hyaline, smooth, I-, 6-7 × 3-3.5 μm. Cystidiaclavate. *Hyphae* with clamps (Imazeki and Hongo 1987; Breitenbach and Kranzlin 1991).

Isolation of L. tigrinus into pure mycelial culture

Collected fruiting bodies of three *L. tigrinus* strains were tissue-cultured. Initially, inner tissues (1 mm^2) were cut and laid on potato dextrose agar (PDA). Fungal mycelia growing out of the explants were sub-cultured on freshly prepared PDA until pure cultures were obtained. All fungal cultures were incubated at room temperature for 7 days or until the medium was totally covered by the mycelia.

Preparation and inoculation of L. tigrinus on indigenous culture media

To evaluate the nutritional requirements of the three *L. tigrinus* strains, different indigenous culture media was used. These media were potato sucrose gelatin (PSG), corn grits decoction gelatin (CDG), and coconut water gelatin (CWG).These were chosen based on their availability in the local Philippine market. To prepare PSG and CDG in a liter of distilled water, 250 g of cubed potatoes and 50 g of corn grits were boiled separately until tender. Then, the decoctions were strained to remove the cooked potato and corn grits, and then reconstituted to 1 liter by adding more distilled water. Then, 20 g of unflavored gelatin and 10 g of sucrose were each dissolved and mixed into the decoctions over low heat fire until the mixture is homogenized. For CWG, 1L of mature coconut water was boiled, then, 20 g unflavored gelatin was dissolved, mixed and stirred constantly over low heat fire until the media is homogenized.

All the prepared media were sterilized at 121 °C for 20 min. Then, a 10-mm mycelial disc from a 7-day old pure culture of the three *L. tigrinus* strains were aseptically inoculated onto petri plates with 15 ml of the indigenous culture media. All culture plates were incubated at room temperature to allow the ramification of its mycelia. The diameter of mycelial growth was measured everyday until full colonization of each medium was attained. Statistical analysis was laid out in a complete randomized design. One-way analysis of variance (ANOVA) was computed to determine significant differences between mean colony growth of the macrofungi.

Preparation and inoculation of L. tigrinus on grain spawning media

Locally available seeds, e.g., unmilled rice seeds, corn grits seeds, and sorghum seeds, were also used to evaluate the mycelial growth performance of three *L. tigrinus* strains. To prepare the culture media, the grains were boiled until tender, drained, and dried until 60% humidity was attained. Grains spawns at 40g each were then dispensed in clearglass bottles. Absorbent cotton was plugged into the opening of the bottles, then, wrapped with aluminum foil, and sterilized at 121 °C for 30 min. After sterilization, the bottled grain spawns were placed in an isolation room to cool down and aseptically inoculated with 10-mm mycelial disc of a 7-day old pure culture of *L. tigrinus* strains. The inoculated grain spawn were incubated at room temperature to allow ramification of the mycelia. The grain spawn which was fully ramified by the mycelia in the shortest period of time was chosen as the best spawning material.

Preparation and inoculation of *L. tigrinus* on rice straw-sawdust based substrate formulations enriched with rice bran and rice grits

Preparation of inocula. Two kilograms of the best grain spawning material was boiled until tender, drained with water, dried and was used to propagate the mycelia of *L. tigrinus* strains ZB12MF03, ZB12MF05 and ZB12MF06. Then, in a small polypropylene (pp) bags, 40g of the spawning materials was dispensed, the opening of the pp bags were plugged with cotton with a polyvinyl chloride (pvc) pipe which served as its neck, then, finally wrapped with aluminum foil. The grain spawns were sterilized for 30 min at 121^{o}C. After sterilization, grain spawns were allowed to cool down and inoculated with 10-mm mycelia dics from 7-day old mycelia as previously discussed. Finally, grain spawns were incubated until the grains were fully colonized by the mycelia. These grain spawns was used as inoculum for the fruiting spawns of *L. tigrinus* strains ZB12MF03, ZB12MF05 and ZB12MF06.

Preparation of enriched substrata. For the fruiting spawns, rice straw was initially composted by soaking it in a water tank for three days to ferment. Then, the tank was drain with water. The rice straw was hauled from the tank, piled and covered with sacks to stimulate the occurrence and growth of natural decomposers. The pile was composted for one week and was aerated by turning with a spading fork every after two day interval. Finally, the composted rice straw was chopped into small pieces, and then 8 parts of the composted rice straw was mixed with 2 parts of sawdust and added with rice bran and rice grit supplementation (Table 1).

The formulated substrates were bagged in 6 x 12 inches pp bags with five replicates. A pvc neck with cotton ball plugged in it was placed at the open end of the bags and pasteurized in a chamber for 5 hours with 60-80 ^{o}C

temperature. After sterilization, the bags were placed in an isolation room to cool down.

Inoculation of enriched substrata for mass production of L. tigrinus. The bagged substrates were aseptically inoculated with 40g of the best grain spawn that was previously prepared. The inoculated bags were then incubated at room temperature to allow the spread of mycelia into the bagged substrates. Bags with fruiting initials were opened at one end to allow maturation of fruiting bodies. The opened fruiting bags were watered using a mister everyday to prevent drying of the fruiting initials. Mature fruiting bodies were harvested, the size of the pileus and stipe was measured using a vernier caliper and then weighed to determine the biological efficiency of *L. tigrinus* strains ZB12MF03, ZB12MF05 and ZB12MF06 based on the following formula:

$$\text{Biological efficiency (\%)} = \frac{\text{total fresh weight of the fruiting bodies per bag}}{\text{total weight of substrate per bag}} \times 100$$

All the treatments were laid out in a complete randomized design under laboratory conditions. Data were analyzed using one-way analysis of variance (ANOVA) to determine significant differences between treatments.

Results and discussions

Mycelial growth of L. tigrinus on different indigenous culture and grain spawning media

Mycelia are the vegetative part of a fungus consisting of a mass of branching, thread-like hyphae. The luxuriant growth of mycelia depends on the nutritional content of the medium where they grow. Mycelial growth is important in mushroom production, because mycelial stock culture served as source of mushroom cell lines. Several studies have reported mycelial growth on various substrata. For example, Adesina *et al.* (2011) assessed the mycelial growth of *L. squarrosulus* on leaves and barks of different fruit trees. Their results showed that the best mycelial growth was observed on *Spondias mombin* leaves supplemented with rice bran (with 10.43 cm linear growth diameter) as opposed to other substrata, e.g. *Psidium guajava* supplemented with oil palm waste fiber with only 5.47 cm linear growth diameter after 7 days incubation. Reyes *et al.* (2009), on the other hand, used indigenous culture media such as rice bran decoction gelatin, rice straw decoction gelatin, and coconut water gelatin in evaluating mycelial growth of *Coprinus comatus*. Results showed that fastest mycelial colonization was observed with rice straw decoction gelatin (7 days) as compared to rice bran (11 days) and coconut water

(8 days), however the mycelial density in rice straw decoction gelatin was thin and scanty as compared to the thick and cottony mycelia produced in the coconut water gelatin. Therefore, the best indigenous culture media for *C. comatus* was coconut water gelatin. The luxuriance and rapidity of growth of a certain mushroom partly depended on the appropriate culture medium used in its cultivation in the laboratory (Reyes *et al.* 2009). It is therefore an important step prior to mushroom cultivation the determination of the best medium/media which favor/s the efficient mycelia growth of the cultivated mushroom. In this study, the mean colony diameter and the mycelia density of the three *L. tigrinus* strains on the different indigenous culture media were significantly higher in CWG. The highest mean mycelial growth was observed in *L. tigrinus* strain ZB12MF03 with 84.16 mm followed by *L. tigrinus* strain ZB12MF06 with 82.85 mm, and finally, by *L. tigrinus* strain ZB12MF05 with 65.87 mm (Table 2). All three cultivated mushroom specimens also exhibited very thick mycelial density on the 7th day of incubation (Fig. 1). On the other hand, the lowest mean mycelial growth was observed for all the specimens in PSG medium, i.e. 28.49 mm for ZB12MF03, 23.49 mm for ZB12MF05, and 79.38 mm for ZB12MF06 (Table 2, Fig.1). This means that the mycelia of *L. tigrinus* strains ZB12MF03, ZB12MF05, and ZB12MF06 grew well on CWG than on CDG and PSG. The superiority of coconut water gelatin as a culture medium for the efficient mycelial growth of the different strains of *L. tigrinus* in this study confirmed previous findings regarding its suitability as a culture medium for the propagation of mycelia of most wild mushrooms including *Ganoderma lucidum*, *Auricularia polytricha*, and *Schizophyllum commune* (Reyes *et al.*, 1992, 1993; Bulseco *et al.,* 2005; Garcia *et al.,* 2004; Tayamen *et al.,* 2004; Reyes *et al.,* 2009). This is perhaps because of the nutritional content of coconut water which has primarily glucose and fructose in the immature coconut, and sucrose in the more mature fruits. Coconut water is also rich in many essential amino acids including lysine, leucine, cystine, phenylalanine, histidine, and tryptophan (Campbell *et al.* 2000). Among the minerals, i.e. K, Na, Ca, P, Cu, S, Fe, Mn, Zn, Cu and Mg present, potassium was the most abundant in young coconut (Fife, 2008). With regards to the protein content of the coconut water, the level increases from 0.13% in young coconut to 0.29% in mature coconut. This level was enough to fulfill the nitrogen requirement of the growing mycelia (Rau, 1999). Thus, coconut water is an ideal medium for the mycelial growth due to its nutrient content that fulfills the nutritional needs of the mushroom.

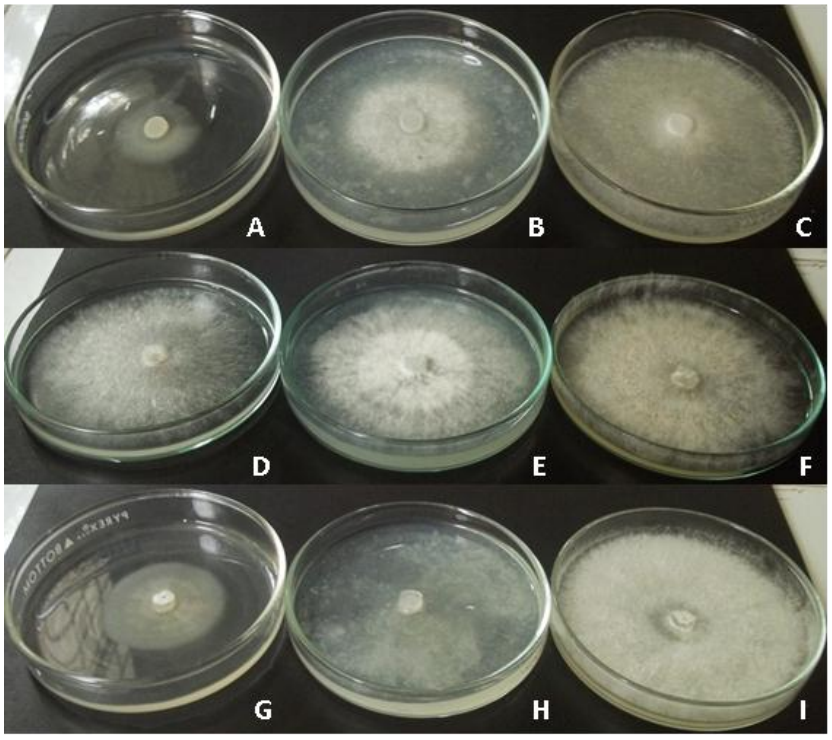

Fig. 1. Mycelial growth performance of the three strains[ZB12MF03 (A, B, C), ZB12MF05 (D, E, F) and ZB12MF06 (G, H, I)] of*L. tigrinus* on different indigenous culture media: potato sucrose gelatin (A, D, G), corn grits decoction gelatin (B, E, H), coconut water gelatin (C, F, I),on the 7th day of incubation.Note the luxuriant and thick growth of mycelia on coconut water gelatin and a very thin growth on potato sucrose gelatin.

Grain spawning medium serves as the planting material to inoculate more massive substrates with mushroom mycelia for mass production of mushroom fruiting bodies. Chang and Miles (1997) noted that the spawning of mushroom is a process of cellular expansion. This is an important process in order to produce more mycelia needed for mass production. In this study, different granulated spawning materials such as unmilled rice seeds, sorghum seeds, and corn grit seeds were evaluated because their availability in the local market. 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 (Fig. 2). This result was not consistent with the data reported by Dulay*et al.* (2012) 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.

However, the results in this study means that *L. tigrinus* mycelia efficiently grew in any type of grain spawning materials so it would be easy to propagate the mycelia of this mushroom for mass production. Still these findings contradicts the report of Dulay *et al*. (2012) where they reported that the incubation period of *L. tigrinus* was significantly affected by the spawning materials used. Dulay*et al.* (2012) reported a 5-day incubation period for unmilled rice seeds as compared to our reported 7-day incubation period for all the evaluated grain spawns. Perhaps, different strains of similar species of mushrooms exhibited different mycelial growth on various substrata. Similar observation was reported by Visscher (1989) wherein different strains of king oyster mushroom (*P. eryngii*) responded differently to different substrates, supplements, supplementation amount, and environmental factors in relation to mycelium run, average mushroom yield and quality.

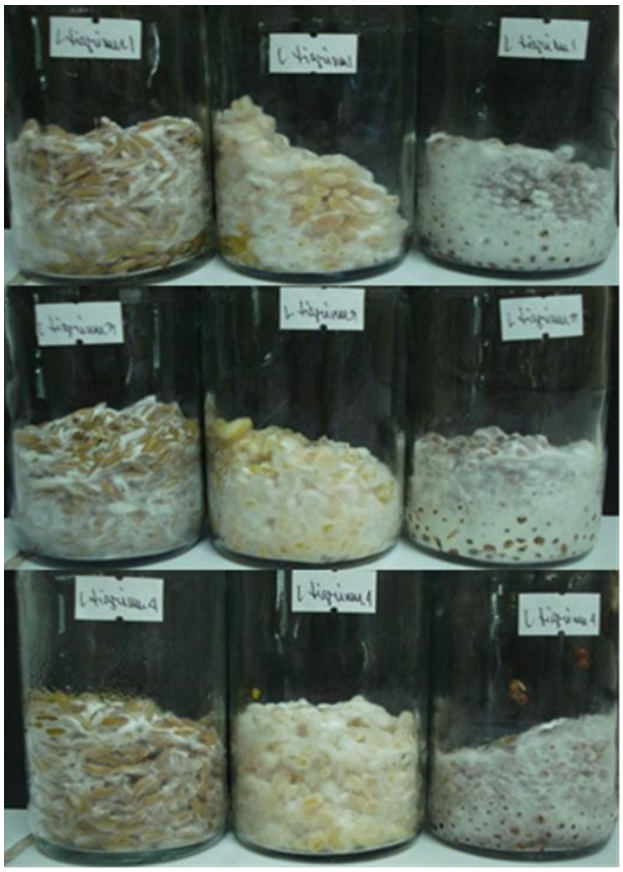

Fig. 2. Mycelial colonization on the different spawning materials: *L. tigrinus* strain ZB12MF03:(A) unmilled riceseeds, (B) corn grits seeds, (C) sorghum seeds; *L. tigrinus* strain ZB12MF05 (D) unmilled riceseeds, (E) corn grits seeds, (F) sorghum seeds and *L. tigrinus* strain

Fruiting body performance of *L. tigrinus* strains on different rice straw-sawdust based substrate formulations enriched with rice bran and rice grits.

Fruiting spawns are usually compacted in pp bags for successful cultivation of mushrooms. These fruiting spawns mimic the natural substrates of the mushrooms from where fruiting bodies directly emerge. Since mushrooms are organism with the ability to utilize various lignocellulosic materials, they could grow in any substrate that supports the nutritional requirements needed for their growth. The substrates used for the cultivation depends on the abundance of materials containing lignin and cellulose that are found in the area. Like, for example, in Hawaii, Tisdale *et al*. (2006) used wood chips as substrate for the cultivation of *Pleurotus ostreatus*. In Cuba, Bermudez *et al*. (2001) used coffee pulp, cocoa shells, and coconut shells as substrate for *P. ostreatus*. Likewise, in Pakistan, Shah *et al.* (2004) utilized saw dust, wheat straw, and leaves as substrate while Vetayasuporn (2006) in Thailand used coconut husk, and bagasse. Both studies cultivated *P. ostreatus*. In Nigeria, Onouha*et al.* (2009) used paddy straw, oil palm fibre, sawdust, and a mixture of oil palm fibre and sawdust in the cultivation of *V. volvacea*. Also from Nigeria, cultivation of *L. squarrosulus* used wood logs and leaves as substrate (Adesina*et al.*2011) while Oghenekaro *et al*. (2008) used saw dust from different tropical tree species, e.g. *B .nigerica, Chlorophora excels, Celtis zenkeri, Guera cedrata,* and *Nesogordenia papaverifera*. In the Philippines, rice straw and saw dust based formulations were used in the cultivation of *S. commune* (Bulseco *et al*., 2005), *L. sajor-caju* (Cuevas *et al*., 2009), *L. tigrinus* (Dulay *et al.,* 2012), and *G. lucidum* (Tayamen*et al.* 2004). In this study, generally, the best treatment formulation for strains ZB12MF03 and ZB12MF06 of *L. tigrinus* was 8 parts rice straw + 2 parts sawdust formulation with 50g RG supplementation (T7) while for strain ZB12MF05, it was noted for 8 parts rice straw + 2 parts sawdust formulation with 75g RG supplementation (T8) (Table 3). In terms of number of flushes, *L. tigrinus* strain ZB12MF03 had the most number of flushes (4.0) at T1 (no supplementation), T2 (with 25g RB), and T7 (with 50g RG). A mean of 3.4 number of flushes at T8 (with 75g RG) was observed with *L.tigrinus* strain ZB12MF05 while a mean of 4.6 number of flushes for both T7 (with 50g RG) and T9 (with 100g RG) was recorded in *L.tigrinus* strain ZB12MF06 (Table 3).

This result was better compared to the 2.33 number of flushes reported by Ayodele *et al.* (2007) in the evaluation of the yield of *L. squarrosulus* on selected economic tree. However, Vetayasuporn (2006) got a higher number of 6.0 flushes in sawdust substrate as well as 5.0 number of flushes in bagasse and coconut husk substrates in the cultivation of *P. ostreatus*. In terms of the number of fruiting bodies, *L. tigrinus* (ZB12MF03) had the most number of

fruiting bodies (32.80) at T2 (with 25g RB). However, this was not statistically different with T1 (no supplementation, 29.0), T6 (with 25g RG, 23.2), and T7 (with 50g RG, 26.6). The lowest mean number of fruiting bodies was 5.00 at T5 (with100g RB) (Table 3). *L. tigrinus* (ZB12MF05), on the other hand, had the highest mean number of fruiting bodies at T8 (with 75g RG) with 31.60. But then again, this was not statistically different with other treatments, e.g. at T1 (no supplementation) with 21.80 and T7 (with 50g RG) with 25.80. The lowest mean number of fruiting bodies was also recorded at T5 (with 100g RB) with 5.00 (Table 3). For *L. tigrinus* (ZB12MF06), T9 (with100g RG) showed the highest mean number of fruiting bodies (37.00) which was not statistically different with treatments 4 to 8. The lowest mean number of fruiting bodies (20.20) was registered at T1 (no supplementation) which was not statistically different with T2 (with 25g RB, 24.40) and T3 (with 50g RB, 21.60) (Table 3).

These results were much higher compared to the 13.3 mean number of fruiting bodies of *V. volvacea* as reported by Onouha *et al.* (2009), and with the 22.11 mean number of fruiting bodies for *P.ostreatus* on sawdust as presented by Shah *et al.* (2004), and the 12.00 mean number of fruiting bodies of *L. squarrosulus*on *S. mombin* supplemented with rice bran as noted in Adesina *et al.* (2011). This means that the three strains of *L. tigrinus* did not show any particular trend with the increase of substrate formulation. This result is in congruence with the report of Oghenekaro *et al.* (2009) where *L. squarrosulus* grown in sawdust of different tropical tree species supplemented with 1% CaCO3, 1% sugar and 20% wheat bran did not also show any difference with the increase of substrate supplementation.

In the study, the sizes of the pileus of *L. tigrinus* were also evaluated (Table 3). *L. tigrinus* strain ZB12MF03 and strain ZB12MF05 registered the highest mean diameter of the pileus with 35.46 mm and 47.13 mm at T6 (with 25g RG). However, *L. tigrinus* strain ZB12MF06 registered the highest mean diameter of the pileus with 43.82 mm at T2 (with 25g RB). On the other hand, for the size of the stipe, *L. tigrinus* strain ZB12MF03 and ZB12MF06 registered the highest mean length of stipe with 34.31 mm and 34.72 mm at T7 (with 50g RG) while for *L. tigrinus* strain ZB12MF05, T5 (with 100g RB) registered the highest mean length of the pileus with 46.76 mm. The highest mean diameter of the stipe of *L. tigrinus* strain ZB12MF03 registered 4.51 mm at T4 (with 75g RB). *L. tigrinus* strains ZB12MF05 and strain ZB12MF06 both registered the highest mean diameter of stipe at T5 (with 100g RB) with 5.98 mm and 5.28 mm, respectively. Sher *et al.* (2011) noted that the major ecological factors that affected stalk height, stalk diameter, and cap size in mushroom were temperature, humidity, air, and compacted materials. Onouha *et al*. (2009) reported a 3.7 cm mean diameter of pileus of *V. volvacea*

cultivated in paddy straw (positive control) and oil palm fibre which was much lower compared to the results obtained in this study. Cultivation of *P. flabellatus* on different substrates, on the other hand, resulted in highest mean pileus diameter on mango sawdust, highest stalk diameter in mahogany tree substrata, and highest stalk length in Kadom sawdust (Islam *et al.,* 2009).

The mean fresh weights of mushrooms harvested throughout the cultivation period at different treatments were also evaluated (Table 3). The two strains *of L. tigrinus* registered highest mean weight at T7 (with 50g RG) for ZB12MF03 and ZB12MF06 with 61.90 g and 75.73 g, respectively. For *L. tigrinus* strain ZB12MF05, highest mean fresh weights were recorded at T8 (with 75g RG) with 84.30 g. The lowest mean weight for all strains of *L. tigrinus* was registered in T5 (with 100g RB), e.g. strain ZB12MF03 (13.54g), strain ZB12MF05 (15.44g), and strain ZB12MF16 (47.31g). These results were higher compared to the 16.3g mean weight of *V. volavacea* cultivated in paddy straw (Onouha *et al.,* 2009), to the 32.10g fresh weight of *L. squarrosulus* on *B. nigeria* (economic tree species) (Ayodele *et al.,* 2007), and to 20.50 g fresh weight of *L. squarrosulus* on *S. mombin* substrate supplemented with rice bran (Adesina *et al.,* 2011). This means that addition of supplementation in the substrate formulations had an effect in the weight of fruiting bodies harvested.

Biological efficiency is an important parameter used to determine the ability of cultivated mushrooms to convert the substrate into fruiting bodies. *L. tigrinus* strain ZB12MF03 and strain ZB12MF06 both registered highest mean biological efficiency at T7 (with 50g RG) with 11.26% and 13.77%, respectively. *L. tigrinus* strain ZB12MF05 got the highest mean biological efficiency of 14.66% at T8 (with 75g RG). The lowest biological efficiency was reported at treatment 5 for all strains. 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 including the findings in this paper were lower compared to the biological efficiency of *L. tigrinus* (15.93%) as reported by Dulay *et al.* (2012).

Perhaps, different species and even different strains of same species exhibited differences in biological efficiency. For example, *P. osteratus* had a 97.9% biological efficiency in the 1st batch and 62.4% biological efficiency in 2nd batch cultivation in wood substrates in Hawaii (Tisdale *et al*., 2006). Shah *et al.* (2004) reported a 64.69% biological efficiency also of *P. ostreatus* while Obodai and Vowotor (2002) reported 50.93% biological efficiency for the same species. This observation was supported by the works of Moonmoon *et al.*

(2010) where cultivation of different strains of *P. eryngii* produced different growth and yield. Peng *et al.* (2010) also reported that different king oyster (*P. eryngii*) mushroom strains response differently to the supplementation amount of rice bran.

Table 1. Treatments showing the formulation of composted rice straw and saw dust with different amounts of rice bran (RB) and rice grits (RG) supplementation

Treatments	**Substrate**	**Supplementation**
	(RS+SD)	RB and RG (g)
T1	8+2	
T2	8+2	25 RB
T3	8+2	50 RB
T4	8+2	75 RB
T5	8+2	100 RB
T6	8+2	25 RG
T7	8+2	50 RG
T8	8+2	75 RG
T9	8+2	100 RG

Table 2. Colony diameter of mycelial growth of *L. tigrinus* strains ZB12MF03, ZB12MF05 and ZB12MF06 on different culture media

Macrofungal species	**Indigenous cuture media**	**Secondary Mycelial Growth Diameter (mm)**							
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8
L. tigrinus ZB12MF03	PSG	8.10±0.13^{b}	9.40±0.90^{b}	13.91±0.94^{b}	17.84±0.88^{b}	20.98±0.51^{b}	23.46±1.51^{b}	26.32±2.94^{b}	28.49±4.41^{b}
	CDG	8.79±0.83^{b}	10.23±1.85^{b}	13.32±1.27^{b}	17.07±1.04^{b}	20.81±1.24^{b}	24.20±1.28^{b}	28.29±0.93^{b}	31.97±1.17^{b}
	CWG	12.81±0.43^{a}	23.53±1.29^{a}	36.69±1.80^{a}	51.54±4.84^{a}	62.30±1.03^{a}	72.24±2.25^{a}	81.92±1.64^{a}	84.16±1.42^{a}
L. tigrinus ZB12MF05	PSG	9.37±0.45^{a}	12.98±1.18^{a}	15.47±1.53^{a}	17.59±1.44^{b}	18.71±1.24^{a}	19.99±1.30^{a}	21.99±1.79^{b}	23.39±2.05^{b}
	CDG	9.15±0.70^{a}	10.44±1.67^{a}	13.77±4.10^{a}	16.52±5.53^{b}	19.72±8.12^{a}	22.55±8.59^{b}	27.03±10.50^{b}	30.77±10.97^{b}
	CWG	9.92±0.33^{a}	16.89±0.78^{a}	24.92±0.99^{a}	33.41±0.46^{a}	37.54±11.84^{a}	50.08±1.66^{a}	60.01±1.58^{a}	65.87±1.60^{a}
L. tigrinus ZB12MF06	PSG	8.54±0.21^{c}	18.80±2.71^{ab}	33.96±3.12^{a}	48.17±3.67^{a}	61.18±2.68^{a}	72.79±2.76^{a}	76.55±1.96^{a}	79.38±2.33^{b}
	CDG	9.17±0.21^{b}	16.81±2.24^{b}	29.83±2.84^{b}	42.20±2.75^{b}	54.85±1.88^{b}	66.77±3.17^{b}	72.77±1.39^{b}	80.87±1.75^{ab}
	CWG	10.49±0.38^{a}	20.50±0.58^{a}	31.94±0.58^{ab}	43.35±0.70^{b}	56.13±0.83^{b}	67.67±0.86^{b}	78.11±2.58^{a}	82.85±2.81^{a}

Table 3. Mean number of flushes, fruiting bodies, size of cap and stipe, weight and biological efficiency of *L. tigrinus* strains ZB12MF03, ZB12MF05 and ZB12MF06 on the different substrate formulations

Mushroom		Substrate	Enrichment	Number of	Number	Size of	Size of Stipe (mm)		Fresh Weight	Biological
Species	Treatments	(RS+SD)	RB and RG (g)	Flushes	of FB	Pileus (mm)	Length	Diameter	mean (g)	Efficiency
L. tigrinus	T1	8 + 2		4±1.22^{a}	29.00±10.12^{ab}	32.52±4.61^{a}	29.34±0.92^{ab}	3.50±0.23^{a}	53.07±11.73^{ab}	10.61±2.35^{abc}
(ZB12MF03)	T2	8 + 2	25 RB	4±1.41^{a}	32.80±11.56^{a}	30.26±3.41^{a}	30.98±1.24^{a}	4.01±0.30^{a}	57.83±10.65^{ab}	11.02±2.03^{ab}
	T3	8 + 2	50 RB	2.4±1.14^{ab}	16.20±3.27^{bcde}	32.94±3.34^{a}	30.95±3.65^{a}	3.99±0.27^{a}	32.32±8.85^{abc}	5.88±1.61^{abcd}
	T4	8 + 2	75 RB	3.2±1.64^{ab}	11.40±3.78^{de}	34.29±9.34^{a}	32.98±4.44^{a}	4.51±0.36^{a}	30.69±13.10^{bc}	5.34±2.28^{cd}
	T5	8 + 2	100 RB	1.8±0.84^{b}	5.00±3.44^{e}	26.44±3.73^{a}	27.51±4.10^{ab}	3.53±0.69^{a}	13.54±6.05^{c}	2.26±1.01^{d}
	T6	8 + 2	25 RG	3.4±1.67^{ab}	23.20±16.12^{abcd}	35.46±8.94^{a}	34.30±7.22^{a}	3.67±0.70^{a}	51.92±33.99^{ab}	9.89±6.47^{abc}
	T7	8 + 2	50 RG	4±1^{a}	26.60±16.55^{abc}	29.25±5.93^{a}	34.31±3.78^{a}	3.87±0.51^{a}	61.90±35.54^{a}	11.26±6.46^{a}
	T8	8 + 2	75 RG	3±2.24^{ab}	13.60±8.56^{cde}	24.88±15.37^{a}	24.35±13.97^{ab}	3.96±2.23^{a}	32.74±27.76^{abc}	5.69±4.83^{bcd}
	T9	8 + 2	100 RG	2.6±2.41^{ab}	11.00±10.56^{de}	27.23±16.19^{a}	20.36±15.59^{b}	3.57±2.24^{a}	34.33±36.13^{abc}	5.72±6.02^{bcd}
L. tigrinus										
(ZB12MF05)	T1	8 + 2		2.8±0.84^{ab}	21.80±11.39^{abc}	38.41±4.57^{ab}	34.60±2.37^{ab}	4.31±0.86^{ab}	49.47±18.74^{bcd}	9.89±3.75^{abcd}
	T2	8 + 2	25 RB	1.6±1.34^{b}	6.40±5.46^{de}	30.92±22.96^{ab}	28.10±16.57^{b}	3.47±2.33^{b}	20.28±17.64^{de}	3.86±3.36^{e}
	T3	8 + 2	50 RB	2.2±1.30^{ab}	10.80±6.83^{cde}	37.75±21.34^{ab}	26.44±14.97^{b}	4.20±2.41^{ab}	32.04±22.29^{cde}	5.83±4.05^{cde}
	T4	8 + 2	75 RB	1.8±1.48^{b}	6.40±5.41^{de}	39.42±23.00^{ab}	31.13±17.98^{ab}	4.14±2.41^{ab}	26.72±21.52^{de}	4.65±3.74^{de}
	T5	8 + 2	100 RB	1.6±0.55^{b}	5.00±2.45^{e}	30.97±12.38^{ab}	46.76±6.65^{a}	5.98±1.10^{a}	15.44±10.13^{e}	2.57±1.69^{e}
	T6	8 + 2	25 RG	2.6±0.55^{ab}	18.80±7.16^{bc}	47.13±13.04^{a}	39.15±4.87^{ab}	4.78±0.12^{ab}	58.18±14.50^{abc}	11.08±2.76^{abc}
	T7	8 + 2	50 RG	2.6±0.55^{ab}	25.80±10.28^{ab}	42.91±4.67^{ab}	35.29±4.99^{ab}	4.65±1.01^{ab}	71.21±13.62^{ab}	12.95±2.48^{ab}
	T8	8 + 2	75 RG	3.4±0.89^{a}	31.60±6.54^{a}	38.63±9.13^{ab}	36.70±2.52^{ab}	4.81±.063^{ab}	84.30±29.28^{a}	14.66±5.09^{a}
	T9	8 + 2	100 RG	2.4±2.30^{ab}	16.40±15.13^{bcd}	22.49±20.65^{b}	24.00±22.00^{b}	3.68±3.50^{ab}	48.12±48.22^{bcd}	8.02±8.04^{bcde}
L. tigrinus										
(ZB12MF06)	T1	8 + 2		4.2±1.10^{ab}	20.20±2.77^{d}	41.71±5.51^{ab}	30.58±4.57^{ab}	4.41±0.75^{ab}	55.06±10.60^{ab}	11.01±2.12^{ab}
	T2	8 + 2	25 RB	4.4±1.14^{ab}	24.40±11.55^{bcd}	43.82±12.03^{a}	33.25±6.68^{ab}	4.94±0.97^{ab}	67.85±11.33^{ab}	12.92±2.16^{a}
	T3	8 + 2	50 RB	4.2±0.84^{ab}	21.60±5.18^{cd}	39.59±10.68^{ab}	32.79±4.77^{ab}	4.35±0.51^{ab}	57.75±5.74^{ab}	10.50±1.04^{ab}
	T4	8 + 2	75 RB	3.2±0.84^{b}	33.20±6.02^{ab}	33.54±3.10^{bc}	28.98±1.15^{bc}	4.31±0.34^{ab}	67.88±7.11^{ab}	11.81±1.24^{a}
	T5	8 + 2	100 RB	3.2±1.30^{b}	29.00±12.67^{abcd}	28.18±5.61^{c}	25.16±2.78^{c}	5.28±1.47^{a}	47.31±19.19^{b}	7.89±3.20^{b}
	T6	8 + 2	25 RG	3.8±0.84^{ab}	32.60±8.29^{abc}	37.02±4.94^{abc}	29.25±3.16^{bc}	3.99±0.61^{b}	63.74±21.88^{ab}	12.14±4.17^{a}
	T7	8 + 2	50 RG	4.6±0.89^{a}	35.00±4.64^{ab}	42.66±4.77^{a}	34.72±4.77^{a}	4.21±0.39^{b}	75.73±14.72^{a}	13.77±2.68^{a}
	T8	8 + 2	75 RG	4.2±1.30^{ab}	31.80±11.12^{abc}	33.36±5.24^{bc}	28.36±2.65^{bc}	4.26±0.62^{b}	73.39±25.11^{a}	12.76±4.37^{a}
	T9	8 + 2	100 RG	4.6±0.55^{a}	37.00±10.68^{a}	36.29±6.15^{abc}	29.39±3.73^{bc}	4.64±0.77^{ab}	72.91±20.75^{a}	12.15±3.46^{a}

In summary, the best substrate formulation for the growth of *L. tigrinus* strain ZB12MF03 and strain ZB12MF06 was at T7 (with 50g RG) and for *L. tigrinus* strain ZB12MF05, it was at T8 (with 75g RG). The best indigenous culture media for mycelial growth of *L. tigrinus* was recorded in CWG. The three strains of *L. tigrinus* in this research also showed that unmilled rice seeds, corn grits seeds, and sorghum seeds produced thick mycelial density at 7 days incubation. Differences observed in the result as compared to those reported by Dulay *et al*. (2012) showed that different strains of mushrooms belonging to the same species exhibited differences in growth responses and fruiting body yield.

The study further showed that agricultural wastes could be used as supplement to improve growth of mushrooms including *L. tigrinus*.

Acknowledgment

This study was supported as a dissertation grant by the Department of Science and Technology under the Accelerated Science and Technology Human Resource and Development Program (ASTHRDP) in the Philippines. The authors thank Dr. SofronioKalaw, Rich Milton Dulay, and the staff of the Center for Tropical Mushroom Research and Development, CLSU for their assistance in the conduct of this study.

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(Received 12 July 2013; accepted 31 August 2013)