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Original Article

Mycelial growth of Philippine mushroom *Lentinus tigrinus* in selected cucurbit-based media and its antioxidant activity

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Abstract

Lentinus tigrinus, a basidiomyceteous fungus, is known to be nutritious and exhibits biological activities. The mycelial growth performance of *L. tigrinus* on selected cucurbit-based solid and liquid media, the ability to scavenge 2,2'diphenyl-1-1picrylhydrazyl (DPPH) free radicals, and the phenolic content of ethanolic extract of mycelia were investigated. Squash decoction *gulaman* (local crude agar) recorded the widest mycelial diameter (71.99 mm) regardless of the amount. However, 500 g/L of all cucurbit species registered the widest colony diameter of 73.35 mm. In liquid culture, both squash decoction and luffa decoction showed the highest mycelial biomass (0.09 g dry wt.). The highest mycelial biomass (0.14 g dry wt.) was noted at 500 g/L of all broth media. Extract of mycelia grown in winter melon decoction showed the highest scavenging activity (51.56%) and phenolic content (25.04 mg GAE/g sample). Therefore, decoction of the cucurbit fruits could be used as main ingredient of culture media for efficient production of mycelial biomass and improvement of antioxidant properties of *L. tigrinus*.

Keywords: *L. tigrinus*, Cucurbit fruits, Antioxidants, Phenolics, Submerged culture

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Introduction

Lentinus tigrinus, also known as *Kabuteng tigre* among Filipinos, is an edible fungus that is usually seen growing on decaying logs, stumps, fence posts, and trunks of dead trees. The mycelia of this mushroom have been successfully domesticated and the optimum culture conditions have been established (Dulay et al., 2012). Liquid cultivation of *L. tigrinus* mycelia using juices of selected tropical fruits such as coconut water, watermelon, honeydew, and grapes (Dulay et al., 2017a), and decoctions of carbohydrate-rich indigenous sources including rice bran, corn grit, and potato (Dulay et al., 2015a) have been demonstrated in our laboratory. These culture media significantly influenced the production of mycelia and antioxidant properties of *L. tigrinus*. Moreover, *L. tigrinus* contains carbohydrates, proteins, dietary fibers, reducing sugars, fats, and minerals, and its lyophilized water extract demonstrates blood glucose-lowering effect in alloxan-induced mice (Dulay et al., 2014). Furthermore, Dulay et al. (2017b) reported that extracts of *L. tigrinus* also show antioxidant and antibacterial activities. Reyes et al. (2013) reported the anti-inflammatory property of this mushroom as shown by inhibition of IL-8 gene expression. It has also been reported that this mushroom has myco-accumulation potential of heavy metals (Soriano et al., 2017).

In our search for alternative media for liquid cultivation and biomass production of *L. tigrinus*, cucurbits were identified in the present work. Cucurbits (Cucurbitaceae) fruits are distinctly characterized by a cylindrically green fruit with thin rind when young and become thicker as they mature, with extremely diverse shape and size but with appropriate ratio of length and diameter of fruit (Bisognin, 2002). In Central Luzon, Philippines, cucurbits such as squash (*Cucurbita maxima*), luffa (*Luffa cylindrica*), bottle gourd (*Lagenaria siceraria*), and winter melon (*Benincasa hispida*) are commonly cultivated by vegetable farmers. Aside from additional source of income, cucurbit farming also provides nutritious foods for Filipinos, as they consumed cucurbit fruits as fresh salads, pickles, vegetable soups, and as among other native vegetable dishes. As nutritious food, they contain valuable nutrients and bioactive compounds. For instance, squash, irrespective of cultivars, has high beneficial fiber and minerals (Czech et al., 2018), whereas luffa contains compounds like phenolics, triterpenoids, ascorbic acids, oleanolic acid, carotenoids, flavonoids, α-tocopherol, chlorophylls, and ribosome-inactivating proteins (Azeez et al., 2013). These bioactive components are highly effective as immunostimulant, anti-inflammatory, anti-diabetic, antitumor, antiviral and oxytocic (Azeez et al., 2013). Kumar et al. (2012) mentioned that bottle gourd has high amounts of carbohydrates, proteins, fats, fibers, calcium, and magnesium, while Al-Snafi (2013) stated that winter melon is rich in saccharides, proteins, carotenes, vitamins, and minerals. Moreover, some cucurbits have also been reported to exhibit anthelmintic, emetic, and purgative properties (Robinson and Decker-Walters, 1997). It is hypothesized that the above-mentioned nutrients are important for the efficient growth of mycelia and could contribute to the nutritional value and functional activities of mushrooms.

Given the rich nutritional status of cucurbits, it is therefore necessary to determine the effect of decoction of selected cucurbits at varying amounts on the mycelial growth of *L. tigrinus* on both solid and liquid culture conditions. It is also of significant interest to determine the antioxidant properties of the extract of the harvested mycelia grown in liquid culture.

Material and Methods

Culture

Mycelia blocks were inoculated on potato dextrose agar plates and incubated for seven days. After incubation, a 10 mm-diameter cork borer was used to prepare the mycelial discs as inoculants in the evaluation of mycelia growth.

Preparation of solid and liquid cucurbit-based media

The cucurbits (squash, luffa, bottle gourd, and winter melon) were purchased from the local market. These were peeled, washed, chopped, and weighed at different amounts (100 g, 250 g, and 500 g). Each amount was boiled into 1 L distilled water and the decoction was filtered using cheesecloth. A 500 mL of decoction was reboiled and 10 g of *gulaman* (agar) was added. This was dispensed into a dry clean 1L-capacity flask. On the other hand, in preparing liquid media, 50 ml (from the remaining 500 mL decoction) decoction was dispensed into a glass bottle. Ten bottles were prepared for each decoction. Media were autoclaved for 30 min at 15 psi and 121°C. Flask media were pour-plated into sterilized petri plates in triplicate and allowed to solidify prior to inoculation.

Evaluation of mycelial growth

Both solid and liquid cucurbit-based media were aseptically inoculated with the mycelial disc. After five days of incubation at 30°C, mycelial growth diameter was measured using a Vernier caliper. The optimum amount of cucurbit and the best cucurbit type were determined based on the colony diameter and density of mycelia. On the other hand, inoculated culture bottles were incubated for 15 days in a static condition at 30°C. The mycelia were harvested, air dried, and weighed. The best cucurbit type and amount were identified based on the dry weight of mycelial biomass. The air-dried mycelia from the best concentration of each cucurbit were pulverized prior to extraction.

Determination of antioxidant property

Five grams of the pulverized samples were extracted in 300 mL of 95% ethanol and concentrated in a rotary evaporator until dryness and were prepared for the assay. The ability of the extracts as scavengers of 2, 2'diphenyl-1-1picrylhydrazyl (DPPH) free radicals was determined following the procedure of Kolak et al. (2006) with minor modifications. However, phenolic content was estimated using the Folin-Ciocalteu method of Sunita and Dhananjay (2010).

Statistical analysis

The SAS System Version 9.0 (SAS Institute Inc. Cary, NC, USA) was used to analyze and compare all the data.

Results

Mycelial growth and biomass production in cucurbit-based media

The mycelial diameter of *L. tigrinus* grown on selected cucurbit-based solid media after five days of incubation is presented in Table 1. It can be seen that from among solid media, squash decoction *gulaman* recorded the widest mycelial diameter, whereas bottle gourd *gulaman* had the smallest. In terms of the amount, 500 g/L showed the widest mycelial diameter, while 100 g/L registered the smallest. Tukey's HSD revealed a significant difference among cucurbit types and cucurbit amounts. At 500 g/L of all media produced very thick mycelial density except bottle gourd *gulaman* (Figure 1). However, the mycelial diameter and mycelial density of *L. tigrinus* were improving in increasing amount of all cucurbits decoction media.

Table-1. Colony diameter of mycelia of *L. tigrinus* grown on selected cucurbit-based solid media on the 5th day of incubation.

Solid Media	Diameter of Mycelial Growth (mm/5 days)			
	100 g/L	250 g/L	500 g/L	Mean
Squash	64.97	72.87	78.14	71.99[a]
Luffa	66.34	69.41	70.48	68.74[b]
Bottle gourd	60.18	64.28	71.85	65.44[c]
Winter melon	61.34	69.87	72.91	68.04[b]
Mean	63.21[c]	69.11[b]	73.35[a]	-

Means with similar letter superscript indicate no statistical difference ($p<0.05$).

The effect of selected cucurbit-based liquid media on the mycelial production of *L. tigrinus* was also evaluated. Table 2 shows the mean dry weight of mycelial biomass after 15 days of incubation. Both squash decoction and luffa decoction recorded the highest mycelial dry weight regardless of the amount.

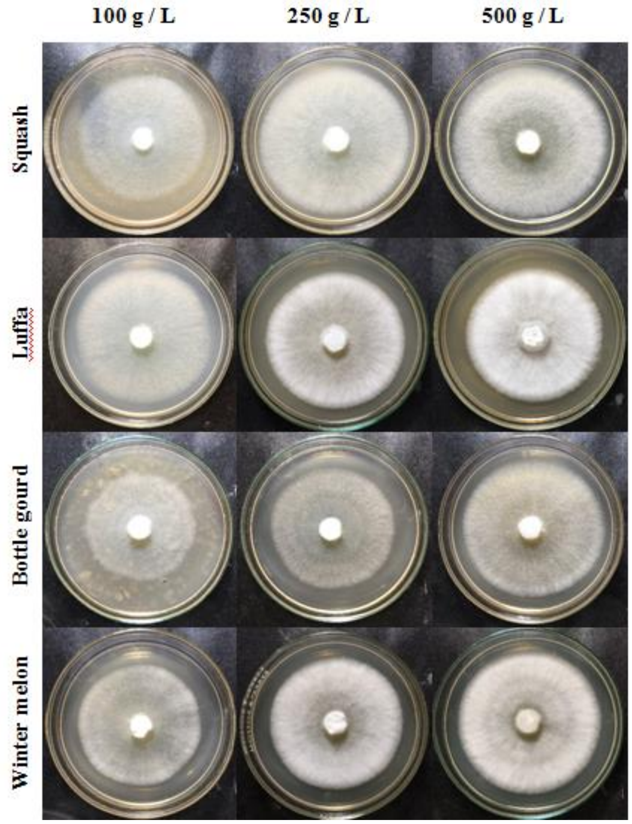

Figure-1. Mycelial growth performance of *L. tigrinus* on the varying amounts (100 g/L, 250 g/L, and 500 g/L) of cucurbits (squash, luffa, bottle gourd, and winter melon) after 5 days of incubation.

Table-2. Yield of mycelia of *L. tigrinus* cultured in selected cucurbit-based liquid media after 15 days of incubation.

Liquid Media	Yield of Mycelial Biomass (g dry wt./15 days)			
	100 g/L	250 g/L	500 g/L	Mean
Squash	0.03	0.08	0.16	0.09[a]
Luffa	0.03	0.05	0.18	0.09[a]
Bottle gourd	0.02	0.06	0.10	0.06[b]
Winter melon	0.04	0.06	0.10	0.07[b]
Mean	0.03[c]	0.07[b]	0.14[a]	-

Means with similar letter superscript indicate no statistical difference ($p<0.05$).

On the other hand, the lowest biomass yield was noted in bottle gourd decoction. Similar to solid media, the mycelial biomass yield increased as the amount of cucurbit increases, and so 500 g/L had the highest biomass yield. The liquid cultures of *L. tigrinus* in the varying amounts of cucurbits are shown in Figure 2.

These results indicate that the mycelial biomass production of *L. tigrinus* is dependent on the amount and type cucurbit as basal media in the liquid culture.

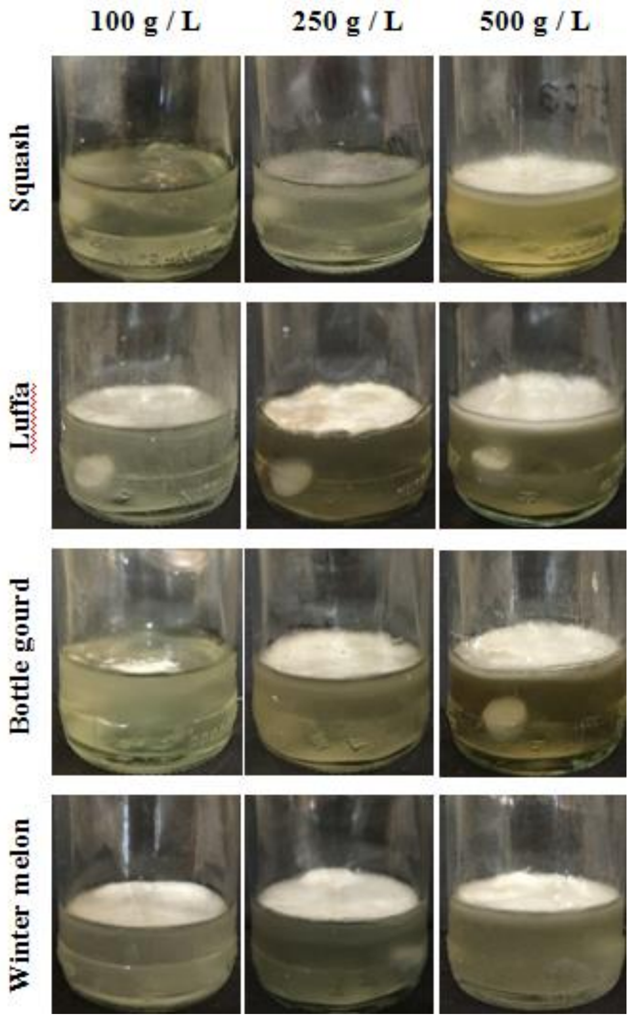

Figure-2. Liquid culture of *L. tigrinus* in the varying amounts (100 g/L, 250 g/L, and 500 g/L) of cucurbits (squash, luffa, bottle gourd, and winter melon) after 15 days of incubation.

Antioxidant property of mycelia

The percentage radical scavenging activity of the extracts of mycelia is presented in Table 3. Apparently, the scavenging activity was significantly affected by the different cucurbit types. Extract of mycelia from winter melon decoction recorded the highest scavenging activity. This was followed by the mycelial extract from luffa decoction. On the other hand, mycelial extracts of both squash broth and bottle gourd broth registered almost the same activity. Phenolic content of the mushroom extracts was also quantified. Results of the analysis revealed that extract of mycelia grown in winter melon had the highest phenolic content, but statistically comparable with the phenolic contents of mycelial extracts from luffa decoction and bottle gourd decoction (Table 3).

Table-3. Antioxidant properties of ethanolic extract of *L. tigrinus* mycelia grown in selected cucurbit-based liquid media at 500 g/L.

Mycelia grown in	DPPH Scavenging Activity (%)	Phenolic Content (mg GAE/g sample)
Squash	39.84^{d}	18.58^{b}
Luffa	43.75^{c}	24.42^{a}
Bottle gourd	38.28^{d}	24.42^{a}
Winter melon	51.56^{b}	25.04^{a}
Cathechin	66.41^{a}	-

Means with similar letter superscript indicate no statistical difference ($p<0.05$).

Discussion

In mushroom production, the growth performance of mycelia is dependent on the type of culture media. Mycelial biomass production in submerged cultivation or liquid culture is of greater interest than the conventional lengthy production process because the former is a fast and efficient alternative of producing biomass and metabolites (Dulay et al., 2015b). In this work, four selected cucurbit-based media at varying amounts were evaluated in both solid and liquid culture conditions. Squash decoction *gulaman* was found to be the most suitable cucurbit type and 500 g/L was the optimum amount of each cucurbit for the luxuriant growth of *L. tigrinus* mycelia in both state of cultivation. The superiority of squash decoction as solid and liquid media for the growth of mycelia of *L. tigrinus* could probably be attributed to its essential chemical components. Squash contains carbohydrates, proteins, reducing sugars, amino acids, tocopherols, carotenoids, and minerals (Sharma and Rao, 2013; Adebayo et al., 2013; Czech et al., 2018; Kim et al., 2012), which are essential for physiological function. Moreover, Karanja et al. (2013) reported that squash has β-carotene (provitamin A) and fatty acids (linoleic acid, oleic acid, and palmitic acid). In our previous work (Dulay et al., 2017c), supplementation of varying amounts of vitamin A in the culture media stimulated the mycelial growths of *L. tigrinus*, *Schizophyllum commune*, and *Ganoderma lucidum*, and the vitamin A-enriched substrate improved the yields and biological efficiencies of *L. tigrinus* and *G. lucidum*. Luffa decoction was also identified as a suitable medium for mycelial biomass production in liquid culture. Luffa contains sugars and lignocellulose (Siqueira et al., 2010). Petre et al. (2016) mentioned that lignocellulose improves the growth of the mycelium through stimulation of the enzymatic activity. In addition, luffa has high amounts of essential mineral elements such as sodium, potassium, magnesium, and calcium, and rich in nutrients like sitosterol, β-tocopherol, phytol, stigmasterol, and hydrocarbons (Adewuyia and Oderinde, 2012). The above-mentioned nutrients of squash and luffa are also present in coconut water, which serve as the most suitable indigenous medium for the luxuriant mycelial growths of *L. tigrinus* and *L. strigosus* (Dulay et al., 2012; Dulay et al., 2017d). Although most of these nutrients are also components of bottle gourd and winter melon, the inferior performance of mycelia in these liquid media could probably be accounted to the presence of phenolics compounds, which are inhibitory to the normal physiology of the fungal cells, thereby affecting the growth and development of the mycelia. Pizzolitto et al. (2015) reported that some natural phenolic compounds showed inhibitory effects on the growth of fungus, *Apergillus parsiticus*.

Several mushrooms have been reported to exhibit antioxidants properties, which neutralize the free radicals that damage the cells resulting to aging and degenerative diseases such as cancer. These antioxidant properties of mushrooms include carotenoids, ascorbic acids, ergosterol, tocopherols, polysaccharides, and phenolics (Sanchez, 2017). In general, the physico-chemical characteristics of mushrooms are dependent on the properties and compositions of medium or substrate (Oyetayo and Ariyo, 2013). The antioxidant properties of the extracts of mycelia grown in 500 g/L of each cucurbit were also determined in our intention to establish the effect of cucurbit-based media not only on the growth performance but also on the antioxidant activity of *L. tigrinus*. Surprisingly, the squash decoction that showed the highest yield of mycelial biomass exhibited the lowest scavenging activity and phenolic content. This observation does not conform to our hypothesis that the mycelia grown in the most suitable medium would exhibit maximum antioxidant properties. Interestingly, mycelia grown in winter melon decoction recorded the maximum antioxidant properties of *L. tigrinus*.

The radical scavenging activity and phenolic content of *L. tigrinus* was found dependent on the type of cucurbit. The same finding was also reported in our previous works. For instance, the scavenging activity of *L. tigrinus* is dependent on the type of liquid media, in which rice bran broth registered the highest radical scavenging activity (18.94%) among indigenous liquid media (Dulay et al., 2015a). In addition, *L. tigrinus* mycelial extract from honeydew juice recorded the highest scavenging activity (79.79%) among selected tropical fruit juices (Dulay et al., 2017a). Moreover, the maximum radical scavenging activity and phenolic content of *Pleurotus djamor* and *G. lucidum* were found in the extracts of mycelia grown in corn grit decoction and coconut water, respectively (Bustillos et al., 2018). These findings of previous and current works clearly suggest that mycelia of *L. tigrinus* and other mushrooms can be manipulated by the culture media to exhibit higher antioxidant properties, which indicates a significant advantage in the development of functional food and nutraceuticals.

Conclusion

Altogether, the present study has shown for the first time that decoction of the fruit of cucurbits could be utilized as alternative basal media for the efficient growth of mycelia of *L. tigrinus* in both solid and liquid culture conditions and for the enhancement of the antioxidant properties. The cultivation technique demonstrated in the present work represents significant contribution in the Philippine mushroom industry as well as in the nutraceutical and pharmaceutical industries. However, it is necessary to further elucidate other biological properties of the *L. tigrinus* extract, which are essential in the development of useful products for the benefits of mankind.

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Contribution of Authors

Liwanag EJ: Contributed in data collection, literature review and manuscript write up
Dulay RM: Contributed in interpretation of the data, writing of manuscript and literature review
Kalaw S: Contributed in data analysis and interpretation, manuscript write up and final approval