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Culture conditions for mycelial growth of eight Philippine wild strains of tiger sawgill mushroom, *Lentinus tigrinus*

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Abstract

Lentinus tigrinus is a wild edible and medicinal mushroom with potential culinary and pharmaceutical applications. In the present work, we evaluated the different culture media, pH and physical conditions such as aeration, illumination and temperature for maximum mycelial growth of eight *L. tigrinus* strains. Mycelial growth of the eight strains respond differently to the nutritional and physical factors. All strains showed maximum mycelial growth on coconut water gulaman (CWG), except TLLt 3, which favored potato dextrose agar (PDA) and malt extract agar (MEA). TLLt 1, 5, 6, 7 and 8 mycelia luxuriantly grew at pH 5.0-8.0 whereas TLLt 2 preferred pH 5.0-7.0, and TLLt 3 and 4 favored pH 5.0-6.5. Sealed condition was found to be more suitable for TLLt 1 mycelia while no significant difference was noted between the two aeration conditions for the mycelial growth of other seven strains. Both TLLt 1 and 2 mycelia favorably grew in lighted condition while the other six favorably grew in both lighted and dark conditions. Room temperature (30^0C) recorded the fastest mycelial growth fo all strains, but found comparable to air-conditioned temperature (23^0C) for the growth of TLLt 3, 6 and 8 mycelia. Therefore, mycelial growth of *L. tigrinus* on the different nutritional and physical parameters may vary depending on the strain type.

Keywords: *Lentinus tigrinus*, mycelia, commercial culture media, laboratory made media.

Introduction

Lentinus tigrinus is a culinary- medicinal mushroom which is popularly known as tiger saw gill mushroom or *kabuteng tigre* due to the appearance of the gills and presence of small brown scales on its basidiocarp. This mushroom is a white rot basidiomycete belonging to Class Agaricomycetes, Order Polyporales and Family Polyporacea. It is characterized by leathery flesh and tough fruiting body and commonly grows singly or in group on fallen logs or rotten branches during the onset or middle part of rainy season (Dulay *et al*., 2012). This mushroom was recently, observed growing in various parts of Luzon, Philippines.

As a culinary food ingredient, the basidiocarp contains proteins, carbohydrates, ash and fibers but low in fat and energy value (Dulay *et al*., 2014; Pourianfar *et al*., 2020). As a

medicine, the extracts contain bioactive compounds that exhibit a number of medicinal attributes such as antioxidant, antibacterial, antifungal (Sevindik, 2018; Dulay *et al*., 2017), antiproliferative and pro-apoptotic (Mohammadnejad *et al*., 2019) and hypoglycemic activities (Dulay *et al*., 2014). Gao *et al*. (2019) identified fungal proteins (Fip-lti 1 and Fip-lti2) from *L. tigrinus* that can protect the liver from Con A induced liver oxidative injury. The dichloromethane extract contains cerevisterol, stellasterol and ergosterol which have been reported to exhibit medicinal activities such as anti - inflammatory, antibacterial and antioxidant activities in other mushroom species (Ragasa *et al*., 2018). Laccase with inhibitory activity against HIV 1 reverse transcriptase was isolated from the broth of mycelial culture (Xu *et al*., 2012). The occurrence of mushrooms in the wild is seasonal so that most of them appear only during rainy season. Moreover, when they appear, the amount is not sufficient. Optimization of culture conditions and domestication of wild mushrooms are necessary for the continuous supply fruiting bodies or mycelia for evaluation of their biological activities. In our desire to search for additional strains of *L. tigrinus* which can be used as source of nutritious food and to develop drugs and functional foods, we evaluated the nutritional and physical requirements for mycelial growth. Therefore, the present study established the suitable culture media and physical conditions for mycelial growth, maintenance of germplasm and cultivation of different strains of *L. tigrinus.*

Materials and Methods

Tissue Culture

Fruiting bodies of eight strains of *L. tigrinus* were collected from the different areas of Luzon Island, Philippines. Small tissue from the inner part of the fruiting body was aseptically cut using sterile scalpel and inoculated into a sterile PDA slant. The tissue was allowed to colonize the medium for one week. Afterward, mycelial block was transferred into PDA plates and allowed to grow for a week. The agar plate culture served as the source of inoculum for the evaluation of nutritional and physical requirements for mycelial growth of the different strains.

Evaluation of culture media

To assess the nutritional requirements of different strains of *L. tigrinus*, various culture media were used i.e. four commercial culture media and four home-made culture media. The commercial media include potato dextrose agar (PDA), malt extract agar (MEA), mycological agar (MA) and sabouraud dextrose agar (SDA). These media were prepared according to the manufacturer's instruction written in the label. On the other hand, the home-made culture media namely: potato sucrose gulaman (PSG), coconut water gulaman (CWG), corn grit decoction gulaman (CGDG) and rice bran decoction gulaman (RBDG) were prepared following the preparations described by Kalaw *et al*. (2016) and Dulay *et al*. (2012). All culture media were sterilized in an autoclave at 121°C, 15 psi for 30 min. Once cooled, approximately 20 ml of the media were dispensed in previously sterilized petri plates and allowed to solidify overnight. Mycelial disc (10 mm diameter) from a week-old *L. tigrinus* culture was inoculated at the center of the plate. The mycelial growth rate was determined and the mycelial density was also observed. Each treatment was replicated thrice.

Evaluation of pH

The pH requirement was assessed using the most suitable culture medium. The pH of the medium was adjusted at 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 using 0.1 M hydrochloric acid and 0.1 M sodium hydroxide. Afterwards, the media were autoclaved at 121°C, 15 psi for 30 min. Media were pour-plated and allowed to cool. Mycelial disc (10 mm diameter) from a week-old culture was inoculated at the center of the plate. The mycelial growth was measured at 7th day incubation. The mycelial density was visually assessed. The experiment was done in triplicate.

Evaluation of physical factors

Aside from nutritional factor, the influence of aeration, illumination and temperature was also determined. The protocol outlined by Kalaw *et al*. (2016) was used in this study. In the determination of the most appropriate aeration condition, the first set of plates was sealed with parafilm while the second set of plates were not sealed with parafilm. In illumination experiment, culture plates were wrapped with black cloth while the other plates were illuminated with fluorescent

light. Finally, to determine the influence of temperature, the culture plates were incubated in three temperature conditions namely: room temperature (30^0C), air-condition temperature (23^0C) and refrigeration temperature (9^0C). All treatments were prepared in triplicate.

Statistical analysis

The data gathered were statistically analyzed using SPSS version 16. Turkeys HSD was used in comparing more than two means while T - test was employed in comparing means of experiments with two treatments at 5% level of significance.

Results and Discussion

Effect of culture media

In-vitro cultivation of mushroom mycelia requires a suitable culture medium that will support efficient growth and development. This culture medium contains the essential nutrients needed by the mushroom organism. Several commercial and laboratory made culture media are now being used as culture medium for the maintenance of the pure culture of mushroom. The influence of four commercially available dehydrated culture media and four indigenous culture media on the mycelial growth of eight *L. tigrinus* strains was evaluated. Table 1 shows the mycelial growth rates of eight strains as affected by the different culture media. It can be seen that each strain of *L. tigrinus* respond differently to the eight culture media. All strains showed the highest mycelial growth rate on CWG, except TLLt 3 which favored PDA and MEA. Aside from CWG, TLLt 2 mycelia also favored SDA, whereas TLLt 5 and 7 also preferred RBDG and TLLt 4 also favored RBDG, MEA and PDA, which showed no significant difference with CWG. These results suggest that each strain has specific culture medium preference.

It was found that among eight strains, mycelia of seven strains exceedingly favored CWG. The superiority of CWG could be attributed to its nutrient compositions. Coconut water contains sugars, inorganic ions, vitamins, minerals, amino acids, enzymes, auxin, cytokinin, diphynelurea and trace elements such as zinc, selenium, iodine, sulfur, manganese, boron, molybdenum etc. (Yong *et al*., 2009; Prades *et al*., 2012). Earlier studies also reported that coconut water is an ideal medium for mycelial growth *Pleurotus citrinopileatus*, *Pleurotus djamour, Pleurotus salmoneostramineus* and *Ganoderma lucidum* (Jacob *et al*., 2015; Magday *et al*., 2014; Zurbano *et al*., 2017). On the other hand, commercially-available media such as PDA and MEA were also found suitable for one strain. Similarly, *Agrocybe aegarita, Pleurotus ostreatus*, and *Volvariella volvacea* exhibited faster mycelial growth on MEA (Muthu and Shanmugasundaram, 2015; Nasim *et al*., 2001). In addition, Kumar *et al*. (2020) observed that optimum mycelial growth of *Pleurotus ostreatus* was obtained in PDA.

Effect of pH

The pH of the medium is an important factor that can influence the rate of mycelial growth and proliferation. Table 1 presents the pH requirement of eight strains of *L. tigrinus*. It is interesting to note that all strains can grow from pH 5.0 to 8.0. Strains TLLt 1, 5, 6, 7 and 8 can grow on a wide range of pH as indicated by the no significant differences in mycelial growth in all pH levels evaluated. However, the other three strains preferred narrower pH range. TLLt 2 required 5.0 to 7.0, whereas TLLt 3 and 4 favored pH between 5 – 6.5 and 5 – 6.0, respectively. All strains showed very thick mycelia except TLLt 1 and 8, which do not possess thick mycelia. The data obtained in the present study imply that the pH requirement of mushroom is not only species specific but also strain dependent. In a similar work, Kalaw *et al*. (2016) reported difference in pH requirements of two *L. tigrinus* strains, CLSU strain A and CLSU strain B which displayed larger mycelial growth at pH 6 to 6.5 and 6, respectively. On the other hand, Dulay *et al*. (2012) disclosed that the optimum pH of *L. tigrinus* was found at 7 to 8. Abon *et al*. (2020) reported that the optimum pH for *Volvariella volvacea* La Clementina strain and Vinces strains was at 6.5 to 7.5 and 6.5 to 7 for Montalvo strain.

Influence of aeration

Oxygen affects cell growth, cellular morphology, nutrient uptake and metabolite biosynthesis (Simonic *et al*., 2008). The aeration requirements of *L. tigrinus* strains were determined by incubating them in sealed and unsealed conditions. It can be noticed that aeration did not influence the mycelial growth of seven strains of *L. tigrinus* as indicated by no significant difference in mycelial growth between sealed and unsealed conditions (Table 2). However, *L. tigrinus* TLLt 1 strain showed faster mycelial growth in sealed condition. In terms of mycelial density, all strains displayed very thick mycelia in both conditions except

TLLt 8, which do not exhibit thick mycelia. Results suggest that aeration was not a major physical factor in most *L. tigrinus* strains, although it is in some strains. The findings corroborate early reports regarding the influence of aeration on mushrooms. For instance, Abon *et al*. (2020) reported that the three Ecuador strains of *Volvariella volvacea* were not significantly influenced by aeration conditions. Same with the mycelial growth of *Cyclocybe cyndracea* and *P. djamour* (Landingin *et al*., 2021; Bumanlag *et al*., 2018. In fact, Reyes *et al*. (1998) found out that *V. volvacea* preferred sealed conditions.

Influence of illumination

Light conditions during mycelial growth are known to influence fungi in many ways (Rangel *et al*., 2011). Although not photosynthetic, mushrooms need light to stimulate the mycelial growth. The influence of light was assessed by incubating the culture plates in lighted and dark conditions. Apparently, TLLt 1 and 2 strains significantly displayed faster mycelial growth and very thick mycelia in lighted condition, whereas the mycelial growth of other six strains showed no significant different between lighted and dark conditions (Table 2). Both conditions also showed very thick mycelial growth of the six strains. These results suggest that the strains evaluated exhibited varied responses to illumination. Variations in mycelial growth response of different species and strains of mushrooms in light and dark conditions have been documented in earlier reports. For example, Abon *et al*. (2020) found out that the mycelia of two strains of Ecuador *Volvariella volvacea* favorably grew in lighted condition while one strain exceedingly favored dark conditions. Similarly, Quiaco *et al*. (2014) observed that light was found not essential for mycelial growth of three strains of *Lentinula edodes*. On the other hand, the mycelial growth of *Auricularia polytricha* and *Auricularia auricula* is better in artificial light compared to darkness (Priya and Geetha, 2016). Lastly, Dulay *et al*. (2021) disclosed that the mycelia of *Trametes elegans* was not influenced by lighted and dark conditions.

Influence of temperature

Among the different physical parameter, temperature is considered as the most fundamental factor. It determines the distribution of fungi (Hudson, 1986). The influence of temperature was also determined by incubating the culture plates in three temperature conditions such as room temperature (30°C), air-condition temperature (23°C) and refrigeration temperature (6°C). It can be seen that TLLt 1, 2, 4, 5 and 7 significantly recorded higher mycelial growth when incubated at 30°C (Table 2). However, mycelial growth of TLLt 3, 6 and 8 showed no significant difference between 30°C and 23°C, suggesting that both temperature conditions are favorable for growth. Contrastingly, no mycelial growth of all strains was observed at 6°C. The results imply that the temperature requirement for mycelial growth of *L. tigrinus* is strain dependent and the strains evaluated are suitable to the tropical conditions of the country. The present study established that the eight strains of *L. tigrinus* are considered tropical mushrooms as they favorably grew at 30°C. This agrees with the observation of Kerketta *et al*. (2017) that the mycelial growth of each strain *Calocybe indica* significantly varied in all temperature evaluated. However, Alam and Rahman (2020) reported that the 25°C was the optimum temperature for mycelial growth of five strains of *Pleurotus salmoneostraminius.*

Table 1. Effect of culture media and pH on the mycelial growth of the eight strains of *Lentinus tigrinus*.

Factors	Mycelial growth rate (day^{-1})							
	TLLt 1	TLLt 2	TLLt 3	TLLt 4	TLLt 5	TLLt 6	TLLt 7	TLLt 8
Media								
CWG	15.00^{a}	15.00^{a}	13.37^{b}	12.86^{a}	15.00^{a}	15.00^{a}	15.00^{a}	10.71^{a}
PSG	12.59^{ab}	8.84^{c}	8.68^{c}	9.23^{b}	8.19^{de}	12.12^{c}	10.19^{c}	6.99^{cd}
CGDG	11.78^{b}	9.01^{c}	8.87^{c}	4.85^{d}	11.42^{b}	12.64^{c}	12.55^{abc}	7.86^{bcd}
RBDG	12.05^{b}	9.55^{c}	12.59^{b}	11.74^{a}	15.00^{a}	13.73^{b}	14.14^{ab}	8.00^{bcd}
PDA	11.61^{b}	13.75^{ab}	15.00^{a}	12.86^{a}	9.34^{cd}	12.87^{c}	11.39^{bc}	8.96^{ab}
MEA	7.47^{c}	12.38^{b}	15.00^{a}	12.86^{a}	10.61^{bc}	12.19^{c}	11.21^{c}	8.60^{bc}
SDA	12.82^{ab}	15.00^{a}	13.07^{b}	9.35^{b}	10.83^{b}	11.00^{d}	4.84^{d}	6.50^{d}
MA	7.26^{c}	8.49^{c}	6.85^{d}	7.60^{c}	7.17^{e}	6.45^{e}	10.41^{c}	4.34^{e}
pH								
5.0	15.00^{a}	17.73^{a}	15.00^{a}	15.00^{a}	12.79^{a}	15.00^{a}	15.00^{a}	9.37^{a}
5.5	12.00^{a}	17.56^{ab}	14.60^{ab}	15.00^{a}	13.46^{a}	15.00^{a}	15.00^{a}	9.54^{a}
6.0	12.00^{a}	17.76^{a}	13.42^{ab}	14.79^{a}	13.01^{a}	15.00^{a}	15.00^{a}	8.88^{a}
6.5	12.00^{a}	18.00^{a}	14.50^{ab}	12.95^{b}	13.45^{a}	15.00^{a}	15.00^{a}	9.69^{a}
7.0	12.00^{a}	18.00^{a}	13.21^{c}	11.90^{c}	13.85^{a}	15.00^{a}	15.00^{a}	8.89^{a}
7.5	14.92^{a}	16.93^{b}	13.65^{bc}	10.00^{d}	15.00^{a}	15.00^{a}	15.00^{a}	8.28^{a}
8.0	15.00^{a}	15.77^{c}	12.72^{c}	9.37^{e}	13.94^{a}	15.00^{a}	15.00^{a}	7.53^{a}

Means with similar superscripts are statistically comparable from each other using Tukeys HSD at 5% level of significance. CWG, coconut water gulaman; PSG, potato sucrose gulaman; CGDG, corn grit decoction gulaman; RBDG, rice bran decoction gulaman; PDA, potato dextrose agar; MEA, malt extract agar; SDA, Sabouraud dextrose agar; MA, mycological agar.

Table 2. Effect of physical factors on the mycelial growth of the eight strains of *Lentinus tigrinus*.

Factors	Mycelial growth rate (day^{-1})							
	TLLt 1	TLLt 2	TLLt 3	TLLt 4	TLLt 5	TLLt 6	TLLt 7	TLLt 8
Aeration								
Sealed	15.00^{a}	18.00^{a}	15.00^{a}	15.00^{a}	22.50^{a}	15.00^{a}	15.00^{a}	9.81^{a}
Unsealed	14.25^{b}	16.78^{a}	14.79^{a}	15.00^{a}	22.50^{a}	15.00^{a}	15.00^{a}	8.66^{a}
Illumination								
Lighted	15.00^{a}	18.00^{a}	15.00^{a}	18.00^{a}	22.10^{a}	18.00^{a}	18.00^{a}	12.06^{a}
Dark	13.76^{b}	17.06^{b}	15.00^{a}	18.00^{a}	22.50^{a}	18.00^{a}	18.00^{a}	11.80^{a}
Temperature								
9^{0}C	ng	ng	ng	ng	ng	ng	ng	ng
23^{0}C	13.76^{b}	14.93^{b}	15.00^{a}	16.25^{b}	18.86^{b}	18.00^{a}	17.51^{b}	12.11^{a}
30^{0}C	15.00^{a}	18.00^{a}	15.04^{a}	18.00^{a}	22.50^{a}	18.00^{a}	18.00^{a}	12.55^{a}

Means with similar superscripts are statistically comparable from each other using Tukeys HSD and t-test at 5% level of significance. ng, no growth.

Conclusion

Each strain has unique nutritional and physical condition requirements for efficient mycelial growth even belonging to the same species. CWG at pH 5.0-8.0 incubated in either sealed or unsealed, either lighted or dark, and at 30^{0}C are found to be the most suitable nutritional and physical conditions for the maximum mycelial growth of most *L. tigrinus* strains evaluated in this study. These information are very vital in maintenance of the germplasms of *L. tigrinus* in the culture collection laboratory and in the generation of production technologies for medicinal and industrial purposes. Evaluation of fruiting body production is currently under investigation in the laboratory.

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References

Abon, M. D., Dulay, R. M. R., Kalaw, S. P., Romero-Roman, M. E., Arana-Vera, L. P., Reyes-Borja, W. O. & Reyes, R. G. (2020). Effects of culture media and physical factors on the mycelia growth of the three wild strains of *Volvariella volvacea* from Ecuador. Journal of Applied Biology and Biotechnology 8(6):60-63.

Alam, N. & Rahman, F. (2020). Optimum vegetative growth conditions and genetic diversity in different strains of *Pleurotus salmoneostramineus* LJN Vassilzeva. Bangladesh Journal of Botany 49(1):125-134.

Bumanlag, C. P. V., Kalaw, S. P., Dulay, R. M. R. & Reyes, R. G. (2018). Optimum conditions for mycelial growth and basidiocarp production of *Pleurotus djamor* on corn-based media. International Journal of Biology, Pharmacy and Allied Sciences 7(4):558-575.

Dulay, R. M. R., Alcazar, A. A., Kalaw, S. P., Reyes, R. G. & Cabrera, E. C. (2021). Nutritional and physical requirements for mycelial growth and basidiocarp production of *Trametes elegans* from the Philippines. Asian Journal of Agriculture and Biology 2021(1).

Dulay, R. M. R., Arenas, M. C., Kalaw, S. P., Reyes, R. G. & Cabrera, E. C. (2014). Proximate composition and functionality of culinary-medicinal tiger saw gill mushroom, *Lentinus tigrinus* (Higher Basidiomycetes) from the Philippines. International Journal of Medicinal Mushrooms 16(1): 85-94.

Dulay, R. M. R., Kalaw, S. P., Reyes, R. G., Cabrera, E. C. & Alfonso, N. F. (2012). Optimization of culture conditions for mycelial growth and basidiocarp production of *Lentinus tigrinus* (Bull) Fr., a new record of domesticated wild edible mushroom in the Philippines. Philippine Agricultural Scientist 95(3):278-285.

Dulay, R. M. R., Miranda, L. A., Malasaga, M. S., Kalaw, S. P., Reyes, R. G. & Hou, C. T. (2017). Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *Lentinus tigrinus* and *Pleurotus djamor*. Biocatalysis and Agricultural Biotechnology 9:141-144.

Gao, Y., Wang, Y., Wu, Y., Chen, H., Yang, R. & Bao, D. (2019). Protective function of novel fungal immunomodulatory proteins Fip-lti1 and Fip-lti2 from *Lentinus tigrinus* in concanavalin A-induced liver oxidative injury. Oxidative Medicine and Cell Longevity 2019:1-15.

Hudson, H. J. (1986). Fungi as inhabitants of animal faeces. Fungal biology. Edward Arnold, London, 146-158.

Jacob, J. K. S., Kalaw, S. P. & Reyes, R. G. (2015). Mycelial growth performance of three species of *Pleurotus* on coconut water gelatin. Current Research in Environmental and Applied Mycology 5(3):263-268.

Kalaw, S. P., De Leon, A. M., Dulay, R. M., Undan, J. R., Alfonso, D. O., Undan, J. Q. & Reyes RG. (2016). Optimization of culture condition for secondary mycelial growth of wild macrofungi from selected areas of Central Luzon. Current Research in Environmental and Applied Mycology 6(4):227-287.

Kerketta, A., Singh, H. K. & Shukla, C. S. (2017). Assessment of Mycelial growth and yield attribute of *Calocybe indica* P and C. International Journal of Current Microbiology and Applied Sciences 6(12):1082-1087.

Kumar PA, Bisht SS, Upadhyay S, & Bahuguna P. (2020). Effect of different media, pH and temperature ongrowth of *Pleurotus ostreatus*. Journal of Biological Innovation 9(2):133-140.

Landingin, H. R. R., Francisco, B. E., Dulay, R. M. R., Kalaw, S. P. & Reyes, R. G. (2021). Mycochemical screening, proximate nutritive composition and

radial scavenging of *Cyclocybe cylinracea* and *Pleurotus cornucopia*. Current Research in Environmental and Applied Mycology 11(1):37-50.

Magday, J. J. C., Bungihan, M. E. & Dulay, R. M. R. (2014). Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *Ganoderma lucidum*. Current Research in Environmental and Applied Mycology 4(2):162-172.

Mohammadnejad, S., Pourianfar, H. R., Drakhshan, A., Jahabaleh, I. & Rezayi, M. (2019). Potent antiproliferative and pro-apoptotic effects of a soluble protein fraction from culinary-medicinal mushroom *Lentinus tigrinus* on cancer cell. Journal of Food Measurement and Characterization 13:3015-3031.

Muthu, N. & Shanmugasundaram, K. (2015). Effect of five different culture media on mycelial growth of *Agrocybe aegerita.* International Journal of Pharmaceutical Sciences and Research 6(12):5193-5197.

Nasim, G., Malik, S. H., Baiwa, R., Afzal, M. & Mian, S. W. (2001). Effect of three different culture media on mycelial growth of oyster and Chinese mushrooms. Journal of Biological Sciences 1(12):1130-1133.

Pourianfar, H. R., Mohammadnejad, S., Shahtahmaseldi, S., Ansari, A. M., Zibael, S., Ghadirian, R., Rezaelian, S. & Dowom, S. A. (2020). Toxicity and nutritional assessment of extracts of medicinal tiger sawgill mushroom, *Lentinus tigrinus* (Agaricomycetes), a newly domesticated in Iran. International Journal of Medicinal Mushrooms 22(11):1079-1088.

Prades, A. M., Dornier, M., Diop, N. & Pain, J. P. (2012). Coconut water, uses, composition and properties: A review. Fruits 67:87-107.

Priya, R. U. & Geetha, D. (2016). Cultural and physiological studies on black ear mushrooms, *Auricularia polytricha* (Mont) Sacc and *Auricularia auricula* (L) Underw. Mushroom Research 25(2):125-131.

Quiacoe, E. H., Amoah, C., Obodai, & Odamtten, T. G. (2014). Nutrient requirements and environmental conditions for the cultivation of the medicinal mushroom (*Lentinula edodes*) in Ghana. International Journal of Scientific and Technology Research 3(12):45-50.

Ragasa, C. Y., Tan, M. C. S., De Castro, M. E., De Los Reyes, M. M., Oyong, G. G. & Shen, C. C. (2018). Sterols from *Lentinus tigrinus*. Pharmacognosy Journal 10(6):1079-1081.

Rangel, D. E. N., Fernandez, E. K. K., Braga, G. U. L. & Roberts, D. W. (2011). Visible light during mycelial growth and conidiation of *Metarhizium robertsii* produces conidia with increased stress tolerance. FEMS Microbiology Letters 315(2):81-86.

Reyes, R. G., Eguchi, F., Iijima T. & Higaki, M. (1998). Physiological considerations for efficient colonization of fukurotake, *Volvariella volvacea.* Journal of Wood Science 44:408-413.

Sevindik, M. (2018). Investigation of antioxidant/oxidant. Status and antimicrobial activities of *Lentinus tigrinus*. Advances in Pharmacological Sciences Article ID 1718025.

Simonic, J., Stajic, M., Glamoclija, J., Vukojevic, J., Duletic-Lausevic, S. & Brceski, I. (2008). Optimization of submerged culture conditions for extra- and intrcellular polysaccharide production by medicinal mushroom Ling Zhi or Reishi mushroom *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst (Aphyllophoromycetideae). International Journal of Medicinal Mushrooms 10(4):351-360.

Xu, L. J., Wang, H. X. & Ng, T. B. (2012). A laccase with HIV reverse transcriptase inhibitory activity from broth of mycelial culture of mushroom *Lentinus tigrinus.* Journal

of Biomedicine and Biotechnology 2012:536725

Yong, J. W. H., Ge, L., Ng, Y.F. & Tan, S. N. (2009). The chemical composition and biological properties of coconut, (*Cocos nucifera*) water. Molecules 14:5144-5164.

Zurbano, L. Z., Bellere, A. D. & Savilla, L. C. (2017). Mycelial growth, fruiting body production and proximate composition of *Pleurotus djamour* on different substrates. CLSU International Journal of Science and Technology 2(1):20-30.