

**MYCELIAL GROWTH PERFORMANCE AND ANTI-OXIDANT
PROPERTIES OF *Lentinus tigrinus* USING SELECTED
ROOT CROP BASED CULTURE MEDIA**

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ABSTRACT

MARTIN, AIRA MARIZ G., Bachelor of Science in Biology, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2019, MYCELIAL GROWTH PERFORMANCE AND ANTI-OXIDANT PROPERTIES OF *Lentinus tigrinus* USING SELECTED ROOT CROP BASED CULTURE MEDIA**

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Lentinus tigrinus is one of the promising mushroom species because of its nutritional value, availability and versatility. This study evaluated the mycelial growth performance of select root crop-based solid and liquid culture media, and antioxidant properties. The DPPH radical scavenging activity and the total phenolic content of the mycelia grown in the select root crop-based liquid media were investigated. Mycelia grown on sweet potato decoction gulaman recorded the highest mycelial diameter with a mean of 64.47 mm, which is statistically comparable to cassava and ube decoction gulaman. However, 500g/L concentration of media registered the highest mycelial diameter with a mean of 57.16 mm, but not significantly different to 250 g/L. In liquid culture, the heaviest mycelial biomass (0.12 g) and maximum volume loss (14.56 mL) were observed in ube decoction which is statistically comparable to sweet potato decoction. Among the concentrations, 500g/L exhibited the highest mycelial dry weight with a mean of 0.13g. Moreover, extract of mycelia in sweet potato decoction showed the highest radical scavenging activity of 30.36 % and total phenolic content of 22.96 mg GAE/g sample.

LITERATURE CITED

- Adejumo, T. O., & Awosanya, O. B. (2005). Proximate and mineral composition of four edible mushrooms species from South Western Nigeria. *African Journal of Biotechnology*, 4(10), 1084-1088.
- Aggarwal, A.K., Siddharth, K., Tripathi A. & Clark, A.M. (2012). Exploring the molecular basis of antifungal synergies using genome-wide approaches. *Frontiers in Microbiology*, 3(15), 2012-2015.
- Amadi, O.C., & Moneke, A.N. (2012). Use of starch containing tubers for the formulation of culture media for fungal cultivation. *African Journal of Microbiology Research*, 6(21), 4527-4532.
- Attarat, J., & Phermthai, T. (2014). Bioactive compounds in three edible *Lentinus* mushrooms. *Journal of Agriculture Technology and Biological Sciences*, 1(14), 491-504.
- Arandilla, J. J. (2017). Optimization of culture conditions for mycelial growth and fruiting body production of *L. tigrinus*. Unpublished undergraduate thesis. Central Luzon State University, Science City of Munoz, Nueva Ecija.
- Bilal, A. W., Bodha, R. H. & Wani, A. H. (2010). Nutritional and medicinal importance of mushrooms. *Journal of Medicinal Plants Research*, 6(4), 2598-2604.
- Buah, J.N., Kawamitsu, Y., Sato, S. & Murayama S. (2015). Effects of different types and concentrations of gelling agent on the physical and chemical properties of media and the growth of banana (*Musa* spp.) in vitro. *Plant Production Science*, 2(2), 138-145.
- Carvajal, A., Koehnlein, E.A., Soares, A.A., Eler, G.J., Nahsima, A.T.A., Bracht, A. & Peralta, R.M. (2012). Bioactives of fruiting bodies and submerged culture mycelia of *Agaricus brasiliensis* (*A. blazei*) and their antioxidant properties. *Food Science and Technology*, 46(1), 439-499
- Chandrasekara, A., & Kumar, T. J. (2016). Roots and tuber crops as functional foods: a review on phytochemical constituents and their potential health benefits. *International Journal of Food Science*, 16(15), 3631-647.
- Chang, S.T., & Miles, P.G. (2004). *Mushrooms: Cultivation, Nutritional Value, Medical Effect, and Environmental Impact (2nd Edition)*. CRC press. USA.
- Charles, A.L., Siroth, K. & Huang, T.C. (2005). Proximate composition, mineral contents, hydrogen cyanide and phytic acid of 5 cassava genotypes. *Food Chemistry*, 92(4), 615-620.

- Cooke, R.D., & Maduagwu, E.N. (1978). The effects of simple processing on the cyanide content of cassava chips. *International Journal of Food Science and Technology*, 13(4), 299-306.
- Costa, C., Henriques, A., Pires, C., Nunes, J., Ohno, M., Chibana, H., Sacorreia, I. & Teixeira, M.C. (2013). The dual role of candida glabrata drug:H⁺ antiporter CgAqr1 (ORF CAGL0J0994f) in antifungal drug and acetic acid resistance. *Front Microbiology*, 4(1), 170.
- Decker, E.A. (2006). Phenolics: prooxidants or antioxidants. *Nutrient Review*, 55(1), 396-398.
- De Leon, A. M., Reyes, R. G. & Dela Cruz, T. (2013). Enriched cultivation of three wild strains of *Lentinus tigrinus* using agricultural waste. *Journal of Agricultural Technology*, 9(5), 1199-1214.
- Dixon, B.M., Oduro, I. & Robert, A. (2009). Nutritional and biochemical composition of *D.alata* (*Dioscorea* spp.) tubers. *Journal of Food Agriculture and Environment*, 7(2), 373-378.
- Dulay, R. M. R., Cabrera, E. C., Kalaw, S. P. & Reyes, R. G. (2012). Optimal growth conditions for basidiospore germination and morphogenesis of Philippine wild strain of *Lentinus tigrinus* (Bull.). *Mycosphere Journal of Fungal Biology*, 3(6) 926-933.
- Dulay, R. M. R., Arenas, M. C., Kalaw, S. P., Reyes, R. G. & Cabrera, E. C. (2014a). Proximate composition and functionality of the culinary-medicinal tiger sawgill mushroom, *Lentinus tigrinus* (Higher basidiomycetes), from the Philippines. *International Journal of Medicinal Mushrooms*, 16(1), 85-94.
- Dulay, R. M. R., Gagarin, W. S., Abell, E. A., Kalaw, S. P. & Reyes, R. G. (2014b). Aseptic cultivation and nutrient compositions of *Coprinus comatus* on *Pleurotus* mushroom spent. *Journal of Microbiology and Biotechnology Research*, 1(4), 1-7.
- Dulay, R. M. R., Flores, K. S., Tiniola, R. C., Marquez, D. H. H., Dela Cruz, A. G., Kalaw, S. P. & Reyes, R. G. (2015). Mycelial biomass production and antioxidant activity of *Lentinus tigrinus* and *Lentinus sajor-caju* in indigenous liquid culture. *Mycosphere Journal of Fungal Biology*, 6(6), 659-666.
- Dulay, R. M. R., Miranda, L. A., Malasaga, J. S., Kalaw, S. P. & Reyes, R. G. (2016a). Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *L. tigrinus* and *P. djamour*. *Journal of Biocatalysis and Agricultural Biotechnology*, 1(9), 141-144.
- Dulay, R.M.R., Vicente, J.J.A., Dela Cruz, A. G., Gagarin, J.M., Fernando, W., Kalaw, S.P. & Reyes, R.G. (2016b). Antioxidant activity and total phenolic content of

- V.volvacea* and *S.commune* mycelia cultured in indigenous liquid media. *Mycosphere*, 7(2), 131-138.
- Dulay, R.M.R., Cardona, E.M., Kalaw, S.P. & Reyes, R.G. (2016c). Optimization of liquid culture conditions of *C.cinerea* as natural source of bioactive compounds. *Der Pharma Chemica*, 8(18), 313-319.
- Dulay, R.M.R., Miranda, L.A., Malasaga, J.S., Hou, C.T., Kalaw, S.P. & Reyes, R.G. (2017). Antioxidant and antibacterial activities of acetonitrile and hexane extracts of *L.tigrinus* and *P.djamour*. *Biocatalysis and Agricultural Biotechnology*, 9, 141-144.
- Dzomeku, M., Narhmensah, D.L., Obodai, M., Prempeh, J., Takli, R.K., Komlaga, G., Dziedzoave, N.A. & Sonnenberg, A. (2014). Vegetative growth of different strains of *Pleurotus* and *Lentinula* species on cassava (*Manihot esculenta*) and yam (*Dioscorea rotundata*) wastes in Ghana. In Proceedings of the 8th International Conference on Mushroom Biology and Mushroom Products, 64(1), 280-285.
- Griensven, V., & Layos, D. (2000). Retrieved from the book science and cultivation of edible fungi education. A.A. Balkema Publishers, 1-2.
- Hausman, M. S. (2012). Environmental condition for mushroom cultivation. Retrieved from <http://www.sciencedirect.com/science>.
- Hibbet, D. S., Tsuneda A. & Murakami, S. (2018). The scotoid form of *Lentinus tigrinus* genetics and development of a fungal morphological innovation. *American Journal of Botany*, 1(8), 466-478.
- Hoa, H.T., & Wang, C.L. (2015). The effects of temperature and nutritional conditions on mycelium growth of two oyster mushrooms (*Pleurotus ostreatus* and *Pleurotus cystidiosus*). *Mycobiology*, 43(1), 14-23.
- Kim, M. Y., Seguin, P., Ahn, J. K., Kim, J. J., Chun, S. C., Kim, E. H., Seo, S. H., Kang, E. Y., Kim, S. L. & Park, Y. J. (2008). Phenolic compound concentration and antioxidant activities of edible mushroom. *Journal of Agricultural and Food Chemistry*, 4(2), 7265.
- Kolak, U., Öztürk, M., Özgökce, F. & Ulubelen, A. (2006). Norditerpene alkaloids from *Delphinium linearilobum* and antioxidant activity. *Phytochemistry*, 67(19), 2170-2175.

- Kosanić, M., Ranković, B. & Dašić, M. (2012). Mushrooms as possible antioxidant and antimicrobial agents. *Iranian Journal of Pharmaceutical Research*, 6(2), 1095–1102.
- Kwoseh, C.K., Asomani-Darko, M. & Adubofour, K. (2012). Cassava starch-agar blend as alternative gelling agent for mycological culture media. *Botswana Journal of Agricultural and Applied Sciences*, 8(1), 8-15.
- Labarere, J., & Menini, G. U. (2000). Collection, characterization, conservation and utilization of mushroom germplasm resources in Africa. In Labarere and Gemini characterization, conservation, evaluation and utilization of mushroom genetic resources for food and agriculture. The global network on mushrooms, under the aegis of FAO, 1(2), 9-13.
- Laurie, S., Faber, M., Adebola, P. & Belete, A. (2015). Biofortification of sweet potato for food and nutrition security in South Africa. *Food Research International*, 76(1), 962-970
- Lechner, B. E., & Alberto, E. (2007b). Optimal conditions for the fruit body production of natural occurring strains of *Lentinus tigrinus*, *Journal of Bioresource Technology*, 98(9), 1866-1869.
- Lindequist, U., Timo, H. J., Niedermeyer B. D. & Dieter Ju" lich, W. (2005). The pharmacological potential of mushrooms. Oxford University Press. 2(3), 285–299.
- Martin, J.F., & Demain, A.L. (1980). Control of antibiotic biosynthesis. *Microbiological Reviews*, 44(2), 230.
- Martinez-Velaverde, I., Periago, M.J. & Demain, A.L. (1980). Nutritional importance of phenolic compounds in the diet. *Life Sciences*, 50(1), 5-18.
- Masoumi, F., Pourianfar, H.R., Masoumi, A. & Mendi, E. M. (2015). A study of mycelium characterization of several wild genotypes of the button mushroom from Iran. *International Journal Of Advanced Research*, (3)2, 236-246.
- Mata, G., Hernandez, D. M. & Andreu, L. G. (2005). Changes in lignocellulolytic enzyme activities in six *Pleurotus* spp. strains cultivated on coffee pulp in confrontation with *Trichoderma* spp. *World Journal of Microbiology and Biotechnology*, 21(2), 143-150.
- Menini, G.U. (2000). Some aspects of mushroom cultivation in developing countries. *Mushroom Sciences*, 13, 777-780.
- Mshandete, A.M., & Mgonia, J.R. (2009). Submerged liquid fermentation of some Tanzanian basidiomycetes for the production of mycelial biomass,

exopolysaccharides and mycelium protein using waste peel media. *ARPN Journal of Agricultural and Biological Science*, 4(6), 260-263.

- Niederpruem, D., & Dennen, D. (1966). Kinetics, nutrition and inhibitor properties of basidiospore germination in *Schizophyllum commune*. *Archives of Microbiology*, 54(2), 91-105.
- Oei, N.Y.L., Elzinga, B.M., Wolf, O.T., De Ruiter, M.B., Damoiseaux J.S., Kuijer, P.A., Veltman, D.J., Scheltens, P.H. & Rombouts, S.A. (2005). Cortisol decreases hippocampal and prefrontal activation during declarative memory retrieval in young men. *Neuropsychologia*, 41, 371-377.
- Okhuaya, J.A., & Okogbo, F.O. (1991). Cultivation of *Pleurotus tuber-regium* (Fr.) Sing on various wastes. *Proceedings of the Oklahoma Academy of Science archive*, 1(7), 1-3.
- Ramesh, V., Kaushal, H., Mishra, A.K. Singh, R. & Salotra, P. (2014). Clinico-epidemiological analysis of post kala-azar dermal leishmaniasis (PKDL) cases in india over last two decades: a hospital based retrospective study. *BMC Public Health*, 15, 1092.
- Reyes, R. G., Eguchi, F., Kalaw, S. K. & Kikukawa, T. (2009). Mushroom growing in the tropics. Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, 1(6), 85-88.
- Rodriguez, A. R., Santoyo, S. & Soler-Rivas, C. (2009). Antioxidant properties of mushroom. *Journal of Functional Plant Science and Technology*, 1(5), 5-6.
- Sandal, L., Bhattacharya, A. & Ahuja, P. S. (2001). An efficient liquid culture system for tea shoot proliferation. *Plant Cell Tissue Organ Culture*, 65(1), 75-80.
- Sawicka, B., Paradowska, K., Slupski, J. & Marczak, B. (2014). Nutrition value of the sweet potato (*Ipomea batatas*) cultivated in south-eastern polish conditions. *International Journal of Agronomy and Agricultural Research*, 4(4), 169-178.
- Schroeter, J. (2002). Attractive *Lentinus* sp. or *Panus* sp. – fungi.7y Retrieved from <http://www.flickr.com/photos/25747229@N00/4436833420/>.
- Shehata, T.E., & Marr, A.G. (1971). Effect of nutrient concentration on the growth of *E.coli*. *American Society for Microbiology*, 1, 210-216.
- Shekhar, T.C., & Anju, G. (2014). Antioxidant activity by DPPH radical scavenging method of *Ageratum conyzoides* Linn. leaves. *American Journal of Ethnomedicine*, 1(4), 244-249.
- Slinkard, K., & Singleton V. (1977). Total phenolic analysis: automation and comparison with manual methods. *American Journal of Enology and Viticulture*, 28, 49-55.

- Stanley, H.O., Odu, N.N. & Onwuka, J.U. (2013). The effect of honey on the mycelial growth of *P. sajor-caju* (Oyster mushroom). *International Research Journal of Environment Science*, 2(12), 13-18
- Sunagawa M., & Magae Y. (2005). Isolation of genes differentially expressed during fruit body development of *P. ostreatus* by differential display of RAPD. *FEMS Microbiology Letters*, 246(2), 279-284.
- Sumita, M. & Dhananjay, S. (2010). Quantitative analysis of total phenolic content in *Adhatoda vasica* nees extracts. 2(4), 2403-2406.
- Tudses, N. (2016). Isolation and mycelial growth of mushrooms on different yam-based culture media. *Journal of Applied Biology & Biotechnology*, 4(5), 33-36.
- Tulin, A.B. (2009). Improvement of the growth, yield, and tuber quality of purple yam through macro and micronutrient fertilization. *Analysis of Tropical Research*, 31(2), 95-119.
- Valverde, M. E., Hernández-Pérez T. & Paredes-López, O. (2015). Review article: edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology*, 3(5), 14.
- Velangi, F., & Wolever, A. (2001). Glycemic index of potatoes commonly consumed in North America. *Journal of the Dietetic Association*, 105(4), 557-562.
- Vikraman, N., Joseph, J., Ragi, P.R. & Anil, K.N. (2014). Media standardization for the mass multiplication of a bio-control agent, *Trichoderma asperellum*. *International Journal of Current Research*, 6(11), 9979-9983.
- Wang, J., & Xu, E. (2014). Isotonic versus hypotonic maintenance iv fluids in hospitalized children: a meta-analysis. *Pediatrics*, 4(7), 334-348.
- Wongjirathiti, A., & Yotakkot, S. (2017). Utilisation of local crops as alternative media for fungal growth. *Tropical Agriculture Science*, 40(2), 295-304.
- Xu, Y. W., & Baojun. (2014). Distribution of antioxidant activities and total phenolic contents in acetone, ethanol, water, and hot water extracts from 20 edible mushroom via sequential extraction. *Austin Journal of Nutrition and Food and Sciences*. 2(1), 1009.
- Yang, F. C., & Liau. (1998). The influence of environmental condition on polysaccharide formation by *Ganoderma lucidium* in submerged culture, *Journal of Process Biochemistry*, 1(33), 547-553.

- Yildiz, S., Yilmaz, A., Can, Z., Kilic, C. & CaferYildiz, U. (2017). Total phenolic, flavonoid, tannin contents and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus citrinopileatus* cultivated on various sawdust. *The Journal of Food*, 42(3), 315-323.
- Young, I.S., & Woodside, J.V. (2001). Antioxidants in health and disease. *Journal of Clinical Pathology*, 54(1), 176-186.
- Zhang, Y., Geng, W., Shen, Y., Wang, Y. & Dai, Y. C. (2014). Edible mushroom cultivation for food security and rural development in China. *Molecular Diversity Preservation International*, 6(5), 2961-2973.
- Zhu, F., Bian, Z. X., Du, B. & Xu, B. (2015). β -Glucans from edible and medicinal mushrooms: Characteristics, physicochemical and biological activities. *Journal of Food Composition and Analysis*, 3(2), 165-173.