EFFECT OF LIGHT EMITTING DIODE (LED) ON THE MYCELIAL GROWTH, MYCELIAL BIOMASS PRODUCTION AND ANTIOXIDANT PROPERTY OF *Ganoderma lucidum*

ABEGAIL A. ALCAZAR

An Undergraduate Thesis Submitted to the Faculty of the Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines
in Partial Fulfillment of the Requirements
for the Degree of

BACHELOR OF SCIENCE IN BIOLOGY

JUNE 2018

ACCEPTANCE SHEET

This undergraduate thesis entitled "**EFFECT OF LIGHT EMITTING DIODE (LED) ON THE MYCELIAL GROWTH, MYCELIAL BIOMASS PRODUCTION AND ANTIOXIDANT PROPERTY OF *Ganoderma lucidum***" prepared and submitted by **ABEGAIL A. ALCAZAR**, in partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOLOGY,** is hereby accepted.

RICH MILTON R. DULAY, M.Sc.
Adviser

5/22/18
Date Signed

SOFRONIO P. KALAW, Ph.D.
Critic

Date Signed

RICH MILTON R. DULAY, M.Sc.
Department Research Coordinator

5/22/18
Date Signed

Accepted as partial fulfillment of the requirements for the degree of **BACHELOR OF SCIENCE IN BIOLOGY**

ANGELES M. DE LEON, Ph.D.
Chair, Department of Biological Sciences

Date Signed

RICH MILTON R. DULAY, M.Sc.
College Research Coordinator

5/22/18
Date Signed

EVARISTO A. ABELLA, Ph.D.
Dean, College of Arts and Sciences

Date Signed

BIOGRAPHICAL SKETCH

The author came from a very simple, contended yet happy family, which originated from Talavera, Nueva Ecija and currently living at Mabalacat, Pampanga. She was born on September 3, 1996 and the youngest among the five daughters of Mr. Alejandro S. Alcazar and Mrs. Gelen A. Alcazar.

She started her formal education in Campus Elementary School then transferred to Calipahan Elementary School and finished her elementary as a student with an academic honor last 2008. She continued her secondary education in Putlod San Jose National High School wherein she was an honor student from first year until third year, then transferred and finished her last year at Talavera National High School in 2012.

For her tertiary education, she enrolled in Central Luzon State University at Science City of Munoz, Nueva Ecija. She is currently pursuing the degree of Bachelor of Science in Biology and hoping to finish her course this year 2018.

ACKNOWLEDGMENT

With genuine and heartfelt thanks, the researcher would like to express her gratitude to the several persons who helped her complete this promising research.

To her beloved parents and sisters, for their immeasurable love, prayers, time, effort, moral and financial support which gave her the motivation to strive hard and determination to reach her goal in life.

To Mr. Rich Milton R. Dulay, her precious adviser, for his guidance, valuable assistance and suggestions which led to the improvement of this research. The knowledge he shared, the time he spent, especially for the continuous remind and encouragement which made her do well to improve this research.

To Dr. Sofronio P. Kalaw, her critic, for his expertise, intelligent suggestions and ideas which greatly contributed to the realization of this study.

To her classmates, co-advisees especially her dear friends, who keeps persuading her work really hard. For the memories they create together outside and inside the laboratory that will never be forgotten and will always remain to the heart of the researcher.

Above all to the almighty God, for the unconditional love he gave to the researcher and to her family. For his infinite wisdom and benevolence that granted her the strength to overcome the problems and hindrances in her life.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

LIST OF APPENDIX TABLES

LIST OF APPENDIX FIGURES

ABSTRACT

ALCAZAR, ABEGAIL A., Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines, **JUNE 2018, EFFECTS OF LIGHT EMITTING DIODE ON THE MYCELIAL GROWTH, MYCELIAL BIOMASS, AND ANTIOXIDANT PROPERTY OF *Ganoderma lucidum***

Adviser: RICH MILTON R. DULAY, M.Sc.

Light is an important factor for the growth of many forms of life including mushroom. *Ganoderma lucidum* is a white rot wood-degrading basidiomycete that typically grows on logs. To assess the effect of illumination condition, this paper highlighted the effect of light emitting diode on the mycelia growth, biomass production and antioxidant activities of *G. lucidum*. Among the different set of LED, *G. lucidum* recorded the highest mean mycelia diameter in fluorescent light condition with a mean of 76.67mm. On the contrary, blue LED condition recorded the lowest mean mycelia diameter with a mean of 62.33mm after 3 days of incubation. In terms of mycelial biomass, *G. lucidum* favors red LED condition which exerted the highest mycelial yield of 7.20g, while fluorescent light condition recorded the lowest mycelial yield with a mean of 4.14g after 15 days of incubation. In terms of DPPH radical scavenging activity and total phenolic content, alternating light and dark condition exerted highest radical scavenging activity of 84.80% and highest TPC of 146.29 GAEg/ sample. Therefore, different illumination conditions are evident to have an effect not only on the growth of *G. lucidum* but also on enhancing its antioxidant property.

LITERATURE CITED

Ambra, R., Grimaldi, B., Zamboni, S., Filetici, P., Macino, G., & Ballario P. (2004). Photomorphogenesis in the hypogeous fungus *Tuber borchii*: isolation and characterization of Tbwc-1, the homologue of the blue-light photoreceptor of *Neurospora crassa*. *Fungal Genetics and Biology*, 41(7), 688-697

Blumenstein, A., Vienken, K., Tasler, R., Purschwitz, J., Veith, D., Frankenberg-Dinkel N., & Fischer, R. (2005). The *Aspergillus nidulans* Phytochrome FphA Represses Sexual Development in Red Light. *Current Biology*, 15(20), 1833-1838.

Boh, B., Berovic, M., Zhang, J., & Zhi-Bin, L. (2007). *Ganoderma lucidum* and its pharmaceutically active compounds. *Biotechnology Annual Review*, 13, 265-301.

Boh, B., Hodzar, D., Dolnicar, D, Berovic, M., & Pohleven, F. (2000). Isolation and quantification of triterpenoid acids from *Ganoderma applanatum* of Istrian origin. *Food Technology and Biotechnology*, 38(1), 11-18.

Borkovich, K.A., Alex, L.A., Yarden, O., Freitag, M., Turner, G.E., Read, N.D., Seiler, S., Bell-Pedersen, D., Paietta, J., Plesofsky, N., Plamann, M., Goodrich-Tanrikulu, M., Schulte, U., Mannhaupt, G., Nargang, F.E., Radford, A., Selitrennikoff, C., Galagan, J.E., Dunlap, J.C., Loros, J.J,, Catcheside, D., Inoue, H., Aramayo, R., Polymenis, M., Selker, E.U., Sachs, M.S., Marzluf, G.A., Paulsen, I., Davis, R., Ebbole, D.J., Zelter, A., Kalkman, E.R., O'rourke, R., Bowring, F., Yeadon, J., Ishii, C., Suzuki, K., Sakai, W., & Pratt, R. (2004). Lessons from the Genome Sequence of *Neurospora crassa*: Tracing the Path from Genomic Blueprint to Multicellular Organism. *Microbiology and Molecular Biology Reviews*, 68(1), 1-108.

Bula, R.J., Morrow, R.C., Tibbitts, T.W., Barta, D.J., Ingnatius, R.W., & Martin, T.S. (1991). Light-emitting diodes as a radiation source for plants. *HortScience*, 26(2), 203-205.

Chang, S., & Miles, P. (1989). Edible mushroom and their cultivation. *CRC Pess. INc*, 41-78

Chen, Y., Xie, M.Y., Nie, S.P., Li,C., & Wang, Y.X. (2008). Purification, composition analysis and antioxidant activity of a polysaccharide from the fruiting bodies of *Ganoderma atrum*. *Food Chemistry*, 107(1), 231-241.

Cheung, L.M., Cheung, P.C., & Ooi, V.E. (2003). Antioxidant activity and total phenolics of edible mushroom extracts. *Food Chemistry*, 81(2), 249-255.

Dong, J. Z., Lei, C., Zheng, X. J., Ai, X. R., Wang, Y., & Wang, Q. (2013). Light wavelengths regulate growth and active components of *Cordyceps militaris* fruit bodies. *Journal of Food Biochemistry*, 37(5), 578-584.

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. *The Philippine Agricultural Scientist*, 95(3), 278-285.

Dulay, R.M.R., Ray, K., & Hou, C.T. (2015). Optimization of liquid culture conditions of Philippines wild edible mushrooms as potential source of bioactive lipids. *Biocatalysis and Agricultural Biotechnology*, 4(3), 409-415.

Dulay, R.M.R., Miranda, L.A., Malasaga, J.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 djamour*. *Biocatalysis and Agricultural Biotechnology*, 9,141-144.

Ellis, R. J., Bragdon, G. A., & Schlosser, B.J. (1999). Properties of the blue light requirements for primordia initiation and basidiocarp maturation in *Coprinus stercorarius*. *Mycological Research*, 103(6), 779-784.

Elmastas, M., Isildak, O., Turkekul, I., & Temur, N. (2007). Determination of antioxidant activity and antioxidant compounds in wild edible mushrooms. *Journal of Food Composition and Analysis,* 20(3), 337-345.

Food and Agricultural Organization (Fao) (2006). – Corporate Document Repository: Wild Edible Fungi: A Global Overview of their Importance to People. www.fao.org.documents.

Furukawa H. (1992). Tokyo: Kyoritsu Shuppan Co. Ltd. *Mushroom Science*, 89-92.

Halliwell, B., & Gutterridge, J.M.C. (1999). The definition and measurement of antioxidant in biological systems. *Free Radical Biology and Medicine*, 18(1), 125-126.

Han, H. F., Nakamura, N., & Hattori, M. (2006). Protective effects of an acidicpolysaccharide isolated from fruiting bodies of *Ganoderma lucidum* against murine hepatic injury induced by Propionibacterium acnes and lipopolysaccharide. *Journal of Natural Medicines*, 60, 295-302.

Hikino, H., & Mizuno, T. (1989). Hypoglycemic actions of some heteroglycans of *Ganoderma lucidum* fruit bodies. *Planta Medica*, 55, 385-389.

Idnurm, A., & Heitman, J. (2005). Light controls growth and development via a conserved pathway in the fungal kingdosm. *PLoS Biology, 3*(4), 95.

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 Environment and Applied Mycology,* 5(3), 263-268.

Jang, M.J., Lee, Y.H. Kim, J.H. & Ju, Y.C. (2011). Primordium Formation, Morphological Properties, Ergosterol Content and Antioxidant Activity of Fruit Body in *Pleurotus eryngii. The Korean Journal of Mycology,* 39(3), 175-179.

Jang, M.J., Lee, Y.H. Ju, Y.C. Kim, S.M., & Koo, H.M. (2013). Effect of color of light emitting diode on development of fruit body in *Hypsizygus marmoreus. Mycobiology*, 41(1), 63-66.

Jia, J., Zhang, X., Hu, Y. S., Wu, Y., Wang, Q. Z., Li, N. N., & Dong X.C. (2009). Evaluation of in vivo antioxidant activities of *Ganoderma lucidum* polysaccharides in STZ-diabetic rats. *Food Chemistry,* 115(1), 32-36.

Kalaw, S.P., Alfonso D.O., Dulay, R.M.R., De Leon, A.M., Undan, J.Q., Undan, J.R., & Reyes, R.G. (2016). Optimization of culture conditions for secondary mycelial growth of wild macrofungi from selected areas in Central Luzon, Philippines. *Current Research in Environmental & Applied Mycology*, 6(4), 277-287.

Kim, D.H., Choi, H.J., Jo, W.S., & Moon, K.D. (2012). Quality characteristics of *Pleurotus eryngii* cultivated with different wavelength of LED lights. *Korean Journal of Food Preservation*, 19(3), 354-360.

Kohda, H., Tokumoto, W., Sakamoto, K., Fujii, M., Hirai, Y., Yamasaki, K., Komoda, Y., Nakamura, H., Ishihara, S., & Uchida, M. (1985). The biologically active constituents of *Ganoderma lucidum* (Fr.) Karst. Histamine release-inhibitory triterpenes. *Chemical Pharmaceutical Bulletin*, 33(4), 1367-1374.

Kurtzman,R.H.Jr. & Carrera, D.M. (2013). Light, what it is and what it does for mycology. *Micologia Aplicada International*, 25(2), 23-33.

Langseth, L. (1996). Oxidants, antioxidants and disease prevention. International life Science Institute, Belgium, *International Journal of Pharmaceutical Science*, 4(2), 359-362.

Leatham , G.F., & Stahmann, M.A. (1987). Effect of light and aeration on fruiting of *Lentinula edodes. Transactions of the British Mycological Society*, 88(1), 9-20.

Lee, Jh. (2000). Cultivation of reishi (*Ganoderma lucidum*).*MushWorld Cultivation*, 12-24, available at http://www.mushworld.com/sub_en.html

Lee, J. M., Kwon, H., Jeong, H., Lee, J. W., Lee, S. Y., Baek, S. J., & Surh, Y.J. (2001). Inhibition of lipid peroxidation and oxidative DNA damage by *Ganoderma lucidum*. *Phytotherapy Research*, 15(3), 245-249.

Lee, J.M., Kwon, H.,Jeong, H., Lee, J.W., Lee, S.Y., Baek, S.J., & Surh,Y.J. (2001). *Phytotherapy Research*, 15, 245-249.

Lin, J. M., Lin, C. C., Chlaen, M. F., Ujiie, T., & Takada, A. (1995). Radical scavenger and antihepatotoxic activity of *Ganoderma formosanum*, *Ganoderma lucidum* and *Ganoderma neo-japonicum*. *Journal of Ethnopharmacology*, 47(1), 33-41.

Liu, W., Wang, H., Pang, X., Yao, W., & Gao, X. (2010). Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Ganoderma lucidum*. *International Journal of Biological Macromolecules*, 46(4), 451-457.

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

Mau, J.L., Lin, H.C., & Chen, C.C. (2002a). Antioxidant properties of several medicinal mushrooms. *Journal of Agricultural and Food Chemistry*, 50, 6072-6077.

Mau, J.L., Lin, H.C., & Song, S.F. (2002b). Antioxidant properties of several specialty mushrooms. *Food Research International*, *35*(6), 519-526.

Mc Cree K.J. (1972). The action spectrum, absorptance and quantum yield of photosynthesis in crop plants. *Agricultural Meteorology*, 9, 191-216.

Mizuno, T., Wang, G., Zhang, J., Kawagishi, H., Nishitoba, T., & Li, J. (1995). Reishi, *Ganoderma Lucidum* and *Ganoderma Tsugae*: Bioactive Substances And medicinal effects. *Food Reviews International*, 11, 151-166.

Musngi, R.B., Abella, E.A., Lalap, A.L., & Reyes, R.G. (2005). Four species of wild *Auricularia* in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. *Journal of Agricultural Technology*, 1(2), 279-299.

Olle, M. & VirŠille A. (2013). The effects of light-emitting diode lighting on greenhouse plant growth and quality. *Agricultural and Food Science*, 22(2), 223-234.

Poyedinok, N.L. M, O.B. S, V.V., Buchalo, A.S., & Negriyko, A.M. (2008). Light regulation of growth and biosynthetic activity of Ling Zhi or Reishi medicinal mushroom, *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.

Aphyllophoromycetideae), in pure culture. *International Journal of Medicinal Mushrooms,* 10, 369-378.

Ramírez, D. A., Muñoz, S. V., Atehortua, L., & F.C. Michel. (2010). Effects of different wavelengths of light on lignin peroxidase production by the white-rot fungi *Phanerochaete chrysosporium* grown in submerged cultures. *Bioresource Technology*, 101(23), 9213-9220.

Reyes, R.G., Eguchi, F., Iijima, T., & Higaki, M. 1997 – *Collybia reinakeana*, a wild edible mushroomfrom the forest of Puncan, Nueva Ecija, Philippines. *Mushroom Science and Biotechnology,* 15(2), 99–102.

Reyes, R. G., Eguchi, F., Iijima, T., & Higaki, M. (1998). Physiological considerations for efficient mycelial colonization of Philippine strains of *Volvariella volvacea*. *Journal of World Science*, 44(5), 408-413.

Reyes, R.G., Abella, E.A., Eguchi, F., Iijima, T.. & Higaki, M. (2004). Physiology of *Collybia reinakeana*, a wild, endemic, edible mushroom from Puncan forest, Carranglan, Nueva Ecija, Philippines. *The Journal of Tropical Biology*, 3, 11-19.

Reyes, R.G., Lopez, L.L.M.A., Kumakura, K., Kalaw, S.P., Kikukawa, T., & Eguchi, F. (2009). *Coprimus comatus*, a newly domesticated wild nutriceutical mushroom in the Philippines. *Journal of Agricultural Technology*, 5(2), 299-316.

Reyes, R. G., Kalaw, S. P., Dulay, R. M. R., Yoshimoto, H., Miyazawa, N., Seyama, T., & Eguchi, F. (2013). Philippine Native and Exotic Species of Edible Mushrooms Grown on Rice-Straw-Based Formulation Exhibit Nutraceutical Properties. *The Philippine Agricultural Scientist*, 96(2).

Santoso, U., Kubo, K., Ota, T., Tadokoro, T., & Maekawa, A. 1996 – Nutrient composition of kopyor coconuts (*Cocos nucifera* L.). *Food Chemistry,* 57, 299-304.

Sone, Y., Okuda, R., & Wada, N. (1985). Structures and anti-tumor activities of polysaccharides isolated from fruiting body and the growing culture of mycelium of *Ganoderma lucidum*. *Agricultural Biology and Chemistry*, 49, 2641-2653.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry*, 40, 945-948.

Slinkard, K., & Singleton, V.L. (1977). Total phenol analyses: automation and comparison with manual methods. *American Journal of Enology and Viticulture,* 28(1), 49-55.

Sliva, D. (2006). *Ganoderma lucidum* in cancer research. *Leukemia Research*, 30(7), 767-768.

Tasaka, K., Akagi, M., Miyoshi, K., Mio, M., & Makino, T. (1988). Antiallergic constituents in the culture medium of *Ganoderma lucidum*. (I) Inhibitory effect of cyclooctasulfur on histamine release. *Agents Actions*, 23, 153-156.

Toth, J. O., Luu, B. & Ourisson, G. (1983). Ganoderic acid T and Z: cytotoxic triterpenes from *Ganoderma lucidum*. *Tetrahedron Letters*, 24, 1081-1084.

Tseng, Y. H., Yang, J. H., & Mau, J.L. (2008). Antioxidant properties of polysaccharides from *Ganoderma tsugae*. *Food Chemistry*, 107(2), 732-738.

Valverde Me, Hernandez-P ´ Erez T. & Paredes-L ´ Opez , O. (2015). Edible mushrooms: improving human health and promoting quality life. *International Journal of Microbiology*, 376-87.

Villaceran Jr, A.B., Kalaw, S.P., Nitural, P.S., Abella, E.A., & Reyes, R.G. (2006). Cultivation of Thai and Japanese Strains of *Pleurotus sajor–caju* on rice straw-based *Volvariella volvacea* mushroom spent and composted rice straw in Central Luzon Region, Philippines. *International Journal of Agricultural Technology*, 2(1), 69-75.

Wang, S. Y., Hsu, M. L., Hsu, H. C., Lee, S. S., Shiao, M. S., & Ho, C.K. (1997). The anti-tumor effect of *Ganoderma lucidum* mediated by cytokines released from activated macrophages and T lymphocytes. *International Journal of Cancer*, 70, 699-705.

Wang, L., Chen, X., Wang, Q., Hao, J., & Lan, L. (2011). Effect of Different light of LED light quality on growth and antioxidant enzyme activities of *Ganoderma lucidum*. *China Journal of Chinese Material Medica*, 36(18), 2471-2474.

Wasser, S. (2002). Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. *Applied Microbiology and Biotechnology*, 60, 258-274.

Wasser, S. P., & Weis, A. L. (1999). Medicinal properties of substances occurring in higher Basidiomycetes mushrooms: current perspective (review). *International Journal of Medicinal Mushrooms*, 1, 31-62.

Wong, J. Y. & Chye, F.Y. (2009). Antioxidant properties of selected tropical wild edible mushrooms. *Journal of Food Composition and Analysis*, 22(4), 269-277.

Wu, J. Y., Chen, H. B., Chen, M. J., Kan, S. C., Shieh, C. J., & Liu, Y.C. (2013). Quantitative analysis of LED effects on edible mushroom *Pleurotus eryngii* in

solid and submerged cultures. *Journal of Chemical Technology and Biotechnology,* 88(10), 1841-1846.

Yan, J., Chen, H.B., Chen, M.J. Kan, S.C. & Shieh, C.J. (2013). Quantitative analysis of LED effects on edible mushroom *Pleurotus eryngii* in solid and submerged cultures. *Journal of Chemical Technology and Biotechnology,* 88(10), 1841-1846

Yang, J. H., Lin, H. C., & J.L. Mau. (2002). Antioxidant properties of several commercial mushrooms. *Food Chemistry, 77*(2), 229-235.

Yang, H. L., Wu, T.X., & Zhang, K.C. (2004). Enhancement of mycelial growth and polysaccharide production in *Ganoderma lucidum* (the Chinese medicinal fungus, 'Lingzhi') by the addition of ethanol. *Biotechnology Letters*, 26, 841-844.

Zapata, P. A., Rojas, D. F., Ramirez, D. A., Fernandez, C., & Atehortua, L. (2009). Effect of different light-emitting diodes on mycelial biomass production of Ling Zhi or Reishi medicinal mushroom *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst.(Aphyllophoromycetideae). *International Journal of Medicinal Mushrooms,* 11(1).