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MYCELIAL BIOMASS PRODUCTION AND RADICAL SCAVENGING ACTIVITY OF *Lentinus tigrinus* IN SUBMERGED CULTIVATION USING SELECTED TROPICAL FRUIT JUICE

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ABSTRACT

Lentinus tigrinus is wood-rotting edible and medicinal mushroom. In this study, we demonstrated the mycelial biomass production and radical scavenging activity of *L. tigrinus* in submerged cultivation using selected tropical fruit juices. The maximum mycelial biomass of *L. tigrinus* was recorded in pure juice of both honeydew (4.65 g) and watermelon (4.34 g) and in 50% juice of both grapes (2.00 g) and coconut (1.39 g). All concentrations of both honeydew and watermelon and the 25% of grapes extract showed primordial development while no primordia was observed in all concentrations of coconut juice. In radical scavenging activity, both mycelia grown in 25% honeydew juice and 25% grapes juice recorded the highest scavenging activities of 79.79% and 73.06%, respectively. The scavenging activity of *L. tigrinus* mycelia grown in watermelon juice was not affected by the varying concentrations. However, mycelia in pure coconut juice registered the highest scavenging activity (77.20%) but statistically comparable with those at 50% coconut juice. Therefore, the four selected tropical fruit juices could be used as media in submerged cultivation of *L. tigrinus* and they could affect its mycelial biomass production and radical scavenging activity in concentration dependent manner.

Keywords: *Lentinus tigrinus*, tropical fruit juice, antioxidant, submerged culture.

INTRODUCTION

Mushrooms are considered functional food because of their nutritional and medicinal value. They are valuable source of diverse natural bioactive components including polysaccharide, protein, polysaccharide-protein complexes, etc., which are known as anticancer, anti-tumor anti-diabetic, anti-inflammatory, antibacterial, antifungal, and antioxidant agents. Although mushrooms have useful components, many Filipinos have not been able to consume mushrooms due to low production and limited number of cultivated mushroom. Therefore, several studies have focused on the development of production techniques in order to improve the mushroom yield. Submerged cultivation is a promising method for efficient production of mushroom biomass and obtaining pharmaceutical agents such as antioxidant. Antioxidants are any natural substance that inhibits the damaging effects of free radicals brought by oxidation.

Lentinus tigrinus is a one the wild basidiomycetous fungi that have been successfully cultured in laboratory condition. Its secondary mycelia grew best on coconut water gulaman at a wide pH range, in either sealed or unsealed, under both dark and room temperature (32 °C) conditions. The maximum yield of fruiting body was attained in 2 parts of sawdust + 8 parts of rice straw substrate formulation with the biological efficiency of 15.93% **[1]**. In addition, this mushroom is rich in carbohydrates, proteins, fibers, minerals, and exhibits hypoglycemic effect in alloxan-induced mice and antibacterial activity **[2]**. In our previous work, the effect of different indigenous liquid culture media on the biomass production and free radical scavenging activity and total phenolics of *L. tigrinus* was evaluated and we found out that the mycelial biomass and antioxidant activity were affected by different liquid media **[3]**.

The aim of the present study was to investigate the effect of selected tropical fruit juices as liquid culture media in the submerged cultivation of *L. tigrinus* and their influence on the radical scavenging activity of biomass mycelia in our intention to develop advanced cultivation technique as source of mushroom biomass for nutraceutical and pharmaceutical purposes.

MATERIALS AND METHODS

Culture Source and Inoculant

Pure culture of *L. tigrinus* was obtained from the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Munoz, Nueva Ecija. Approximately 10 mm x 3

mycelial block from the pure culture was aseptically inoculated on Potato Dextrose Agar plates. These were incubated in inverted position at 30°C to allow mycelial ramification. After 7 days of incubation, mycelial discs were prepared using a flame-sterile 10 mm-diameter cork borer. These served as inoculant in the evaluation.

Evaluation of Mycelial Growth

Four tropical fruits namely honeydew, grapes, watermelon, and coconut were selected based on the availability in the market. Each fruit was peeled and squashed using a blender except coconut. Coconut juice was obtained from a newly cracked mature coconut. The fruit puree and coconut juice were diluted in distilled water to prepare the 25%, 50%, and 100% concentrations of the fruit juices. Fifty ml of each concentration of fruit juices was individually dispensed into bottles, plugged with cotton and covered with recycled paper. Bottled fruit juices were sterilized using an autoclave at 121˚C, 15 psi for 30 minutes. After cooling, mycelial discs were aseptically inoculated in sterile fruit juice media. Inoculated bottles were incubated at 30˚C in static condition for 15 days until complete mycelial ramification were attained. The mycelia were harvested and weighed and the volume loss of culture spent was measured.

Extraction of Mycelia

Mycelia from the different concentrations of each fruit juice were subjected to methanol extraction. These were homogenized using a food processor, soaked in methanol for 24 hours, and filtered using Whatman filter paper No. 2. Filtrate was concentrated to dryness at 50°C in waterbath prior to radical scavenging activity assay.

Radical Scavenging Activity Assay

The free radical scavenging activity of the samples was estimated using the stable 2,2'diphenyl-1-1picrylhydrazyl (DPPH) radical following the standard method of Shimada et al. (1992) with minor modifications. A 100 µl of test sample in ethanol was added with 5 µl DPPH solution (5 mg DPPH powder in 2 ml of ethanol) in 96-well microtitter plates. The mixture was shaken vigorously and left to stand for 30 min in the dark, and the absorbance was then measured at 517 nm. The inhibition of DPPH free radicals was calculated.

Statistical Analysis

Data were analyzed using Analysis of Variance (ANOVA). Treatment means were compared using Duncan Multiple Range Test (DMRT) at 5% level of significance.

RESULTS AND DISCUSSION

Mycelial Biomass Production

Mycelial biomass production in liquid culture using different indigenous sources of nutrient medium is simple and fast alternative method for bioactivity evaluation. In the present study, the effect of selected tropical fruit juices on the mycelial biomass production of *Lentinus tigrinus* was investigated. Figure 1 shows the mycelial biomass of *L. tigrinus* influenced by the different concentrations of the four fruit juices in submerged cultivation. Apparently, 100% of both honeydew and watermelon significantly produced the maximum mycelial biomass having means of 4.62 g and 4.34 g, respectively, followed by 50%. The 25% of both fruit juices had the lowest biomass yield. Pure grapes juice did not show any mycelial growth. The highest yield (2.00 g) was found at 50% of grapes juice and its 25% produced 1.58 g. In coconut juice, 50% registered the highest yield (1.39 g) but statistically comparable with pure juice (1.36 g). Moreover, it was also observed in the present study that fruit juices stimulated the formation of primordia of *L. tigrinus* (Figure 2). All concentrations of both honeydew and watermelon and the 25% of grapes extract showed primordial development. However, no primordial was observed in all concentrations of coconut juice. The results strongly indicate that the concentration of fruit juices influenced the mycelial growth and production of *L. tigrinus*. The superiority of pure juice of both honeydew and watermelon could be attributed to nutrient contents of these fruits particularly sugars. This could also be explained by the buffering capacity of these fruit juices, thus, the effect of pH in these media on the mycelia biomass production of *L. tigrinus* must be evaluated. In our previous work, the maximum mycelial biomasses of *G. lucidum*, *P. cystidiosus*, *V. volvacea* and *S. commune* favourably produced in Sabouraud dextrose broth at pH 7, 7, 6 and 8, respectively, when incubated at 28°C and 30°C **[4]**. No growth was found in pure juice of grapes probably due to its high phytochemicals and pH that inhibited the growth of mycelia.

Radical Scavenging Activity of *L. tigrinus* Mycelia

Mushrooms have long been recognized for their unique umami flavour and important biological activities such as antioxidant **[5]**. Antioxidant is any substance that inhibits the damaging effects of free radicals brought by oxidation. The most common antioxidants present in foods are phenolic acids, flavonoids, carotenoids, tocopherol and ascorbic acids **[6]**. In this study, the antioxidant activity of *L. tigrinus* grown on different concentrations of selected fruit

juiceS was investigated. Figure 3 shows the results of DPPH radical scavenging assay. Apparently, the concentration of the fruit juices significantly influenced the radical scavenging activity of *L. tigrinus* mycelia. Both mycelia grown in 25% honeydew juice and 25% grapes juice recorded the highest scavenging activities of 79.79% and 73.06%, respectively. However, no significant difference of scavenging activity of *L. tigrinus* mycelia was noted when grown on the different concentrations of watermelon juice. In coconut juice, pure juice mycelia registered the highest scavenging activity (77.20%), but this was statistically comparable with the mycelia from 50% coconut juice. Dulay et al. **[3]** reported that *Lentinus tigrinus* hold a promising antioxidant which is influenced by different culture media. However, the percentage radical scavenging activity obtained in the previous work is far lower when compared to the percentage scavenging activity presented in this study. This could be attributed to the natural antioxidants such as phenolics present in the fruits that contributed to the scavenging activity of the mycelia. The total phenolic contents and radical scavenging activities of *Lentinus tigrinus, Lentinus sajor-caju, S. commune* and *V. volvacea* varied when cultured in the different indigenous culture broth **[3, 5]**. Several mushrooms are also reported for their important radical scavenging activities including *Ganoderma lucidum, Grifola umbellata, Coriolus versicolor, Tricholoma lobayense, Tremella fuciformis, Pleurotus florida*, and *Panaeolus antillarium* **[7, 8, 9]**.

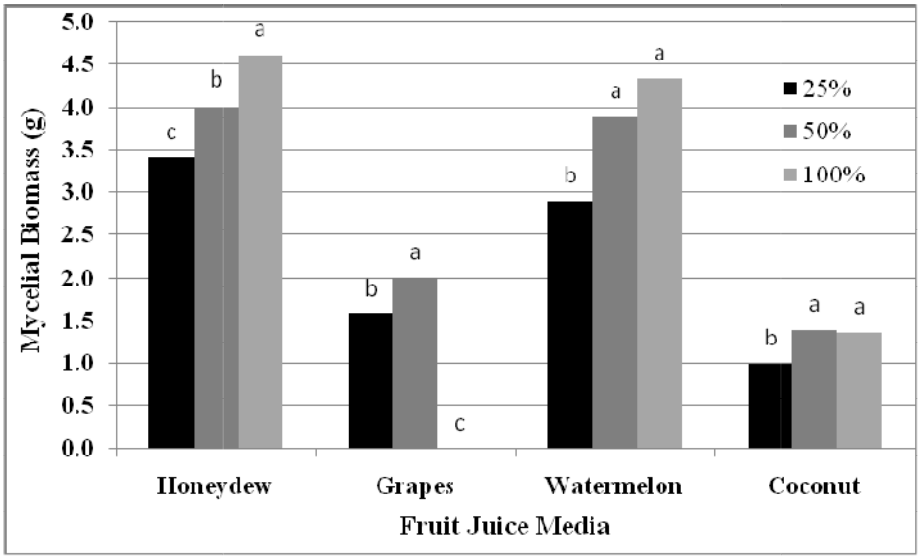

Figure 1: Mycelial biomass of *L. tigrinus* influenced by the different concentrations of tropical fruit juices in submerged cultivation after 15 days of incubation. In each fruit juice, treatment means with the same letter are not significantly different from each other at 5% level using DMRT

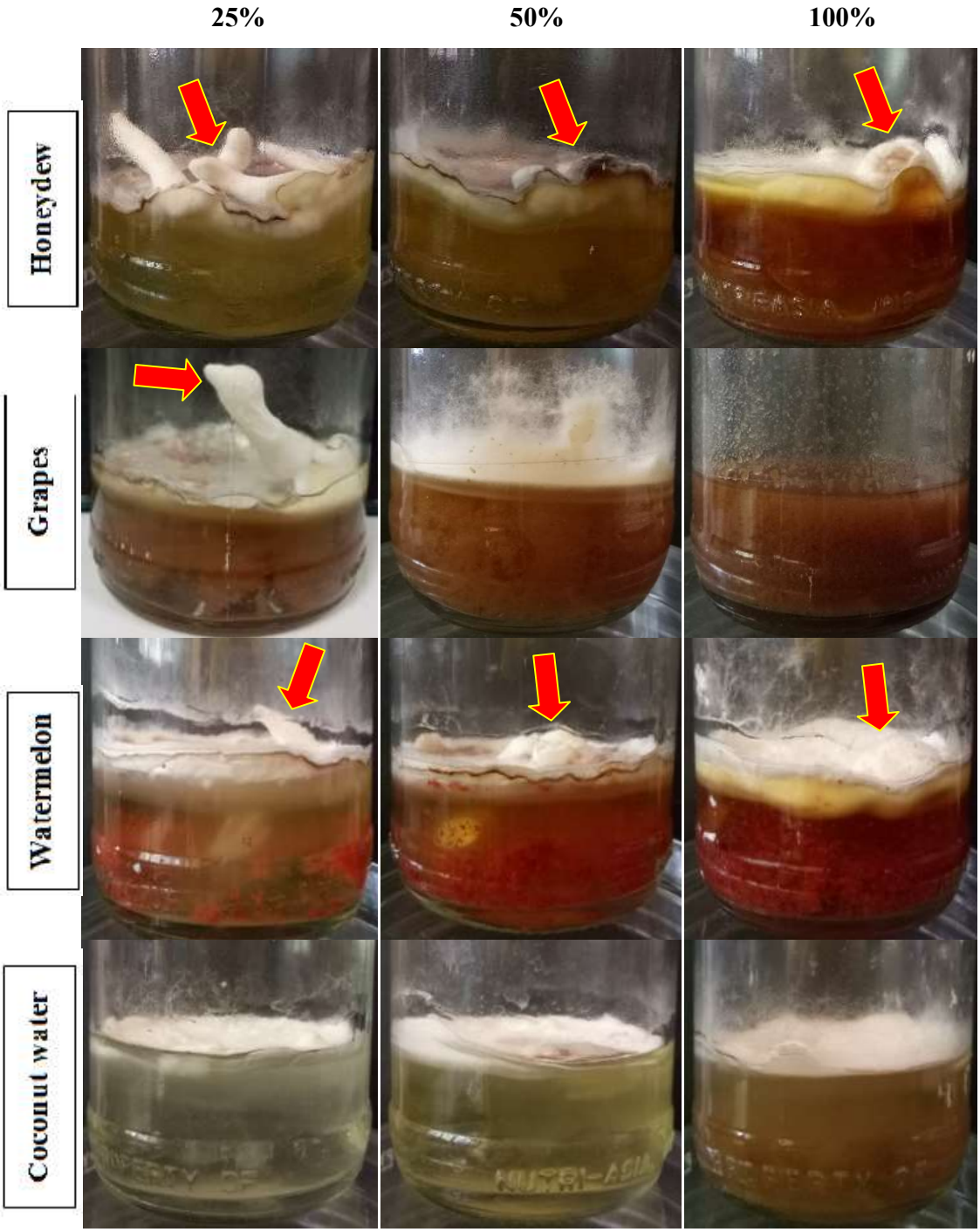

Figure 2: Mycelial culture of *L. tigrinus* influenced by the different concentrations of tropical fruit juices in submerged cultivation after 15 days of incubation. Red arrows pointed the primordial development

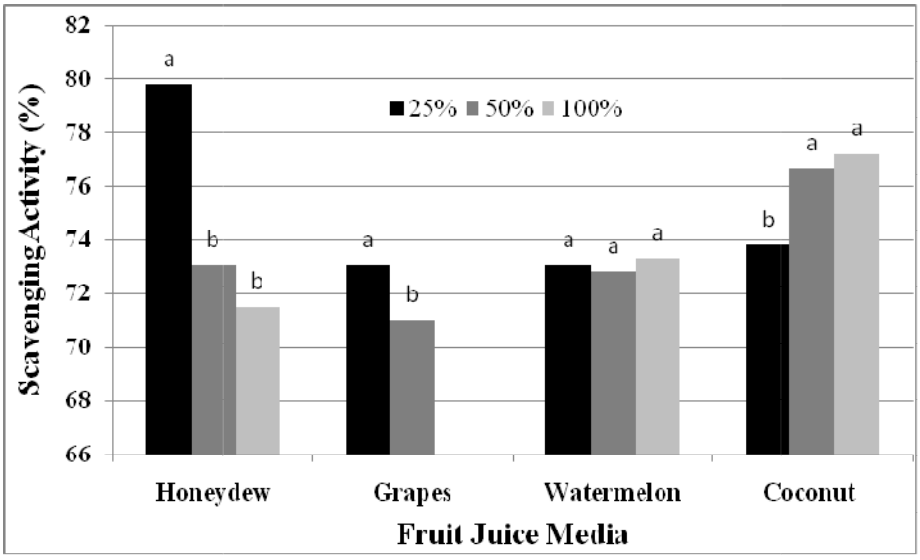

Figure 3: Radical scavenging activity of *L. tigrinus* mycelia grown in different concentrations of fruit juices

CONCLUSION

In conclusion, the mycelial biomass production and radical scavenging activity of *L. tigrinus* were affected by the selected tropical fruit juices in submerged cultivation system and were dependent on the concentration and type of the fruits.

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