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Moringa oleifera leaf extract improves the mycelial biomass and antioxidant activity of mushroom *Pleurotus florida* (Mont.) singer

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Abstract

Addition of supplements in culture media enhances the production of mycelia and even the biological activities of mushroom. In the present work, the effects of *Moringa oleifera* leaf extract (MOLE) supplemented in coconut water as culture media on the mycelial production and antioxidant properties of *Pleurotus florida* were investigated. Coconut water supplemented with 9% MOLE significantly produced the maximum biomass yield of 0.35 g, followed by 6% MOLE (0.34 g). However, the highest scavenging activity against DPPH free radicals was obtained in extracts of mycelia grown in coconut water with 6% and 9% MOLE. Non-supplemented media recorded the lowest mycelial yield and scavenging activity. Increasing mycelial yield and scavenging activity was observed as the concentration of MOLE increases. This work demonstrates the utilization of *M. oleifera* leaves as supplement of culture media for the improved production of mycelia and enhanced antioxidant properties of *P. florida*.

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Introduction

Pleurotus florida (Mont.) Singer (Family Pleurotaceae) is a basidiomycetous mushroom, characterized by clumps of fruiting bodies with light gray, convex with an incurved margin at young stage and expanding to white, flat, fan-shaped cap and with white, tough, velvety at base, and usually curved stipe. It is an edible mushroom and considered to be highly nutritious. *P. florida* contains both essential and non-essential amino acids, having threonine and glutamine as the most abundant, and exhibits inhibitory effects against platelet aggregation (as mediated by platelet activating factor, arachidonic acid Na and adenosine diphosphate) and IL-8 gene expression (Reyes *et al*., 2013). Alam *et al.* (2007) reported that *P. florida* is rich in proteins, fibers, carbohydrates, minerals, and low amount of lipid. However, ergosterol, ergosterol peroxide, cerevisterol, dilinoleoyloleoylglycerol, and a mixture of linoleic acid, palmitic acid, stearic acid, and oleic acid are isolated in the dichloromethane extract of fruiting bodies of *P. florida* (Ragasa *et al*., 2015). Its methanolic and ethanolic extracts showed potent antioxidant activity (Menaga *et al*., 2013; Rahman *et al*., 2013).

P. florida is the most widely cultivated in the Philippines, particularly in Central Luzon in which the fruiting bodies are mass produced in solid state fermentation using rice straw and sawdust-based substrate formulation in fruiting bags. The conventional cultivation technique is a lengthy process in producing mushroom biomass for food and pharmaceutical purposes. Thus, many studies have introduced the use of submerged culture or liquid cultivation for the production of mycelial biomass and metabolites (e. g. Kim *et al*., 2003; Dong and Yao, 2005). Dulay *et al*. (2015a) reported the optimization of culture conditions of Philippine strains of edible mushrooms *Schizophyllum commune*, *Ganoderma lucidum*, *Volvariella volvacea*, and *Pleurotus cystidiosus* in submerged culture. Moreover, liquid cultivation is employed in the production of mycelial biomass for the evaluation of the effects of culture media on the radical scavenging activities and total phenolic contents of *Lentinus tigrinus*, *Lentinus sajor-caju*, *Schizophullum commune*, and *Volvariella volvacea* (Dulay *et al*., 2015b; Dulay *et al*., 2016). Submerged cultivation is more advantageous than solid state fermentation because it is fast, very efficient, less contamination, and it can easily add supplements in the medium. Supplementation is also practiced to make the culture media more nutritious for the mushrooms, thereby producing higher mycelial biomass yield.

Moringa oleifera Lam (Moringaceae) is fast-growing, softwood, perennial tree which is also distributed in the Philippines. It is a high-valued plant for nutritional, pharmacological, and other purposes. As medicinal food, it is as good source of vitamins, minerals, amino acids, proteins, and phenolics, and possess antitumor, anti-inflammatory, antihypertensive, antioxidant, antidiabetic, antibacterial, and other biological properties (Anwar *et al*., 2007; Richa *et al*., 2005). Tannins, saponins, alkaloids, flavonoids, cardiac glycosides, reducing sugars, anthraquinones and phenols, steroids and volatile oils are also components of this plant (Abubakar and Usman, 2016; Patel *et al*., 2014).

In the Philippines, *M. oleifera* leaves are consumed to increase production of woman's breastmilk (Estrella *et al*., 2000). The ability of *M. oleifera* leaf extract to enhance mycelial growth of mushroom *P. florida* was evaluated in the present work.

We report herein the effects of *M. oleifera* leaf extract (MOLE) supplement on the mycelial biomass production of *P. florida* using coconut water as basal medium in submerged cultivation technique. Mycelia were extracted using ethanol and the radical scavenging activity was determined.

Materials and method

Mushroom strain

Culture plates of seven-day old mycelia of *P. florida* from the Center for Tropical Mushroom Research and Development, Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippine were used in

this study. A flame-sterilized 10 mm diameter cork borer was used to prepare the mycelial discs, which served as inoculants in the evaluation of the growth performance of *P. florida*.

M. oleifera leaf extract

M. oleifera leaves were collected from Central Luzon State University, Science City of Muñoz, Nueva Ecija, Philippines. Collected samples were washed, blot dried, pulverized using food blender, and squeezed to obtain the fresh extract. Extract was placed in a sterile amber bottle prior to preparation of the culture media.

Preparation of liquid culture media

Coconut water obtained from a newly cracked coconut was used as the basal medium. MOLE was diluted in coconut water following the different concentrations (0%, 1%, 3%, 6%, and 9%) as treatments. A total of 250 ml volume was prepared for each concentration. pH was maintained at pH 6. Fifty ml of the prepared culture media was dispensed into clean glass culture bottles with cotton plug. Each treatment concentration was replicated five times. These were sterilized in an autoclave at 15 psi, 121°C for 30 min. After sterilization, culture media were allowed to cool prior to inoculation of mycelial discs.

Evaluation of mycelial growth in submerged culture

Mycelial discs were aseptically inoculated into culture bottles containing the liquid media. These were incubated in static condition at 30°C for 15 days to allow the mycelial growth. Mycelia were harvested, air-dried and weighed. The best concentration of MOLE was determined.

Ethanol Extraction and radical scavenging assay

The air-dried mycelia were pulverized and soaked in 80% ethanol for 24 hours. This was filtered using Whatman filter paper No.1 and concentrated under reduced pressure at 45°C using a rotary evaporator up to dryness. Extract was obtained and kept in a clean vial, closed tightly and stored in a cool dry place. The extract was assayed using 2, 2'diphenyl-1-1picrylhydrazyl (DPPH) radical scavenging to determine the antioxidant property. The procedure established by Kolak *et al.* (2006) was followed with minor modification.

The absorbance readings were monitored at 517 nm using a UV-VIS spectrophotometer and the percentage DPPH radical scavenging effect was calculated. The SAS System Version 9.0 (SAS Institute Inc. Cary, NC, USA) was used to analyze all the data.

Results and discussion

In *P. florida* fruiting body production, supplementation of substrate is practiced by mushroom growers to improve the yield and biological efficiency. Some of the supplements used are molasses, rice bran, corn grit, fertilizer, leguminous plant leaves, and other nutritious materials. In the present work, the effects of different concentrations of MOLE as supplement of coconut water-based media on the mycelial biomass production of *P. florida* in submerged culture were determined. Figure 1 depicts the mean weight of mycelial biomass of *P. florida* after 15 days of incubation. Apparently, the mycelial biomass yield had increased in increasing concentration of MOLE in the media. Coconut water with 9% MOLE recorded the maximum mycelial biomass yield of 0.35 g, but this was not statistically different with 6% MOLE (0.34 g). These mean values were significantly higher when compared with non-supplemented media. The results of the present study suggest that augmentation of MOLE in the media could improve mycelial biomass production of *P. florida*.

The favourable response of mycelia of *P. florida* to MOLE could probably be attributed to the growth promoting components of *M. oleifera* leaves. Foidl *et al.* (2001) reported that ethanol extract of *M. oleifera* leaves contain growth promoters, which used as foliar spray to enhance the growth and improve the yield of different crops such as peanut, soya bean, corn, sorghum, onion, tomato, cantalope, bell pepper, coffee, sugarcane, and black bean. In addition, application of extract of *M. oleifera* leaves enhanced

the nodulation of black gram, *Vigna munga* (Bose, 1980). However, further studies on the elucidation of growth promoters and/or phytohormones present in *M. oleifera* leaves, if any, are necessary to verify these reported claims. Phytohormones such as gibberellins at 0.1 ppm, indole acetic acid at 0.1 ppm, and 2, 4 dichlorophenoxy acetic acid at 1 ppm significantly produced the highest mycelial dry weight of *P. florida* (Adenipekun and Gbolagade, 2006).

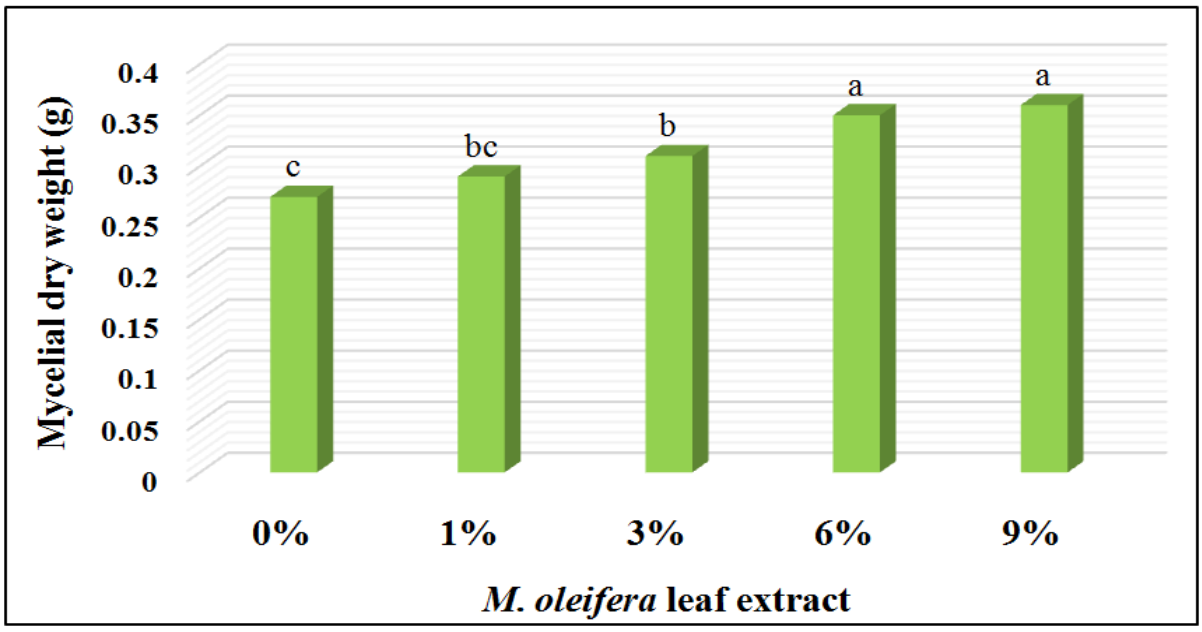

Fig. 1. Mean weight of mycelial biomass of *P. florida* grown on coconut water supplemented with varying concentrations of *M. oleifera* leaf extract in submerged culture for 15 days.

Aside from the potential phytohormones, the suitability of MOLE for the efficient mycelial biomass production of *P. florida* could also be due to the nutritional and chemical compositions of *M. oleifera* leaves.

It contains high concentrations of iron, copper, phosphorous, calcium, vitamins A, B, and C, beta-carotene, folic acid, nicotinic acid, alpha-tocopherol, riboflavin, pyridoxine, and amino acids (Makkar and Becker, 1996), which are essential requirements for the growth of mycelia of *P. florida*. Adenipekun and Gbolagade (2006) reported that thiamine, followed by pyridoxine were the most suitable vitamins and calcium was the best macronutrient for mycelial growth of *P. florida*.

After evaluating the effect of MOLE on the mycelial growth, the mycelial biomasses were extracted using ethanol and the extracts were analyzed in DPPH radical scavenging assay to further determine the effect of MOLE on the antioxidant activity of *P. florida*. Similar to mycelial growth response, the radical scavenging activity had increased as the concentration of MOLE increases (Figure 2).

The maximum radical scavenging activity of 68% was obtained in extracts of mycelia grown in both 6% and 9% MOLE. This was followed by mycelial extract from 3% MOLE, which showed no statistical difference. On the other hand, the non-supplemented media recorded the lowest radical scavenging activity. These results clearly indicate that MOLE affects not only the mycelial growth but also the antioxidant activity of *P. florida*. The improved antioxidant property of the mycelia by MOLE could be explained by the fact that *M. oleifera* leaves is rich in natural antioxidants including beta-carotene, ascorbic acid, calcium, potassium, and proteins (Siddhuraju and Becker, 2003). The accumulation these important components could probably have played major role increasing in antioxidant activity of *P. florida*.

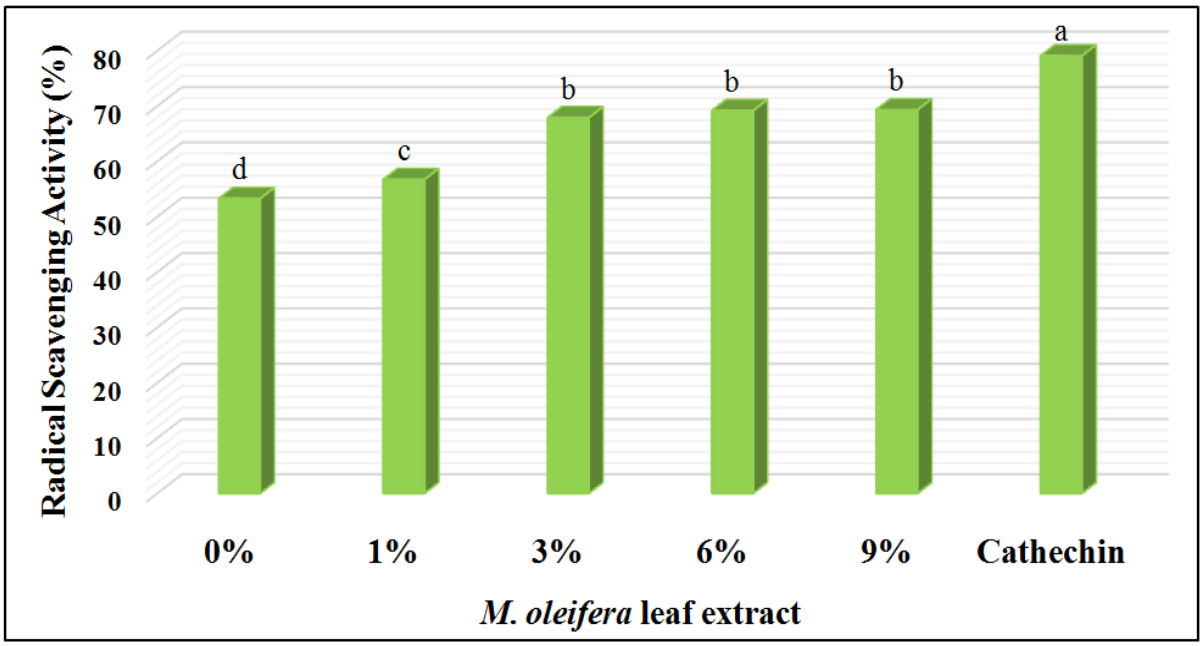

Fig. 2. Radical scavenging activity of extracts of *P. florida* mycelia grown on coconut water supplemented with varying concentrations of *M. oleifera* leaf extract.

Recently, Fasoranti *et al.* (2019) studied the effect of selenium fortified substrate on the nutrient and antioxidant properties of *Pleurotus ostreatus* and *Pleurotus pulmonarius* and they found out that extract of selenium-fortified mushrooms showed higher antioxidant properties. Moreover, corncob substrate supplemented with three herb residues (CKI, QC1, QC2) at 30% concentration significantly improved the scavenging activity against DPPH free radicals and phenolic content (Jin *et al.*, 2018). Li *et al.* (2017) mentioned utilization of stalks of perilla as substrate produced fruiting bodies of *P. ostreatus* with high antioxidant activities.

Conclusion

Taken the results together, *M. oleifera* leaf extract could be utilized as very good supplement of culture media in submerged cultivation for the luxuriant mycelial growth and higher antioxidant property of *P. florida*.

This practical, innovative, and easy cultivation technique for *P. florida* could be used by mushroom growers and nutraceutical industries to efficiently produce mushroom biomass and extracts with healthful benefits. Further works on elucidation of growth promoting components of *M. oleifera* leaves in our interest to verify the claim of the present that this plant enhances mycelial growth must be done.

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