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MYCELIAL GROWTH PERFORMANCE OF *Pleurotus ostreatus* ON CULTURE MEDIA SUPPLEMENTED WITH NATURAL CALCIUM SOURCES

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ABSTRACT

Cultivation of *Pleurotus* mushroom is common throughout the world due to its simple and inexpensive production cost resulting to high yield. Accordingly, substrate supplementation has become a common practice used to improve biological efficiency, yield, and nutritional value of mushroom. The present study is focused on the influence of natural calcium sources including agricultural lime, eggshell, and oyster shell powder on mycelia growth performance of *Pleurotus ostreatus* on culture plates. The mycelial diameter and density was evaluated. Results revealed that the addition of agricultural lime and oyster shell powder in all level of concentrations (2, 4, 6, 8, and 10%) was suppressive to mycelial growth. Meanwhile, supplementation of 2% and 4% eggshell powder was comparable to the mycelial diameter and density of *P. ostreatus* on non-supplemented media. This finding suggests that eggshell powder can be used as a substrate additive without compromising the mycelial growth response, and a potential source of Ca.

Keywords: *Pleurotus ostreatus*, substrate supplementation, calcium sources, mycelial growth

INTRODUCTION

Oyster mushroom is the second most commercially cultivated edible mushrooms in the world [1]. They can successfully grow in variable temperature conditions, making them ideal to cultivate throughout the year in different regions of tropical countries [2]. Several lignocellulosic wastes are commonly used as substrates in mushroom production. Species of *Pleurotus* is an efficient lignin-degrading mushroom and can be cultivated on several types of such materials [3] like banana waste [4], corn, bean, coffee, soybean straw, cotton stalks, pigeon pea stalks and sugar cane remnants [5]. The growth stage of fungal mycelium in the substrate is essential for mushroom production. An appropriate substrate enables mycelium colonization and prevents potential risks of contamination during the process, primarily by other fungi and bacteria that can affect the production [6]. It was reported that the types of substrate used have significant influence on the growth performance of oyster mushroom [7]. Successful mushroom growth primarily depends on its uptake of nutrients and physiochemical environment in the medium or substrate [8]; hence, substrate supplementation is an important method to improve growth, yield, and nutritional content of mushrooms [9]. For the past years, several materials have been used as growth substrate additives to improve oyster mushrooms. These substrate supplements include urea, ammonium sulphate, gram flour, soybean meal, mustard cake, cotton seed cake, and molasses [10]. In this study, natural Ca sources including agricultural lime, eggshells, and oyster shells were used as a potential additive for *P. ostreatus* cultivation. We aimed to evaluate the role of Ca supplementation on mycelial growth response of *P. ostreatus* on culture plates.

MATERIALS AND METHODS

Source of Pure Culture

Pure culture of *P. ostreatus* was acquired from the Center for Tropical Mushroom Research and Development (CTMRD), Central Luzon State University, Science City of Munoz, Nueva Ecija. Mycelial discs of *P. ostreatus* were transferred using a sterile inoculating needle into the sterilized potato dextrose agar (PDA) culture plates. These were then incubated at room temperature until complete mycelial colonization.

Preparation of Ca Sources

Agricultural lime was already obtained in powder form. Eggshells were washed with purified water and crushed into pieces. Oyster shells were also cut into pieces and soaked in distilled water for 24 hours. These were then rinsed several times with purified water to remove impurities and unwanted odor. These materials were then sun-dried for several hours. Dried eggshells and oyster shells were pulverized using a blender and sifted accordingly.

Preparation of Ca Supplemented Culture Media

One hundred ml of PDA media was prepared for each concentration (0, 2, 4, 6, 8 and 10%) of agricultural lime, eggshell powder, and oyster shell powder and dispensed in small bottles. After the addition of varying levels of the Ca sources into the media, pH levels were obtained using a digital pH meter. All treatments were adjusted to pH level 6.0. The bottles were then covered, properly labeled, and sterilized in an autoclave at 121°C, 15 psi for 30 minutes.

Evaluation of Mycelial Growth Response

After full ramification of *P. ostreatus* mycelia in the culture media, mycelial discs were prepared using a sterile 10-mm cork borer which served as the inocula to evaluate growth response. The mycelial discs were transferred to PDA culture plates supplemented with varying concentrations of agricultural lime, eggshell powder, and oyster shell powder in three replicates for each treatment. These culture plates were incubated at room temperature for seven days. Mycelial growth rate was recorded daily by measuring the colony diameter (in mm) using a digital vernier caliper. Mycelial density was described as very thin (+), thin (++), thick (+++), very thick or cottony (++++).

Statistical Analysis

Data were analyzed using analysis of variance (ANOVA) in one way classification analysis using SPSS software version 20. Least Significant Difference (LSD) was used to compare the significant differences among treatments.

RESULTS

Mycelial diameter and density

Mycelial growth performance of mushrooms can be influenced by supplementing culture media with particular mineral. Since mushrooms are known to possess an effective mechanism to absorb trace elements from the substrate (Kala & Svoboda, 2000), this study evaluated the influence of different Ca sources on mycelial growth responses of *P. ostreatus*. Table 1 presents the mycelial growth diameter and mycelial density of *P. ostreatus* on Ca-enriched PDA media at varying concentrations after seven days of incubation. In media with agricultural lime (AGL), significant differences on mean between treatments were noted. Highest mycelial growth (86.09±3.42 mm) was recorded on non-supplemented media (control).

Table1: Mycelial growth diameter and mycelial density of *P. ostreatus* on potato dextrose agar at varying concentrations of Ca sources after 7 days of incubation at room temperature

Calcium sources	Concentration (%)	Mycelial growth (mm)	Mycelial Density
	0	86.09±3.42[a]	++++
AGL	2	74.75±1.29[b]	++++
	4	67.51±2.57[c]	++++
	6	52.86±1.14[d]	++++
	8	37.28±2.49[e]	+++
	10	10.00±0.00[f]	-
	0	86.09±3.42[a]	++++
ESP	2	86.70±4.17[a]	++++
	4	83.40±1.87[a]	++++
	6	75.38±3.39[bc]	+++
	8	74.77±1.05[c]	+++
	10	73.36±2.62[c]	+++
	0	86.09±3.42[a]	++++
OSP	2	69.64±1.11[b]	+++
	4	71.13±2.37[b.]	+++
	6	68.30±1.62[b]	+++
	8	70.00±2.63[b]	+++
	10	70.73±2.22[b]	+++

Values presented are means and SD
Treatment means in each Ca source with the same letter of superscript in each column are not significantly different from each other at 5% level of significance using LSD. Mycelial density column: very thin (+), thin (++), thick (+++), very thick or cottony (++++)

Interestingly, as the concentrations were increased, the mycelial growth diameter decreased. Supplementation of 10% of AGL completely suppressed mycelial growth in culture media (Figure 1). Despite the decreased mycelial diameter in supplemented media, mycelial density was observed to be very thick and cottony in 2%, 4% and 6% concentrations similar with the control. This can signify that the nutrients present in the media might be adequate to nourish luxuriant growth. Meanwhile, eggshell powder (ESP)-supplemented media with 2% (86.70±4.17 mm) and 4% (83.40±1.87 mm) were both statistically comparable to the control at 5% level. Also, the mycelial density for both levels was noted to be very thick and cottony (Figure 1). However, enrichment with 6%, 8% and 10% of ESP revealed a significant difference with the latter concentrations, having mycelial diameter averages of 73.36-75.38 mm.

Correspondingly, their mycelial density was observed to be thick. Finally, addition of OSP in different concentrations significantly differed in mycelial growth response with the control. The mycelial density was also thick.

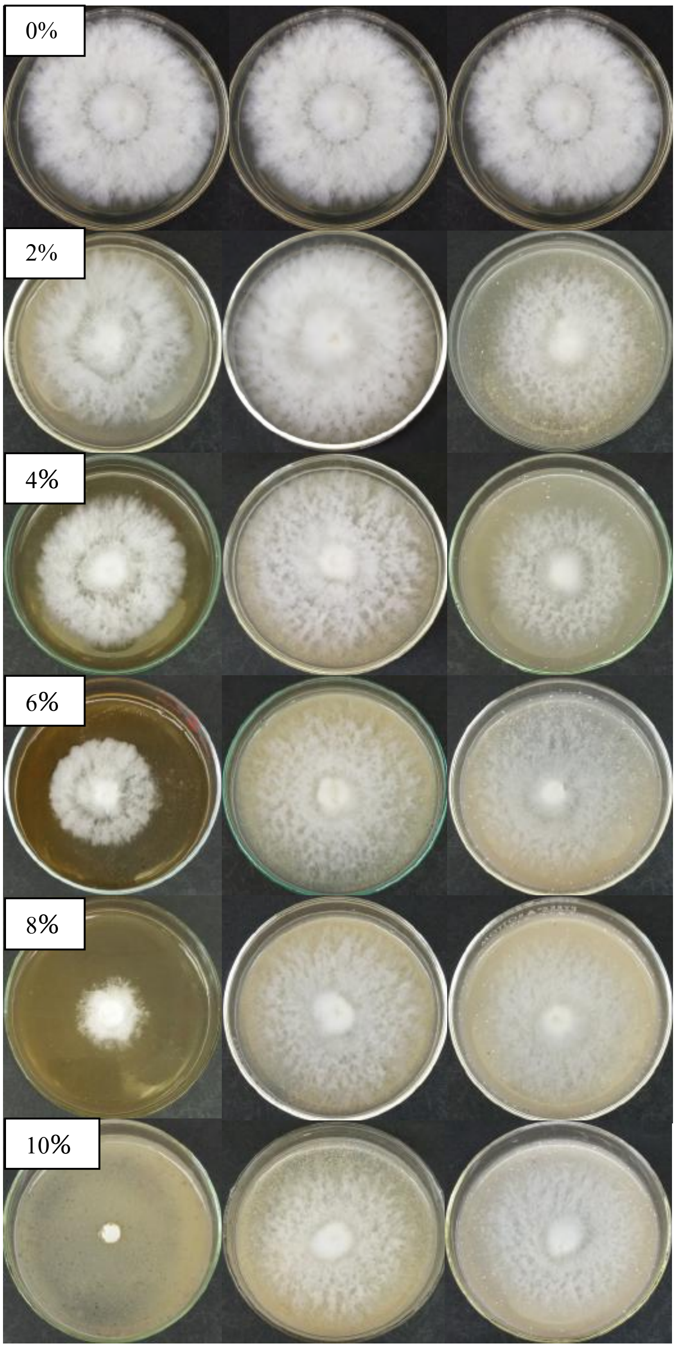

Figure 1: Culture plates of *P. ostreatus* on potato dextrose agar with varying concentrations of Ca sources after 7 days of incubation

DISCUSSION

Nutrient content of substrate was one of the factors limiting the saprobiotic colonization of cultivated mushrooms [11]. Thus, different levels of supplementation influence the quality, growth, and yield of cultivated mushrooms [12]. The findings were similar with the previous report that higher OSP concentrations reduced mycelial growth in *Pleurotus florida* [13]. Meanwhile, supplementation of 5% calcium carbonate ($CaCO_3$) on potato sucrose agar (PSA) medium totally inhibited mycelial growth of *Hypsizygus marmoreus* [14]. One study also concluded that mycelial growth rate of *Pleurotus eryngii* decreased on sawdust medium with 1 to 5% oyster shell powder [15]. Addition of high levels of Ca sources was suppressive to mycelial growth which might be caused by the accumulation of mineral at high amounts. Moreover, supplementation of additives increased the temperature of substrate which could contribute to the decrease on mycelial growth rate [16]. On the other hand, previous studies were focused on improving nutritional value of mushrooms by adding minerals to the substrate such as selenium [17] and $CaCO_3$ [18]. High level of Se in culture medium decreased fungal growth rate, hyphae diameter and septum distance and caused alteration in color of colony. However, at a lower Se concentration, the mycelial growth response was efficient [17, 19]. Meanwhile, a study evaluated the ability of *P. ostreatus* mycelium to absorb iron from iron-supplemented culture medium. It was concluded that bioaccumulation in *P. ostreatus* mycelium has a potential alternative to produce non-animal food sources of Fe [20].

CONCLUSION

This present study signified the influence of varying concentrations of natural Ca sources on mycelial growth performance of *P. ostreatus*. Agricultural lime and oyster shell powder decreased mycelial growth on culture plates while 2 to 4% eggshell powder showed comparable mycelial growth and density with *P. ostreatus* mycelia grown on non-supplemented media. This result recommends further research on the effect of Ca sources on growth performance of *P. ostreatus* including the yield and biological efficiency, and nutritional content of the fruiting bodies to determine whether Ca enrichment can improve growth and Ca level in mushroom.

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