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Mycelial Growth Performance of three *Pleurotus* species on Corn Varieties in the Philippines

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ABSTRACT

Three varieties of corn (sweet, glutinous, and yellow) were assessed as possible corn-grit decoction gelatin media for three *Pleurotus* species. Mycelial growth for each species of *Pleurotus* was measured daily and mycelial density was also considered. Physical factors such as pH, aeration, illumination and temperature affecting mycelial growth were also evaluated. The best corn-grit decoction gelatin medium was assessed to be species-specific. *P. pulmonarius* had luxuriant mycelial growth on sweet corn-grit decoction gelatin medium with largest mycelial diameter of 90mm and shortest incubation period of eight days. *P. florida*had prolific growth on glutinous corn-grit decoction gelatin medium with largest mycelial diameter of 90mm and shortest incubation period of eight days. Lastly, *P. sajor-caju* was best grown in yellow corn-grit decoction gelatin medium with largest mycelial diameter of 90mm after seven days incubation period. Physical parameters were also evaluated as factors which influence growth of mycelia. Rich mycelial growth was evident on pH 8.0 for *P. pulmonarius* and *P. florida* while *P. sajor-caju* grew optimally at pH 7.0 with a corresponding mean mycelial growth of 86.83mm, 90mm and 86.23mm, respectively. All three strains of *Pleurotus* had verdant mycelial growth on sealed, dark and room-temperature incubation conditions. The results of the recent study was a benchmark in proving the ability of the three cornvarieties in supporting the efficient mycelial growth of three species of *Pleurotus* under appropriate physical conditions.

KEYWORDS: *Pleurotus*, corn, grits, sweet, glutinous, yellow

INTRODUCTION

Pleurotus species, commonly known as oyster mushrooms, are edible fungi cultivated worldwide especially in South East Asia, India, Europe and Africa [1]. The utilization of different locally available substrates for the growth of various *Pleurotus* species has been the focus of many researches worldwide.

The bioconversion of solid wastes which are generated from the industry and in agriculture into edible biomass, functional food or as a source of medicine and pharmaceuticals is one of the main contributions of mushroom cultivation [2]. In the Philippines, corn or maize (*Zea mays*) is the second most important crop and is also a staple food for Filipinos specifically those from the Southern areas of the country [3]. Specifically, the objectives of this study were:

1) to evaluate the mycelial growth of *Pleurotus* species on corn grit decoction gelatin medium (CDG), and;

2) to determine the appropriate physical conditions such as pH level, aeration, illumination and temperature for mycelial growth of *Pleurotus.*

Since agricultural wastes can be utilized as potential substrates in mushroom cultivation, by means of locally indigenous materials such as corn as culture medium for the efficient and low-cost cultivation of *Pleurotus* species; hence, this study was conducted.

MATERIALS AND METHODS

Source of pure culture:

Pure cultures of *P. pulmonarius, P. florida,* and *P. sajor-caju* were obtained from the culture collection of Center for Tropical Mushroom Research and Development (CTMRD) of the Department of Biological Sciences, College of Arts and Sciences, Central Luzon State University, Science City of Munoz, Nueva Ecija, Philippines.

Influence of three varieties of corn as culture media:

Corn grit decoction gelatin (CDG) of three varieties of corn; sweet corn (SCDG), glutinous corn (GCDG) and yellow corn (YCDG) were used as the culture media for the mycelial growth of three *Pleurotus* species. To prepare CDG in a liter of distilled water, 50 grams of corn grits (*i.e.* sweet, glutinous and yellow) was boiled separately until tender. Then, the decoction was strained to remove cooked corn remainders, and reconstituted to a liter by adding more distilled water. Ten grams of sucrose and ten grams of white *gulaman*bar were dissolved and mixed over low flame until mixture is homogeneous. Approximately 10 mm^2 x 3 mm mycelial disc the pure stock culture of the three *Pleurotus* species was aseptically transferred to the center of a sterilized CDG plates using a sterilized cork borer. CDG plates were incubated at room temperature, and the diameter of mycelia was measured daily using digital Vernier caliper until full ramification. Mycelial density as well as incubation period were also considered.

Influence of physical factors:

The best CDG medium for each species of *Pleurotus* with the shortest incubation period and thickest mycelial density was used in the evaluation of physical factors, *i.e.* pH level, aeration, illumination and temperature. Measurements of mycelial growth in diameter was measured using digital Vernier caliper daily and recorded until total colonization of the mycelia was obtained. The most optimal CDG medium for each *Pleurotus* species was adjusted to different pH levels from 5.5 to 8.0 with 0.5 intervals by adding either 0.1 M of NaOH or 0.1 M HCl prior to sterilization and inoculation of mycelial discs. From the most suitable pH of each CDG medium, aeration requirement was considered. To create sealed (unaerated) condition, the CDG plates with inoculated mycelial discs of each *Pleurotus* species were covered with parafilm twice; while those devoid of any seals were incubated as unsealed (aerated). From the best pH and aeration conditions, illumination requirement was evaluated. For light condition, the culture plates were placed inside a disinfected chamber with artificial light (322.92 lumens m^{-2}) under room temperature while dark condition was induced by covering the inoculated plates with a clean black garbage bag. Finally, the optimum temperature for mycelial growth was evaluated from the best culture media and physical conditions being previously assessed. The cultured plates were incubated at various temperature ranges; *i.e.* room-temperature, air-conditioned and refrigerated. Measurement of the actual temperature during the entire study was recorded three times a day, *i.e.* early morning, noon time and late afternoon.

Statistical Analyses of Data Obtained:

The data of this research study were laid out in a completely randomized design (CRD). Analysis of variance (ANOVA) was employed to test the overall significance of data while the least significance difference (LSD) test was used to compare the differences among treatment means. T-test was also employed to test differences among means of aeration and illumination conditions.

RESULTS AND DISCUSSION

Evaluation of Corn-based Culture Media:

The nutritional content present in a particular medium or substrate used can greatly affect the growth of mycelia. An evaluation of the suitability of a certain medium is necessary to reflect the efficiency of mushroom growth. Since corn was considered to be the second most important crop in the Philippines, it is therefore imperative to evaluate its suitability in growing mushrooms. The mycelial growth of*P. pulmonarius, P. florida*and *P. sajor-caju* were evaluated and enhanced using three indigenous corn-grit decoction gelatin (CDG) media. Table 1 shows the daily mycelial diameter (mm), incubation period (days) and mycelial density of three *Pleurotus* species on different CDG media.

After 8 days of incubation, *P. pulmonarius* grown in sweet corn-grit decoction gelation (SCDG) medium recorded the widest mycelial diameter with a mean of 90.00 mm, very thick mycelial density and shortest incubation period. Analysis of variance revealed significant effect of CDG medium on the mycelial diameter of *P. pulmonarius.* In case of *P. florida*, the largest mycelial diameter was observed in SCDG medium butgrew luxuriantly in glutinous corn-grit decoction gelatin (GCDG) medium with very thick mycelial density compared to other CDG media (Fig.1). Finally, in *P. sajor-caju*, YCDG medium recorded the largest mycelial growth (90 mm) with thick mycelial density and shortest incubation period of 7 days.

The suitability of corn as a culture media on *Pleurotus* species can be attributed to the chemical components of each variety of corn. Specifically, corn or maize, has several cultivars which vary according to the kernel color (yellow, white, and bicolor) and by its sugar content (sweet) [4]. One of the factors which could affect the growth of mycelia is the sugar content of the corn variety. While sweet corn variety has higher sucrose content compared to the other two corn varieties considered in this study,the nutritional components can be attributed for the growth performance of *P. pulmonarius.*

Table 1: Diameter of daily mycelial growth, incubation period and mycelial density of *Pleurotus* species on three indigenous corn-grit decoction gelatin (CDG) media

Mushroom species		Mycelial Diameter (mm)								M. D.
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	
Pleurotus pulmona-rius	SCDG	12.65^{a}	19.90^{a}	29.68^{a}	38.80^{a}	51.87^{a}	61.61^{a}	72.82^{a}	90.00^{a}	++++
	GCDG	11.42^{a}	18.59^{a}	29.03^{a}	36.91^{a}	48.55^{a}	58.86^{a}	72.05^{a}	88.07^{a}	+++
	YCDG	10.84^{a}	16.86^{b}	23.18^{b}	29.78^{b}	38.31^{b}	47.13^{b}	55.36^{b}	64.87^{b}	++
Pleurotus florida	SCDG	10.66^{a}	19.98^{a}	30.51^{a}	41.15^{a}	56.13^{a}	68.27^{a}	79.52^{a}	90.00^{a}	++
	GCDG	10.59^{a}	18.06^{b}	26.84^{ab}	37.72^{a}	49.71^{ab}	61.99^{ab}	73.02^{ab}	90.00^{a}	++++
	YCDG	10.47^{a}	16.18^{c}	25.69^{b}	33.68^{a}	47.28^{b}	56.95^{b}	67.13^{b}	90.00^{a}	+
Pleurotus sajor-caju	SCDG	10.69^{a}	17.12^{b}	28.37^{a}	41.39^{a}	56.27^{a}	67.96^{a}	83.52 ^{a}	-	++++
	GCDG	10.38^{a}	14.12^{b}	17.93^{b}	25.31^{b}	34.65^{b}	41.71^{b}	47.31^{b}	-	++
	YCDG	10.59^{a}	15.16^{b}	25.11^{a}	35.89^{a}	54.41^{a}	66.58^{a}		-	++++

SCDG – sweet corn-grit decoction gelatin; YCDG – yellow corn-grit decoction gelatin; GCDG- glutinous corn-grit decoction gelatin. Means of the same superscript in a column are not significantly different at 5% level using LSD.
M.D. - Mycelial density: very thin (+); thin (++); thick (+++), very thick (++++)

The nutritional value of sweet corn, *Zea mays var. saccharata*, (per 100 grams) has an energy value of 86 kcal, carbohydrates (18.70g), protein (3.27g), total fat (1.35g), cholesterol (0mg), dietary fiber (2g), vitamins (folates, niacin, pantothenic acid, pyridoxine, riboflavin, thiamin, vitamin A, C, E and K); minerals (calcium, copper, iron, magnesium, manganese, selenium, and zinc); electrolytes (sodium and potassium) as well as phytonutrients (Carotene α & β, cryptoxanthin-β and lutein-zeaxanthin) [5]. On the other hand, waxy (glutinous) corn has little or very low content of amylose (<5%) in grain starch, total fat (4.7g), saturated fat (0.7g), polyunsaturated fat (2.2g), monounsaturated fat (1.3g), cholesterol (0mg), sodium, potassium, total carbohydrate, protein, vitamins (vitamins A, C, D, B-6 and B-12), calcium, iron and magnesium [5, 6]. Moreover, the endosperm of yellow corn contains 88.4% starch, 8.0 %, protein, 0.8% oil, 0.3% ash, 0.6% sugars and 1.9% fiber (Bunge, 2011). It was also reported that significantly lower fat percentage was noticed in sweet corn (3.0%) in comparison with the fat percentage of white (4.8%) and yellow (4.5%) corns [7].Successful growth and colonization of mycelia of the three *Pleurotus* species on three varieties of indigenous corn-grit decoction gelatin media was achieved in this study. Corn grits also yielded very luxuriant mycelial growth at the shortest incubation period for *Lentinussquarrosulus*and *Polysporusgrammocephalus*[8]. This was not in congruence with the results of *L. tigrinus*and *P. cyanescens*were poorest mycelial growth was observed in corn grits [9, 10].

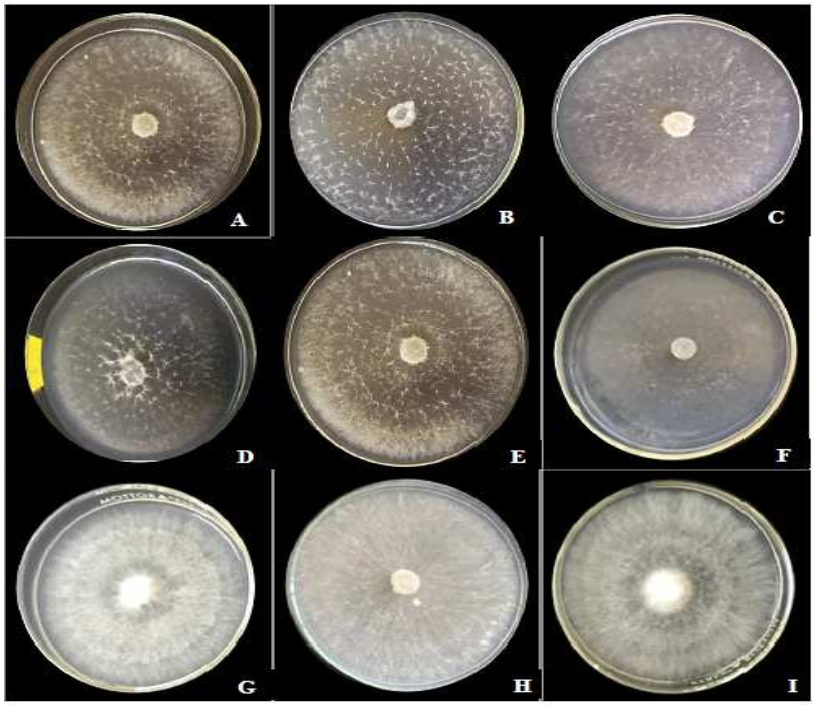

Fig. 1: Secondary mycelial growth performance of three *Pleurotus* species on different indigenous corn-decoction grit (CDG) media. *P. pulmonarius* grown in (A) SCDG, (B) GCDG, and (C) YCDG. *P. florida*grown in (D) SCDG, (E) GCDG, and (F) YCDG. *P. sajor-caju* grown in (G) SCDG, (H) GCDG and (I) YCDG

Influence of Physical Factors on Mycelial Colonization:

The best corn- grit decoction gelatin (CDG) culture medium for a specific species of *Pleurotus* was further used as an assay medium for the evaluation of physical parameters. The largest and fastest mycelial growth of *P. pulmonarius* was noted in SCDG medium and produced luxuriant mycelial growth at pH level of 8.0 (7 days incubation), incubated at sealed (8 days incubation), dark (8 days incubation), and room temperature conditions (8 days incubation) (Table 2). On the other hand, GCDG medium registered as the best indigenous CDG medium for *P. florida* and resulted to optimal growth of mycelia at pH level of 8.0 (7 days incubation), incubated at sealed (8 days incubation), dark (8 days incubation), and room temperature (8 days incubation) conditions. However, *P. sajor-caju* grew luxuriantly in YCDG medium recorded to have prolific mycelial growth at pH level of 7.0 (6 days incubation), incubated at sealed (6 days incubation), dark (5 days incubation), and room temperature (8 days incubation) conditions.

There is a specific range of pH of the medium which can make a fungus grow opulently. However, the pH is not a unitary factor with mechanism its of action varying at different hydronium ions [H_3O^+] which reflects that part of a pH growth curve may reflect the effect of a low pH on enzyme systems, or another high pH on metal solubilities[11]. Likewise, pH of the media influences the maximum growth rate and biomass yield as well as the minimum duration of the lag phase of the growth of mushroom together with inoculum size and nutrient composition [12]. In this study, *P. pulmonarius* and *P. florida* grew optimally at pH 8.0, while the *P. sajor-caju* had opulent mycelial growth at a pH 7.0. Highest percentage of germination was recorded on *L. tigrinus*at pH level of 7.5 [12] while it was suggested an optimum pH of 7.5 for the germination of basidiospores of *S. commune* [13].

Among the three strains of *Pleurotus* used in this study, all showed significant mycelial growth when oxygen was deprived. All three *Pleurotus* species favored sealed or unaerated conditions for fast and thicker mycelial growth, which was similar with the oxygen requirements of *Volvariellavolvacea*[14], *Coprinuscomatus*[15] and *Pleurotus pulmonarius* Spanish strain [16]. This, however, was in contrary to the oxygen requirements of *Agaricusblazei*[17] and *Agrocybeaegrita*[18] which preferred aerated conditions. Mycelial growth of *P. ostreatus*and *P. florida*are stimulated mainly by high CO_2 concentrations ranging up to 22% - 28% [19].

Light or illumination is one of the four factors that condition mushroom aside from temperature, humidity and concentrations of CO_2[20]. The reactions of fungi to visible and ultraviolet are of three main types: inductive, inhibitive and trophic [11]. *Pleurotus* species exhibit inductive and trophic responses to light [20]. Results of this study was also in agreement with the findings on *L. tigrinus*[9], on *C. reinakeana*[21], on *V. volvacea*[14], on *C. comatus*[15], on *A. blazei*[17] and on *P. pulmonarius* Spanish strain [16].

Table 2: Growth performance of three *Pleurotus* species as affected by different physical factors

Physical Factors	Secondary Mycelial Diameter (mm)		
	Pleurotus pulmonarius	*Pleurotus Florida*	*Pleurotus sajor-caju*
pH level			
5.5	76.65[a]	83.77[a]	77.02[c]
6.0	79.11[a]	52.28[b]	78.53[bc]
6.5	81.24[a]	82.09[a]	79.44[bc]
7.0	80.15[a]	90.00[a]	86.24[a]
7.5	84.23[a]	86.19[a]	79.07[bc]
8.0	86.83[a]	90.00[a]	82.63[ab]
Aeration			
Unsealed	78.33[b]	81.40[b]	65.82[b]
Sealed	90.00[a]	90.00[a]	90.00[a]
Illumination			
Lighted	81.76[b]	88.52[a]	65.03[b]
Dark	90.00[a]	90.00[a]	90.00[a]
Temperature			
RT (27-32ºC)	87.24[a]	90.00[a]	90.00[a]
R (9-11ºC)	10.00[c]	10.00[c]	10.00[c]
AC (18-21ºC)	38.12[b]	55.41[b]	69.46[b]

Means of the same superscript in a column are not significantly different at 5% level using LSD.
Mycelial density: very thin (+); thin (++); thick (+++), very thick (++++)
RT – Room Temperature; R – Refrigerated; AC – Air-Conditioned

Finally, temperature is one of the cardinal factors which determine the distribution of fungi in different ecological niches[22]. Fungi can be classified as either temperate, semi-temperate or tropical depending on the mycelial growth [23]. Each *Pleurotus* species in this study grew luxuriantly at room temperature (27-32ºC) with very thick mycelial density and shortest incubation period. Such findings can be attributed to the fact that each *Pleurotus* species have characteristic atmospheric temperature of most tropical countries, whereby belonging to tropical mushroom species. No growth of mycelia was recorded on refrigerator-incubated plates (7ºC to 11ºC), likewise no mycelial ramification was also observed under refrigerated conditions of *L. tigrinus*[9].

Conclusion:

The mycelial growth of three *Pleurotus* species on different corn grit decoction gelatin (CDG) media evaluated was species-specific. Also, all *Pleurotus* species had luxuriant mycelial growth at sealed, dark and room-temperature conditions. At present, this study was able to promote the effective growth of mycelia of three *Pleurotus* species using sweet, glutinous, and yellow corn varieties which was not previously reported. Hence, this study recommends the utilization of three varieties of corn as culture media for the cultivation of *Pleurotus* species.

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