Mycelial Performance and Primordial Development of *Volvariella volvacea* in Different Leaf Litter

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Abstract - *Volvariellavolvacea commonly known as paddy straw mushroom is a tropical mushroom that is widely grown in Southeast Asia due to its favorable taste and ideal tropical condition for its proliferation. One of the most vital factor in V. volvacea production is its spawning material. This are commonly plant materials and considered as agro industrial waste. V. volvacea belong to leaf litters which thrive in rice straw and banana leaves. Thus, this study specifically intends to: determine the mycelial performance and primordial development of V. volvacea in the* combination of dried leaves of cacao (*Theobroma cacao), acacia (Samaneasaman), rice (Oryza sativa) straw and banana (Musa spp.). Each combination was added with 30% of sawdust. Completely randomized design was used for the experimental design. The ideal combination for spawning substrate were noted in treatment eight (35% acacia leaves + 35% cacao leaves + 30% sawdust) since it elucidated luxuriant mycelial run with zonation pattern. In addition, the shortest period of primordial development was noted with a mean of 6.33 days of incubation in treatments five (35% banana leaves + 35% cacao leaves + 30% sawdust), six (35% banana leaves + 35% acacia leaves + 30% sawdust) and seven (35% banana leaves + 35% rice straw + 30% sawdust). Moreover, this study revealed that all the test combination was colonized with mycelia which indicates that the evaluated materials which are considered as locally available and abundant in the farming community of CBSUA-Sipocot can be utilized as spawning materials for the production of V. volvacea. This is an economically eco-friendly approach in dealing with agro industrial waste of the locality.*

Keywords - *mycelial run, primordial development, zonation pattern*

INTRODUCTION

Volvariellavolvacea commonly known as the edible paddy straw mushroom has been described as a primary homothallic basidiomycete [1]-[3]. Its homokaryotic mycelium that arises from the germination of a single basidiospore can convert to dikaryotic form which complete the sexual cycle without mating; however, its dikaryotic mycelia does not contains clamp connections [4].

Mushrooms have been received significant interest in recent years since it is a delicious food with high nutritional attributes and great medicinal values [5]. Additionally, it can produce mycopharmaceuticals andmyco Nutraceutical [6]. For instance, immunomodulatory protein (Fip) has been purified from *V. volvacea* [7]. Being identified as healthy food due to its dietary and medicinal attributes [8], *V. volvacea* became popular in Malaysia, Thailand, Southern China and the Philippines [9]. Due to higher demand in healthy food annual production of *V. volvacea* has increased in recent years. Additionally, Royse et.al. [10] claimed that mushroom market represented 63 billion US dollar in 2013. Since *V. volvacea* is a tropical species of mushroom which requires relatively high temperatures (28–35°C) for vegetative growth and fruiting, the tropical climate of the Philippines provides a suitable climatic condition favorable to its production. Thus, study on its mycelial production prior to fruiting is highly significant.

Dried leaves are abundant and considered waste in the locality of CBSUA-Sipocot. During rainy season it causes clogging of drainage and encourages stagnant water wherein pest such as mosquitos thrives in this environment. Thus, utilizing it as viable spawning material is ideal to address leaf litter problem in the community.

OBJECTVE OF THE STUDY

Most tropical species of mushrooms can be classified as leaf litter and wood rotter. For instance,*V.*

volvacea belong to leaf litters which thrive in rice straw and banana leaf. Thus, this study specifically intends to: (1) determine the mycelial performance of *V. volvacea* on dried leaves of cacao, acacia, rice straw and banana (2) determine which combination of dried leaves of cacao, acacia, rice straw and banana is suitable as spawning material for *V. volvacea*, and; (3) evaluate occurrence of primordial development in combination of dried leaves of cacao, acacia, rice straw and banana as spawning material.

MATERIALS AND METHODS

Source of Mushroom

Fruiting body of *V. volvacea* was obtained from the vicinity of CBSUA – Sipocot campus and tissue cultured in coconut water media.

Collection of Substrates

Dried rice straw (*Oryza sativa*), dried leaves of banana (*Musa* spp.), acacia (*Samaneasaman*) and cacao (*Theobroma cacao*) were collected at the vicinity of Central Bicol State University of Agriculture, Sipocot Campus. It was put in a pile for one week to homogenize the dryness of each substrate. Sawdust was obtained free from furniture makers at barangay Bolo Sur, Sipocot, Camarines Sur. It was piled up and allowed to decompose for four weeks. D1 rice bran was obtained from Sipocot Market, SipocotCamarines Sur.

Soaking, Chopping and Bagging

Each substrate was soaked in a separate drum for 24 hrs. Subsequently, it was drenched with clean running water until the foul odor was removed. Then it was air dried to remove excess water. Once water was partially removed, rice straw, banana leaves and cacao leaves were chopped at approximately one inch in length except acacia leaves since its size is relatively one inch each. The chopped substrates were mixed with the appropriate ratio given in the treatment. It was mixed thoroughly until homogenize. Prior to sterilization, its moisture content was analyzed using moisture meter to attain 65% moisture content.

Pasteurization, Incubation and Inoculation of Substrates

Five grams of the substrate were placed in each petri plate, sealed with cling wrapped and autoclaved at (121 ^{O}C/15 psi) for 45 minutes. Subsequently, it was allowed to cool and transferred to inoculation area. Then, five mm mycelial block of seven-day old *V. volvacea*pure culture was aseptically inoculated at the center of the petri plates. The newly inoculated substrates were placed in the incubation room and allowed to fully ramify at room temperature. Each treatment was replicated three times.

Figure 1. Substrate for Spawn production

Table 1. Treatment of different substrates

TRT	%(BL)	%(AL)	%(CL)	%(RS)	%(SD)
T1	70				30
T2			70		30
T3		70			30
T4				70	30
T5	35		35		30
T6	35	35			30
T7	35			35	30
T8		35	35		30
T9			35	35	30
T10		35		35	30
T11	23.3	23.3	23.3		30
T12	23.3		23.3	23.3	30
T13		23.3	23.3	23.3	30
T14	17.5	17.5	17.5	17.5	30

**note: BL – banana leaves, AL –acacia leaves, CL – cacao leaves, RS – rice straw and SD – sawdust*

Statistical Analysis

Data were analyzed using (STAR) Statistical Tool for Agricultural Research using ANOVA under Completely Randomized Design. Comparison of means utilized pairwise mean comparison of treatments and Tukeys' Honest Significant Difference (HSD) Test.

RESULT AND DISCUSSION

Mycelia are threadlike mass of branching hyphae which is the vegetative part of fungus. Fastest

colonization of mycelia provides significant positive factor in mushroom production and farming sector since this can be considered as parameter of healthy substrates. The fastest number of days recorded for full mycelial ramification was noted in (T2) 70% CL+ 30% SD, (T8) 35% AL + 35% CL + 30% SD and (T11) 23.3% BL + 23.3% AL + 23.3% CL + 30% SD wherein, it took only five days to fully ramify the substrate. However, (T6) 35% BL + 35% AL + 30% SD was recorded with the longest day of ramification of nine days among the substrates. The fast mycelial colonization in treatments (T2) 70% CL + 30% SD, (T8) 35% AL + 35% CL + 30% SD and (T11) 23.3% BL + 23.3% AL + 23.3% CL + 30% SD can be attributed to the cacao leaves components wherein it contains 70, 35% and 23.3% respectively. As stated by Aikpokpodion [11], cacao leaf contains 0.9% N, 0.2% P, 2.0% K, 0.6% Ca and 0.5% Mg. In addition, treatment 11 contains 23.3 % banana leaf wherein, according to Yang et al. [12], banana leaves on its dry matter contains 50% nitrogen free extract and 5% water soluble carbohydrate which can be a contributory factor for mycelial production of mushroom. Furthermore, luxuriant mycelial growth with zonation pattern was noted in (T3) 70% AL + 30% SD and (T8) 35% AL + 35% CL + 30% SD.

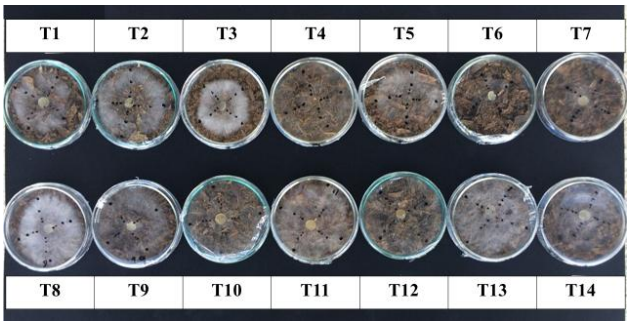

Figure 2. Three days' mycelial run in different substrate.

Fast mycelial ramification equates to fast pace of production in mushroom farming. Results on the number of days of full mycelial ramification is shown in table two, wherein on the second day the fastest mycelial run were noted in (T12) 23.3% BL +23.3% CL + 23.3% RS + 30% SD while the lowest run was recorded in (T1) 70% BL + 30% SD and (T3) 70% AL + 30% SD with 14.33 and 13.00 mean respectively. On the third day the faster mycelial run was noted in (T12) 23.3% BL + 23.3% CL + 23.3% RS + 30% SD, (T2) 70% CL + 30% SD and (T8) 35% AL + 35% CL + 30% SD with 39.33, 38.33 and 37.33 mean respectively. The consistent mycelial run of treatment (T12) 23.3% BL + 23.3% CL + 23.3% RS + 30% SD from day two and three can be attributed to banana leaves since according to Oliveiraet.al. [13], banana leaf sheaths contain 37.3% cellulose, 24.3 % lignin in leaf blade, 0.4 % starch in petioles and midrib and 10.5% in rachis. Wherein, leaf blade and leaf sheath are both present in the substrate.

Table 2. Total number of days of mycelial full ramification

	Treatment	Number of Days		Mycelial density
1	70% BL + 30% SD	6.33	b c	++
2	70% CL + 30% SD	5.00	c	++
3	70% AL + 30% SD	6.67	b	+++
4	70% RS + 30% SD	6.67	b	+
5	35% BL + 35% CL + 30% SD	6.67	b	+
6	35% BL + 35% AL + 30% SD	9.00	a	+
7	35% BL + 35% RS + 30% SD	6.67	b	+
8	35% AL + 35% CL + 30% SD	5.00	c	+++
9	35% CL +35% RS + 30% SD	6.33	b c	++
10	35% AL + 35% RS + 30% SD	5.00	c	+
11	23.3% BL + 23.3% AL + 23.3% CL + 30% SD	5.00	c	++
12	23.3% BL + 23.3% CL + 23.3% RS + 30% SD	6.33	b c	+
13	23.3% AL + 23.3% CL + 23.3% RS + 30% SD	6.67	b	++
14	17.5% BL + 17.5% AL + 17.5% CL + 17.5% RS + 30% SD	7.00	b	++

**note: Means with the same letter are not significantly different. Mycelial density = + thin, ++ thick, +++ very thick*

Moreover, treatment 2 and 8 contains 70% and 35% cacao leaves respectively. Cacao leaves contain large amounts of nutrients due to dust transported from rain wash [14]. This dust that are being included in the leafcan be a key factor in the mycelial ramification of mushrooms since it can contain minerals and nutrients which can be utilized by mushroom. This is also correlated in the fourth day wherein treatment nine which contain 35% cacao leaves elucidated to be highly significant in term of mycelial colonization among the rest of the treatment except (T10) 35% AL + 35% RS. The fast colonization of mycelia in treatment 10 can be attributed to acacia leaves that contains 27% crude

protein [15]; 0.2 Ca ,0.3 P, 36.9 % ADF and 47.5 NDF [16]; ADF contains cellulose and lignin, on the other hand, NDF contains hemicellulose, cellulose and lignin [17] which is vital in mushroom growth. Additionally, rice straw has high amount of potassium and phosphorus both at 15%, iron 0.8%, 80% carbohydrates, 28% calcium, 25% magnesium, 7.1% protein, and 0.12 % sugar [18]. Xiao et al. [19], claimed that it has 35.7% hemicellulose, 32% cellulose, and 22.3% lignin. However, all the treatment was able to sustain mycelial run which can be attributed to partially degraded sawdust that was present in all the evaluated media at 30 %. As stated by Tejano, [20] sawdust usually contains 54.78 % lignin and 35 to 45% cellulose [21]. This components of sawdust are being utilized by mushroom for its growth and development. Moreover, it has a moisture content of 8.25% (wet basis), 42.38% carbon, 76.23% of volatile matter and 1.49% of ash on dry basis. This attributes can be a factor that can be utilized by mushroom in its developmentfor the proliferation of its mycelial growth.

Table 3. Mycelial growth for 3 day of incubation

	Treatment	Mycelial growth in (mm) Day 2		Day 3		Day 4	
1	70% BL	14.33	c	26.66	abc	46.33	bcde
2	70% CL	17.00	abc	38.33	a	55.00	abcd
3	70% AL	19.33	abc	33.67	abc	48.67	bcde
4	70% RS	17.33	abc	20.33	abc	52.33	abcde
5	35% BL + 35% CL	18.67	abc	31.33	abc	45.33	bcde
6	35% BL + 35% AL	13.00	c	28.00	abc	27.67	e
7	35% BL + 35% RS	19.67	abc	34.67	abc	29.67	de
8	35% AL + 35% CL	17.67	abc	37.33	a	48.33	bcde
9	35% CL +35% RS	15.67	bc	14.67	c	65.33	ab
10	35% AL + 35% RS	16.00	bc	15.67	bc	79.00	a
11	23.3% BL + 23.3% AL + 23.3% CL	22.33	abc	35.67	ab	57.00	abc
12	23.3% BL + 23.3% CL + 23.3% RS	28.67	a	39.33	a	40.00	bcde
13	23.3% AL + 23.3% CL + 23.3% RS	27.33	ab	32.33	abc	53.33	abcde
14	17.5% BL + 17.5% AL + 17.5% CL + 17.5% RS	20.00	abc	29.33	abc	37.33	cde

**note: each treatment was added with 30% sawdust; Means with the same letter are not significantly different.*

Primordia is the initial fruiting body of mushroom. It determines the possible fruiting bodies that will eventually mature. The earliest primordial development was elucidated in (T5) 35% BL + 35% CL + 30 % SD, (T6) 35% BL + 35% AL + 30% SD and (T7) 35% BL + 35% RS + 30% SD with six days of occurrence. However, the longest primordial development was noted in (T12) 23.3% BL + 23.3% CL + 23.3% RS + 30% SD and (T13) 23.3% AL + 23.3 % CL + 23.3 % RS + 30% SD with 21 and 22 days respectively. The early primordial development of the *V. volvacea*in treatment (T5) 35% BL + 35% CL + 30% SD, (T6) 35% BL + 35% AL + 30% SD and (T7) 35% BL + 35% RS + 30% SD can be due to the presence of banana leaf. As stated by Rochana et al. [22], banana leaves contain 9.80% dry matter, 8.30% crude protein, 8.10% crude fat and 35.20 % hemicellulose. Furthermore, the ability of all the tested media to serve as spawn carrier can be attributed to the sawdust since it contains higher amount of lignin [23]. It has a moisture content of 8.25% (wet basis), 42.38% carbon, 76.23% of volatile matter and 1.49% of ash on dry basis.

Table 4. Primordial development of test substrate

	Treatment	Number of days of primordial development	
1	70% BL + 30% SD	11.00	c
2	70% CL + 30% SD	11.33	c
3	70% AL + 30% SD	11.00	c
4	70% RS + 30% SD	14.00	bc
5	35% BL + 35% CL + 30% SD	6.33	d
6	35% BL + 35% AL + 30% SD	6.33	d
7	35% BL + 35% RS + 30% SD	6.33	d
8	35% AL + 35% CL + 30% SD	11.33	c
9	35% CL +35% RS + 30% SD	18.00	ab
10	35% AL + 35% RS + 30% SD	11.00	c
11	23.3% BL + 23.3% AL + 23.3% CL + 30% SD	14.33	bc
12	23.3% BL + 23.3% CL + 23.3% RS + 30% SD	21.00	a
13	23.3% AL + 23.3% CL + 23.3% RS + 30% SD	22.00	a
14	17.5% BL + 17.5% AL + 17.5% CL + 17.5% RS + 30% SD	11.00	c

**note: Means with the same letter are not significantly different.*

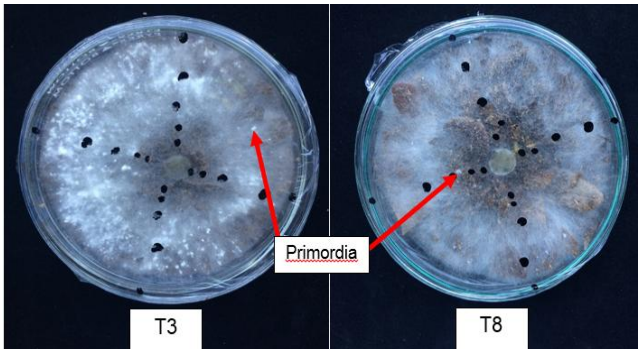

Figure 3. Primordial development of treatment 3 and 8.

CONCLUSION AND RECOMMENDATION

Cacao leaf, banana leaf, rice straw, and acacia leaf litter under CBSUA-Sipocot campus were utilized in this study to serve as spawn material for *V. volvacea*. Results revealed that all the combination of the test substrate were able to colonized by the *V. volvacea*mycelia. However, the luxuriant mycelia with zonation pattern was noted in treatment (3) 70% AL + 30% SD and (8) 35% AL + 35% CL + 30% SD. On the other hand, treatment (2) 70% CL + 30% SD, (8) 35% AL + 35% CL + 30% SD, (10) 35% AL + 35% RS + 30% SD and (11) 23.3% BL + 23.3% AL + 23.3% CL + 30% SD revealed the shortest number of days of mycelial ramification. In terms of primordial development, the shortest number of primordial occurrence were recorded in treatment (5), 35% BL + 35% CL + 30% SD, (6) 35% BL + 35% AL + 30% SD and (7) 35% BL + 35% RS + 30% SD with 6.33 days of incubation while the longest number of days for primordial development was noted in treatment (13) 23.3% AL + 23.3% CL + 23.3% RS + 30% SD with 22 days of incubation period.

Combination of 35% acacia leaves + 35% cacao leaves + 30% sawdust was the ideal spawning material of *V. volvacea*since it elucidated shorter period of mycelial colonization and luxuriant mycelia with zonation pattern. The abundance and availability of the materials in the locality makes it an ideal spawning materials for the local farmer. This will also promote an environmental economical friendly approach in harnessing agro industrial waste in the community.

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