MYCELIAL GROWTH AND FRUCTIFICATION OF AURICULARIA POLYTRICHA ON DIFFERENT SUBSTRATES

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Abstract: *Auricularia polytricha* is a wood-rotting mushroom known for being one of the edible mushrooms, and recognized for its nutraceutical and pharmaceutical properties. To domesticate this species, the most favorable conditions for its mycelial growth and yield was evaluated in various culture media, grain spawn and fruiting substrates. The results revealed that *A. polytricha* cultured in coconut water gelatin (CWG) had the fastest mycelial ramification (6.33 days), and the thickest and highest mycelial growth at 13.17 mm/day. For spawn grain production, sweet sorghum produced the fastest mycelial run among grains at 5.0 days, had the highest mycelial growth at 16 mm/day, and had the thickest mycelia. For fruiting bodies production, the combination of good lumber sawdust, rice bran and lime (GLS:RB:L) had the fastest mycelial run at 30.33 days, highest mycelial growth (10.0 mm/day), yield (254.0 g) and biological efficiency (30.79%).

Keywords: Auricularia polytricha, culture media, grain spawn, fruiting substrates, mycelial ramification

1. INTRODUCTION

Auricularia polytricha also known as earwood fungus is one of the edible mushrooms in the world and used as a major additive for several Chinese dishes. It is domesticated for cultivation in tropical and temperate regions because of its mycelium which can grow at temperatures ranging from 10°C to 40°C (Irawati *et al.*, 2012). Moreover, its worldwide cultivation is also due to its nutraceutical and pharmaceutical properties (Kirk *et al.*, 2001). The protein, vitamin, and carbohydrate content of wood ears are reported to be higher than that of many vegetables and fruits, and the caloric content is relatively low (Cheng & Tu, 1978), so they make a nutritious ingredient of soups and other dishes (Schenck & Dudley,1999). *A. polytricha* contains around 8%–10% protein, 0.8%–1.2% fat, 84%–87% carbohydrate, 9%–14% fibre and 4%–7% ash (Ying, 1987). Furthermore, it has long been used in traditional Chinese medicine for increasing the fluidity of blood and improving blood circulation. It is also reported that *Auricularia spp*. exhibits antioxidant and hypoglycaemic activities and lowers cholesterol levels (Chang, 1999; Kho *et al.*, 2009).

Commercial production of fresh edible mushrooms is a fast growing industrial activity that can be carried out in a large or small scale. Its efficient and relatively short biological process of food protein recovery from negative value lignocellulosic materials, utilizing the degrading capabilities of mushrooms (Martínez-Carrera *et al.*, 2000), can convert the huge lignocellulosic waste materials into a wide diversity of products (edible or medicinal food, feed and fertilizers), protecting and conserving the environment (Chang, 1999). Likewise, producing mushrooms using agricultural wastes and residues such as rice straw, coco peat, rice bran, coconut husk and banana leaf litters can be considered as economically suitable solution to the excessive presence of agricultural waste materials since it is highly regarded as one of most efficient biological ways to recycle and reuse these wastes and by-products (Madan *et al.*, 1987). Unlike other

mushroom species, *A. polytricha* is easier to cultivate and yield fruiting bodies faster without requiring expensive facilities. In addition, there is a deficiency of protein-rich sources of food in developing countries. Mushroom cultivation simultaneously addresses two important issues the world is faced today: reducing agricultural waste and sustaining sources of human nutrition (Razak *et al.*, 2012).

In the Philippines, *A. polytricha* is called "tengang daga" and are found in the wild, wood rotters and not well known to many of the local people. Although listed as one of the top culinary mushrooms, the production of earwood mushroom by our local growers is still insufficient. Most of the dried *A. polytricha* mushroom in the Philippine market is imported from China, and fresh form of this mushroom can hardly be found (Razak *et al.*, 2012) which indicates that there is no steady supply of *Auricularia* in the Philippine market.

It is usually cultured in potato dextrose agar (PDA) and spawn in sorghum. Moreover, they are commonly cultivated in an artificial log. However, problems on the availability of the culture media, grain spawn, and artificial log arises since PDA is expensive, while sorghum and good lumber sawdust are not readily available. The nationwide log ban imposed in the country makes it hard to acquire good lumber sawdust. Furthermore, competition from other industries such as wood based particle boards and charcoal briquettes production arises since these industries offer higher price for sawdust supplies from its supplier causing the price of sawdust to increase. Also, sawdust supplies are often mixed up with chemicals used in the processing industry thus, the tainted supply of sawdust negatively affects mushroom growth and yield (Razak, 2012). Hence, alternative substrates to replace sawdust are needed by our local mushroom industry. This study therefore aimed to use different locally available growing media, grain spawn and fruiting substrate and determine the most suitable substrate for faster mycelial ramification and attain optimum biological efficiency that would serve as basis for earwood mushroom production.

2. METHODOLOGY

2.1 Source of strain

Auricularia polytricha was gathered from the logs and tree trunks in the wilderness of Lopez, Quezon, Philippines. It was tissue cultured and grown in Potato Sucrose Gelatin (PSG). The collected mushroom was identified based on the description made by Musngi *et al.* (2005) on the different species of *Auricularia*. The specimen gathered has a strongly convex dorsal surface with a dense pileus and rounded margin.

2.2 Experimental design

The study was divided into three phases wherein the treatments in each phase were laid out in Completely Randomized Design (CRD).

2.2.1 Phase I: Evaluation of mycelial growth on different culture media

The following treatments were replicated three (3) times and laid out in CRD: T1 (Control) – Potato Sucrose Gelatin (PSG), T2 – Sweet Potato Sucrose Gelatin (SPSG), T3 – Rice Bran Sucrose Gelatin (RBSG), T4 – Corn Grit Sucrose Gelatin (CGSG), T5 – Coconut Water Gelatin (CWG) and T6 – Cassava Sucrose Gelatin (CSG).

PSG served as the control since it is widely used as substitute for the expensive PDA. PSG, SPSG and CSG used 250 g of potato, sweet potato cubes, cassava cubes and 50 g for corn grit and rice bran (D1) as the standard amounts used (Reyes *et al.*, 2005). Washed potato, corn grits, sweet potato, cassava and rice bran were boiled separately in 1L of tap water for about 15 to 20 minutes and strained. The decoctions were added with water to make it one liter. The decoctions were boiled again. On the other hand, 1L of coconut water (from matured coconut) was strained and boiled. Then, 10 g of sugar (except for coconut water) and 20 g of agar were added to all of the decoctions. The prepared media were sterilized at 15 psi for 15 minutes. Then they were dispensed in sterile petri plates and allowed to cool.

The petri plates with culture media were aseptically and individually inoculated with tissue from the *A. polytricha* fruit. The media were incubated under dark condition at room temperature until the mycelia fully branched out and occupied the whole petri plate.

Mycelial growth in each petri dish was determined by measuring the average diameter of the mycelia colony every day for 2 weeks. The average reading was plotted against time (day) to obtain the mycelial growth in mm/day (Razak *et al.*, 2012). The number of days it took for total mycelial ramification was also recorded. Analysis of Variance (ANOVA) was performed to determine if there are any significant differences on the number of days and mycelial growth on the various culture media used. Mycelial thickness was also observed and photographed.

2.2.2 Phase II: Grain spawn production

The culture media with the best fungal growth and very fast ramification were used for the evaluation of mycelial growth on different substrates. The following treatments were prepared and replicated three (3) times: T1 (Control) – sorghum seeds, T2 – corn grits and T3 – palay seeds. Sweet sorghum served as the control since it is the commonly used spawn for mushroom production (Stamets, 2000). Faster mycelial ramification is also observed in sorghum than in any other grains (Sahu *et al.*, 2014). The grains were boiled until slightly swelled and drained. Moisture was determined by using a moisture meter and adjusted to 65% moisture content. One hundred (100) grams of each grain was dispensed in clean catsup bottle and secured with cotton plug and used paper. They were sterilized for 45 minutes at 15 psi.

A 7-day old 10 mm mycelial block of *A. polytricha* culture from the best isolation media was inoculated on the grains. It was stored at room temperature to allow mycelial ramification. Mycelial ramification was determined by measuring mycelia extension at 4 sides of the bottles at 2-day intervals for 14 days. The average reading was plotted against time (day) to obtain the average growth in mm/day

(Razak *et al.*, 2012). The number of days it took for total mycelia ramification was also recorded.

2.2.3 Phase III: Mycelial ramification of A. polytricha in different fruiting substrates

The grains which have better mycelial run was used in inoculating the fruiting substrates. Six substrates served as treatments and replicated three times which were laid out in CRD: T1 (Control) - 79% good lumber sawdust: 20% rice bran: 1% lime; T2 - 79% coco peat: 20% rice bran: 1% lime; T3 - 79% coco lumber sawdust: 20% rice bran: 1% lime; T4 - 79% coco husk: 20% rice bran: 1% lime; T5 - 79% dried banana leaves: 20% rice bran: 1% lime; and T6 - 79% rice straw: 20% rice bran: 1% lime.

100% good lumber sawdust served as the control treatment since it is the commonly used substrate by wood ear mushroom growers in the Philippines. Rice straw, banana leaves, cocohusk, cocopeat and coco lumber sawdust were used as treatments and substitute for good lumber sawdust since they are also ligno-cellulosic and readily available in the locality. Good lumber sawdust was not readily available and supply is not stable because of the total log ban imposed in most parts of the country. The cellulose and lignin contents are important components of any substrate since the lignocellulytic enzymes of oyster mushrooms convert it into carbohydrates which serve as the energy source (Custodio, 2004). Cellulose rich substrates give better yields and help in more enzyme production, which is correlated with higher yield (Arisha, 2010).

Rice straw, dried banana leaves and cocohusk were soaked overnight in a separate drum; it was rinsed with clean water afterwards. They were manually chopped into approximately 1 inch length. The substrates were mixed in weight basis. Seven hundred fifty (750) grams of the substrates were put in each fruiting bag. Rice bran supplements the organic nitrogen which helps in getting higher yields (Arisha, 2010). Lime is used as a pH buffer in substrate that holds the pH steady as the mushrooms grow to ensure the substrate does not go too acidic during the growth cycle (Kwon & Kim, 2004). Water was sprinkled to attain 65% moisture content. Moisture meter was used to determine the moisture level of the substrates. The premix substrates were pasteurized for eight hours at 60°C - 80°C and allowed to cool.

Mycelial growth was determined by measuring mycelia extension at four (4) sides of the bag at 2-day intervals for 30 days. The average reading was plotted against time (day) to obtain the average growth in mm/day (Razak *et al.*, 2012). The number of days it took for total mycelia ramification was also recorded.

2.3 Evaluation of fruiting performance of A. polytricha

Biological efficiency (BE) of *A. polytricha* was evaluated. The fruiting bags were cut into two and both sides were allowed to fruit. To determine the BE, the formula below was used:

Biological Efficiency (%) = $\frac{\text{weight (g) of mushrooms produced}}{\text{weight (g) of substrates used}} \times 100$

2.4 Statistical analysis

All the data obtained was subjected to one-way analysis of variance (ANOVA) and the mean differences were determined by Scheffe multiple comparison post hoc test. Differences at p<0.05 were considered statistically significant.

3. RESULTS AND DISCUSSION

3.1 Mycelial ramification of A. polytricha on different culture media

Mycelia are the vegetative part of a fungus consisting of a mass of branching, thread-like hyphae. The luxuriant growth of mycelia depends on the nutritional content of the medium where they grow. Mycelial growth is important in mushroom production because mycelia stock culture served as source of mushroom cell lines (De leon *et al.*, 2013).

At temperatures 26°C to 30°C, and relative humidity (RH) of 87% to 95%, the number of days it took for mycelial ramification was recorded and its growth was observed (Table 1). *A. polytricha* in CWG had full ramification on day 6.33 which was significantly different from the rest of the treatments (Figure 1). It was followed by SPSG and CGSG which were both ramified on day 9.67 and 10.33, respectively. The result was not significantly different with one another. On the other hand, CSG and PSG were ramified in 11.67 and 12.67 days. The number of days for mycelial ramification was not significantly different with one another and was not significantly different to CGSG. Conversely, *A. polytricha* inoculated in RBSG had the slowest mycelial run, having ramified on day 22.67, which was significantly different from the rest of the treatments.

It is evidently shown that mycelial growth was fastest in CWG at 13.17 mm/day which was significantly different from the rest of the treatments. It was followed by SPSG and CGSG with an average mycelial growth of 9.17 and 8.39 mm/day, which were not significantly different from each other. On the other hand, growth in CGSG was not significantly different with the mycelial growth in CSG and PSG at 7.56 and 7.00 mm/day, respectively. Again, mycelial growth was slowest in RBSG at 3 mm/day. This was significantly different from the rest of the treatments.

Treatment	Number of days of mycelial ramification	Mycelial growth (mm/day)	Mycelial thickness
T1 – (Control) Potato Sucrose Gelatin (PSG)	12.67 ^b	7.00 ^c	+
T2 - Sweet Potato Sucrose Gelatin (SPSG)	9.67°	9.17 ^b	++
T3 - Rice Bran Gelatin (RBSG)	22.67 ^a	3.00 ^d	+++
T4 - Corn Grit Sucrose Gelatin (CGSG)	10.33 ^{bc}	8.39 ^{bc}	+
T5 - Coconut Water Gelatin (CWG)	6.33 ^d	3.17 ^a	+++
T6 - Cassava Sucrose Gelatin (CSG)	11.67 ^b	7.56 ^c	++
Coefficient of Variation	4.70	5.84	

 Table 1. Number of days of mycelial ramification, mycelial growth (mm/day), and thickness of A. *polytricha* inoculated in different culture media.

*In a column with the same letters indicate that the values are not significantly different by Scheffe multiple comparison post hoc test (p>0.05).

*The lowest degree of mycelia thickness is marked as +, intermediate degree as ++, and the highest degree as +++ (Razak, 2012)

In terms of mycelial thickness, CWG had the thickest followed by SPSG, RBSG and CSG. PSG and CGSG had thin mycelial growth. Coconut water contains minerals, essential nutrients and growth hormone such as cytokinin for the induction of morphogenesis. i.e. induce fungal cells to divide and grow rapidly (Magday et al., 2014). Thus, its nutritional content might be the main factor why it has the ability to stimulate rapid mycelial growth. and thicker and denser mycelial mat, as shown in Figures 2 and 3. In the locality and in other parts of the Philippines, coconut is always abundant and mature coconut water is just a waste product compared to young coconut water. Many researches use coconut water as an effective medium that supports mycelial growth of different mushroom species (Radenahmad et al., 2009). As stated in Santoso et al. (1999), mature coconut water contains 92% sucrose, making it suitable for the cultivation of mycelia. Moreover, the protein content of coconut water increases as it matures, from 0.13% to 0.29%; just enough to fulfill the nitrogen requirement of the growing mycelia (Rau, 1999). The results of the study are in congruence with the study of Jacob et al. (2015) who reported that CWG supported the luxuriant mycelial growth of Pleurotus citrinopileatus, Pleurotus djamor, and Pleurotus salmoneostramineus. Moreover, Magday et al. (2014) also reported that CWG of pH 6.0, and in sealed and lighted conditions at room temperature (32°C), yielded the most efficient mycelial growth. The results are also consistent with the findings of De Leon et al. (2013) who reported the suitability of mature coconut water as a culture medium for Lentinus squarrosulus and Polyporus grammocephalus.

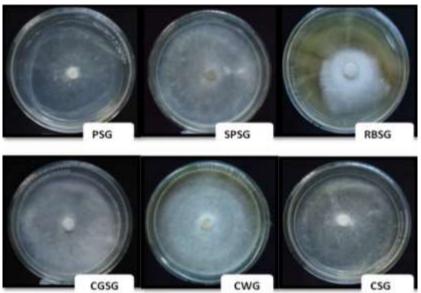


Figure 1. Mycelial growth of *A. polytricha* on different culture media 12 days after inoculation.

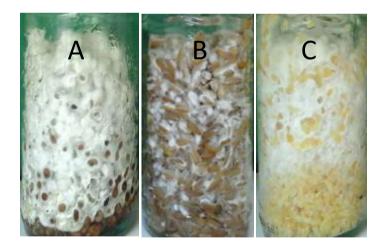


Figure 2. Mycelial growth of *A. polytricha* on the different spawning materials, namely: (A) sweet sorghum, (B) rice grain, and (C) corn grits, after 10 days inoculation.

3.2 Mycelial ramification on different grain substrates

Mushroom spawn serves as the planting material for a given substrate. According to Goltapeh & Purjam (2003), spawn quality is considered as the most important part in mushroom production. Bahl (1988) mentioned that grain spawn is in common use because of its ability to ramify the substrate faster and, when with an additive such as rice bran, to stimulate fruiting. Mushroom mycelia block inoculated in rice bran sucrose gelatin incubated at dark condition served as inoculants in the various grain spawn. Furthermore, Chang *et al.*, (1997) noted that mushroom spawning is a process of cellular expansion in order to produce more mycelia for mass production. In this study, different spawning materials such as sweet sorghum seeds, unmilled rice grains and corn grits were evaluated. Mycelial ramification and growth on the grain substrates, at temperatures of 30°C to 33°C and RH of 87°C to 90%, were observed and recorded.

Results revealed that among the grain spawns used in the study, sorghum grains were fully ramified with mycelia five days after incubation (Figure 2), whereas corn grits and rice seeds were ramified on the 7th and 8th day after incubation. The results clearly proved that the best spawn grain to use is sorghum. The faster ramification of mycelia in sorghum could be attributed to the fact that it contains more carbohydrates and/or starch which is around 93% as compared to corn grits and palay seeds at just 82% and 80%, respectively (www.grainza.com).

In addition, faster mycelial growth was also observed in sorghum since it had an average mycelial growth of 17.67 mm/day compared to corn grits and palay seeds with mycelial growth rate of 12.61 mm and 10.76 mm, respectively (Table 2). Analysis of variance (ANOVA) showed that there are significant differences among the treatments, proving that sorghum seeds are still the best spawn for faster mycelia growth of *A. polytricha*.

Furthermore, it was also observed that thick and dense mycelia grew on sorghum seeds (Figure 2); whereas, corn grits and rice grains exhibited an intermediate and lowest degree of mycelial thickness. It was also noted that at the latter part of incubation, there was rapid loss of moisture in rice seeds as compared to sorghum and corn grits.

The high carbohydrate, fats and protein component of sorghum can stimulate faster mycelial growth. Furthermore, larger surface area and pores of substrates support faster mycelium growth (Tinoco *et al.*, 2001). This could account for the significant differences among the mycelial growth recorded for the rice grains and corn grits. Sorghum grains have a larger surface area compared to the others. Since smaller particles are generally more compact than larger particles, sorghum would have larger air spaces than rice grains and corn grits, and thus would mean increased ventilation within the sorghum, resulting in improved respiration by the mycelia, and hence, significantly inducing higher growth rate (Nahar *et al.*, 2011).

The result of the study is in congruence with the study of Onyango *et al.* (2011) where rapid mycelial growth was observed in sorghum grains which may be attributed to a greater food reservoir. Also, Rathaiah & Surargiary (1994), Sahu *et al.* (2013), Mathew *et al.* (1996), and Chaurasia (1997) reported sorghum as the most suitable for

spawn development of *P. sajor caju, P. djamor*, and *P. columbines*. Moreover, Khatri & Agrawal (2002), Hafeez *et al.* (2000), Asghar *et al.* (2007), and Saayir & Yildiz (2004) reported early and superior spawn development in sorghum compared to other grains.

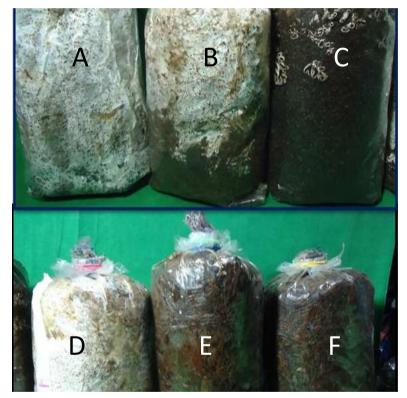


Figure 3. Mycelial run of *A. polytricha* (day 31) on different fruiting substrates namely (A) good lumber sawdust + rice bran + lime; (B) coconut peat + rice bran + lime; (C) coconut lumber sawdust + rice bran + lime (D) coconut husk + rice bran + lime (E) banana leaves + rice bran + lime; and (F) rice straw + rice bran + lime.

mooulated in various grain spawn.					
Treatment	Number of days of mycelial ramification	Mycelial growth per day (mm/day)	Mycelial thickness		
Sorghum	5.00 °	16.00 ^a	+++		
Corn Grits	7.00 ^b	10.93 ^b	++		
Rice Seeds	8.00 ^a	8.93 °	+		
coefficient of variation	3.70	0.00			

Table 2. Mycelial growth (mm/day) and thickness of *A. polytricha* inoculated in various grain spawn.

*In a column with the same letters indicate that the values are not significantly different by Scheffe multiple comparison post hoc test (p>0.05).

* The lowest degree of mycelia thickness is marked as +, intermediate degree as ++, and the highest degree as +++ (Razak, 2012)

3.3 Mycelial run, mycelial growth, fresh yield and biological efficiency on different fruiting substrates

Fruiting of *A. polytricha* under cultivated conditions can occur in 25-40 days after grain spawn inoculation into pasteurized bulk substrates (Stamets, 2000).

Combination of GLS:RB:L had the fastest mycelial run which was fully ramified in 30.33 days after incubation (Table 3), though this was not significantly different with the mycelial run on CP:RB:L and CH:RB:L. Mixture of CLS:RB:L, on the other hand, had the slowest mycelial run, which completed ramification in 38.33 days after inoculation. This significantly differed from the rest of the treatments. In contrast, mycelial run was not observed in BLL:RB:L and RS:RB:L combinations.

Furthermore, highest mycelial growth of 10.00 mm/day was observed in GLS:RB:L, which was not significantly different to CH:RB:L and CP:RB:L that had an average mycelial growth of 9.26 and 9.24 mm/day, respectively. The average mycelial growth of 7.86 mm/day for CLS:RB:L was significantly different from the rest of the treatments. Conversely, since no mycelial run was observed in BLL:RB:L and RS:RB:L combinations, no growth was recorded.

In terms of total yield and biological efficiency (BE), GLS:RB:L had the highest fresh yield (254 g) and BE (30.79%), which are significantly different from the rest of the treatments. It was followed by CH:RB:L with a yield of 54.33 g and BE of 6.59%. This was not significantly different from the yield of 51.00 g and BE of 6.18% obtained from CP:RB:L. CLS:RB:L had a yield of 29.67 g and BE of 3.60% which was significantly different from other treatments. However, no yield was obtained from BLL:RB:L and RS:RB:L combination.

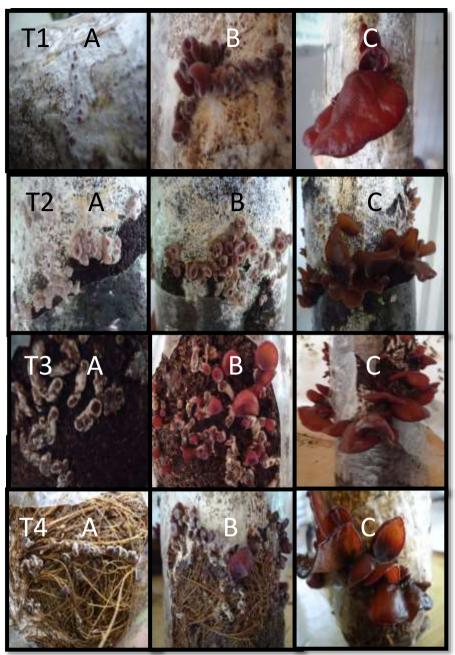


Figure 4. A) Primordia, B) cup-shaped bodies and C) fruit bodies of A. *polytricha* on different substrates. (T1) - good lumber sawdust + rice bran + lime; (T2) coconut peat + rice bran + lime; (T3) coconut lumber sawdust + rice bran + lime; and (T4) coconut husk + rice bran + lime.

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Fruiting Substrates	Time for complete mycelial run (day)	Mycelial Growth (mm/day)	Fresh Yield (g)	Biological efficiency (%)
79% good lumber sawdust (GLS) + 20% rice bran (RB) + 1% lime (L)	30.33 ^b	10.00 ^a	254.00 ^a	30.79 ª
79% coconut peat (CP) + 20% rice bran (RB) + 1% lime (L)	32.33 ^b	9.24 ^a	51.00 ^b	6.18 ^b
79% coconut lumber sawdust (CLS) + 20% rice bran (RB) + 1% lime (L)	38.33 ª	7.86 ^b	29.67 °	3.60 °
79% coconut husk (CH) + 20% rice bran (RB) + 1% lime (L)	32.67 ^b	9.26 ^a	54.33 ^b	6.59 ^b
79% banana leaf litters (BLL) + 20% rice bran (RB) + 1% lime (L)	0.00 °	0.00 ^c	0.00 ^d	0.00 ^d
79% rice straw (RS) + 20% rice bran (RB) + 1% lime (L)	0.00 ^c	0.00 °	0.00 ^d	0.00 ^d
Coefficient of Variation	5.22	5.60	9.43	9.42

 Table 3. Time for complete mycelial run, mycelial growth, fresh yield, and biological efficiency of substrates (%) for different fruiting substrates.

*In a column with the same letters indicate that the values are not significantly different by Scheffe multiple comparison post hoc test (p>0.05).

Faster mycelial ramification, higher mycelial growth, fresh yield and BE in good lumber sawdust could be attributed to its high cellulose, lignin and carbon components which is around 35% to 45% cellulose and 20% to 25% lignin (Sjostrom, 1993). It also contains high amount (42.38%) of carbon. It could be noted that *Auricularia* mushrooms grow best in a substrate with a carbon:nitrogen (C:N) ratio of 35:1 and might exhibit slower mycelia growth if this ratio becomes higher or lower (Chang *et al.*, 1997). Thus, the higher yield, faster mycelial run and higher mycelial growth could be attributed not only to its lignin and cellulose components but also to its carbon content, which is relatively much higher than the carbon component of other fruiting substrates.

However, the result is comparatively lower to the study of Petcharat (1996), in which spawn running takes 55.8 days in 1 kg of substrate. Also, it is in contrast with the study of Ahila devi *et al.*, (2013), where *A. polytricha* grown in paddy straw in combination with wheat and rice bran in a 3:1 ratio had the highest yield as compared to paddy straw combined with sawdust in a 3:1 and 1:1 ratio.

Likewise, Ediriweere *et al.* (2015) showed that *A. polytricha* grown in media with coconut leaves exhibited no fructification, though it had the highest mycelial growth per day. Also, banana leaves-containing substrate gave the highest yield while paddy straw and coir dust containing media had intermediate yields. Also, fructification of *A. polytricha* occurred within 40-45 days of inoculation. Meanwhile, *A. polytricha* did not grow in sawdust substrate.

However, the result is in congruence with the study of Veeralakshmi *et al.* (2014), in which mycelial run takes 21.3 - 46.1 days. Moreover, the result is in consonance with the study of Kushwaha *et al.* (2006) showing that paddy straw in combination with rice bran had the lowest yield compared to other fruiting substrates such as wheat straw, rape seed and maize stalk.

4. CONCLUSIONS

A. polytricha grown in coconut water gelatin as culture media exhibit the fastest mycelial run, and the highest and thickest mycelial growth compared to those grown in potato sucrose gelatin (control). Among grain substrates, sweet sorghum gives the fastest mycelial ramification and had the highest density of mycelial growth. For fruiting bodies production, the fastest mycelial ramification, highest mycelial growth, yield and biological efficiency were obtained in mushrooms grown in 79% good lumber sawdust, 20% rice bran and 1% lime combination.

5. **RECOMMENDATIONS**

Further studies on the use of other culture and spawn media, and fruiting substrates are highly recommended for more understanding of the growth of *A. polytricha*. Furthermore, longevity of coconut water gelatin as a culture medium and proximate analysis of its components should be determined. Analysis on the components of the fruiting substrates should also be determined and proximate composition of mushroom grown in different substrates should be established.

6. REFERENCES

Ahila Devi P., Veeralakshmi, S. Prakasam, V. & Vinothini, M. (2013). Saw dust and wheat bran substrates for the cultivation of new wood ear mushroom (*Auricularia polytricha* (Mont.) Sacc. *American-Eurasian J. Agric. & Environ. Sci.*, 13 (12): 1647-1649.

- Arisha, M. (2010). Optimum medium for oyster mushroom production. (Unpublished Thesis for M.S. Agriculture). Zagazig University. Retrieved from http://www.academia.edu.
- Asghar, R., Tariq, M. & Rehman, T. (2007). Propagation of *Pleurotus sajor-caju* (oyster mushroom) through tissue culture. *Pak. J. Bot.*, 39(4): 1383-1386.
- Bahl, N. (1988). Handbook on mushrooms (2nd Ed.). Oxford and IBH Publishing Co. Ltd., New Delhi, Bombay, pp. 52.
- Chang S.T. (1999). Global impact of edible and medicinal mushrooms on human welfare in the 21st century: nongreen revolution. *Int J Med Mushroom*, 1:1–7.
- Chang, S. T., Buswell, J. A. & Miles, P. G. (Eds.) (1997). Genetics and breedings of edible mushrooms. Gordon and Breach Science Publisher. Philadelphia. USA pp. 324.
- Chaurasia, V. K. (1997). Studies on production technology of *Pleurotus columbines* at Raipur. (Unpublished Master of Science Thesis) Submitted to I.G. K. V., Raipur:95
- Custodio, Christopher D. (2004). Coco lumber sawdust. Mushroom Growers Handbook. Retrieved from http://Alohamedicinal.com
- De Leon, A. M., Reyes, R.G. & Dela Cruz, T. (2013). Enriched cultivation of three wild strains of *Lentinus tigrinus* (Bull.) Fr. Using agricultural wastes. *Journal of Agricultural Technology*, 9(5), 1199-1214.
- Ediriweere S.S., Wiijesundera, R. L. C., Nanayakkara, C. M. & Weerasena, O. V. D. S. J. (2015). Comparative study of growth and yield of edible mushrooms, *Schizophyllum commune Fr., Auricularia polytricha (Mont.) Sacc.* and *Lentinus squarrosulus* on lignocellulosic substrates. *Mycosphere* 6(6), 760-765 (2015).
- Hafeez, F. Y., Shah, N. H. & Malik, K. A. (2000). Field evaluation of lentil cultivars inoculated with *Rhizobium leguminosa rumbv. viciae* strains for nitrogen fixation using nitrogen-15 isotope dilution. *BiolFertil Soils*, 31,65-69.
- Irawati, D. Hayashi, C. Takashima, Y. Wedatama, S. Ishiguri, F. Iizuka, K. Yoshizawa, N. & Yokota, S. (2012). Cultivation of the edible mushroom *Auricularia polytricha* using sawdust based substrate made of three Indonesian commercial plantation species, Falcataria moluccana, Shorea sp., and Tectona grandis. *Micología Aplicada International, Puebla, México.* 24, (2), 33-41.
- Jacob J.K.S., Kalaw, S.P., & Reyes, R.G. (2015). Mycelial growth performance of three species of *Pleurotus* on coconut water gelatin. *Current Research in Environmental* & *Applied Mycology* 5(3), 263–268. Doi 10.5943/cream/5/3/9
- Khatri, R. K. & Agrawal, K. C. (2002). Studies on mushroom flora of Madhya Pradesh and Chhattisgarh with special reference to wild edible bamboo mushroom (*Cantharellus* Spp.) Indira Gandhi KrishiVishwavidyalaya; Raipur.
- Kirk. P. M., Cannon, P.F., David, J. C. & Salpers, J. A. (2001). Dictionary of the fungi, 9th edition. CAB International, Wallingford, UK.

- Kushwaha, K. P. S., Bhatt, P. & Singh, R. P. (2006). Evaluation of different substrate for yield performance of *Auricularia polytricha* a medicinal mushroom. *Internat. J. Agric. Sci.* 2(2), 389-391.
- Kwon, H. & KIM, B.S. (2004). Bag cultivation. Mushroom Growers Handbook I. Retrieved from <u>http://alohamedicinals.com</u>
- Madan M, Vasudevan P. & Sharma, S. (1987), Cultivation of *Pleurotus sajor-caju* on different wastes. *Biol Wastes* 22, 241–250.
- Magday, J.R., Bungihan, M.E. & Dulay, R.M.R. (2014). Optimization of mycelial growth and cultivation of fruiting body of Philippine wild strain of *Ganoderma lucidum*. *Current Research in Environmental & Applied Mycology* 4 (2), 162–172
- Martínez-Carrera D, Aguilar A, Martínez W, Bonilla M, Morales P, & Sobal M. (2000). Commercial production and marketing of edible mushrooms cultivated on coffee pulp in Mexico. In Coffee Biotechnology and Quality (ed. T Sera, C Soccol, APandey,S Roussos). Kluwer Academic Publishers, Dordrecht, Neterhlands. pp. 471–488.
- Mathew, A.V., Mathai, G. & Suharban, M. (1996). Performance evaluation of five species of *Pleurotus* (Oyster mushroom) in Kerala. *Mushroom Research*, 5 (1), 9-12.
- Mohammadi Goltapeh, E. & Purjam, E. (2003). Principles of mushroom cultivation. Tarbiat Modarres University Press, UK., pp: 604.
- Musngi, R. B., Abella, E. A., Lalap, A. L. & R. G. Reyes. (2005). Four species of wild *Auricularia* in Central Luzon, Philippines as sources of cell lines for researchers and mushroom growers. *Journal of Agricultural Technology*, 1(2), 279-299.
- Nahar, D. L., Obodai, M., Baka, D. & Dzomeku. (2011). The efficacy of sorghum and millet grains in spawn production and carpophore formation of *Pleurotus ostreatus* (Jacq. Ex. Fr) Kummer. *International Food Research Journal*, 18(3), 1143-1148 M.
- Onyango, B.O., Palapala V.A., Arama P.F., Wagai S.O. & Gichimu B.M. (2011). Morphological characterization of Kenyan native wood ear mushroom [*Auricularia auricula* (L. ex Hook.) Underw.] and the effect of supplemented millet and sorghum grains in spawn production. *Agric. Biol. J. N. Am.*, 2(3), 407-414.
- Petcharat, V. (1996). Cultivation of Wild Mushroom: VI Hed Hu Hnu (*Auricularia spp.*) Thai National Agris Centre. Food and Agriculture Organization of the United Nations.
- Radenahmad, N., Saleh, F., Swangjaroen, K., Rundorn, W., Withyachumnarnkul B., & Connor, J.R. (2009). Young coconut juice significantly reduce histopathological changes in the brain that are induced by hormonal imbalance: a possible implication to postmenopausal women. *Histology and Histopathology*. 24(6), 667 – 74.

- Rau, U. (1999). Production of Schizophyllan. In: Methods in Biotechnology, Carbohydrate Botechnology Protocols, C. Bucke ed. HumanaPress, Inc. Totowa, New Jersey, USA., 10, 43-55.
- Rathaiah, Y and Surargiary, M. (1994). Use of parboiling paddy as spawn substrate for oyster mushroom. *Mushroom Res.* 1 (1), 1-3
- Razak, D.L.A.B.D, Abdullah, N., Mohd N., Johari, K. & Sabaratnam, V. (2012). Comparative study of mycelia growth and sporophore yield of *Auricularia polytricha* (Mont.) Sacc on selected palm oil wastes as fruiting substrate. *Appl Microbiol Biotechnol* DOI 10.1007/s00253-012-4135-8.
- Saayir, A. & Yildiz, A. (2004). Growth of mycelium of *Pleurotus spp*. on different grains and determination of their competition with some contaminant fungi. *Acta Alimentaria*. 33 (3), 249-257.
- Sahu, S., Singh, K.D.P., Patel, R., & Indira, K.D.P., Vishwavidhyalaya, G. K. & Raipur, C.G. (2013). Screening of suitable grains substrates for Spawn development, growth and yield of *Pleurotus eous* INDIA. *American International Journal of Research in Formal, Applied & Natural Sciences*. 5(1), 86-89.
- Santoso, U., Kubob, K., Otac, T., Tadahiro, T., & Akio, M. (1999). Nutrient composition of kopyor coconuts (*Cocos nucifera L*.). Elsevier Journal Article.
- Sjostrom, E. (1993), Wood Chemistry. Academic Press, San Diego.
- Stamets, P. (2000). Growing gourmet and medicinal mushrooms; 3rd Ed. Ten speed press. Berkeley-Toronto, 153-167.
- Tinoco, R., Pickard, M. A. & Vasquez-Duhalt, R. (2001). Kinetic differences of purified lacasses from six *Pleurotus ostreatus* strains. *Lett. Appl. Microbiology*. 32(5), 331-335.
- Veeralakshmi, S., Ahila Devi, P., Praksam, V. & Thiribhuvanamaia, V. (2014). Molecular characterization and standardization of cultivation for wood ear mushroom (Auricularia polytricha (Mont.) Sacc. International Journal of Biotechnology Research, 2(5), 60-64.