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Mycelial Growth and Fructification of Earwood Mushroom (*Auricularia polytricha*) on Different Substrates

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Abstract

Auricularia polytricha is wood-rotting mushroom known as one of the edible mushrooms in the world 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), growth rate (13.17 mm/day) and had the thickest mycelial growth. For spawn grain production, mycelial run on sweet sorghum grains was fastest at 5.0 days, had the highest growth rate at 16 mm/day and had the thickest mycelia. For fruiting bodies production, combination of good lumber sawdust, rice bran and lime (GLS:RB:L) had the fastest mycelial run at 30.33 days, highest growth rate (10.0 mm/day), yield (254.0 g) and biological efficiency (30.79%).

Keywords: *Auricularia polytricha*, culture media, grain spawn, fruiting substrates, domestication

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1. Introduction

Auricularia polytricha also known as earwood fungus is one of the edible mushrooms in the world and use as major additives 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 [1]. Moreover, its worldwide cultivation is also due to its nutraceutical and pharmaceutical properties [2]. 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 so they make a nutritious ingredient of soups and other dishes [3]. *A. polytricha* contains around 8–10 % protein, 0.8–1.2 % fat, 84–87 % carbohydrate, 9–14 % fibre and 4–7 % ash [4]. Furthermore, it has long being 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 [5].

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 [6] 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 [5]. 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 too much presence of agricultural waste materials since it is highly regarded as one of most efficient biological ways to both recycle and reuse these wastes and by-products [7]. Unlike other mushroom species, cultivation of *A. polytricha* is easier and yield fruiting bodies faster without requiring expensive facilities. In addition, there is a deficiency problem of protein-rich source of food in developing countries and mushroom cultivation offers solution to both problems [8].

In the Philippines, it is locally called tengang daga and are found in the wild, wood rotters and not well known for many of the local people. Although listed as one of the top culinary mushrooms, the production of Wood ear 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 [11] 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. Hence, alternative substrates to replace sawdust are needed by our local mushroom industry.

2. Objectives of the Study

This study 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.

3. Materials and Methods

3.1. Source of strain

A. polytricha was gathered from the logs and tree trunks in the wilderness of Lopez, Quezon. It was tissue cultured and grown in Potato Sucrose Gelatin. The species were identified based on the description made by [9] on the different species of *Auricularia*. The specimen gathered has a strongly convex dorsal surface with a dense pileus and rounded margin.

3.2. Experimental design

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

Phase I: Evaluation of mycelial growth on different culture media

The following treatments was replicated 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 [10].(Reyes, et al., 2009). Washed potato, corn grits, sweet potato, cassava and rice bran were boiled separately in one liter 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, one l of coconut water (from matured coconut) was strained and boiled. All of the decoctions were added with 10 g of sugar (except for coconut

water) and 20 g of agar. The prepared media was sterilized at 15 psi for 15 minutes. Then it was 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 [8]. Number of days of 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.

Phase II: Grain spawn production

The culture media with best fungal growth and very fast ramification was used for the evaluation of mycelial growth on different substrates. The following treatments was prepared and replicated 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 [4]. Faster mycelial ramification is also observed in sorghum than any other grains [11]. 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 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 [8]. Number of days of total mycelia ramification was also recorded.

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 [12]. Cellulose rich substrates give better yields and helps in more enzyme production, which is correlated with higher yield [13].

Rice straw, dried banana leaves and cocohusk were soaked overnight in a separate drum; subsequently it was rinsed with clean water. They were manually chopped into approximately 1 inch length. The substrates were mixed in weight basis. Seven hundred fifty grams of the substrates was put in each fruiting bag. Rice bran supplements the organic nitrogen which helps in getting higher yields [13]. 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 acid during the growth cycle [17]. 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 - 80°c and allowed to cool.

Mycelial growth was determined by measuring mycelia extension at 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 [8]. Number of days of total mycelia ramification was also recorded.

3.3. 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 ff. formula below was used:

$$\text{BE}(\%) = \frac{\text{Wt (g) of mushrooms produced}}{\text{Weight (g) of substratesused}} \times 100$$

3.4. 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.

4. Results and Discussion

4.1. 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 [15].

At temperatures 26 to 30°C, and RH of 87 to 95%, number of days of mycelial ramification was recorded and its growth was observed (Table 1). *A. polytricha* in CWG had full ramification at day 6.33 which was significantly different with the rest of the treatments. It was followed by SPSG and CGSG which were both ramified at 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 at day 11.67 and 12.67. The number of days of 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 which was ramified at day 22.67 which was significantly different with the rests 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 rests 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 one another. On the other hand, growth in CGSG was not significantly different with the mycelial growth in CSG and PSG which were 7.56 and

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

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 c	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	13.17 a	+++
T6 - Cassava Sucrose Gelatin (CSG)	11.67 b	7.56 c	++
CV (%)	4.70	5.84	

*In a column the same letters indicate that the values are insignificantly different by Scheffe multiple comparison post hoc test (P= 0.05). CV = coefficient of variation

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

7.00 mm/day respectively. Again, mycelial growth was slowest in RBSG at 3 mm/day and significantly different with the rests of the treatment.

In terms of mycelial thickness, CWG had the thickest (Fig. 3 and 4) 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) [16].Thus, its nutritional content might be the main factor why it has the ability to stimulate rapid mycelial growth, thicker and denser mycelial mat as shown in Fig. 2 and 3. In the locality and other parts of the Philippines, coconut is always abundant and mature coconut water is just a waste product as compared to young coconut water. Many researches use coconut water as an effective media that supports mycelial growth of different mushroom species [17]. As reference [18] cited, 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%, thus just enough to fulfill the nitrogen requirement of the growing mycelia [19].The result of the study is in congruence with the study of [20] who reported that CWG supported the luxuriant mycelial growth of *Pleurotus citrinopileatus*, *Pleurotus djamor* and *Pleurotus salmoneostramineus.* Moreover, reference [16] 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 were also consistent with the findings of reference [15] who reported the suitability of mature coconut water as a culture medium for *Lentinus squarrosulus* and *Polyporus grammocephalus.*

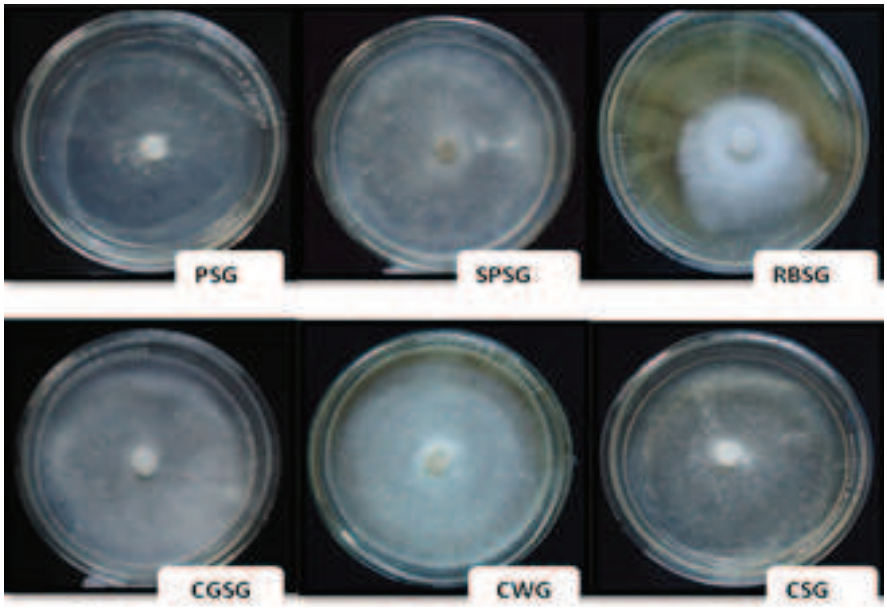

Figure 1: Mycelial growth of *A. polytricha* on different culture media 12 days after inoculation.

4.2. Mycelial Ramification on Different Grain Substrates

Mushroom spawn serves as the planting material for a given substrate. According to reference [21], spawn quality is considered as the most important part in mushroom production. As mentioned by reference [22], grain spawn is in common use because of its ability to ramify the substrate faster and added with additive such as rice bran to stimulate fruiting. Mushroom mycelia block inoculated in CWG incubated at dark condition served as inoculants in the various grain spawn. Furthermore, reference [23] 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. At temperatures 30 to 33°c, and RH of 87 to 90%, number of days of mycelial ramification on grain substrates was recorded (Table 2) and its growth was observed.

Results revealed that among the grain spawns used in the study, sorghum grains were fully ramified with mycelia five days after incubation (Fig. 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/starch which is around 93% as compared to corn grits and palay seeds which is just 82% and 80% respectively [24].

In addition, faster mycelial growth was also observed in sorghum since it had an average mycelial growth of 17.67 mm/day as compared to corn grits and palay seeds

wherein the mycelial growth rate is 12.61 mm and 10.76 mm respectively (Table 2). Analysis of variance 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.

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

Treatment	Number of days of mycelial ramification	Mycelial growth per day (mm/day)	Mycelial thickness
Sorghum	5.00 c	16.00 a	+++
Corn Grits	7.00 b	10.93 b	++
Rice Seeds	8.00 a	8.93 c	+
CV	3.70	0.00	

*In a column the same letters indicate that the values are insignificantly different by Scheffe multiple comparison post hoc test ($P \geq 0.05$). CV = coefficient of variation

**The lowest degree marked as + of mycelia thickness, intermediate degree marked as ++, and the highest degree marked as +++ of mycelia thickness (Fazal, 2013)

Furthermore, it was also observed that thick and dense mycelia grew on sorghum seeds (Fig. 1), whereas, an intermediate and lowest degree of mycelial thickness was observed in corn grits and rice grains. It was also noted that at the latter part of incubation, rapid loss of moisture was observed in rice seeds as compared to sorghum and corn grits which believed to be utilized by the mycelia.

The high carbohydrate, fats and protein component of sorghum could stimulate faster mycelial growth. Furthermore, larger surface area and pore of substrates support faster mycelium growth [25]. 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 latter. Since smaller particles are generally more compact than larger particles, sorghum would have larger air spaces than rice grains and corn grits, thus would mean increased ventilation within the sorghum resulting in improved respiration by the mycelia, hence, signifcantly higher growth rate [26].

The result of the study is in congruence with the study of reference [27] where rapid mycelial growth was observed in sorghum grains which may be attributed to a greater food reservoir. Also, reference [11,28-30], reported sorghum as the most suitable for spawn development of *P. sajorcaju, P. djamor* and *P. columbines* respectively. Moreover, reference [31-34] Saayir and Yildiz (2004) reported early spawn development in sorghum grains and found to be superior to other grains.

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

4.3. Mycelial run, mycelial growth, fresh yield and BE on different fruiting substrates

Fruiting of *A. polytricha* under cultivated conditions can occur for 25-40 days after grain spawn inoculation into pasteurized bulk substrates [4].

Combination of GLS:RB:L had the fastest mycelial run which was fully ramified at 30.33 days after incubation (Table 3) which 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, was completely ramified at 38.33 days after inoculation and significantly different from the rest of the treatments. In contrast, mycelial run was not observed in BLL:RB:L and RS:RB:L combination.

Furthermore, highest mycelial growth was observed in GLS:RB:L which was 10.00 mm/day, which was not significantly different to CH:RB:L and CP:RB:L which had an average mycelial growth of 9.26 and 9.24 mm/day respectively. It was followed by

CLS:RB:L with an average mycelial growth of 7.86 mm/day which was significantly different from the rest of the treatments. Conversely, since no mycelial run was observed in BLL:RB:L and RS:RB:L combination, no growth was recorded.

In terms of total yield and BE, GLS:RB:L had the highest fresh yield (254 g) and BE (30.79%) and significantly different from the rests of the treatments. It was followed by CH:RB:L with a yield of 54.33 g and BE of 6.59%. It was not significantly different from the yield and BE obtained from CP:RB:L which was 51.00 g and 6.18% respectively. 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.

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 – 25% lignin [35]. It also contain high amount of carbon which is 42.38%. It could be noted that *Auricularia* mushrooms grows best in a substrate with a C:N ratio of 35:1 and might slow its mycelia growth if it becomes higher or lower [23]. Thus, the higher yield, faster mycelial run and higher mycelial growth could be attributed not only for its lignin and cellulose components but also for 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 [36], in which spawn running takes 55.8 days in 1 kg of substrate. Also, it is in contrast with the study of [37], where *A. polytricha* grown in paddy straw in combination with wheat and rice bran in a 3:1 ratio had the highest yields as compared to paddy straw combined with sawdust in a 3:1 and 1:1 ratio.

Likewise, reference [38] showed that *A. polytricha* grown in media with coconut leaves exhibited no fructification though 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 reference [39], in which mycelial run takes 21.3 – 46.1 days. Moreover, the result is in consonance with the study of [40] showing that paddy straw in combination with rice bran had the lowest yield as compared to other fruiting substrates such as wheat straw, rape seed and maize stalk.

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

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 c	3.60 c
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 c	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 c	0.00 d	0.00 d
CV	5.22	5.60	9.43	9.42

*In a column the same letters indicate that the values are insignificantly different by Scheffe multiple comparison post hoc test ($P>0.05$). CV = coefficient of variation

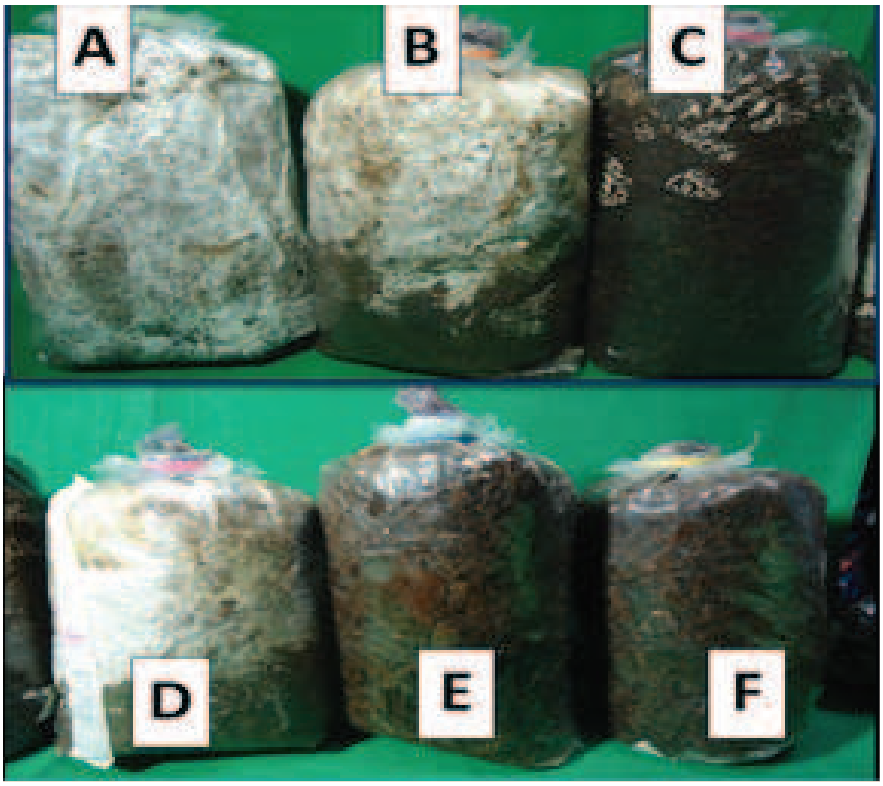

Figure 3: Mycelial run of *A. polytricha* (day 31) on different fruiting substrates namely, (A) good lumber sawdust + rice bran + lime (B) coconut peat + rice bran + lime (C) coconut lumber sawdust + rice bran + lime (D) coconut husk + rice bran + lime (E) banana leaves + rice bran + lime and (F) rice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

5. Conclusion and Recommendation

A. polytricha grown in coconut water gelatin as culture media exhibits the fastest mycelial run, highest and thickest mycelial growth than potato sucrose gelatin (control). Sweet sorghum as grain substrate gives the fastest mycelial ramification and had the highest and densest mycelial growth. 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. 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 also be determined. Analysis on the components of the fruiting substrates should also be determined and proximate composition of mushroom grown in different substrates should also be established.

Author's Note

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