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Research Article
Effect of Different Culture Media, Grain Sources and Alternate Substrates on the Mycelial Growth of *Pleurotus eryngii* and *Pleurotus ostreatus*

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Abstract

Background and Objective: Oyster mushroom *Pleurotus* is one of the most aromatic edible mushrooms. This study evaluated a few selected determinants for promoting mycelial growth and spawn production of *P. eryngii* and *P. ostreatus* such as culture media, grain sources and alternate substrates. **Materials and Methods:** Seven different substrate formulations were evaluated, viz: TS1 (100% wheat straw-S), TS2 (100% cardboard-C), TS3 (100% spent coffee ground-SCG), TS4 (50% S+50% C), TS5 (50% S+50% SCG), TS6 (80% S+20% C) and TS7 (80% S+20% SCG). The efficiency of different culture media potato dextrose agar (PDA), yeast malt agar (YMA) and malt extract agar (MEA) and selected grains (wheat, rye, barley and oat) was investigated. Each study was arranged in the complete randomized design with 4 replicates. **Results:** PDA media was the most suitable for mycelial growth of *P. eryngii* while *P. ostreatus* had a better mycelial growth on YMA and MEA media. Barley and rye grains were the most favourable for the mycelium growth of *P. eryngii* while oat grains were the best source that enhanced both of mycelial extension and density levels of *P. ostreatus*. The supplement of wheat straw (S) with SCG substrate improved mycelial extension while the substrate containing 50% S+50% C was the most favourable for both of mycelial growth and primordia formation in *P. eryngii* and *P. ostreatus*. **Conclusion:** The results revealed the feasibility of using recyclable wastes of cardboards and spent coffee ground for *Pleurotus* mushrooms cultivation. This would alleviate accumulation of urban generated wastes thus protecting the environment.

Key words: *Pleurotus eryngii*, *Pleurotus ostreatus*, mushroom cultivation, potato dextrose agar, yeast malt agar, malt extract agar, mycelial extension

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mushroom cultivation is an appropriate approach for agro-industrial residues management. Mushrooms have been appreciated for their nutritional properties, economical and ecological values and medicinal properties. They produce many useful secondary metabolites, high protein content with essential amino acids, vitamins, minerals and exopolysaccharides[1]. Many mushroom species contain a wide range of metabolites as antitumour, antigenotoxic, antioxidant, antihypertensive, antiplatelet aggregating, antihyperglycemic, antimicrobial and antiviral activities[2]. The consumer demand for mushrooms has increased over the years worldwide and mushroom cultivation become a highly efficient method for recycling the agricultural residues and producing nutritious food[3]. Just as seed quality is important to crop production, quality of spawn is important in mushroom production. The success of mushroom cultivation and its yield depends to a large extent on the quality of the spawn used[4]. Many previous studies reported that different culture conditions had promising results on the yield of mycelium and metabolic substances contained in the mushrooms[5,6]. Currently, sterilized grains such as cereals and millets are the most popular supporting substrates for mushroom spawn production due to its bio-chemical properties and practical performance over other materials[7]. Therefore, the alternative of suitable grain sources and culture media for each specialty mushroom species is important for mycelial growth of mushroom.

Pleurotus sp. is an aromatic edible fungus and of which some species constitute a very valuable protein source[8,9]. The mycelium growth of *Pleurotus* sp. mushrooms is affected strongly by many factors such as cultural media, temperature, sources of carbon and nitrogen, grain sources and lignocellulosic substrate sources[10,11]. The main nutritional sources for oyster mushroom *Pleurotus* sp. are cellulose, hemicellulose and lignin and most of such substrate materials need the supplementation of nitrogen source such as wheat and rice bran to meet optimal C/N ratio[7]. A wide variety of agricultural and industrial wastes is used for growing *Pleurotus* species such as cereal straw, cotton waste, sawdust, cardboard, coffee industry residue, wastepaper, etc.[12-18]. The use of agro-industrial residues in bioprocesses may be one of the solutions to bioconversion of inedible biomass residues into nutritious protein rich food in the form of edible mushrooms[19]. The cultivation of mushroom on solid urban wastes such as cardboard and spent coffee ground (SCG) combination can be viewed as an effective means to utilize bio-resource residue and a sound environmental protection strategy[20]. So far, sawdust and straw are widely used as the main substrate for mushroom cultivation[21] but very little work has been conducted to find out the suitability of urban solid wastes for the cultivation of *P. eryngii* and *P. ostreatus*. Furthermore, the slow growth rate is usually one of the main issues which increases the cost of production and thus becomes of profound interest for many researchers to study and develop new substrates to enhance the growth rate of mushrooms. The aims of this study were to evaluate and determine optimal culture media and suitable grain sources for spawn production. Alternate substrates including cardboard and spent coffee ground in comparison with wheat straw for the mycelial growth of *P. eryngii* and *P. ostreatus* was also studied.

MATERIALS AND METHODS

Collection and maintenance of *P. eryngii* and *P. ostreatus* culture: The study was conducted from February-August, 2019 at Sietalab in Vantaa, Finland. The pure cultures of *P. eryngii* and *P. ostreatus* were colonized from fresh mushroom samples which were sold from Finnish market and were used for mass culture production. The fresh mushroom samples after alcohol sterilization were cut longitudinally into two halves and bits from collar region (i.e., at the junction of cap and stalk) were transferred to pre-sterilized PDA culture medium (at 121°C and 17 psi for 15 min), which were, incubated at 24°C in the incubator for 1 week. Mycelium from growing edges was carefully transferred to PDA slants and again incubated for 2-3 weeks to obtain pure cultures. The mycelial culture was maintained in glass tubes on medium PDA and covered with sterile liquid Vaseline and sub-cultured every three months.

Media preparation and mycelium growth test: Following 3 media were used for the purpose: (1) PDA-Potato Dextrose Agar medium: potato (250 g), dextrose (20 g), agar (20 g) and water (1000 mL), (2) MEA-Malt Extract Agar medium: malt extract (30 g), agar (15 g) and water (1000 mL), (3) YMA-Yeast Malt Agar medium: malt (20 g), yeast (2 g), agar (15 g) and water (1000 mL). The flasks having media were sterilized in the autoclave at 121°C and17 psi for 15 min and then poured into petri dishes (diameter of 85 mm) under the laminar flow hood to avoid contamination. Media were allowed to cool to 37°C. The treatment medium was inoculated with a 14 days-old agar mycelial plug (5 mm) of *P. eryngii* and *P. ostreatus* under aseptic conditions. An inoculum plug was placed in the middle of each petri dish and

special care was taken to keep the mycelium cylinder in direct contact with each substrate. Radial growth of mycelium of different portions was observed until the petri dishes were filled with it. The experiment was repeated for four times. The plates were incubated at 24°C and observed for 7 days during which the vegetative growth characteristics of mycelium and mycelial density of *Pleurotus* spp. were recorded. The growth rate was computed by the formula:

$$\text{Growth rate} = \frac{\text{Colony diameter on the last day (cm)}}{\text{Number of day's}}$$

the measurement was taken after inoculation. Daily mycelial growth was determined using a ruler across the petri-dish horizontally.

Different grain sources: The study used grain sources consisting of wheat, rye, oat and barley grains. The grains were washed 3 times in clean water to do away with chaff, dust and different particles contained in and then soaked in water for 12-14 h for full absorption of water. Soaked grains were boiled in water for 20 min. Water was decanted and the grains were spread on a wire-mesh for drying the outer surface of grains. Each grain was supplemented with 1.3% $CaCO_3$. Supplemented grains were filled into petri dishes containing spawns with 30 g grain/petri dish and sterilized by autoclaving at 121°C and 17 psi for 60 min. After cooling, each petri dish was once inoculated with 5 mm mycelium disc of each *P. eryngii* and *P. ostreatus*. An inoculum plug was placed in the middle of each petri dish and special care was taken to keep the mycelium cylinder in direct contact with each substrate and petri dishes were incubated at room temperature (22±2°C) under dark conditions. For each test, each treatment was replicated 4 times. The diameter of the mycelium growth was measured after 3 days for 7 days. Levels of mycelial density among the treatments were compared visually and categorized as T (thin), ST (somewhat thin), Co (compact) and SC (somewhat compact).

Substrate preparation: Different substrates namely, wheat straw (S), cardboard (C) and spent coffee ground (SCG) were prepared in 7 formulations: TS1 (100% S), TS2 (100% C), TS3 (100% SCG), TS4 (50% S+50% C), TS5 (50% S+50% SCG), TS6 (80% S+20% C) and TS7 (80% S+20% SCG). Wheat straw (S) and cardboard (C) were obtained from Vantaa, Finland while spent coffee ground (SCG) was collected from a local coffee shop. The principal substrate used for commercial production of *Pleurotus* spp., is cereal straws[22-24] and therefore, wheat straw (TS1) was used as a control treatment. The collected lignocellulosic substrates of S and C were chopped into small pieces (2-4 cm long) and soaked in tap water for overnight. The excess water in the substrates was allowed to runoff until the required moisture level reached. The substrates were added 5% $CaSO_4$ and 0.2% $CaCO_3$. Wheat bran was used as supplementary substances to increase mushroom yield and to achieve faster mycelial growth. Each substrate was supplemented by 20% (w/w) of wheat bran and mixed thoroughly. Water was added to adjust moisture content to 65%. The substrate mixture was filled into petri dishes (85 mm diameter) and sterilized at 121°C and 17 psi for 60 min. After allowing to cool down to room temperature the substrates were inoculated with the mother culture (5 mm plugs) of the selected strains for testing separately. These inoculated petri dishes were incubated in a dark room at 24±2°C temperature for mycelium growth. The mycelial growth was measured after 7 days of growth for 2 weeks and images recorded photographically until the end of experiment. Mycelial density among the treatments were compared visually and categorized as T (thin), ST (somewhat thin), Co (compact) and SC (somewhat compact). The time taken for overgrowth of these mycelia in the whole petri dish and primordial formation was tracked which was begun when the first pin heads were verified, was also recorded. The experiments used four replicates.

Statistical analysis: The experiment was conducted in a Complete Randomized Design with 4 replicates. Therefore, data were analyzed and subjected to analysis of variance and the means were compared using Duncan's multiple-range test. The software SPSS 16 was used for data analysis. Differences at $p \leq 0.05$ were considered significant.

RESULTS

Effect of different culture media on the mycelium growth: Different media, i.e., PDA, MEA and YMA were used to identify their effects on mycelium growth of *P. eryngii* and *P. ostreatus* after 1 week of inoculation. The results showed that 3 different media significantly affected on the mycelial extension of various mushroom species ($p<0.05$) (Table 1). Mycelium densities of both *P. eryngii* and *P. ostreatus* were compact in all media (Fig.1). The highest diameter and growth rate of *P. eryngii* (5.89 cm and 0.84 cm) were found in PDA media, followed by YMA media (5.42 cm and 0.78 cm) and the lowest values were found in MEA media (4.29 and 0.61 cm, respectively). The lowest mycelium

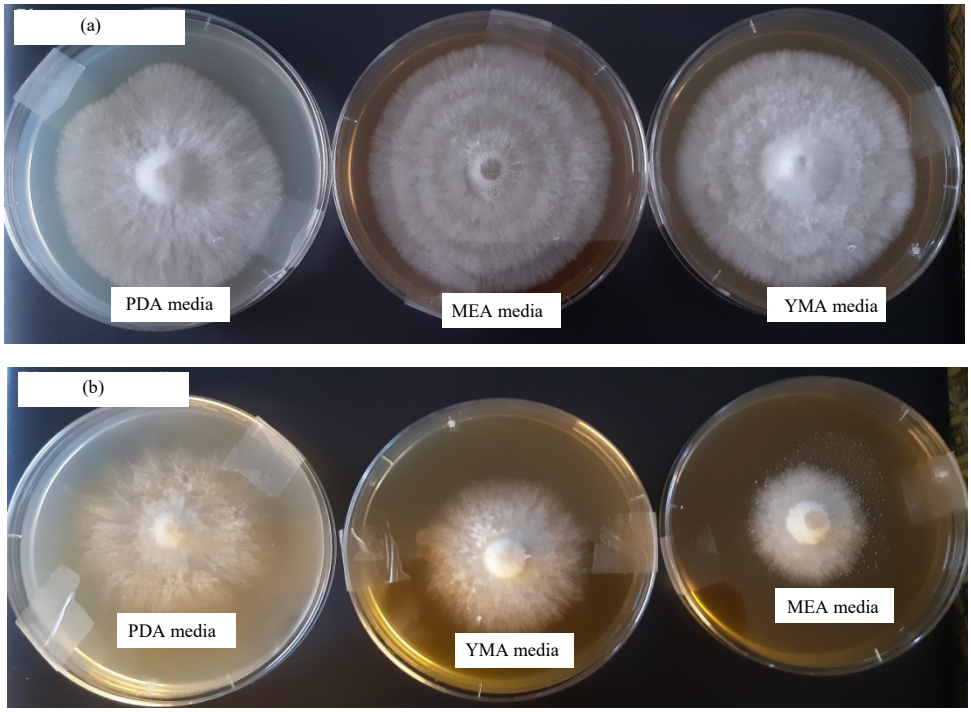

Fig. 1: Mycelium density of both (a) *P. ostreatus* and (b) *P. eryngii* on different culture media

Table 1: Mycelium diameter and growth rate of *P. eryngii* and *P. ostreatus* on different media after 1 week of inoculation

	Pleurotus eryngii		*Pleurotus ostreatus*	
Media	Diameter (cm)[1/]	Growth rate (cm/day)	Diameter (cm)	Growth rate (cm/day)
PDA	5.89±0.26[a2/]	0.84±0.04[a]	6.43±0.41[b]	0.92±0.06[b]
MEA	4.29±0.23[c]	0.61±0.03[c]	7.00±0.62[a]	1.00±0.09[a]
YMA	5.42±0.39[b]	0.78±0.06[b]	7.13±0.25[a]	1.01±0.04[a]

[1/]Means followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test, [2/]±Standard deviation

Table 2: Mycelium diameter, growth rate and density level of tested mushrooms on different grain sources after 1 week of inoculation

	Pleurotus eryngii			*Pleurotus ostreatus*		
Grains	Diameter (cm)[1/]	Growth rate (cm/day)	Density level	Diameter (cm)	Growth rate (cm/day)	Density level
Wheat	3.66±0.62[b2/]	0.52±0.09[b]	SC	5.70±0.55[c]	0.81±0.08[c]	SC
Rye	4.23±0.28[a]	0.60±0.04[a]	Co	7.48±0.41[b]	1.06±0.06[b]	Co
Oat	4.58±0.54[a]	0.65±0.08[a]	SC	7.86±0.47[ab]	1.12±0.07[ab]	Co
Barley	4.27±0.21[a]	0.60±0.03[a]	Co	8.25±0.29[a]	1.17±0.04[a]	SC

[1/]Means followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test, [2/]±Standard deviation

diameter and growth rate of *P. ostreatus* were found in PDA media (6.43 and 0.92 cm, respectively) while YMA and MEA did not show significant differences on the mycelial diameter and growth rate.

Effect of different grain sources on the mycelium growth: The different grain sources had significant effects on the mycelial growth of oyster mushroom *P. eryngii* and *P. ostreatus* at $p<0.05$ after 1 week of inoculation (Table 2). The mycelial growth had 2 types of mycelia compact (Co) and somewhat compact (SC) according to the mycelial density. The substrate with wheat grains gave SC type of mycelial density while mycelial diameter and the growth rate were the lowest compared to other grain substrates on both of *P. eryngii* and *P. ostreatus*. The highest mycelial diameter and growth rate of *P. ostreatus* were found in barley grains (8.25 and 1.17 cm, respectively) with SC type of mycelial density, followed by oat grains (7.86 and 1.12 cm, respectively) and rye grain substrates (7.48 and 1.06 cm, respectively) with Co type of mycelial density. The results also showed that there

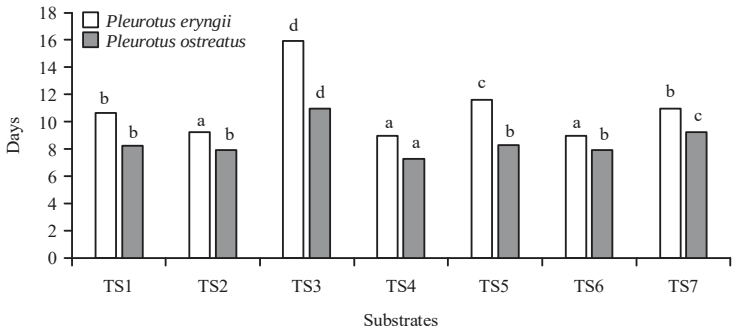

Fig. 2: Full growth time of *P. eryngii* and *P. ostreatus* on different tested substrates
Means followed by the same letters are not significantly different (p<0.05) by Duncan's multiple-range test

Table 3: Mycelium diameter, growth rate and density level of tested mushrooms on different substrates after 1 week of inoculation

	Pleurotus eryngii			*Pleurotus ostreatus*		
Media	Diameter (cm)[1/]	Growth rate (cm/day)	Density level	Diameter (cm)	Growth rate (cm/day)	Density level
TS1	5.20±0.62^{cd} [2/]	0.74±0.08^{bc}	SC	7.40±0.20^{c}	1.06±0.03^{c}	SC
TS2	5.67±0.21^{bc}	0.81±0.03^{b}	SC	8.10±0.10^{b}	1.16±0.02^{b}	Co
TS3	3.33±0.15^{e}	0.47±0.02^{d}	Co	5.30±1.73^{d}	0.75±0.23^{d}	Co
TS4	6.57±0.12^{a}	0.94±0.17^{a}	SC	8.43±0.16^{a}	1.20±0.01^{a}	Co
TS5	4.77±0.15^{d}	0.68±0.02^{c}	Co	7.17±0.15^{c}	1.02±0.02^{c}	Co
TS6	6.17±0.29^{ab}	0.88±0.04^{a}	SC	7.93±0.12^{b}	1.13±0.02^{b}	SC
TS7	5.13±0.15^{cd}	0.73±0.25^{c}	SC	7.30±0.26^{c}	1.04±0.04^{c}	SC

[1/]Means followed by the same letters are not significantly different (p<0.05) by Duncan's multiple-range test, [2/]±Standard deviation

were not significant differences among rye, oat and barley grain substrates on mycelial extension of *P. eryngii* but rye and barley grain substrates gave the compact type (Co) of mycelial density while oat grains had somewhat compact type (SC).

Effect of different alternate substrates on the mycelium growth: The mycelium growth of *P. eryngii* and *P. ostreatus* in various substrates were significantly affected by substrate formulations at p<0.05 (Table 3). There were 2 types of mycelial growth according to the level of mycelial density including compact (Co) and somewhat compact (SC). The highest mycelial diameter and growth rate of *P. eryngii* and *P. ostreatus* were observed in the substrate TS4 with 50% S+50% C, followed by the substrate TS6 with 80% S+20% C and the lowest values were found in 100% SCG (TS3) substrate. It was also observed that mycelial extension of tested mushrooms on 100% C (TS2) substrate was better than the control with 100% S (TS1) substrates. On the other hand, there were no significant differences on mycelial growth of tested mushrooms in substrates TS5 (50% S+50% SCG) and TS7 (80% S+20% SCG) when compared with the control TS1 with 100% wheat straw (S).

The time to reach the full growth of *P. eryngii* and *P. ostreatus* in different substrate formulations are given in Fig. 2. In case of *P. eryngii*, the shortest time to reach the full growth was 9 days which occurred in TS4 and TS6 substrates, followed by 9.3 days in the substrate TS2 with the difference in time been non-significant. The full growth mycelium occurred in 11-11.7 days in the other 2 substrates of TS5 and TS7 compared to the control TS1 (10.7 days) while the longest time of 16 days was observed in the substrate TS3 (100% SCG). The longest time taken for the full growth was 11 days for *P. ostreatus* which occurred in TS3, followed by TS7 of 9.3 days, while the shortest time was 7.3 days in TS4 substrates. The time for full growth of time (8-8.3 days) of *P. ostreatus* were not significantly different among the substrates TS2, TS5 and TS6 compared to the control with 100% of wheat straw (TS1). Healthy and outstanding mycelium is a vital part which reflects the overall growth and productivity and thus substrate components are critical for mushroom cultivation. Lignocellulosic materials containing cellulose, hemicellulose and lignin are a suitable substrate for *Pleurotus* mushrooms. The result showed that the substrates containing higher cardboard percentage in the substrate promoted better mycelial extension than the rest of substrates.

The time primordia appearance of *P. eryngii* and *P. ostreatus* in different substrate formulations are given in Fig. 3. The shortest time for primordia appearance of

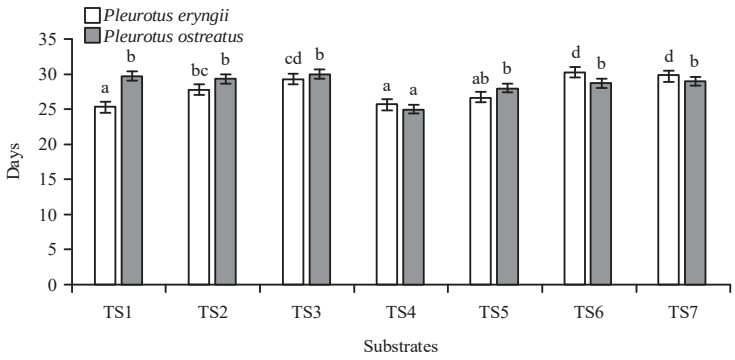

Fig. 3: Primordia appearance time of *P. eryngii* and *P. ostreatus* on different tested substrates
Means followed by the same letters are not significantly different ($p<0.05$) by Duncan's multiple-range test

P. ostreatus of 25 days occurred in the substrate TS4. There were not significant differences (approximately 28-30 days) among the rest of the substrates. In case of *P. eryngii*, the longest time taken for primordia appearance was 30 days which was in the substrates TS6 and TS7, followed by TS3 in 29.3 days while there were not significant differences (approx. 25.3-26.7 days) among substrates TS4, TS5 and the control (TS1).

DISCUSSION

Growth medium is the most important factor in mushroom production because it supplies necessary nutrients for the growth of mushroom mycelia. The results showed that PDA was the most suitable media for mycelial growth of *P. eryngii* while *P. ostreatus* mycelium had better extension on YMA and MEA media. According to Shrestha *et al.*[25], nutritionally rich media produced abundant mycelium growth. This may be because of the sufficiency of all the nutritional requirements and optimal physical conditions for the vegetative growth. Similarly, some previous studies reported that PDA was the most suitable for the mycelial growth of *Macrolepiota procera* and *Cordyceps militaris*[25,26] while yeast malt extract medium was favourable for the mycelial growth of *M. procera* and *Ganoderma lucidum*[26,27]. Based on the results of these studies and of the current study, it was evident that different media support the growth of different *Pleurotus* mushroom species. The colony characteristics of the mycelium depend on the medium composition, efficacy of bio compounds as well as substrate concentrations[28]. Grain spawn is used as a substrate commonly based on its ability to fast and ease of planting[4]. So far mother spawn production as a pure culture of growing mycelium on cereal grains has a fundamental role in agricultural productivity. From the results, barley and rye grains were found to be the most favourable to the mycelium growth of *P. eryngii* while barley and oat grains were a good option for *P. ostreatus*. According to Tinoco *et al.*[29], the larger surface area and pore characteristics of substrate favoured increased the mycelium growth rate. For this reason, grain size of barley and oat is larger than wheat and rye and consequently, mycelial extension of the tested *Pleurotus* species on barley and oat grains was faster than those on wheat and rye grains. In a previous study, Khare *et al.*[30] reported that wheat, barley, sorghum and millet grains could be equally used in the production of good quality spawn for the cultivation of oyster mushrooms.

Wastes are rich in cellulosic and lingo cellulosic pulp residues and which can be used as a novel substrate for the cultivation of mushrooms[31]. In this study, substrates containing 50% and 20% cardboard+wheat straw gave better mycelia extension on both of *P. eryngii* and *P. ostreatus* compared to cardboard or wheat straw alone as well as other mixed substrates. Specially, the substrate containing 50% S+50% C (TS4) was by far the most favourable not only for mycelial growth but also for primordia formation in *P. eryngii* and *P. ostreatus*. This could be attributed to the presence of fine pulp residues in cardboard which can be easily decomposed by *Pleurotus* for its growth. In contrast, the presence of spent coffee ground (100% SCG) in the substrate decreased the extension of mycelial growth compared to control and other substrate formulations, which could partly be attributed to the presence of caffeine residue in SCG which could delay the growth of *P. eryngii* and *P. ostreatus*. Carrasco-Cabrera *et al.*[32] also reported the inhibition of the mycelium growth of *P. ostreatus* on agar and in liquid culture in the presence of caffeine. The addition of wheat straw (S) to the SCG substrate was found to be a good option because it provides cellulose and improves the structure of the substrate. The larger particle size and pores of wheat straws may have

facilitated quicker development of mycelium thus showing higher mycelium growth rate. The substrates with a larger surface area facilitate adequate gas exchange, particularly for higher O_2 and lower CO_2 levels around the mycelium which stimulates the growth[33]. Consequently, the supplement of wheat straw with SCG substrate helps to improve mycelial extension when compared to SCG alone. The mycelial growth analysis revealed that sustainability of edible mushrooms grown on urban wastes and their combination with wheat straw had better growth in a shorter duration.

The level of culture media and grain coverage by mycelium varied depending upon substrate components and mushroom species. The use of cardboard and SCG wastes for the production of *Pleurotus* mushrooms can resolve the most prominent issue of solid waste and its disposal and providing economical gains while providing a nutritious food source for mushrooms. Future research work may also be focused on testing further effects of wheat straw combined with cardboard and SCG for the productivity of *Pleurotus* mushroom and the testing of nutritional profiles of mushrooms. Such findings would be imperative and necessary for commercial cultivation of *Pleurotus* as well as food safely of consumers.

CONCLUSION

Different culture media had differences in supporting the growth of different *Pleurotus* mushroom species. Barley, oat and rye grains produced good quality spawn for the cultivation of oyster mushrooms including *P. eryngii* and *P. ostreatus.* The possibility of utilizing urban wastes includes cardboards and spent coffee ground for the growth of mushroom that is edible to humans is promising and potential.

SIGNIFICANCE STATEMENT

The results of this study suggested the possibility of high-quality fungal inoculum production using different recyclable residues and the need for future research extension for evaluating the inoculative effectiveness for obtaining fruiting bodies. Utilization of these recyclable materials for edible mushroom cultivation will contribute to opportunities for saving land surfaces for high value food production and reducing an ever-increasing issue of urban waste management and protecting environment.

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