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ECO-FRIENDLY MANAGEMENT COMMON LAB CONTAMINANT *Trichoderma* spp IN OYSTER MUSHROOM PRODUCTION USING AGRO-BASED INDUSTRY'S BY-PRODUCTS

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Abstract: An abundant supply of low-cost substrate and management of green mold disease-causing fungus *Trichoderma* are the major hurdles in successful mushroom production. This study aimed to identify the best Agro-based industry's by-products as a substitute for oyster mushroom production (*Pleurotus ostreatus*) while managing fungal contaminants eco-friendly. Two sets of *In-Vitro* [containing 20% extracts, from agro-based industries, such as coffee waste powder, tea dust and Mahua oil cake] and *In-Vivo* experiments [four substrates such as paddy straw, wood sawdust, paddy husk and banana leaves were incorporated with coffee powder, tea dust and Mahua oil cake] were prepared separately. All the experiments were conducted using a complete randomized design with three replicates. The *In-Vitro* data [mycelial growth and sporulation of both fungi], *In-Vivo* data [mycelial mushroom run, pinhead formation and yield] were subjected to ANOVA and DMRT mean separation using SAS 9.1 statistical package at $P < 0.05$. *In-Vitro* results showed that the *Trichoderma* mycelial growth was significantly minimum in Mahua (2.5 cM) and coffee (3.6 cM) in comparison to control, whereas, with decreasing concentration of coffee, tea, and Mahua extract *P. ostreatus* showed enhanced growth. *Trichoderma* sporulation had significantly affected coffee treatment, and even not sporulate in Mahua treated plants. The *In-Vivo* experiment proved that spawn run was consistent and significant among the treatments when mixed tea (20 days) and coffee (21 days), respectively, at $P < 0.05$. Treatment wise coffee treated spawn bags took an average of 32.5 days, whereas, in tea-treated substrates, it was more than 36 days to form pinhead. Mahua treated trials showed poor spawn run in all substrates, longer days of pinhead formation, and lower yield. In contrast, the paddy straw + coffee treatment produced a significantly highest yield of 200.67g. When sawdust was the substrate, the addition of tea showed a significantly higher yield of 185.00g than coffee (145.00g). In conclusion, coffee and tea extracts have a significant effect on yield with paddy straw and sawdust while minimizing the growth of *Trichoderma*.

Keywords: *Pleurotus ostreatus*, eco-friendly, plant extract, substrate, coffee, paddy straw

Introduction

Oyster mushroom (*Pleurotus ostreatus*) is considered nutritious food because it consists of around 20-25% of protein and rich sources of vitamins and minerals essential for human health. Mushroom is good for health due to its low carbohydrate content and low potassium content favorable for hypotension patients. It has high fiber content, and it facilitates the digestion process of the human

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gut. Oyster mushroom is rich in folic acid, and it is beneficial for reducing signs of anemia in human (FAO, 2004). As oyster mushroom can be purchased at low prices in most of the developing countries like Sri Lanka, this mushroom is known as poor man's protein, colloquially. Oyster mushroom is famous among farmers because of low-cost cultivation and easy maintenance (Nadir, 2014).

Oyster mushroom prefers to grow in an environment which has high relative humidity, low light, rich carbon and nitrogen source and ambient temperature. Oyster mushrooms on paddy straw and basal wheat substrate show a faster mycelial growth rate than other substrates (Yang *et al*., 2013). As these materials are easily available in the farms and surroundings, farmers can easily access them at low cost and can begin mushroom farming in a cottage or commercial level with low investment and earn more profit.

Green mold disease, which causes by *Trichoderma* spp is a serious threat to the mushroom industry, and it causes economic losses to mushroom farmers (Jayalal and Adikaram, 2007). *Trichoderma* fungus can grow on any lignocellulose substrate and produces spores rapidly at an ambient temperature. The management of this disease is very difficult because both are fungi; this disease can be identified when the *Trichoderma* is in the sporulation stage with the green colour mycelium. *Trichoderma* can suppress the mycelia growth of mushroom by producing enzymes that are antagonistic to many micro-organisms (Choi *et al*., 2018). Due to the green mold fungi attack, Oyster mushroom mycelium will not grow as expected and doesn't produce any fruiting body resulting in economic losses to the mushroom farmers. Controlling green mold disease in mushroom using fungicide is impossible because both are fungi, and unwanted fungicides application can damage the environment, biodiversity and raise unwanted health issues to humans.

Agricultural (Plant) by-products (wastes) are good substrates for mushroom spawn and mushroom production. By-products from urban and agro-based industries can be converted into organic fertilizer using mushrooms quickly and ecofriendly. For example, Sri Lanka is famous for tea and coffee production. While processing tea and coffee, tremendous by-products are generated as waste annually. Composting these generated wastes is an ideal method to convert these wastes into organic fertilizers. Mushroom-based composting is a quicker and least cost eco-friendly method.

Tea and coffee both consist of chemical compounds called caffeine. Caffeine has been known for its antifungal properties (Nasrollahi and Yadegari, 2016), and it has been proved the antifungal activities against myco-pathogens (Rakatama *et al*., 2018). The coffee husk and coffee by-products are suitable for cultivating Oyster mushroom varieties (Sugiyama *et al*., 2016). This research experiment was conducted to propose a solution to manage the lab fungal contaminants, and investigate mushroom growth, reproduction, and yield performance to confirm the potential and possibilities to use coffee husk and tea dust as a substitute for paddy straw or other recommended bedding materials.

Materials and Method

For the *In-Vitro* experiment, three types of plant extracts were selected to evaluate the growth rate, observe sporulation of *T. viridae* and growth rate and percentage inhibition of radial growth (PIRG) of *P. ostreatus*. Plant extracts of fresh Mahua oil cake (*Madhuca longifolia*), coffee powder (*Coffea arabica*), tea dust (*Camelia sinensis*) were used to prepare culture medium separately. Test

concentrations of 20% plant extracts were obtained. The plant extracts media was prepared by adding an appropriate amount of plant extract, distilled water, and commercial Potato Dextrose Agar.

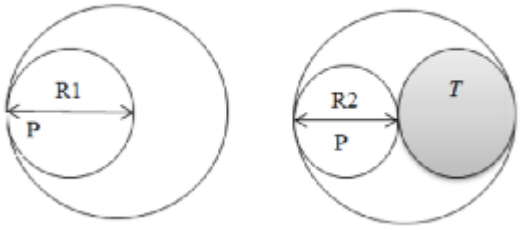

Figure 1: Measurement of radial growth of P.ostreatus

Where R1: radius of *P. ostreatus* in control plate, R2: radius of *P. ostreatus* in dual culture plate

Readings which collected were transformed into percentage inhibition of radial growth (PIRG) using the formula developed by Skidmore and Dickinson (1976).

$$PORG = \frac{R1-R2}{R1} \times 100\ \%$$

To prepare mushroom spawn, paddy seeds were washed and soaked in hot water for about 30 minutes. After that, soaked seeds were filled into sterilized glass bottles and plugged with cotton wool at the top of the bottle and autoclaved at 121°C 15 psi pressure for 20 minutes. Then the sterilized bottles were inoculated with Oyster mushroom mycelium under aseptic condition and incubated at room temperature 25°C in dark condition and kept in a slant position.

Spawn packets were prepared by using four substrates such as paddy straw, wood sawdust, paddy husk, and banana leaves. First, paddy straw, Paddy husk, and banana leaves were soaked overnight in the 2% of calcium carbonate solution and excess water was drained off and allowed for drying under shade. For wood sawdust, 2% of calcium carbonate was mixed in powder form. Then rice bran and green gram powder were mixed with substrates at the rate of 20%, and $MgSO_4$ solution was mixed at a 0.2% rate to the mixtures. Finally, each substrate was treated with 2% coffee grounds, tea grounds, mahua oil cake, and non-treated substrates used to compare the effect of plant grounds treatments.

Polypropylene bags were prepared at the dimensions of 22 cM height × 12 cM bottom diameter. 500g of mixed substrate were tightly filled into the bags with three (3) replicates for each treatment and control. The neck of the bag was prepared using a 1inch width PVC pipe, and the opening was sealed using a cotton plug and covered with paper tied with a rubber band. Then all the bags were autoclaved under 121°C temperature, 15 psi pressure for 20 minutes. Then the mother spawn of *P. ostreatus* was inoculated at the rate of 1 teaspoon to the cooled bags under aseptic condition.

The inoculated bags were incubated at 27±1°C temperature. Spawn Packets were cut in several places in the bags after completion of mycelium colonization. During this period, spawn run days in different substrates and treatments, days of pinhead formation, the yield of each treatment were recorded. Experiments were designed in a complete randomized design. Data analysis of this study has been carried out by using SAS 9.1. Mean separation was performed to identify the best treatment combination using DMRT at $P < 0.05$.

Results and Discussion

Mycelial growth rate of *Trichoderma* and *Pleurotus* in the different plants was extracted. The In-Vitro result shows that the *Trichoderma* mycelial growth was significantly minimum in Mahua (2.5 cM) and coffee (3.6 cM) in comparison to control after eight (8) days of inoculation, whereas, with decreasing concentration of coffee, tea, and Mahua extract P. ostreatus showed enhanced growth. The highest growth rate of *Trichoderma* was observed in the control (Potato Dextrose Agar), and it reached full growth in the plate within four (4) days. The lowest linear growth was observed in Mahua oil cake extract treatment with time. After eight (8) days of culturing both Mahua oil cake extract treatment and coffee powder extract, the treatment showed nearly the same mycelial growth. This observation revealed that Mahua and coffee have some suppression effect on green mold fungi *Trichoderma*. At the same time, the growth and sporulation of Mushrooms were very low than *Trichoderma*.

Pervez *et al* (2017) reported that *T. harzianum* growth was inhibited differently by different concentration of plant extracts, and maximum inhibition percentage of 50.2 and 52.3 at 5% and 10% concentration of Lantana (*Lantana camara*). Nirosha *et al* (2018) stated further that Mahua oil cake extract caused the least mycelial growth in *T. viridae*. But there is no any study related to the antifungal effect of Tea and coffee on *Trichoderma* spp. Further, Fan *et al*., (2006) reported that an increase in caffeine concentration reduces the mycelial growth and the biomass production of *P. ostratus*, and no growth was observed when the concentration of caffeine was 2500 mg/L. *Pleurotus* could not degrade the caffeine but absorbed it. Tannin under 100 mg/L in the medium stimulated the growth of mycelia, and also, *Pleurotus* had the capacity of degrading tannic acid. Fructification occurred after 20 days of inoculation, and the biological efficiency reached about 97% after 60 days. These findings validate the current investigation.

The results revealed that treatments significantly affect spawn run, pinhead formation, and yield of mushrooms in different combinations at $P < 0.05$. The number of days took for spawn run was varied from 20-44 days, but the number of days took for spawn run was consistent and significant among the treatments when mixing tea (20 days) and coffee (21 days), respectively (Table 1). Substrate wise paddy straw, banana leaves have the lowest spawn run days.

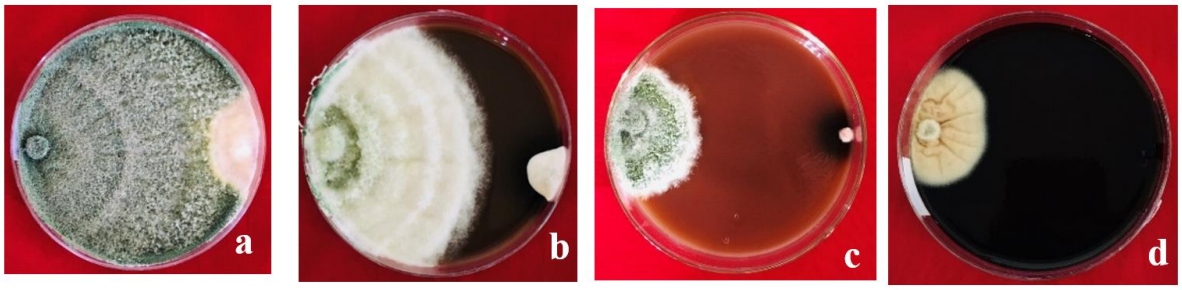

Figure 2: Trichoderma vs P.ostreatus dual culture in 20% of (a) Control, (b) Tea extract, (c) Mahua extract and (d) coffee extract at eight days of inoculation.

Pinhead formation was significant among treatments at $P < 0.05$. Treatment wise coffee treated spawn bags took an average of 32.5 days, whereas, in tea-treated substrates, it was more than 35 days to form pinhead. Mahua treated trials showed poor spawn run and longer days of pinhead formation in all substrates with a minimum of 42 days. Considering the effectiveness of mushroom substrate on

pinhead formation, paddy straw has higher effectiveness within a shorter number of days after inoculation (29 days). The highest and lowest average yield was obtained from coffee and mahua treated substrates in each experiment, respectively, except sawdust. Paddy straw + coffee treatment produced a significantly highest yield of 200.67g compared to other treatments (Table 1).

When Paddy husk, banana leaves, and paddy straw mixed with coffee were used as bedding material, the mushroom yield was higher, but in sawdust, the addition of tea gave a higher yield of 185.00g than coffee (147.00g) with significant difference (Figure 3). Addition of mahua oil cake impaired the mushroom growth and reproduction in all experiments.

Table 1: Effectiveness of Substrate on mushroom

Treatment/ Substrates	Spawn run (days)	Pin head formation (days)	Yield (g)
Wood saw dust	22^{b}	35^{b}	68.00^{c}
Wood saw dust + Coffee	21^{b}	36^{b}	147.00^{b}
Wood saw dust + Tea	20^{b}	35^{b}	185.00a
Wood saw dust + Mahua	44^{a}	$<60^{a}$	$>05.00^{d}$
Paddy husk	25^{b}	35^{b}	33.00^{b}
Paddy husk + Coffee	21^{c}	35^{b}	53.00^{a}
Paddy husk + Tea	20^{c}	52^{a}	50.80^{a}
Paddy husk + Mahua	27^{a}	52^{a}	24.00^{c}
Banana leaves	20^{b}	34^{b}	62.00^{a}
Banana leaves + Coffee	21^{b}	33^{b}	64.67^{a}
Banana leaves + Tea	20^{b}	35^{b}	57.70^{b}
Banana leaves + Mahua	25^{a}	52^{a}	09.00^{c}
Paddy Straw	20a	29bc	122.00b
Paddy Straw + Coffee	21a	32b	200.67a
Paddy Straw + Tea	20a	35b	111.00b
Paddy Straw + Mahua	22a	42a	63.67c

(Values with the same alphabets are not significantly different according to the DMRT at $\alpha = 0.05$)

Figure 3: Mushroom bearing (a) coffee incorporated paddy straw (b) tea incorporated wood saw dust (c) coffee incorporated banana leaves

This investigation confirms that the addition of coffee and tea to the universal oyster mushroom bedding materials such as Paddy straw and wood dust induces mushroom growth and reproduction, therefore, can get higher yield. Furthermore, mushroom production is an economically cheaper and environmentally friendly method to decompose the paddy straw than other agricultural residues (Dubey *et al*., 2019).

Yang *et al* (2015) reported that the addition of 40-60 % of the tea waste to the paddy straw as a substitute to increase mushroom yield and efficiently recycle the tea waste from tea industries. Martínez-Carrera *et al* (2000) reported that coffee pulp is an agro-based waste suitable for growing oyster mushrooms and increasing the yield of other substrates. The addition of tea and coffee waste has a synergistic effect on mushroom growth and controls the release of moisture; therefore, suitable humidity will prevail in the bedding material throughout the mushroom growing.

Conclusion

This study found that Mahua, coffee, and tea have potential antifungal effects. When adding tea and coffee extracts to the bedding material has a synergistic effect. The paddy straw treated with coffee and wood sawdust treated with Tea grounds was found most suitable for mushroom cultivation to yield a higher yield than other substrates treatments. This study recommends that the coffee and tea wastes are suitable substrates to cultivate oyster mushrooms in combination with paddy straw and sawdust, respectively. After harvesting mushrooms, these utilized substrates can be directly used as organic fertilizer for crop production.

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