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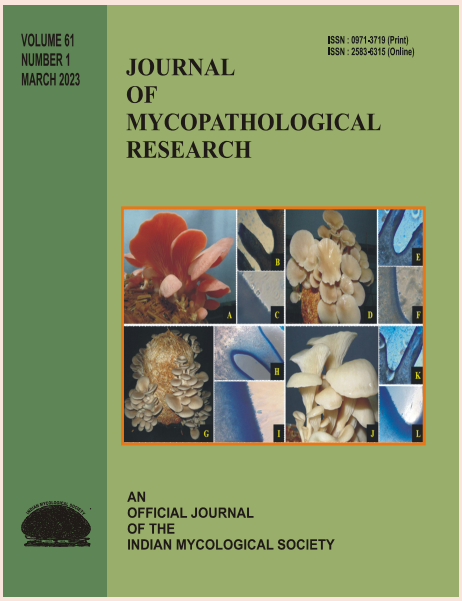

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Department of Botany,
University of Calcutta,
Kolkata 700 019, India

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RAHUL SAHA[1], KRISHNA KANTA PANDEY[1], SANJIT DEBNATH[1], KRIPAMOY CHAKRABORTY[2], PANNA DAS[2], AND AJAY KRISHNA SAHA[1*]

[1]Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura - 799022

[2]Microbiology Laboratory, Department of Botany, Tripura University, Suryamaninagar, Tripura – 799022

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Mushroom cultivation on a cost-effective and easily accessible substrate is one of the key areas of research of mycologists. In this present investigation, agro-industrial wastes have been used as a substratum to produce protein-enriched mushrooms having different medicinal potentiality with respect to growth, productivity, and biological efficiency. Mycelium running rate, development of fruiting bodies and productivity of *Pleurotus florida* was evaluated. The total running of mycelium in days, maximum primordial formation in three flushes, and total numbers of fruit bodies in three flushes were noted. In the grass substratum, fruiting body size was bigger as well as exhibited higher productivity (18.42 %) and biological efficiency (97.58%) in all three flushes and the least productivity and biological efficiency observed in sugarcane bagasse. The current study reveals that the various types of substrates affect mushroom growth, productivity, and biological efficiency.

Key words: Basidiomycetes, *Pleurotus florida*, agro-industrial waste, sugarcane bagasse, primordia

INTRODUCTION

Mushrooms are achlorophyllous, spore-bearing fruiting bodies of basidiomycete that grow above or under the ground on soil or other food sources. The Food and Agriculture Organization (FAO) recommends edible mushrooms as a protein supplement (Alam *et al*. 2007). Edible mushrooms are enriched with high carbohydrates and protein, and low in fat, high in vitamins B, D, K, A, and C (Alam *et al.* 2007, Debnath *et al.* 2017; Barman *et al*., 2018). Mushrooms possess enormous medicinal properties and are effective against certain life-threatening diseases.

Major medicinal properties attributed to mushrooms include anticancer, antidiabetic, antiviral activities, immunity and blood lipid lowering (Thakur and Singh, 2020). *Pleurotus florida* has also antioxidant and antitumor activities (Nayana and Janardhanan, 2000; Manpreet *et al.* 2004). Hence, mushrooms have gained a lot of attention as a functional food supplement and can be used as a preventive measure for the disease (Khan *et al.* 2009). Among the robust number of edible mushrooms, only a few are cultivated commercially on large scale throughout the world (Debnath *et al.* 2020). At the present scenario, mushroom cultivation has become popular globally as it has the potential to ensure income generation along with the nutritional and medicinal efficacy (Kalaè, 2012). The majority of the edible white rot fungi use a variety of lignocellulosic residues such as agro-industrial residues as substrates to grow mushrooms. Numerous environmental assets were used as substrates for mushroom cultivation viz., agricultural crop residue, and industrial by-products. In this connection, plant biologists are in search of suitable and feasible technologies for the cultivation of wild edible mushrooms in a larger scale (Hölker and Lenz, 2004) as it is considered

*Correspondence :akasha.58@gmail.com

as a dependable and beneficial alternative of natural food resources (Pathmashini *et al.* 2008). It is an indoor crop that can be cultivated without sunshine and does not require fertile land and can also be grown in a small space and cost-effective way because and low initial investment. Mushroom cultivation may improve the socioeconomic status of farmers and their families, as well as solve unemployment issues in rural and semi-urban areas, especially for women (Shahi *et al.* 2018). Due to culinary, nutritional, and health benefits, along with waste management potentiality mushroom market has been reported to be growing steadily (Beetz and Kustidia, 2004). Based on the above considerations the present study was focused on the development of cost-effective cultivation techniques along with the evaluation of productivity and biological efficiency of *P. florida*.

MATERIALS AND METHODS

Sample collection and identification

Pleurotus florida was collected from local Lake Chowmuhani Market (23°502 31.522 2 91°162 55.462 2 E 17 m) of Tripura during the rainy season. Identification of the specimen was done based on morpho-anatomical characteristics (Fig. 1) and then compared with accessible literature (Pegler, 1977; Purkayastha and Chandra, 1985). Dried specimen was preserved as herbarium material for future use (MCCT- F1) in the Mycology and Plant Pathology Laboratory, Department of Botany, Tripura University.

Culture preparation and preservation

The pure mycelial culture was obtained from the basidiocarp and maintained on potato dextrose agar (PDA) medium. The specimen was dried in hot air oven (Model: ROV/DG) at a constant temperature of 40–45°C for 24 hours and sealed in a marked polyethylene bag with 1, 4-dichlorobenzene for further analysis (Debnath *et al.* 2017).

Mother spawn preparation

1 kg of wheat grain was procured from the local market for the preparation of mother spawn. Healthy grains were first washed with running tap water for surface cleaning. The grains were boiled with an equal volume of double distilled water for15–20 mins and excess water is drained off. After removing excess water, the grains were surface dried in shade for 4 hrs. The grains were then thoroughly mixed with calcium sulphate (2%) w/w and calcium carbonate (0.5%) (w/w). After that, 300 g grains were filled in each 500 ml conical flask and sealed with cotton plug. This preparation was sterilized at (121^{0}C at 15 Psi) for 1 hour and allowed to cool at room temperature. Prepared containers were inoculated with the mushroom mycelium under laminar airflow (SNS Make Laminar). Then the containers were incubated in a BOD incubator (BOD-1 SNS) at 28°C for 15–20 days for mycelium running.

Substrate preparation

Four types of substrates namely, paddy straw, grass [*Imperata cylindrical* (L.) P. Beauv], sugarcane bagasse and sawdust were collected from farmers' fields, local sugar cane juice sellers, and sawmills, respectively. Substrates were soaked in hot water (100^{0}C) and then air-dried. After drying, the substrates were thoroughly mixed with 2% of calcium carbonate (2:1 ratio) and sprinkled with distilled water for maintenance of the moisture of the substrates.

Preparation and culture of spawn packet

The spawn packets were prepared individually for each type of substrate (1 kg) in a polyethylene bag (22.5 × 30 cm). The prepared mother spawn amount was placed in each packet. The inoculated packets were again sealed and wrapped with aluminum foil and kept on a rack in an incubation room (25±2 °C) and relative humidity was also maintained (70–80%). The mycelium running rate of each type of substrate was observed daily basis and data was noted. After the total spreading of mycelium throughout the substrates, spawn packets were opened in the upper portion and some holes were made by using sterilized needle for proper aeration and sprayed with water twice daily.

Harvesting, Productivity, and biological efficiency of mushroom

The different forms of mushroom yield were evaluated. Mycelium running in the substrate in days, the time required to complete mycelium spreading throughout the surface of substrate

packets (RT) in days, total primordia formation at three flushes, the time required to complete fruit body formation (PT) in days, number of the mature fruit body, flush number, time required to primordial development to harvesting (HT) in days, duration of cropping (T = RT + PT + HT) in days, fruit body size, total fresh and dry weight of harvested mushrooms (g/packet), productivity (P%) and biological efficiency (BE%) were evalauted. The first primordia formation and harvesting time mainly depended on the substrates used for the cultivation of mushrooms. The matured mushroom was identified when pileus turned down with the curled edge of the cap and carefully harvested by twisting the stipe to uproot from the base. Productivity (P) was determined by the method of Andrade *et al*.(2008): Productivity (P) was determined by the method ascribed by Andrade *et al.* (2008) [P% = FWM/FWC × 100]; where FWM fresh weight of mushroom and FWC fresh weight of compost.

Biological efficiency (BE) was determined by employing the method of Chang *et al*. (1981),
[BE% = FWM/DWC × 100];
where DWC compost dry weight.

Statistical analysis

Each experiment was carried out in the triplicate form. The results were expressed as mean values ± standard error (SE) and the significance was tested. The data were then subjected to one-way ANOVA followed by Tukey's post – hoc test for multiple comparisons among pairs of unions. All analyses were performed using R- statistical software (R Core Team, 2020).

RESULTS AND DISCUSSION

This study was conducted for the cultivation of *Pleurotus florida* mushroom (Fig.2) in different agro-wastes viz. sugarcane bagasse (Fig.2A), Grasses (Fig.2B), Paddy straw (Fig.2C), and Saw dust (Fig.2D). From this present study it is evident that the mushroom growing on grass and paddy straw showed a higher colonization rate per day compared to the other two substrates but the colonization rate differs significantly in sawdust (df=3 F value=73.3 p<0.001). First harvest (in terms of day) was done from both grass and paddy followed by bagasse of sugarcane and sawdust.

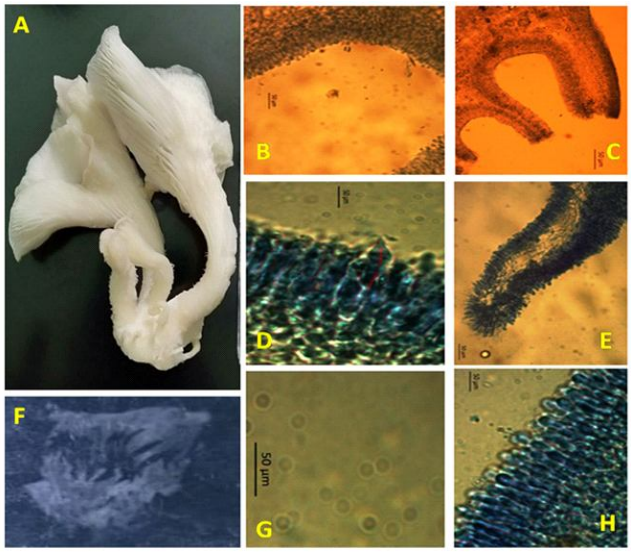

Fig.1: Morphological and anatomical features of *Pleurotus florida* (A) morphology, (B)&(C) pileus section showing basidium and basidiospores with hymenium layer, (D) basidium with basidiospores, (E) pleurocystidia and cheilocystidia, (F) spore print, (G) Spores and (H) basidium with basidiospores.

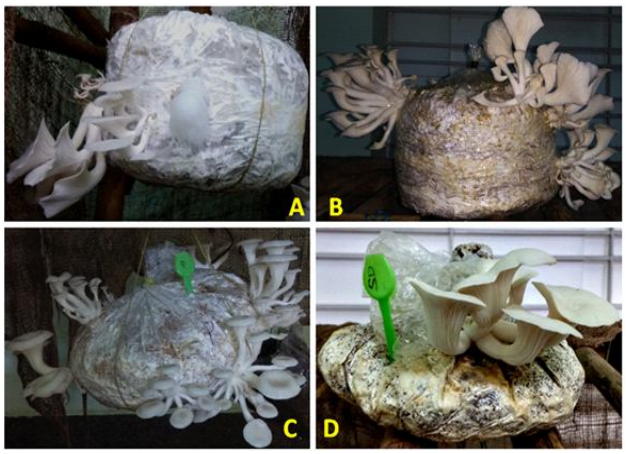

Fig.2: Cultivation of *Pleurotus florida* in different agro-waste substrates. (A) Sugarcane bagasse (B) Grasses (C) Paddy straw (D) Saw dust.

The first harvest in all substrates differs significantly from sawdust (df=3 F value =97.89 p<0.001). The first harvest (day) of mushrooms was observed in grass and paddy straw substrates as compared to the other two substates i.e., bagasse of sugarcane and sawdust (df=3, F value =184.2 p <0.001). Mushroom growing on the paddy straw has the least harvesting period (day) than the other substrates and was found to be significantly different.(df=3, F value =134.6 p <0.001; Table 1). There was no significant difference among the substrates according to the stipe length. However, the longer stipe was found in sawdust as compared to the other substrates i.e., bagasse of sugarcane, paddy

Table1: Total colonization period, first harvest, harvesting period, cap diameter, stipe length, No. of fruiting body and Mushroom weight of *Pleurotus florida*

Substrates	Total colonization period (day)	First harvest (day)	Harvesting period (day)	Cap diameter (mm)	Stipe length (mm)	No. of effective fruiting bodies/ bunch	Mushroom weight (g/bunch)
Sugarcane bagasse	19.00±1.00b	21.00±1.00b	25.33±1.52b	63.22±3.42c	8.7±.40ab	2.80±0.45b	27.00±1.00d
Grass	18.33±1.15b	20.67±0.57b	24.00±1.00b	106.53±1.60a	7.72±0.35c	4.48±0.27a	65.22±1.25a
Paddy straw	18.33±0.58b	20.67±0.57b	23.33±0.57b	86.52±3.47b	8.09±0.10bc	4.06±0.35a	59.82±0.49b
Saw dust	28.00±1.00a	30.67±1.15a	41.67±1.15a	79.75±1.52b	9.27±0.25a	3.32±0.58ab	53.97 ± 1.05c

Table 2: Comparison of biological efficiency of *Pleurotus florida* on different substrates

Substrates	1st flush	2nd flush	3rd flush	Productivity (%)	Biological Efficiency %
Grasses	0.35±0.01a*	0.24±0.02a	0.24±0.02a	18.42±4.20a	97.58±1.67a
Paddy straw	0.33±0.02a	0.15±0.01a	0.15±0.01b	14.48±5.28ab	77.87±12.30ab
Sawdust	0.28±0.00b	0.15±0.00b	0.15±0.00b	12.93±5.00ab	51.73±20.00 bc
Sugarcane bagasse	0.08±0.00c	0.04±0.00c	0.04±0.00c	4.04±1.37b	21.82±7.39 c

*Different letters showed significant differences at p<0.005, after Tukey's multiple comparison test.

straw and grass (df=3, F value=15.8 p <0.001).From the analytic view, there was a less significant difference in Grass, Paddy straw and Saw dust (ab) whereas bagasse of sugar showed a few differences (df=3, F value=9.256 p<0.001). The results have showed that the mushroom weight has the significant difference between the substrates. The highest weight of mushrooms was found in the grass and the least in the bagasse of sugarcane respectively (df=3, F value=882.9 p<0.001)**.** The mushroom flushes of yield varied among different substrates (Table 2). There were three flushes for all of the substrates during the cultivation period. In the first flush, the highest yield of mushroom was seen in grass (0.35±0.01) which has a significant difference with paddy straw (0.33±0.02), Sawdust (0.28±0.00), and bagasse of sugarcane (0.08±0.00). In the second flush, the highest yield of mushroom was found in grass (0.24±0.02) which showed a significant difference with paddy straw (0.15±0.01), Sawdust (0.15±0.00),and bagasse of sugarcane (0.04±0.00).In the third flush, we found the highest yield in grass (0.24±0.02) but no significant difference was found in comparison with paddy straw (0.15±0.01)and sawdust (0.15±0.00). Analysis of variance showed a highly significant result (pd<0.001) between the final mean yield and final substrate weight of oyster mushroom using different substrates. The highest final mean with was obtained by grass (a) (97.58%)followed by paddy straw (77.87%) and, sawdust (51.73%). The lowest final yield of *P. florida* mushroom was obtained in Sugarcane (21.82 gm).(df=3, F =10.26, p <0.001). Oyster mushrooms is a common cultivable mushroom in various substrates for the last few decades around the world because of their faster mycelium running, improved early production, and yield on different agro wastes in comparison with other cultivated mushroom genera (Debnath *et al.* 2019; Philippoussis *et al.* 2001). The biological efficiency of *P. florida* in the present study was ranged from 15-to 98% which showed similarity with the finding of Yang *et al.* (2013), the values obtained for biological efficacy was higher than the finding of Liang *et al.* (2011) and was lower than the finding of Bhattacharjya *et al.* (2014). According to Olfati and Peyvast (2008), time to fruiting was faster in Lawn clippings (grass)

substratum and lawn clippings along with rice straw was the best for fruit body production of *P. florida*.

CONCLUSION

This study was an effort to explore a suitable, easily accessible, and cost-effective agro-industrial waste as a substrate for the cultivation of wild edible *P. florida*. Among the four substrates used for this experimental study, grass was found to be more suitable in term of productivity and biological efficacy as compared to other substrates, although large-scale production is dependent on substrate availbility. In addition, this study may also aid in having a positive impact on economy generation of the small-scale farmers in this region.

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