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Substrate dependent growth optimization of *Pleurotus florida* mushroom on inexpensive substrates

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Abstract

The oyster mushroom (*Pleurotus florida*) is one of the cultivated edible mushrooms and has certain medicinal properties as well as economic and ecological values. Every year, massive amounts of ligno-cellulosic agricultural crop residues are produced worldwide, and not all of them are properly utilized. There are many ways of managing agricultural waste, one of which is mushroom cultivation. This study was conducted to compare the influence of selected consortiums of agricultural residues on the growth of *Pleurotus florida*. This species was cultivated on different combinations of substrates, viz., dried bread grass (*Brachiaria brizantha*), soybean straw, sawdust, rice straw, wheat straw, cotton straw, and sugarcane bagasse. Parameters such as height of stipe, girth of stipe, width of pileus, biological efficiency, and moisture content of fruiting bodies were evaluated. All the experiments were performed in triplicate. The supreme combination of substrates was found to be dried bread grass (8%), soybean straw (15%), saw dust (25%), rice straw (20%), wheat straw (15%), cotton straw (10%), and sugarcane bagasse (7%). This combination has given the best yield in terms of mean fresh weight of fruiting bodies (56.5 g), height of stipe (3.86 cm), girth of stipe (18.33 mm), and width of pileus (5.60 cm). The maximum biological efficiency was obtained 75.55%.

Keywords: Agricultural residues; Biological efficiency; Mushroom; *Pleurotus florida*; Spawn

1. Introduction

Unemployment has drastically increased in the post-lockdown period after the COVID-19 pandemic [1]. Most of the people in India are dependent on farming wages [2]. Considering the current scenario, mushroom farming could be the best option for farmers and entrepreneurs for setting up their new pilot scale business along with routine activities, or they can run this business as a side business [3, 4]. Agricultural wastes like bread grass, soybean husk, green gram husk, lentil husk, wheat husk, etc. are often produced during farming activities and can be utilised as inexpensive substrates for mushroom cultivation [5, 6, 7, 8, 9, 10]. As a result, the farmers will not need to rely on any other major outsourced substrates. This would be an effective bioconversion technology for transforming wastes into potentially valuable resources and could also be an important part of sustainable agriculture development [11, 12, 13]. Thus, mushroom cultivation will undoubtedly assist entrepreneurs in improving their current economic situation. Mushroom cultivation and its farming have tremendous scope since edible species of mushrooms like *Pleurotus florida* exhibit nutritional and medicinal properties [14, 15]. Mushrooms are rich sources of proteins, fibers, amino acids, vitamins, and minerals [16]. Mushrooms are known to be wonderful sources of nutraceuticals, antioxidants, anticancer, immunomodulating, anti-inflammatory, cardiovascular, antimicrobial, and antidiabetic agents [3, 17].

In fact, mushroom cultivation technology is a scientific art because it is unlike seed sowing and growing the plant; rather, mushroom cultivation technology requires monitoring and maintaining environmentally controlled conditions [18, 19]. The substrates should be sterilised to remove contaminants that could harm the fruiting bodies and adversely affect the

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yield [20]. Moisture retention is a critical factor on which 90 percent of the yield is dependent. To provide quality mushrooms to consumers, post-harvest preservation is required prior to selling [21, 22]. The used substrates could be useful for composting and biofertilizer formulations [23, 24]. This is a good approach to bioconversion technology in a sustainable environment [25].

In the present investigation, various consortiums of selected agricultural residues were prepared, and their influence on the growth of *Pleurotus florida* was evaluated.

2. Material and methods

2.1. Arrangement of minimum required infrastructure for oyster mushroom cultivation

Necessary chemicals, equipment and instruments (such as the CentriFog humidifier), consumables, and accessories related to the project were purchased. Infrastructure was arranged with racks, ropes, a ventilation system, and sensors for temperature and humidity monitoring [18].

2.2. Purchasing and processing of the selected agro-industrial residues

Selected agro-industrial residues, viz. dried bread grass (*Brachiaria brizantha*), soybean straw, sawdust, rice straw, wheat straw, cotton straw, and sugarcane bagasse, were purchased from the local farmer and market. Except for sawdust, all substrates were dried, cut into fine pieces, and pulverised with a grinder (Remi make, Mumbai) [8, 9, 17].

2.3. Procurement of spawn sample and development of pure culture

Pleurotus florida spawns were purchased from Peeper Agro Industry, Vimanapura, Bengaluru, India, for the first time, and then its pure culture was obtained by repetitively sub-culturing on potato dextrose agar plates (pH 7.2) [26]. Morphological and microscopic characteristics of *Pleurotus florida* were recorded [22, 27]. Healthy and fresh wheat seeds weighing 200 g were selected for spawn preparation. Seeds were washed for 2–3 times, and excess water was decanted. Fresh water was added and put on the stove to boil for 25 to 30 minutes. Excess water was removed after boiling, and the seeds were allowed to air dry in the shade for 2 to 3 hours. Calcium carbonate (lime) (1.5%) and gypsum ($CaSO_4\ 2H_2O$) (0.5%) were added and mixed carefully with the seeds. Seeds were transferred into conical flasks, sealed with cotton plugs, and sterilised at 121 °C for 20 minutes in an autoclave. The suspension culture of *Pleurotus florida* was mixed aseptically in the seeds. All the flasks were incubated at 20 to 25 °C for 7 to 10 days [21, 28].

2.4. Substrate dependent growth optimization of oyster mushroom

Among the many factors involved in mushroom cultivation, substrate dependent growth optimization is the most critical step. The influence of different substrates on the growth of *Pleurotus florida* was evaluated by formulating various consortiums of selected substrates, viz. dried bread grass (*Brachiaria brizantha*) (X_1), soybean straw (X_2), saw dust (X_3), rice straw (X_4), wheat straw (X_5), cotton straw (X_6) and sugarcane bagasse (X_7) [29]. These substrates have been reported in the literature for the growth of *Pleurotus* spp. However, no scientific study was conducted to evaluate the effects of these substrates in consortium on the growth of *Pleurotus florida* [30,31,32]. The selected substrates were mixed to form a specific consortium design as given in Table 1, and thus a total of 15 formulations were prepared. Each formulation was soaked in fresh water for 8 hours individually. The excess water was drained. Each formulation was individually packed in a piece of cloth and sterilized in an autoclave at 121 °C for 20 min. The sterilized contents of the cloths were opened in a chamber of laminar air flow. An equal quantity of spawns was added while filling the sterilised substrates into each polypropylene bag of 100-gauge thickness. In this way, all the bags were packed and tied with pieces of thread. Then, with the tip of a dull pen, uniform holes were made in all the bags. The holes were approximately 2-3 mm in size. Bags were incubated at 28 °C and a relative humidity of 80-85% was maintained in the cropping room under dark conditions until a complete spawn run was achieved [28].

Table 1 Formulation of different consortiums of selected substrates for evaluation of their impact on the growth of *Pleurotus florida*

Consortium/Formulation number	Substrate mixture ratio						
	X_1 (%)	X_2 (%)	X_3 (%)	X_4 (%)	X_5 (%)	X_6 (%)	X_7 (%)
1	100	0	0	0	0	0	0
2	0	100	0	0	0	0	0
3	0	0	100	0	0	0	0
4	0	0	0	100	0	0	0
5	0	0	0	0	100	0	0
6	0	0	0	0	0	100	0
7	0	0	0	0	0	0	100
8	14.29	14.29	14.29	14.29	14.29	14.29	14.29
9	8	15	25	20	15	10	7
10	20	25	15	8	7	10	15
11	25	15	8	20	15	7	10
12	3	12	17	32	16	9	11
13	13	11	15	12	7	3	39
14	10	10	10	10	10	40	10
15	7	10	15	20	25	15	8

Each attempt has been performed in triplicate (n = 3). Arithmetic mean and standard deviation were calculated using MS-Excel software.

2.5. Harvesting of mushroom and Measurement of selected parameters

The fruiting bodies of *Pleurotus florida* were harvested by twisting to uproot them from the base. The yield of *Pleurotus florida* in terms of the, fresh weight of a fruiting body, height of stipe, girth of stipe, and width of pileus was recorded by using either thread or a Vernier caliper. The maximum girth of a stipe was considered to be 100%, and the relative girth of a stipe was calculated for each consortium. The maximum recorded width of pileus was assumed to be 100%, and the relative width of pileus was calculated for the mushrooms from each consortium. The biological efficiency (BE) for each substrate consortium was calculated as follows [28, 33, 34].

$$\text{Biological efficiency (\%)} = (\text{Fresh weight of mushroom} / \text{Dry weight of substrate}) \times 100$$

The greatest biological efficiency was considered to be 100%, and the relative biological efficiency was calculated for each consortium.

3. Results and discussion

3.1. Development of pure culture and spawn preparation

Colonies of *Pleurotus florida* appeared to be white, filamentous, and circular (Fig. 1). The mean colony diameter was 2.2 cm. The spawns of *Pleurotus florida* prepared in a mushroom cultivation laboratory are shown in Fig. 2.

Figure 1 Colonies of *Pleurotus florida* on potato dextrose agar (PDA)

Figure 2 Spawns of *Pleurotus florida* prepared in mushroom cultivation laboratory

3.2. Substrate dependent growth optimization of *Pleurotus florida*

The mean height of the stipe was 3.86 cm (Fig. 3). The mean girth of the stipe was 18.33 mm. Relative girth of the stipe was greatest on consortium number 9, followed by consortium numbers 12, 10, 8, 11, 13, 5, 3, 14, 2, 7, 6, and 1. Relative girth of the stipe was found to be the same in consortium numbers 11 and 15 (81.82%) and in consortium numbers 2 and 4 (72.73%) (Fig. 4). The mean width of the pileus was 5.60 cm. Relative pileus width of *Pleurotus florida* was recorded greatest on consortium number 9, followed by 14, 12, 10, 11, 8, 2, 6, 1, 5, 3, and 4. Relative pileus width was found to be the same in consortium numbers 10, 13, and 15 (92.86%) and consortium numbers 6 and 7 (66.07%) (Fig. 5). Maximum biological efficiency was obtained 75.55%. Relative biological efficiency was recorded as being greatest on consortium number 9, followed by 13, 14, 12, 11, 10, 8, 15, 4, 3, 6, 5, 2, 1, and 7 (Fig. 6). Selected images of grown mushrooms are displayed in Figs. 7 and 8.

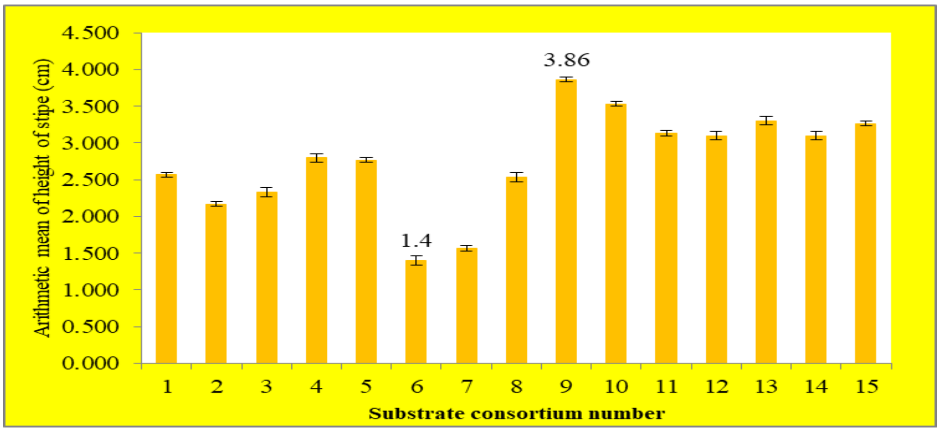

Figure 3 Measurement of mean height of stipe of *Pleurotus florida* grown on different consortiums of substrates

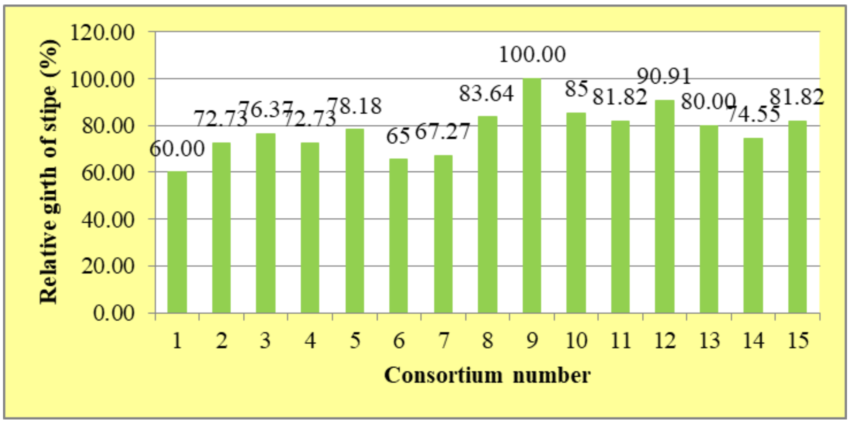

Figure 4 Measurement of relative girth of stipe of *Pleurotus florida* grown on different consortiums of substrates

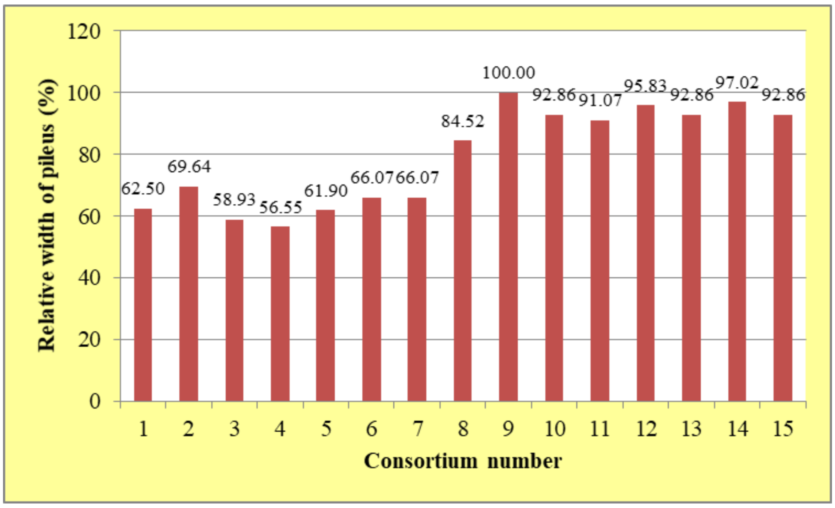

Figure 5 Measurement of relative width of pileus of *Pleurotus florida* grown on different consortiums of substrates

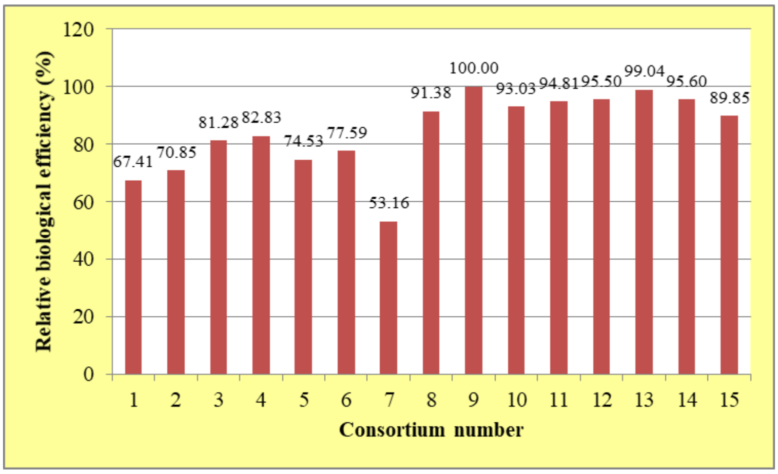

Figure 6 Measurement of relative biological efficiency of *Pleurotus florida* grown on different consortiums of substrates

Figure 7 Fruiting body of *Pleurotus florida* cultivated on consortium no. 9

Figure 8 The basal portion of *Pleurotus florida*'s exterior

The results showed that the changes in stipe length, pileus width, and stipe girth of *Pleurotus florida* grown on different consortiums depended on the types of substrates used to formulate each consortium. The highest mean fresh weight was obtained 56.5 g for the giant fruiting bodies grown on consortium no. 9, indicating the best performance level of the combination of the selected substrates when compared with other consortiums. Similarly, high values of stipe length, pileus width, and stipe girth were obtained from consortium no. 9. Diana et al. [35] reported that agricultural wastes make the most ideal form of materials needed for substrate formulation and that nearly all types of agricultural wastes are useful for mushroom production. The different agricultural wastes contain different types and amounts of nutrients and minerals; however, cellulose, hemicellulose, and nitrogenous compounds are the main biomolecules required by the oyster mushroom for its growth and fructification. As a result, in the current study, consortiums containing mixed substrates supported the growth and fructification of *Pleurotus florida* well. On the other hand, the use of single agricultural waste has aided mushrooms' relatively slow growth and low efficiency. This is in agreement with Chukwurah et al. [36].

4. Conclusion

Among all the designed consortiums, the consortium no. 9 composed of dried bread grass (8%), soybean straw (15%), saw dust (25%), rice straw (20%), wheat straw (15%), cotton straw (10%), and sugarcane bagasse (7%) has given the best yield of *Pleurotus florida* in terms of the, mean fresh weight of fruiting bodies 56.5 g, mean height of stipe 3.86 cm, mean girth of stipe 18.33 mm, and mean width of pileus 5.60 cm. The maximum biological efficiency was obtained 75.55%.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares that there is no conflict of interest.

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Author’s short Biography

Dr. Mukundraj Govindrao Rathod (Ph.D. & MH-SET) At Yeshwant College of Information Technology in Parbhani, Maharashtra, India, Dr. Mukundraj G. Rathod (Ph.D. in Biotechnology & MH-SET) is the in-charge Principal. In addition, he heads this college's Biotechnology and Bioinformatics department. More than 77 research papers, including review articles in various peer-reviewed international and national journals and proceedings of conferences in various research fields, have indeed been published by the author. He has 300 citations in Google Scholar, an i10 index of 10, and a H index of 10, which is impressive. He has an amazing H index of 4, with 31 Scopus citations. He had given oral talks and posters at numerous conferences and seminars. He has also reviewed publications for a number of reputable scientific publishers. He had donated seven crucial industrial cultures to the National Center for Cell Science's Microbial Culture Collection in Pune, Maharashtra, India, for use by the general public. He is currently the lead researcher on a study supported by Swami Ramanand Teerth Marathwada University in Nanded through the Rajiv Gandhi Science and Technology Commission's application scheme for science and technology (Government of Maharashtra). According to the AD Scientific Index 2023, he recently held the 17th rank among the top 20 scientists at Swami Ramanand Teerth Marathwada University, Nanded, and its jurisdiction.