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Original Article

Effect of agricultural substrates mixture on yield and growth performance of two different species of *Pleurotus*

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Abstract:

Mushroom cultivation provides a sustainable way to manage agricultural waste by utilizing organic residues as substrates. This approach not only promotes recycling but also improves agricultural productivity by reusing available resources. This study examines the growth and yield performance of *Pleurotus florida* and *P. ostreatus* on three substrates such as paddy straw (A), banana leaves (B), and a 1:1 mix of both (C). *Pleurotus florida* colonizes the substrate faster, while *P. ostreatus* outperforms in yield, biological efficiency, and organic mass loss. Both species produced higher yields on substrates B and C than on A. Moreover, both species reduced substrate pH effectively. *Pleurotus ostreatus* acidified the substrate more efficiently than *P. florida*. The findings emphasize the importance of selecting appropriate substrates to optimize oyster mushroom cultivation, improving both yield and substrate utilization efficiency.

Key words:
oyster mushroom, agriculture waste, paddy straw, banana leaves

Apstrakt:

Efekat mešavine poljoprivrednih supstrata na prinos i performanse rasta dve različite vrste roda *Pleurotus*

Gajenje pečuraka predstavlja održiv način upravljanja poljoprivrednim otpadom korišćenjem organskih ostataka kao supstrata. Ovaj pristup ne samo da podstiče reciklažu, već i poboljšava poljoprivrednu produktivnost ponovnim korišćenjem dostupnih resursa. Ova studija ispituje rast i performanse prinosa vrsta *Pleurotus florida* i *P. ostreatus* na tri supstrata: pirinčana slama (A), listovi banane (B) i mešavina ova dva supstrata u odnosu 1:1 (C). *Pleurotus florida* brže kolonizuje supstrat, dok *P. ostreatus* daje bolje rezultate u pogledu prinosa, biološke efikasnosti i gubitka organske mase. Obe vrste su produkovale veće prinose na supstratima B i C u poređenju sa supstratom A. Pored toga, obe vrste su efikasno smanjile pH supstrata, s tim što je vrsta *P. ostreatus* to postigla efikasnije od vrste *P. florida*. Rezultati naglašavaju važnost odabira odgovarajućih supstrata za optimizaciju uzgoja bukovača, poboljšanje prinosa i efikasnost korišćenja supstrata.

Ključne reči:
bukovača, poljoprivredni otpad, pirinčana slama, listovi banane

Introduction

Mushroom cultivation has gained significant attention in recent years due to its potential for sustainable food production, waste recycling, and health benefits. Among the various species cultivated, *Pleurotus florida* and *P. ostreatus* are particularly popular due to their rapid growth, high nutritional value, and adaptability to a wide range of substrates. These oyster mushrooms are known for their unique flavors, medicinal properties, and economic importance, making them valuable in both culinary and medicinal markets.

Oyster mushrooms are esteemed as a food source today not only for their unique flavor and texture (Mowsurni & Chowdhury, 2010). Individuals are increasingly prioritizing their dietary choices and physical activity in contemporary society (Tagde et al., 2021). Beneficial chemical, nutritional, and therapeutic attributes (Sifat et al., 2020) as well as their associated health benefits add more interest towards oyster mushrooms (Roy et al., 2015). *Pleurotus* has emerged as the most widely consumed and favored mushroom due to its appealing taste, distinctive flavor, substantial nutritional profile, and therapeutic benefits. These fungi provide a healthy alternative that contains minimal calories, fats, and sugars, yet offers a wealth of fiber, protein, chitin, and vital minerals.

Agricultural waste, consisting of residues left after crop harvest and processing, represents a significant and often underutilized resource in many farming systems. This waste includes straw, husks, leaves, stalks and other plant materials that are typically discarded or burned, contributing to environmental pollution and greenhouse gas emissions (Iscia & Demirer, 2007). However, these agricultural wastes hold considerable potential as substrates for mushroom cultivation. By converting agricultural waste into a growth medium for mushrooms, farmers can enhance the sustainability of their operations, reduce waste disposal costs and generate an additional source of income (Rachna & Sodhi, 2013).

Understanding the growth performance, morphological characteristics, and substrate interactions of these species is crucial for optimizing cultivation practices and maximizing yield. This study focuses on comparing *P. florida* and *P. ostreatus* across two substrates such as paddy straw and banana leaves. The study also delves into the morphological aspects of the mushrooms, examining pileus and stipe dimensions, which are important for marketability and consumer preference. Additionally, the changes in substrate pH provide insights into the metabolic activities of the fungi and their ability to utilize and degrade the substrate efficiently.

Materials and Methods

Collection of Agricultural Waste Material

Agro-waste materials such as paddy straw and banana leaves were used for the present study. Paddy straw and banana leaves were collected from the farmers. These substrates were dried and stored for further use.

Purchase of spawn

Sorghum grain-based spawns of *P. florida* and *P. ostreatus* were procured from the Indian Institute of Horticulture Research, Bangalore and used for the experiment.

Experimental Design

Selected species were cultivated by bag method using two different agro-waste as substrates.
Substrate A: Paddy straw
Substrate B: Banana leaves
Substrate C: 1:1 ratio of paddy straw and banana leaf

Cultivation of mushrooms

In the present study, selected edible oyster mushrooms were cultivated by following the standard procedure of Tamil Nadu Agricultural University. The selected agro wastes were cut into small pieces (6-10 cm) and soaked in water for 12–14 h. The soaked substrates were sterilized at 121 °C for 20-30 min by using an autoclave and excess amount of water content was removed. Before starting the packing of mushroom growing bags, hands were washed thoroughly with the help of antiseptic lotion. A polypropylene bag with a size of 60×30 cm and with a thickness of 80 gauge was used for the cultivation. The bottom end of the bag was tied with the help of thread and turned toward the inside. The sterilized substrate was filled in the bag to a height of 3 inches. A handful of grain-based spawn was sprinkled over the layer. Likewise, a few layers were placed on the bag. Finally, the bag was pressed gently and tied with a thread. A few holes were made in the bags to facilitate ventilation. The spawned bags were kept in a dark room for 1 week to facilitate the spawn running and colonization. Then the bags were transferred to the cropping room. The optimum temperature of 22 °C to 25 °C and required humidity of 85% was maintained by spraying water on the walls of the mushroom unit four to five times a day. After colonization, the mycelium starts to produce its reproductive structure called fruiting bodies. Initially, it looked like a pinhead, and it was transformed into a fully matured fruiting body within two days. After maturity, the pileus edges start to shrink towards the inside. At this stage, the fruiting bodies were collected manually and used for further experiments.

Growth parameters

Colonizing period (spawn run): Colonizing period, also known as pawn running or mycelium running, is a crucial phase in mushroom cultivation. The spawn run period is the time span from spawning to the first appearance of pinheads, indicating that the mycelium has fully colonized the substrate (Pandey et al., 2023). The number of days required for the colonization of fungal mycelium is counted from the day of packing.

Pinheads and fruitbodies developed: Pinheads and fruiting bodies developed on the substrate were counted manually.

Yield: After the maturation, the fruitbodies were handpicked and immediately weighed.

Biological efficiency (Carvalho et al., 2012): Biological efficiency is a term frequently used in the mushroom industry to describe the potential of the macro fungus to yield a fruiting body (mushroom) from a known dry weight of substrate.

Biological efficiency (%) = (Fresh weight of mushroom/Dry weight of the substrate) × 100

Organic mass loss (Carvalho et al., 2012): The organic mass loss of the substrate was calculated by using the following formula.

Organic mass loss (%) = (Initial substrate dry mass – residual/Initial substrate dry mass) × 100

Morphological parameters: Length and width of stipe and pileus were measured immediately after harvesting with the help of thread and measuring scale.

pH analysis of the substrate (Jackson, 1965): The pH of the substrates was determined using a digital pH meter. Ten grams of sample were taken in 100 ml distilled water and stirred continuously for 30 min with a glass rod. The pH of the suspension was recorded after half an hour of settling by the pH meter pre-calibrated using standard buffers of pH 4.0, 7.0 and 9.0.

Results and discussion

Table 1 presents the comparative data on the growth and yield of two species of mushrooms, *P. florida* and *P. ostreatus*, grown on three different substrates labelled A, B, and C. The key metrics provided include the colonizing period, yield, number of pinheads, percentage of pinheads that develop into fruiting bodies, biological efficiency and organic mass loss.

Table 1. Effect of different substrate on the growth, morphology and yield performance of *Pleurotus florida* and *Pleurotus ostreatus*

Species	Substrates	Spawn run period (Days)	Pinhead Initiation (Days)	Yield (gram)	No. of pin heads	Fruiting body Developed From Pinheads (%)	Biological Efficiency (%)	Organic Mass Loss (%)
P. florida	A	18	23	425	70	49.5	85	30
	B	21	26	460	55	64.2	92	35
	C	21	24	445	59	71.2	89	32
P. ostreatus	A	24	29	510	51	55.2	102	38
	B	27	31	565	42	53. 9	113	43
	C	26	31	571	47	63.8	114.2	42

Colonizing period (spawn run) and pinhead initiation

During this phase, the mushroom mycelium, which is the vegetative part of the fungus, colonizes the substrate. The process begins with the inoculation of the substrate with mushroom spawn. The spawn contains mycelium, which acts similarly to seeds in plants. For *Pleurotus* species spawn run duration differs depending on the species type and substrate used.

Pleurotus florida consistently shows a shorter spawn run period compared to *P. ostreatus* across all substrates, with the shortest time on Substrate A (18 days) and the longest on Substrates B and C (21 days). *Pleurotus ostreatus* has a longer spawn run period, ranging from 24 days on substrate A to 27 days on substrate B. Pinhead initiation is the stage where the first mushroom primordia or „pinheads" emerge. It is a critical phase in mushroom cultivation, signifying the transition from vegetative growth to the fruiting stage. This phase is pivotal because it directly influences the subsequent development and yield of the mushrooms. In the current study, *P. florida* demonstrated faster pinhead initiation across all substrates compared to *P. ostreatus*. For *P. florida*, pinheads appeared after 23 days on Substrate A, 26 days on substrate B, and 24 days on substrate C. In contrast, *P. ostreatus* exhibited a longer time to pinhead initiation, with 29 days on substrate A, and 31 days on both Substrates B and C. These differences highlighted the quicker adaptation and readiness of *P. florida* to fruit across various substrates, which could be advantageous for growers seeking shorter production cycles. Understanding the factors that influence pinhead initiation, such as substrate composition, environmental conditions, and species-specific growth characteristics, is essential for optimizing cultivation practices to maximize yield and efficiency. Our results were found to be similar to the results of Pandey et al. (2023) who reported colonization and pin head formation around 20 to 28 days in which banana leaves take maximum days. The faster mycelium growth rate in rice straw can be linked to its balanced composition of alpha cellulose, hemicellulose, and lignin (Mondal et al., 2010). The time required for pinhead formation varies based on the substrate and is affected by factors like moisture content and nutrient availability (Iqbal et al., 2016; Muswati et al., 2021), a trend reflected in our study as well. The differences in pinhead formation times across various substrates can be explained by the variations in nutrient and moisture availability.

Number of pinheads and percentage of fruitbodies developed from pinheads

In terms of the number of pinheads, *P. florida* outperforms *P. ostreatus* across all substrates. The higher number of pinheads for *P. florida* suggests a greater initial fruiting potential, which could lead to higher overall yields if a significant proportion of these pinheads develop into mature fruiting bodies.

The conversion of pinheads into fruiting bodies, however, presents a more nuanced picture. On Substrate A, *P. ostreatus* has a higher conversion rate of 55.2%, indicating that a larger proportion of its initial pinheads develop into mature fruiting bodies compared to *P. florida,* which has a conversion rate of 49.5%. Conversely, on Substrate B, *P. florida* demonstrates a superior conversion rate of 64.2%, significantly higher than the 53.9% observed for *P. ostreatus*. This trend is further accentuated in Substrate C, where *P. florida* achieves a conversion rate of 71.2%, outperforming *P. ostreatus,* which has a conversion rate of 63.8%.

Yield, Biological Efficiency and Organic Mass Loss

When comparing the yield, *P. ostreatus* performs better than *P. florida* across all substrates. For Substrate A, *P. ostreatus* yields 510 grams, whereas *P. florida* produces 425 grams. This trend continues with Substrate B and C. This data highlights *P. ostreatus* as the more productive species in terms of yield across all tested substrates.

Biological efficiency, which measures the ratio of the weight of the harvested mushrooms to the weight of the dry substrate, expressed as a percentage, also shows a clear advantage for *P. ostreatus.* On Substrate A, B and C *P. ostreatus* achieves a greater biological efficiency than *P. florida*. These results indicate that *P. ostreatus* is more efficient in converting substrate into mushroom biomass.

Organic mass loss, indicating the percentage of organic matter in the substrate that is consumed during the cultivation process, varies between the two species. *P. ostreatus* tends to have higher organic mass loss across all substrates, which suggests a more extensive utilization of the substrate. The higher organic mass loss for *P. ostreatus* could correlate with its higher yield and biological efficiency, reflecting a more complete breakdown and utilization of the substrate components.

Pleurotus ostreatus demonstrates superior performance in terms of yield, biological efficiency, and organic mass loss compared to *P. florida* across all substrates. *Pleurotus ostreatus* yields more mushrooms, exhibits higher biological efficiency, and incurs greater organic mass loss, indicating a more effective conversion of substrate into biomass. Thomas et al. (2022) determined the biological efficiency of *P. ostreatus* which falls between 54.5% and 130.9%. The findings of Neupane et al. (2018) also reported highest yield on banana leaf substrates than rice straw, wheat straw, rice straw + wheat straw and sawdust. Our results didn't agree with the results of Mahat et al. (2022) who reported lowest yield in banana leaf substrates and the reason might be the use of semi-dried leaves in their experiment which contain low lignocellulose to hydrolyze into sugar. Tirkey et al. (2017) reported highest yield with a biological efficiency of 91% in the banana leaf substrates.

Length and width of pileus and stipe

Pleurotus florida and *P. ostreatus* exhibit different trends in pileus dimensions across the substrates (**Tab. 2**). On Substrate A, *P. florida* shows a slightly larger pileus length (6.9 cm) and width (7.7 cm) compared to *P. ostreatus* (6.3 cm length, 7.2 cm width). Substrate B results in *P. ostreatus* having a slightly longer pileus (6.5 cm) than *P. florida* (6.2 cm), but *P. florida* maintains a broader pileus width (7.4 cm vs. 7.9 cm for *P. ostreatus*). On Substrate C, both species show increased pileus dimensions, with *P. ostreatus* having a slightly longer pileus (6.9 cm) compared to *P. florida* (6.6 cm), while *P. florida* has a wider pileus (9.2 cm) compared to *P. ostreatus*

Table 2. Length and width of the pileus and stipe

Treatment	*Pleurotus florida*				*Pleurotus ostreatus*			
	Pileus		Stipe		Pileus		Stipe	
	Length (cm)	Width (cm)	Length (cm)	Width (cm)	Length (cm)	Width (cm)	Length (cm)	Width (cm)
A	6.9±0.3	7.7±0.4	3.4±0.4	1±0.4	6.3±0.4	7.2±0.3	2.8±1.1	1.7±0.4
B	6.2±0.5	7.4±0.8	3.1±0.2	1.1±0.3	6.5±0.6	7.9±0.5	1.9±0.9	1.5±0.5
C	6.6.0±0.9	9.2±1.2	2.7±0.2	1.5±0.3	6.9±0.4	8.1±0.4	2.2±0.5	1.8±0.2

(8.1 cm).

Regarding the stipe dimensions, *P. florida* shows longer stipe lengths across all substrates compared to *P. ostreatus*. In terms of stipe width, *P. ostreatus* generally has a broader stipe across all substrates. *Pleurotus florida* generally shows longer pileus lengths and widths on Substrates A and C compared to *P. ostreatus*, while *P. ostreatus* demonstrates slightly longer pileus lengths on Substrate B and consistently broader pileus widths across all substrates. For stipe dimensions, *P. florida* consistently exhibits longer stipe lengths, whereas *P. ostreatus* shows greater stipe widths across all substrates. These morphological differences can influence the visual appeal and marketability of the mushrooms, as well as their suitability for different culinary applications. Our report corroborated with the findings of Dubey et al. (2019) who reported highest stipe length in rice straw substrates.

pH changes in substrates

During the mushroom cultivation process, significant changes in substrate pH were observed for both species (**Tab. 3**). On Substrate A, the initial pH of 6.3 was reduced to 5.2 by *P. florida* and to 5.5 by *P. ostreatus*, indicating that *P. florida* was more effective in acidifying the substrate. For Substrate B, which had an initial pH of 5.2, both species lowered the pH, with *P. florida* achieving a final pH of 4.6 and *P. ostreatus* reaching 4.5, showing that *P. ostreatus* had a slightly greater capacity to reduce the pH. On Substrate C, starting with an initial pH of 5.6, P. *florida* decreased the pH to 5.1, while *P. ostreatus* reduced it further to 4.9, highlighting the superior ability of *P. ostreatus* to acidify the substrate. These results demonstrate the impact of both mushroom species on substrate pH, with *P. ostreatus* generally achieving lower pH levels compared to *P. florida*. The pH of the substrate plays a crucial role in oyster mushroom cultivation. The reduction in pH may be due to the metabolic activity and decomposition process of the substrate by the fungus. Research conducted by Zubairi et al. (2022) found that the pH values of the substrate before spawning were between 8 and 9, which dropped to between 4 and 6 after spawning, demonstrating a significant reduction and acidification of the substrate.

Table 3. pH changes in the substrate used for *Pleurotus florida* and *Pleurotus ostreatus*

Substrate	Initial pH	Final pH	
		P. florida	*P. ostreatus*
A	6.3	5.2	5.5
B	5.2	4.6	4.5
C	5.6	5.1	4.9

Conclusion

This study highlights the effectiveness of paddy straw, banana leaves and their combinations in optimizing the growth, yield, and biological efficiency of *P. florida* and *P. ostreatus*. *Pleurotus ostreatus* demonstrated higher yield and biological efficiency, while *P. florida* exhibited faster growth rates, making both species viable for different cultivation goals. The results underscore the potential of using readily available agricultural waste to reduce costs and promote sustainable practices. Future research should focus on refining substrate combinations and investigating more agricultural residues to improve yield, growth efficiency and improved nutrient content. Additionally, understanding the genetic mechanisms behind substrate utilization could lead to even more effective cultivation techniques. Farmers are encouraged to explore local agricultural waste as substrates, particularly rice straw and banana leaves which showed promising results in this study. Adopting these sustainable cultivation methods can lower production costs, increase yields, and contribute to environmental conservation. Farmers should also monitor moisture and nutrient levels in substrates to optimize mushroom growth and maximize their economic returns.

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