



RESEARCH ARTICLE

Optimising growth and yield of oyster mushroom (*Pleurotus florida* Mont. Singer) using different substrate types and spawning rates

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Abstract

The IHRPF-1 strain of oyster mushroom (*Pleurotus florida* Mont. Singer) is gaining popularity in India due to its distinctive flavour, high nutritional value and increasing consumer preference, often surpassing the commonly cultivated button mushroom (*Agaricus bisporus*). Optimisation of cultivation parameters, particularly substrate type and spawn rate, is essential for improving growth, fruiting and yield. This study evaluated the growth performance and yield of *P. florida* on three different substrates rice straw, powdered rice straw and sawdust using three spawn rates (40, 50 and 60 g kg⁻¹ of dry substrate). Cultivation was carried out under controlled conditions with relative humidity maintained at 57–70 % through a sprinkler system to ensure favourable conditions for pinhead initiation and fruiting body development. Among the tested combinations, rice straw at a spawn rate of 50 g kg⁻¹ produced the highest yield of 193.33 g kg⁻¹ dry substrate and the highest biological efficiency of 50.09 %. This treatment also resulted in the earliest pinhead initiation, the most rapid fruiting body formation and the shortest interval between successive flushes. The findings indicate that rice straw combined with a moderate spawn rate not only optimises growth and yield but also ensures efficient and sustainable mushroom production. Furthermore, this approach promotes the utilisation of agricultural residues, providing an environmentally friendly strategy for mushroom cultivation. These results offer practical insights for small and large-scale farmers aiming to improve the productivity and profitability of *P. florida* cultivation.

Keywords: biological efficiency; oyster mushroom cultivation; *Pleurotus florida*; spawn rate; substrate optimisation

Introduction

Mushrooms, classified as horticultural crops, are increasingly recognised as a progressive and sustainable component of Indian agriculture, owing to their low input requirements, high market value and efficient recycling of agricultural residues (1, 2). Their exceptional nutritional and medicinal properties have generated growing interest in both domestic and international markets. Fresh mushrooms contain 93–95 % water, higher than most vegetables (~92 %) and lean meat (~70 %) and provide moderate protein (1.5–3.0 g/100 g fresh weight), low fat (0.3–0.4 g) and 1.5–6.7 g carbohydrates, with 5.3–14.8 g dry matter. On a dry weight basis, mushrooms contain approximately 56 % carbohydrates, 30 % protein, 2 % fat and 10 % ash, values comparable to dried peas, beans and yeast (3, 4). Beyond nutrition, mushrooms exhibit various therapeutic benefits, including antioxidant, anti-cancer, anti-diabetic and cholesterol-lowering properties (5).

Mushrooms naturally proliferate in fields, forests, manure heaps, irrigation channels and hilly regions during the monsoon due to high humidity and rainfall (6). Globally, China dominates

production, accounting for approximately 93 % of total fresh mushrooms, followed by Japan and Poland (~0.01 % each), while India ranks sixth, producing ~0.24 million tons annually (7, 8). India's mushroom production has increased nearly tenfold over the past decade, with a compound annual growth rate (CAGR) of approximately 8 %, which is higher than the reported growth rate of 3.81 % for China during 2012–2021 (7). However, China continues to remain the largest global producer in terms of total production volume. Improved commercial strains and cultivation techniques have contributed to India's growing share of global mushroom production, rising from 7 % in 1970 to 25 % in 2022. Among Indian states, Bihar leads production (~35.6 kt), followed by Odisha, Maharashtra, Uttar Pradesh and Uttarakhand (8). Andhra Pradesh and Karnataka produced 3515 and 1180 tonnes, respectively, in 2017–18 (9).

White button mushrooms (*Agaricus bisporus*), oyster mushrooms (*Pleurotus* spp.), paddy straw mushrooms (*Volvariella volvacea*), shiitake (*Lentinus edodes*) and black ear mushrooms (*Auricularia auricula-judae*) are the most widely cultivated species

globally (6). Among these, *A. bisporus* holds the largest market share (70 %), followed by oyster mushrooms (17 %) and paddy straw mushrooms (9 %) (8, 10). Oyster mushrooms are particularly valued for their biochemical composition, comprising 25–50 % protein, 2–5 % fat, 17–47 % sugars, 7–38 % micro cellulose and 8–12 % minerals (11). They contain approximately 90 % moisture and 10 % dry matter (12). Fresh mushrooms typically contain approximately 90 % moisture and 10 % dry matter on a fresh weight basis (12). Growth of oyster mushrooms is strongly influenced by environmental factors such as temperature, humidity, CO₂ concentration and light (13). Among winter-growing species, *P. florida* has gained preference due to its gourmet flavour and culinary appeal (14, 15) and was therefore selected for this study.

Traditionally, *Pleurotus* species are cultivated on lignocellulosic substrates such as rice straw, wheat straw, cottonseed hulls, corncobs, bagasse, water hyacinth, coffee pulp, cassava peels, oil palm fibre, paper and cardboard (4). India generates approximately 126.6 million tons of rice straw annually, a substantial fraction of the global total (731 Mt), yet only ~20 % is utilised for fodder, paper, or ethanol; the remainder is often burned or left to decompose, contributing to greenhouse gas emissions (16, 17). Recycling rice straw through mushroom cultivation offers an environmentally sustainable and economically viable alternative. Rice straw is particularly suitable due to its porosity, moisture retention and favourable chemical composition (18, 19).

Despite the availability of various lignocellulosic substrates for oyster mushroom cultivation, comparative evaluation of substrate type and spawning rate under controlled environmental conditions remains limited, particularly for *P. florida*. Optimising these factors is crucial for enhancing biological efficiency, reducing production time and improving economic returns. Therefore, the present study was undertaken to evaluate the influence of different substrate types and spawning rates on growth parameters, yield performance and biological efficiency of *P. florida* under controlled cultivation conditions.

Materials and methods

The experiment was conducted during 2023–24 at the Mushroom Research Laboratory, ICAR–Indian Institute of Horticultural Research (IIHR), Bengaluru, Karnataka, India (12.97° N, 77.64° E), under controlled laboratory and cropping-room conditions.

Substrate preparation and sterilization

For the rice straw substrate, straw was chopped into 30–50 mm pieces using a sickle or straw chopper, soaked in tap water for 2–3 hr, drained and sun-dried to approximately 65 % moisture content (wet basis) (20). The prepared straw was packed into 1 kg polypropylene (PP) bags (300 × 420 mm; 150-gauge thickness), fitted with a polypropylene/PVC ring and a non-absorbent cotton plug. These loosely packed bags were autoclaved at 121 °C and 1.5 kg/cm² pressure for 20 min and cooled for 4–5 hr before inoculation.

For rice straw powder and sawdust substrates, water was added at substrate-to-water ratios of 1:2.5 and 1:1.5, respectively and moisture was adjusted to 65 % (wet basis). The substrates were packed into 1 kg PP bags–250 × 410 mm (220-gauge) for rice straw powder and 200 × 405 mm (220-gauge) for sawdust–selected for their structural strength during prolonged sterilisation. Bags were sealed with the same ring–cotton plug setup. Rice straw

powder bags were autoclaved for 30 min, while sawdust bags required 90 min, followed by cooling for 4–5 hr (20, 21). Sterilisation duration varied due to substrate texture and bulk density: loose rice straw allowed faster heat penetration, whereas denser and more moisture-retentive rice straw powder and sawdust required extended sterilisation to minimise contamination risks (22–24).

Spawn inoculation

Spawn of *P. florida* was obtained from the Mushroom Research Laboratory, ICAR–IIHR, Bengaluru and prepared on sorghum grains that supported faster growth (6.55 days) than other grains, such as maize, wheat, barley, or rice (25). Spawning rates of 40, 50 and 60 g kg⁻¹ of dry substrate were tested across all three substrates. Inoculation was carried out aseptically. For manual inoculation, the required quantity of spawn was measured, the PP ring and cotton plug were removed, spawn was evenly spread on the substrate surface and the ring and plug were replaced (Fig. 1a). A mechanical inoculation method was also employed using a manually operated spawn-spreading machine comprising a conical hopper, dual ball-valve metering system and supporting frame (Fig. 1b). The hopper had a holding capacity of approximately 2–3 kg of spawn and enabled controlled and uniform discharge into substrate bags. The machine was locally fabricated using mild steel components and is commercially available through agricultural machinery suppliers. The approximate fabrication cost ranges between ₹ 20000 and ₹ 30000. The system facilitated faster and more hygienic inoculation compared to manual spreading, with an operational capacity of approximately 20–25 bags hr⁻¹, depending on operator efficiency. In this process, spawn was fed into the hopper, metered into a jar via the first valve, discharged into the PP bag using the second valve and the cotton plug was replaced. Mechanical inoculation was faster, more hygienic, reduced contamination risk and ensured more uniform spawn distribution compared to manual methods (26).

Three substrates–rice straw, rice straw powder and sawdust–were evaluated under the specified spawning rates. Growth parameters recorded included pinhead initiation period, fruiting body formation time, flush interval, total yield and biological efficiency. Cultivation was carried out using the standard 1 kg polypropylene bag method to ensure uniform handling, contamination control and consistent yield (19, 27). Additionally, mechanical and manual spawn inoculation techniques were compared to assess their influence on growth and yield performance.



Fig. 1. Manual and spawn spreading machine inoculation: **a.** Inoculation of mushroom spawn manually, **b.** Inoculation of mushroom spawn by a spawn spreading machine.

Cropping and watering method

Following spawn inoculation, bags were incubated in a spawn-running chamber maintained at 18–28 °C for 25–30 days until complete colonisation by white mycelium. Before shifting to the cropping room, holes were punctured in the polypropylene (PP) bags to allow fruiting body emergence. Cropping was carried out under controlled environmental conditions, with humidity maintained between 57–70 % using a sprinkler system that moistened the floor and indirectly supplied adequate moisture to the substrate bags. These conditions ensured favourable pinhead initiation and healthy fruiting body development across all substrate types (Fig. 2). The cropping cycle lasted 25–30 days, during which mature mushrooms were harvested regularly at the optimum stage.

Morphological parameters

To evaluate the growth and productivity of *P. florida*, a set of morphological and yield-related parameters were recorded during the experimental period. The following observations were systematically documented:

Pinhead appearance

The time interval from the date of spawning to the initial emergence of pinheads was recorded and expressed as mean days. Pinheads represent the early developmental stage of mushrooms, characterised by small, rounded structures resembling tiny pins or dots (28).

Fruiting body formation

The duration from pinhead initiation to the maturation of fruiting bodies was measured in days. Maturity was defined as the stage at which fruiting bodies were fully expanded and ready for harvesting, with a distinct pileus and stipe structure (29).

Period between flushes

The interval between successive flushes, i.e., from the first flush to the last flush, was computed to assess the cropping cycle dynamics. The period between flushes was determined using the formula described by (30):

$$\text{Period between flushes} = \frac{\text{Days taken from first harvest to last}}{\text{Total number of flushes (harvest)}} \quad (\text{Eqn. 1})$$

Total yield

The cumulative mushroom yield was obtained by harvesting, weighing and recording the fresh weight of fruiting bodies from each bag across three flushes. The mean yield kg^{-1} of dry substrate was calculated to enable comparison among treatments (31).

Biological efficiency

Biological efficiency (BE), which indicates the conversion efficiency of substrates into fruiting bodies, was expressed as the ratio of the total fresh mushroom yield to the dry weight of the substrate, multiplied by 100. It was calculated using the formula described by (31):

$$\text{B.E (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100 \quad (\text{Eqn. 2})$$

Statistical analysis

The experiment was laid out in a two-factorial Completely Randomized Design (CRD) to evaluate the effects of substrate type and spawning rate on the growth and yield of *P. florida*. Each treatment combination was replicated three times ($n = 3$). The data were subjected to analysis of variance (ANOVA) to test the significance of treatment differences, following the procedure described in (32, 33). The statistical model used for the two-way ANOVA was:

$$Y_{ijk} = \mu + S_i + R_j + (SR)_{ij} + \epsilon_{ijk} \quad (\text{Eqn. 3})$$

Where μ represents the overall mean, S_i the effect of substrate type, R_j the effect of spawning rate, $(SR)_{ij}$ the interaction effect and ϵ_{ijk} the experimental error. Statistical significance was tested at $p \leq 0.05$. All statistical computations were carried out using Minitab® Statistical Software (Minitab 21.2, Minitab LLC, USA).

Results

Pinhead appearance and fruiting body formation

The time required for pinhead appearance decreased significantly with increasing spawning rate ($p = 0.0333$), with the earliest initiation observed at 60 g kg^{-1} (Fig. 3). Substrate type also showed a strong influence ($p < 0.0001$), with rice straw consistently supporting the fastest pinning. The porous structure of rice straw enhances aeration and enzymatic activity, whereas powdered straw delays pinning due to poor porosity and gas exchange. Fruiting body formation followed a similar trend (Fig. 3). Spawning rate



Fig. 2. Fruiting body emergence of oyster mushroom (*Pleurotus florida*) on different substrate types: **a.** Rice straw substrate, **b.** Rice straw powder substrate, **c.** Sawdust substrate.

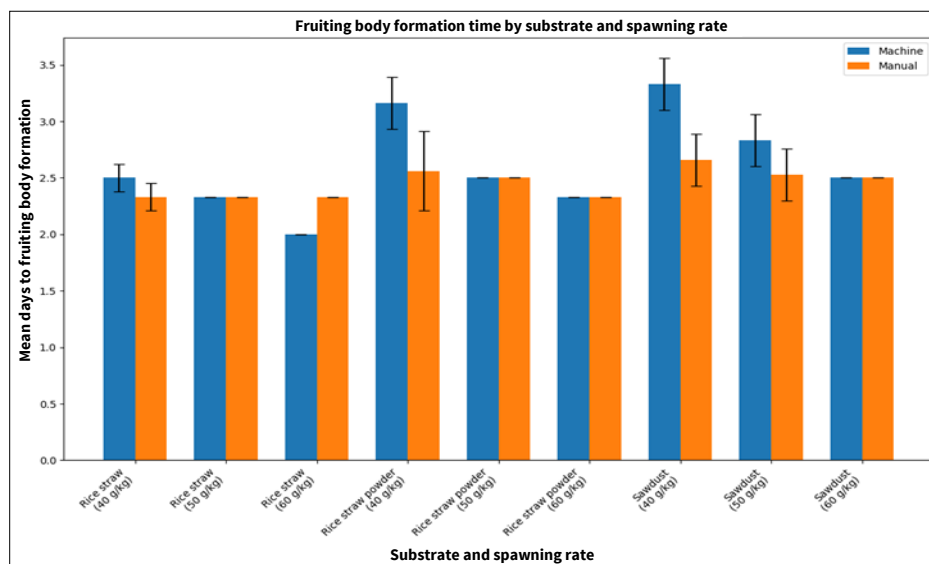


Fig. 3. Effect of spawn rate and substrate on pinhead and fruiting body formation.

significantly reduced the time to fruiting ($p = 0.0077$) and rice straw again outperformed other substrates. The ANOVA results supporting these observations are presented in Table 1. Research indicates that higher inoculum densities accelerate lignocellulose degradation and promote faster fruiting. In contrast, substrates with compact or powdery textures tend to retain excessive moisture, thereby reducing gaseous exchange and delaying primordia initiation (34). This highlights the importance of maintaining both

Table 1. ANOVA summary for pinhead and fruiting body formation.

Factor	df	F-value	p-value	Significance
Spawn rate	2	4.14	0.0333 *	Significant
Substrate	2	49.64	<0.0001 **	Highly significant
Interaction	4	0.59	0.6736	ns

structural integrity and porosity in substrates to ensure timely fructification.

Period between flushes

Flush intervals were primarily affected by substrate type ($p < 0.0001$) and marginally by spawning rate ($p = 0.0789$) (Table 2). The shortest intervals were obtained with sawdust at 50 g/kg spawn, followed by

rice straw (Fig. 4). Powdered straw caused longer intervals due to excessive moisture retention, which slows metabolic recovery. The ANOVA results supporting these effects are summarised in Table 2. Model adequacy ($R^2 = 0.8721$; Adj. $R^2 = 0.8153$) indicated strong predictive capacity. Research indicates that balanced substrate-spawn combinations shorten the overall cropping cycle (35). The relatively better performance of sawdust in reducing flush intervals suggests that fine-textured substrates may favour faster recovery between flushes, though not necessarily higher yields. This indicates

Table 2. ANOVA summary for the period between flushes.

Factor	df	F-value	p-value	Significance
Spawn rate	2	2.93	0.0789	Trend
Substrate	2	56.44	<0.0001 **	Highly significant
Interaction	4	1.00	0.4343	ns

that flush management strategies should consider not only yield potential but also production frequency to optimise overall output.

Total yield

Total mushroom yield was strongly influenced by both spawn rate and substrate ($p < 0.01$). The highest yield (193.33 g kg⁻¹ dry substrate) was recorded with rice straw at 50 g kg⁻¹ spawn, followed by 60 g kg⁻¹ on rice straw (Fig. 5). Lower yields were associated with

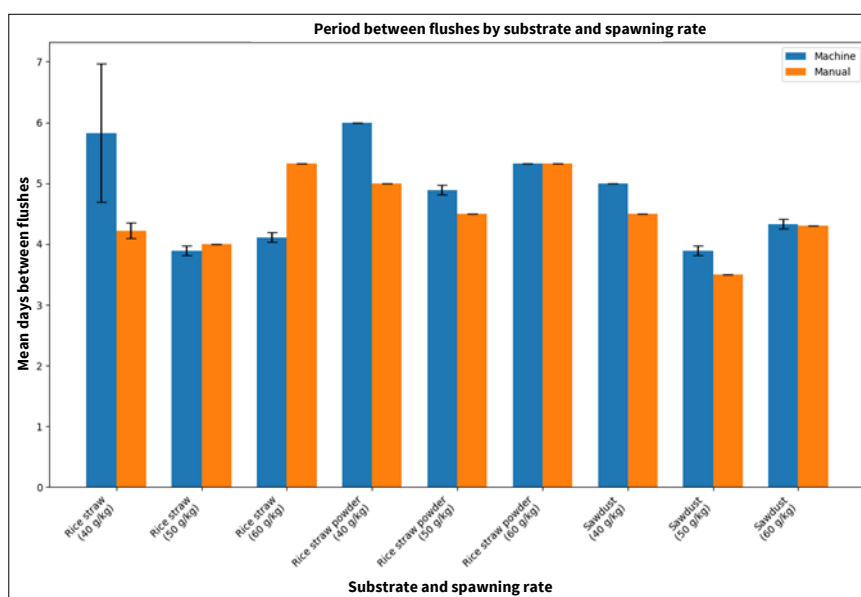


Fig. 4. Effect of substrate and spawning rate on period between flushes.

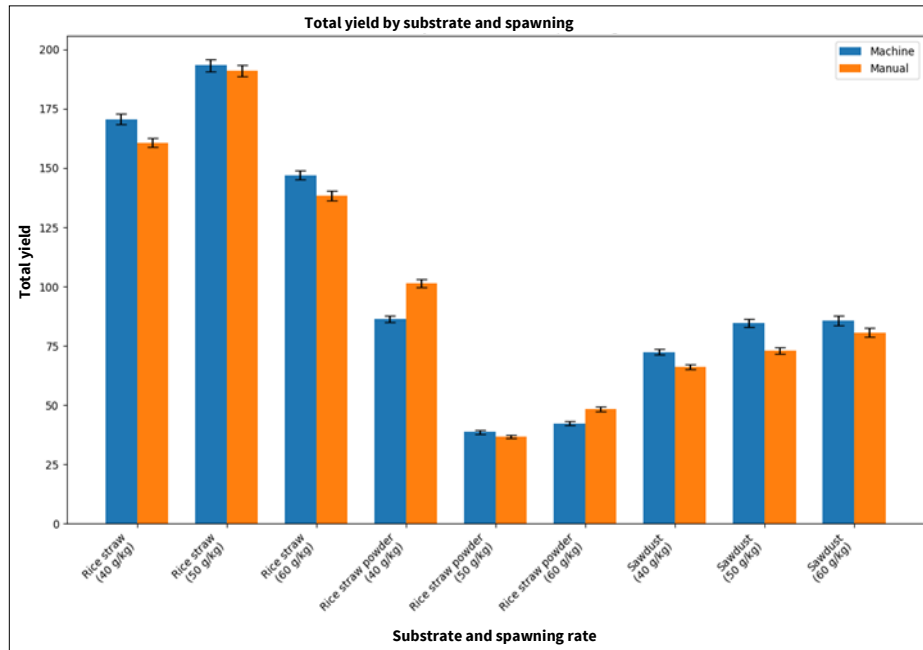


Fig. 5. Effect of substrate and spawning rate on total mushroom yield.

sawdust and powdered straw, which provide denser matrices with reduced aeration. ANOVA results confirmed highly significant differences among substrates ($p < 0.0001$) and a moderate effect of spawning rate (Table 3). Research indicates that the importance of balanced moisture and substrate structure for maximising yield (31). The results suggest that increasing the spawn rate beyond an optimal threshold (50 g kg^{-1}) does not proportionally improve yield, possibly due to rapid nutrient depletion or microbial competition. Thus, rice straw combined with moderate spawn supplementation provides an agronomically and economically favourable strategy for maximising productivity.

Biological efficiency

Table 3. ANOVA summary for total yield.

Factor	df	F-value	p-value	Significance
Spawn rate	2	5.92	0.0054 **	Significant
Substrate	2	42.15	<0.0001 **	Highly significant
Interaction	4	1.21	0.3134	ns

Biological efficiency (BE) varied significantly across treatments, reflecting differences in yield relative to substrate utilisation. The maximum BE (50.09 %) was observed with rice at 50 g kg^{-1} spawn, while sawdust exhibited the lowest values (Fig. 6). ANOVA (Table 4) confirmed significant main effects of both substrate and spawn rate, consistent with the results for total yield. These findings demonstrate that rice straw remains the most efficient substrate for *P. florida* cultivation (24, 28). The optimal spawning rate (50 g kg^{-1}) balanced colonisation speed, nutrient availability and contamination risk, producing superior BE compared to higher or lower rates. Importantly, the consistency of rice straw across yield,

Table 4. ANOVA summary for biological efficiency.

Factor	df	F-value	p-value	Significance
Spawn rate	2	6.87	0.0032 **	Significant
Substrate	2	47.92	<0.0001 **	Highly significant
Interaction	4	0.84	0.4985	ns

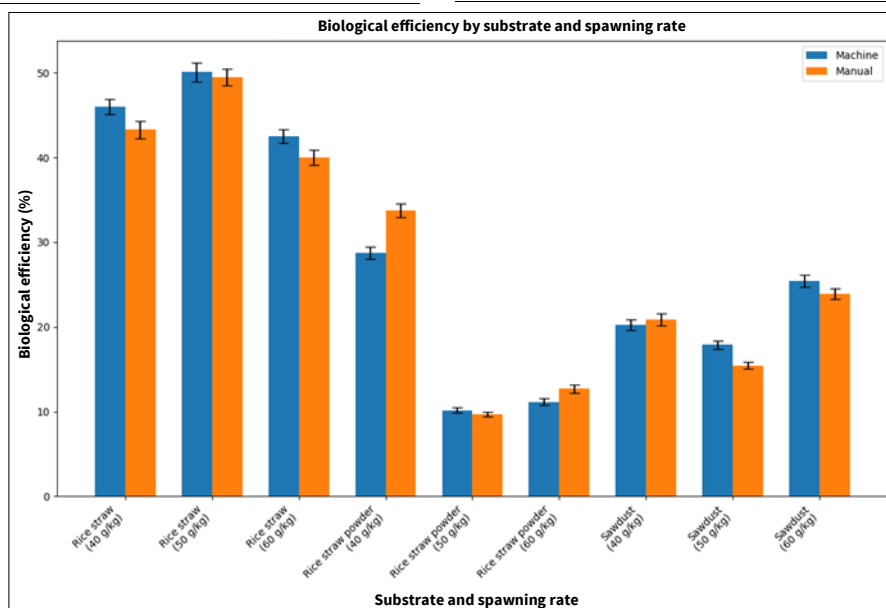


Fig. 6. Effect of substrate and spawning rate on biological efficiency.

efficiency and flush performance indicates its suitability as a reliable substrate in large-scale mushroom production systems.

Discussions

The emergence and development of fruiting bodies of *P. florida* were markedly influenced by substrate type. In the present study, rice straw consistently supported faster and more uniform pinhead initiation and fruiting body formation compared to rice straw powder and sawdust. The superior performance of rice straw can be attributed to its balanced structural integrity and porosity, which facilitate adequate aeration, enzymatic activity and gas exchange during mycelial colonisation and fructification. In contrast, rice straw powder exhibited delayed pinhead initiation and comparatively lower yield. The finer particle size likely reduced substrate porosity and restricted gaseous exchange, leading to excessive moisture retention and slower metabolic differentiation of primordia. Similarly, sawdust, due to its denser structure and comparatively lower nutrient availability, resulted in delayed fruiting and reduced biological efficiency.

These results are consistent with previous findings, where substrate composition, texture and nutrient balance were identified as critical factors governing the rate of mycelial growth and fruiting in *Pleurotus* species (35, 36). Substrates rich in cellulose and hemicellulose, such as rice straw, provide an optimal carbon-to-nitrogen ratio that supports enzyme secretion and energy generation required for fruiting body formation (10, 37, 38). Additionally, the cropping room environment was carefully maintained to ensure optimal fruiting conditions. Relative humidity was regulated between 57–70 % to promote proper pinhead differentiation, as inadequate humidity often results in aborted or malformed fruiting bodies. The temperature was maintained between 24–28 °C during the fruiting phase. Diffused light conditions were provided for approximately 8–10 hr day⁻¹ to facilitate normal fruit body development. Carbon dioxide levels were kept below 1000 ppm through adequate ventilation to prevent elongation of stipes and ensure uniform cap formation (38, 39).

The observed differences among substrates can also be justified by their water-holding capacity and aeration properties, which directly influence gas exchange and nutrient translocation within the substrate matrix. Although rice straw showed superior overall performance in terms of yield and biological efficiency, substrate pre-processing may improve certain physical characteristics such as uniformity and handling convenience. However, excessive reduction in particle size, as observed with rice straw powder, can adversely affect substrate aeration and gas exchange, thereby delaying pinhead initiation and reducing yield performance. Previous studies have similarly emphasised that maintaining an optimal balance between substrate structure and porosity is essential for efficient mushroom growth and fructification (24, 37). Hence, substrate particle size optimisation plays a crucial role in achieving consistent and high-quality fruiting in *Pleurotus* cultivation systems.

The present findings are consistent with recent reports indicating that substrate type and spawning rate significantly influence growth performance and biological efficiency in *P. florida*. Previous studies have demonstrated improved yield and colonisation efficiency when rice straw and optimised spawn rates were employed (11, 34). Similarly, substrate optimisation studies

conducted during 2022–24 reported that lignocellulosic substrates with appropriate structural porosity enhance fruiting body development and yield performance (14, 39). However, limited studies have systematically evaluated the combined influence of substrate type and spawning rate interaction under controlled conditions. Therefore, the present study contributes to the existing literature by providing a comparative evaluation of three substrates under multiple spawning rates, offering practical insights for improved cultivation strategies.

Economic feasibility analysis

In addition to biological performance, economic feasibility is a crucial consideration for commercial mushroom cultivation. A partial budget analysis was conducted based on prevailing local market prices. The cost of rice straw, rice straw powder and sawdust was approximately ₹ 4, ₹ 6 and ₹ 5 per kg, respectively. Spawn cost was estimated at ₹ 80 per kg and at a spawning rate of 50 g kg⁻¹ substrate, the spawn cost per bag was approximately ₹ 4. Additional labour and operational expenses were estimated at ₹ 10 per cultivation bag. Under the best-performing treatment (rice straw at 50 g kg⁻¹ spawning rate), an average yield of 193.33 g kg⁻¹ dry substrate was recorded. At an average market selling price of ₹ 180 per kg of fresh oyster mushroom, the gross return per bag was economically favourable. Compared to rice straw powder and sawdust, rice straw offered lower input cost and higher yield, resulting in improved profitability and benefit–cost efficiency. These findings suggest that optimising substrate selection and spawning rate not only enhances biological efficiency but also improves economic sustainability, particularly for small-scale and resource-limited growers.

Conclusion

This study demonstrated that both substrate type and spawning rate critically affect the growth, development and yield of *Pleurotus florida*. Among the substrates tested, rice straw consistently outperformed rice straw powder and sawdust, owing to its optimal porosity, moisture retention and nutrient composition. A spawning rate of 50 g kg⁻¹ dry substrate on rice straw produced the highest yield (193.33 g kg⁻¹ dry substrate) and biological efficiency (50.09 %), while higher spawn rates accelerated colonisation without further improving productivity. These findings indicate that rice straw combined with a moderate spawning rate provides the most effective and economically viable strategy for *P. florida* cultivation. Moreover, this approach promotes sustainable utilisation of agricultural residues, offering an environmentally friendly solution to reduce crop waste. Overall, adopting rice straw at 50 g kg⁻¹ spawn can optimise mushroom production efficiency and support scalable, eco-conscious cultivation practices.

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Authors' contributions

S¹, KRY, S², DJS, N, SJ and BSM contributed equally to the conception, experimental design, data collection, statistical analysis, interpretation of results and preparation of the manuscript. All authors read and approved the final manuscript. [S¹ - Shashikumar and S² - Shriramulu].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used grammar-check software and generative AI tools (ChatGPT – GPT-4o/5.1) to assist in improving clarity, grammar and language. After using these tools, the authors thoroughly reviewed and edited the content and take full responsibility for the final version of the manuscript.

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