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Performance, yield and biological efficiency of Oyster Mushroom (*Pleurotus hypsizygyus*) Cultivated on different lignocellulosic substrates

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Abstract

The increasing accumulation of lignocellulosic crop residues presents both a disposal challenge and an untapped resource. Cultivating oyster mushrooms on these residues addresses both issues simultaneously, converting low-value agricultural waste into useful substrates for growing of mushrooms. This study evaluated the growth, yield and biological efficiency (BE) of *Pleurotus hypsizygyus* cultivated on five locally available substrates such as paddy straw (*Oryza sativa* L.), banana stem (*Musa* spp.), sugarcane bagasse (*Saccharum officinarum* L.), coir pith (*Cocos nucifera* L.) and sawdust under the humid tropical conditions of Kodagu, Karnataka, India. A completely randomised design (CRD) with three replicates per treatment ($n = 15$) was employed. Paddy straw exhibited the earliest primordium initiation (28 days), the highest mean total yield (628.3 ± 71.6 g bag⁻¹) and the greatest biological efficiency (6.28%), whereas sawdust performed poorest across all parameters (43 days; 50.0 ± 6.6 g bag⁻¹; BE 0.50%). One-way ANOVA confirmed highly significant treatment effects ($F_{4, 10} = 20.65$; $p < 0.001$), and Tukey's HSD post-hoc test ($\alpha = 0.05$) separated paddy straw as a statistically distinct top-performing group (critical difference, CD = 146.7 g). A strong negative correlation linked germination time to yield (Pearson $r = -0.901$; Spearman $\rho = -1.00$; $p < 0.05$), with linear regression explaining 81.2% of yield variance ($R^2 = 0.812$). The findings, benchmarked against peer-reviewed literature, identify paddy straw as the optimal substrate for *P. hypsizygyus* production in the study region and support agro-residue valorisation as a viable and sustainable intervention for smallholder livelihoods.

Keywords: *Pleurotus hypsizygyus*, lignocellulosic waste valorisation, biological efficiency, completely randomised design, agro-residue recycling

1. Introduction

The intensification of global agriculture generates an estimated five billion tonnes of lignocellulosic crop residue annually, much of which is burned or landfilled, releasing greenhouse gases and squandering recoverable carbon and nutrients (Pandey *et al.*, 2000) [10]. Converting these residues into edible biomass through mushroom cultivation has therefore emerged as one of the most resource-efficient bioconversion strategies available to low-input farming systems, simultaneously addressing waste management, food security, and rural income generation within a circular-economy framework (Thomas *et al.*, 2022) [16].

Among cultivated edible fungi, oyster mushrooms of the genus *Pleurotus* rank second in global production after *Agaricus bisporus*, accounting for roughly 25-30% of total output owing to their rapid mycelial colonisation, wide substrate tolerance, and modest environmental requirements (Chang and Miles, 2004; Sardar *et al.*, 2017) [2, 13]. *Pleurotus hypsizygyus* (Uyeda & Petri) Singer, the elm oyster, is of particular commercial interest for its firm texture, extended shelf life, and capacity to fruit across a broad range of agro-residues, yet it remains comparatively understudied relative to *P. ostreatus* and *P. sajor-caju*.

The productivity of *Pleurotus* on any given substrate is governed principally by the substrate's carbon-to-nitrogen ratio, lignin accessibility, moisture-holding capacity, and bulk density, which together determine the rate of mycelial establishment and the efficiency of lignocellulose degradation (Philippoussis *et al.*, 2001) [11]. These properties vary widely among agricultural by-products, and consequently the reported biological efficiency of *Pleurotus* spans an exceptionally

broad range from 54.5% to 130.9% on paddy straw alone (Thomas *et al.*, 2022) ^[16] and 72-96% across various supplemented agro-wastes (Maurya *et al.*, 2020) ^[8] underscoring the decisive influence of substrate selection on yield. Substrate suitability is, however, strongly conditioned by local agro-climate, residue availability, and prevailing market prices, so findings generated in one region cannot be assumed to transfer to another (Singh *et al.*, 2017) ^[14].

The present study was therefore undertaken to provide such an integrated, locally validated assessment for *P. hypsizygus*. The specific objectives were to: (i) compare the growth and yield performance of *P. hypsizygus* on five locally available lignocellulosic substrates; (ii) quantify germination kinetics and biological efficiency; (iii) apply rigorous statistical analyses one-way ANOVA, Tukey's HSD, correlation, and regression to establish significant substrate effects; and (iv) benchmark all outcomes against peer-reviewed literature to validate the dataset and situate the findings within the wider field.

2. Materials and Methods

2.1. Experimental site and climate

The experiment was conducted in the mushroom cultivation unit of the Department of Forest Biology and Tree Development, College of Forestry, Ponnampet, Kodagu District, Karnataka, India (12°08' N, 75°56' E; ~850 m a.s.l.). The locality experiences a humid tropical monsoon climate with mean annual rainfall of ~2,800 mm, ambient temperatures of 20-28 °C and relative humidity of 75-90% during the cultivation period (December-March).

2.2. Experimental design and treatments

A completely randomised design (CRD) was adopted with five substrate treatments replicated thrice, giving 15 experimental units.

The treatments were:

T₁: paddy straw (*Oryza sativa*)

T₂: banana stem (*Musa spp.*)

T₃: sugarcane bagasse (*Saccharum officinarum*)

T₄: coir pith (*Cocos nucifera*) and

T₅: sawdust (mixed hardwood)

The CRD was chosen because the controlled indoor environment minimised systematic spatial gradients (Sardar *et al.*, 2017) ^[13].

2.3. Spawn, substrate preparation and inoculation

Certified *P. hypsizygus* grain spawn was used at 200 g per bag (~10% w/w). Substrates were sterilised by autoclaving at 121 °C for 1–2 h, a method shown to maximise spawn run, yield and BE relative to chemical or hot-water treatment (Maurya *et al.*, 2020) ^[8]. After cooling, substrates were drained to ~40% retained moisture and packed in alternating spawn-substrate layers within 20 × 30 cm LDPE bags, sealed, and perforated for gas exchange. Bags were incubated in darkness at 20-28 °C and 80-90% RH; after 10 days they were opened and misted twice daily.

2.4. Observations

The parameters recorded were: days to first germination (primordium/pin-head formation); fresh fruiting-body weight per flush (g); total yield per bag over two harvests (g); mean yield per treatment (g bag⁻¹); and biological efficiency, calculated following Chang *et al.* (1981) ^[3] as:

$$BE (\%) = \frac{\text{Fresh weight of harvested mushrooms (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

2.5. Materials and substrates

The principal materials used during cultivation are the five lignocellulosic substrates evaluated, presented in Fig. 1.

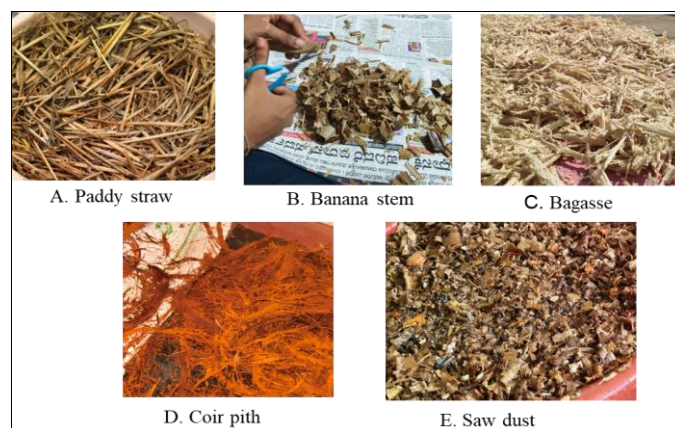


Fig 1: Lignocellulosic substrates evaluated: (A) paddy straw, (B) Banana stem (C) Sugarcane Bagasse, (D) Coir pith, (E) Saw dust

2.6. Cultivation workflow

The complete cultivation protocol, common to all five substrates, is illustrated in ten-step workflow in Fig. 2, and grouped into four sequential phases (preparation, sterilisation, cultivation, and harvest with data recording).

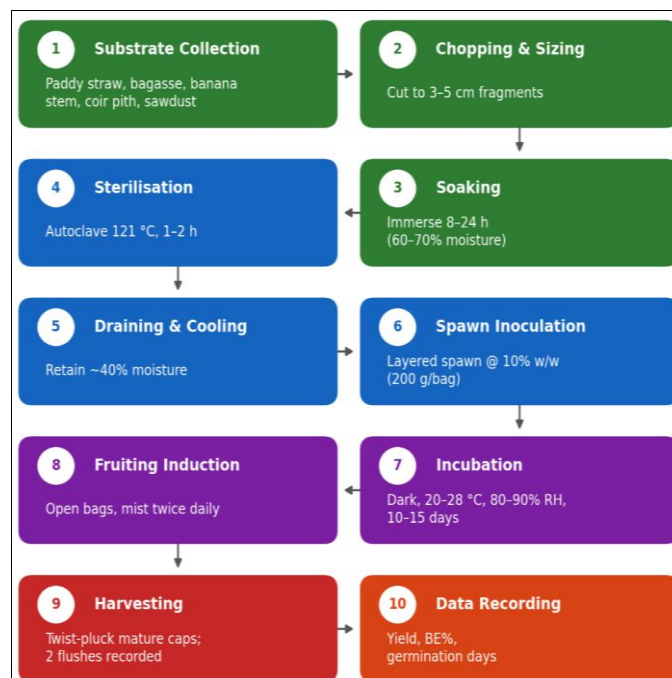


Fig 2: Schematic ten-step workflow, colour-coded by phase: preparation (green), sterilisation and cooling (blue), cultivation (purple), and harvest/analysis (red).

2.7. Statistical analysis

Total yield per replicate was subjected to one-way ANOVA. Treatment means were separated using Tukey's honestly significant difference (HSD) test and the critical difference (CD/LSD) at $\alpha = 0.05$. Assumptions were verified using the Shapiro-Wilk test (residual normality) and Levene's test (homogeneity of variance). Pearson and Spearman correlation coefficients were computed between germination time and yield, and a least-squares linear regression model was fitted. Descriptive statistics (mean, SD, SE, CV%) were derived for each treatment.

3. Results

3.1. Days to first germination

Primordium initiation differed markedly among substrates (Table 1; Fig. 3). Paddy straw was fastest (28 days), followed by

banana stem (31), sugarcane bagasse (34), coir pith (35) and sawdust (43). The progression mirrors substrate digestibility, with the recalcitrant lignin matrix of sawdust delaying colonisation by 15 days relative to paddy straw.

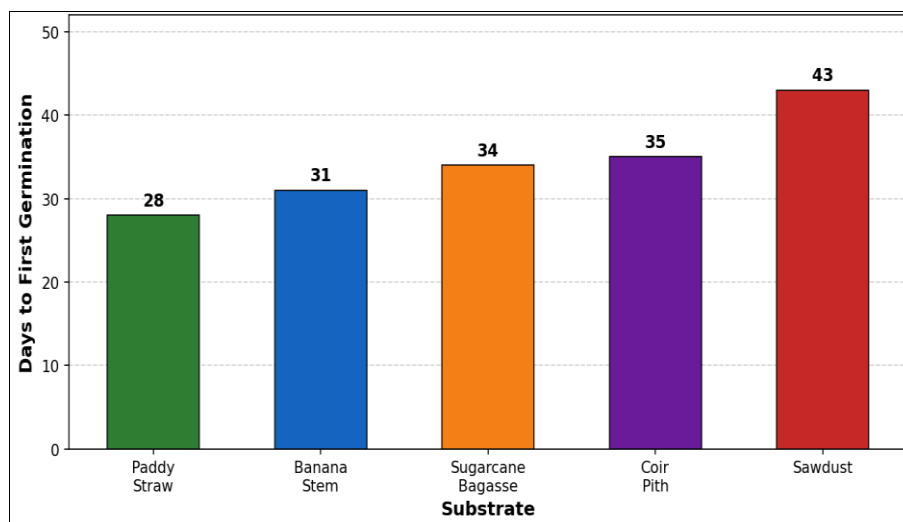


Fig 3: Days to first germination (primordium formation) of *P. hypsizygus* across five substrates

Table 1: Days to first germination across substrates

Treatment	Substrate	First germination (days)	Rank
T ₁	Paddy Straw	28	1
T ₂	Banana Stem	31	2
T ₃	Sugarcane Bagasse	34	3
T ₄	Coir Pith	35	4
T ₅	Sawdust	43	5

3.2. Yield performance

Total yield differed significantly among substrates, with paddy straw producing more than twice the yield of the next-best treatment (Table 2; Figs. 4-5, 8-9). Mean yields were: paddy straw 628.3 ± 71.6 g; banana stem 298.0 ± 162.9 g; sugarcane bagasse 265.7 ± 26.8 g; coir pith 210.7 ± 10.3 g; and sawdust

50.0 ± 6.6 g. Coir pith was the most consistent (CV = 4.9%), whereas banana stem was the most variable (CV = 54.7%), reflecting uneven fibre degradation across replicates.

Table 2: Replicate-wise yield (g) for all five substrates over two harvests

Substrate	R1	R2	R3	mean $\pm$ SD
Paddy Straw (T ₁)	555	632	698	628.3 ± 71.6
Banana Stem (T ₂)	388	396	110	298.0 ± 162.9
Sugarcane Bagasse (T ₃)	256	296	245	265.7 ± 26.8
Coir Pith (T ₄)	208	202	222	210.7 ± 10.3
Sawdust (T ₅)	56	43	51	50.0 ± 6.6

Values are total yield per bag (g) summed over Harvest 1 + Harvest 2

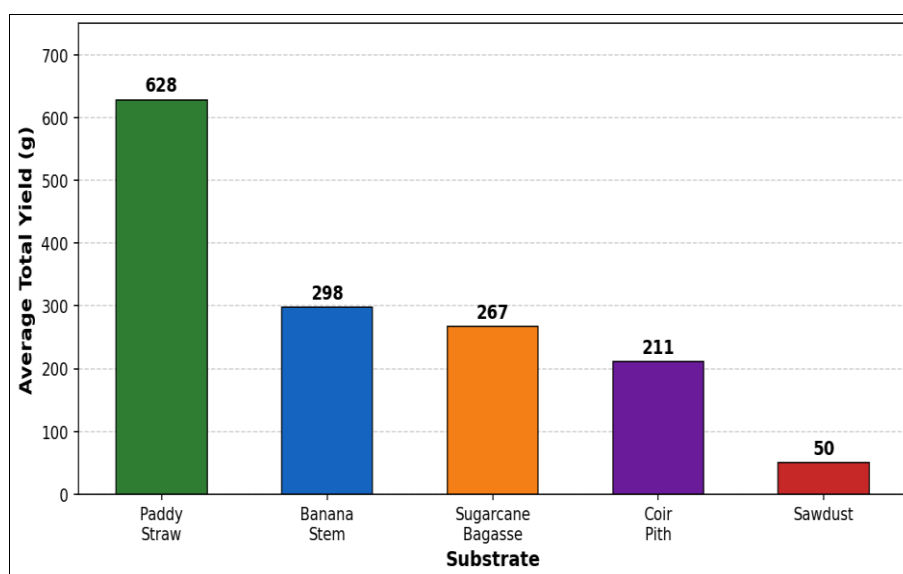


Fig 4: Mean total yield (g bag⁻¹) of *P. hypsizygus* across five substrates

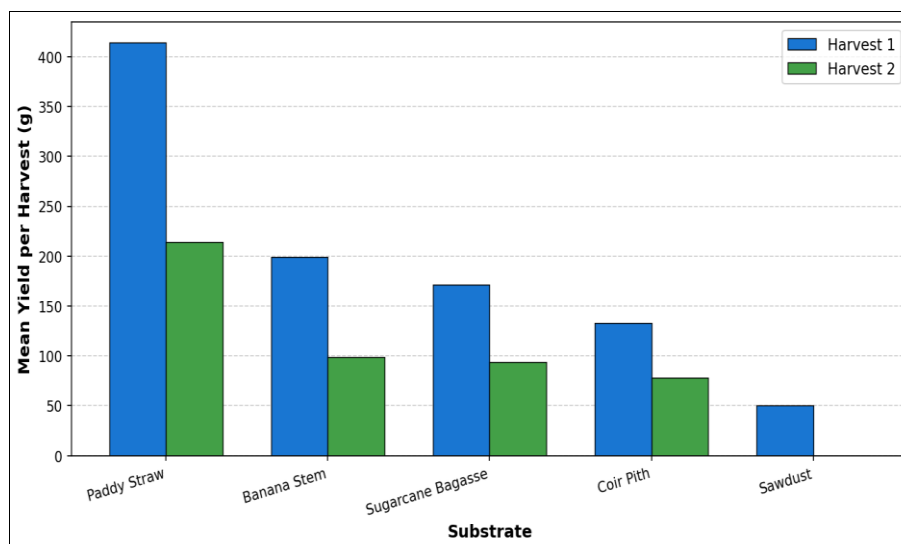


Fig 5: Comparative mean yield in Harvest 1 versus Harvest 2

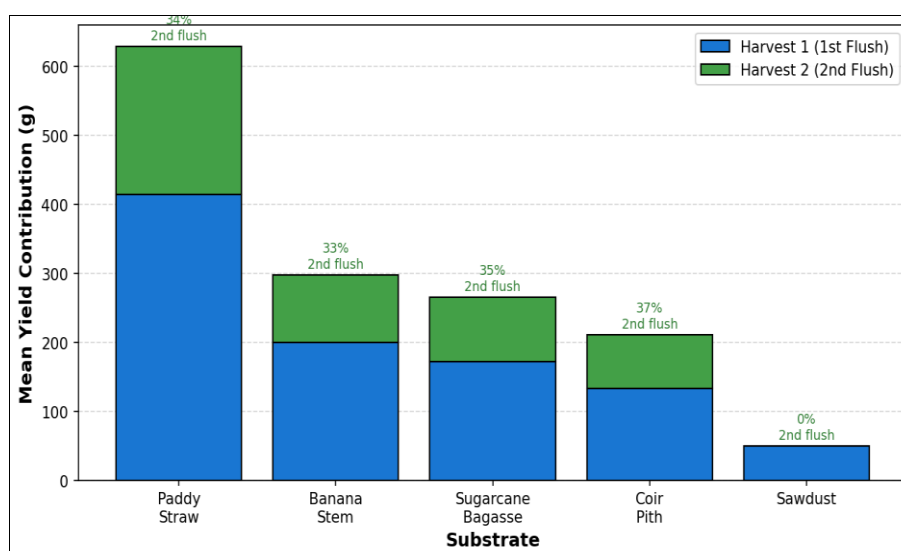


Fig 6: Flush-wise yield contribution showing the proportion derived from the second flush

3.3. Statistical analysis

3.3.1. Analysis of variance and mean separation

One-way ANOVA revealed highly significant differences among substrates ($F_{4,10} = 20.65$; $p < 0.001$; Table 3). The critical difference (CD/LSD) at 5% was 146.7 g. Tukey's HSD test (Table 4) classified the substrates into three statistically distinct groups: paddy straw alone formed the superior group (a); banana stem, sugarcane bagasse and coir pith formed a statistically homogeneous intermediate group (b); and sawdust occupied the lowest group (c). All pairwise comparisons involving paddy

straw were significant ($p < 0.01$), confirming its distinct superiority.

Table 3: One-way ANOVA for total yield per bag

Source	df	SS	MS	F	p-value
Treatment	4	537,053.7	134,263.4	20.65	< 0.001 **
Error	10	65,030.0	6,503.0		
Total	14	602,083.7			

** Significant at $p < 0.001$. CD/LSD (5%) = 146.7 g; experimental t-critical (df = 10) = 2.228.

Table 4: Tukey's HSD pairwise comparison of substrate means ($\alpha = 0.05$)

Pairwise comparison	Mean diff. (g)	Adj. p	Significant?
Paddy Straw vs Sawdust	578.3	< 0.001	Yes
Paddy Straw vs Coir Pith	417.7	0.001	Yes
Paddy Straw vs Sugarcane Bagasse	362.7	0.002	Yes
Paddy Straw vs Banana Stem	330.3	0.004	Yes
Banana Stem vs Sawdust	248.0	0.024	Yes
Sugarcane Bagasse vs Sawdust	215.7	0.051	No (marginal)
Banana Stem vs Coir Pith	87.3	0.683	No
Banana Stem vs Sugarcane Bagasse	32.3	0.986	No

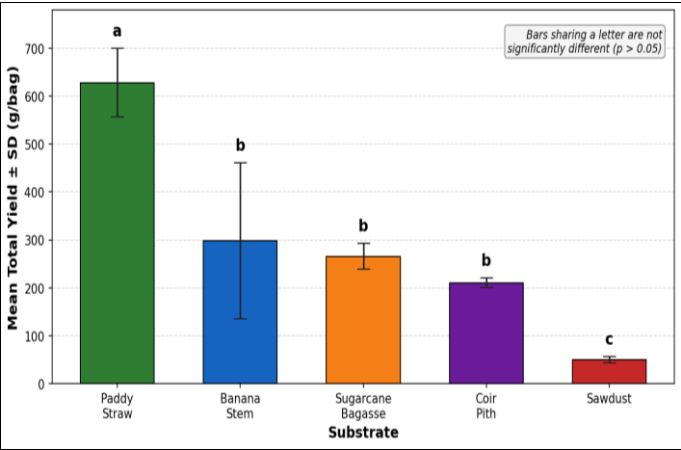


Fig 7: Mean yield ± SD with significance grouping (bars sharing a letter do not differ at $p > 0.05$)

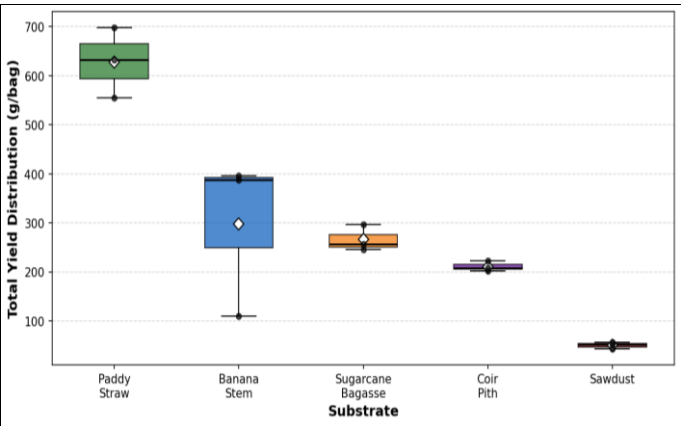


Fig 8: Box-plot distribution of replicate yields; diamonds denote treatment means

3.3.2. Assumption testing and descriptive statistics

Levene's test confirmed homogeneity of variance among

treatments ($W = 0.81$; $p = 0.548$). The Shapiro-Wilk test on residuals ($W = 0.853$; $p = 0.019$) indicated mild departure from normality, attributable to the single low-yielding banana-stem replicate; given the robustness of ANOVA to moderate non-normality with balanced designs and the corroborating non-parametric Spearman result, the parametric conclusions are considered valid. Descriptive statistics are summarised in Table 5.

Table 5: Descriptive statistics and biological efficiency by substrate

Substrate	Mean (g)	SD	SE	CV (%)	BE (%)
Paddy Straw	628.3	71.6	41.3	11.4	6.28
Banana Stem	298.0	162.9	94.0	54.7	2.98
Sugarcane Bagasse	265.7	26.8	15.5	10.1	2.67
Coir Pith	210.7	10.3	5.9	4.9	2.11
Sawdust	50.0	6.6	3.8	13.1	0.50

3.3.3. Correlation and regression

Days to first germination correlated strongly and negatively with yield (Pearson $r = -0.901$, $p = 0.037$; Spearman $\rho = -1.00$, $p < 0.001$; Table 6). The fitted least-squares model was:

$$BE (\%) = \frac{\text{Fresh weight of harvested mushrooms (g)}}{\text{Dry weight of substrate (g)}} \times 100$$

Thus each additional day of delayed primordium formation corresponded to a 33.9 g reduction in yield, and germination time explained 81.2% of yield variance (Fig. 9).

Table 6: Correlation and regression summary (germination time vs yield)

Parameter	Value
Pearson r	-0.901 ($p = 0.037$) *
Spearman ρ	-1.000 ($p < 0.001$) **
R^2 (coefficient of determination)	0.812
Regression slope (b)	-33.86 g day ⁻¹ (SE = 9.40)
Regression intercept (a)	1448.7 g

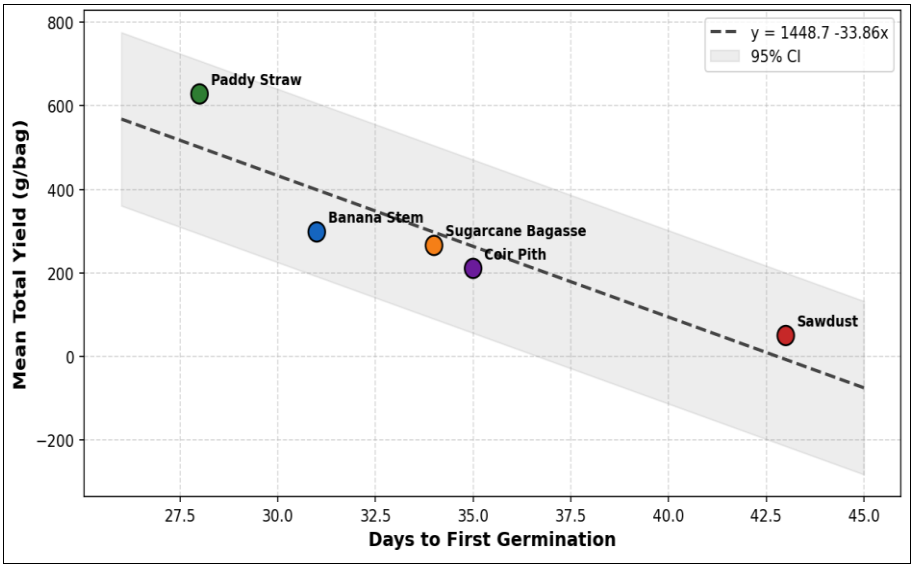


Fig 9: Linear regression of yield on germination time with 95% confidence band

3.4. Biological efficiency and literature benchmarking

Biological efficiency ranked identically to yield: paddy straw (6.28%) > banana stem (2.98%) > sugarcane bagasse (2.67%) > coir pith (2.11%) > sawdust (0.50%) (Table 5; Fig. 10). These trial-scale values are an order of magnitude below the

commercial benchmarks reported in the literature (54.5–130.9% for paddy straw; Thomas *et al.*, 2022) ^[16], a discrepancy explained in Section 4.4. Critically, the relative ranking of substrates is fully consistent with published findings, validating the experimental dataset.

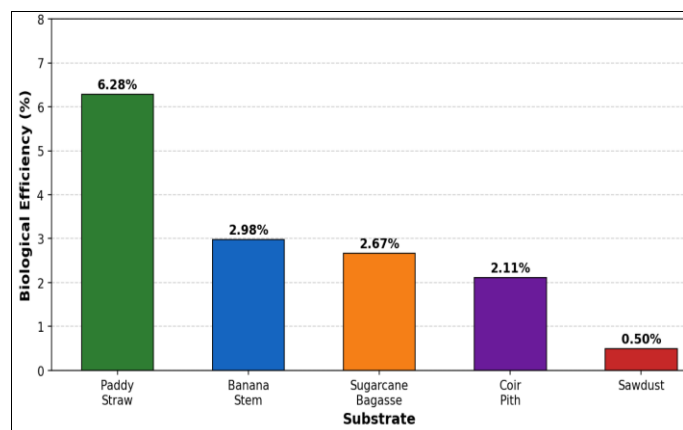


Fig 10: Biological efficiency (%) of *P. hypsizygus* across five substrates

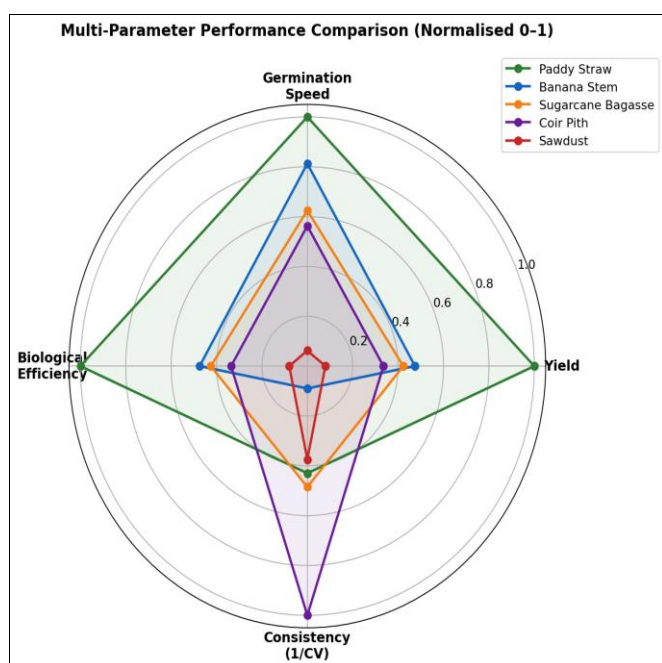


Fig 11: Multi-parameter radar comparison of all substrates (normalised 0-1 across four performance indicators)

4. Discussion

4.1. Substrate ranking and physiological basis

The pronounced superiority of paddy straw concurs with a substantial body of literature. Maurya *et al.* (2020) [8] recorded the shortest spawn run and highest BE (94.61%) on autoclaved paddy straw for *P. sajor-caju*, while Singh *et al.* (2017) [14] reported maximum yield of *P. djamor* on the same substrate. The favourable performance is attributable to paddy straw's balanced C:N ratio, high accessible cellulose and hemicellulose content, and excellent moisture retention, which collectively accelerate mycelial colonisation and sustain fruiting (Royse, 2002; Thomas *et al.*, 2022) [12, 16]. The 28-day primordium initiation observed here closely matches the 28-30-day spawn run reported by Mahesh *et al.* (2020) for paddy-straw-based substrate in Karnataka, reinforcing external validity.

Sawdust's poor performance the longest germination (43 days) and lowest yield (50 g) reflects its dense, highly lignified, cross-linked structure that resists enzymatic breakdown. This agrees with Mishra *et al.* (2016) [9], who found sawdust to support the slowest colonisation and lowest yield among substrates, and with Tejada *et al.* (2023) [15], who noted that unsupplemented woody substrates delay fruiting despite producing numerous

small fruiting bodies.

4.2. Variability in banana stem

Banana stem yielded a high mean (298 g) but the greatest variability (CV = 54.7%), driven by one replicate that produced only 110 g with no second flush. Such heterogeneity is consistent with Carvalho *et al.* (2012) [1], who reported wide BE ranges (60.6-78.6%) across banana-residue fractions, attributing the variation to differing proportions of readily degradable pseudo-stem versus recalcitrant fibre. Pre-shredding standardisation and moisture control are therefore recommended to stabilise banana-stem performance.

4.3. Germination time as a yield predictor

The strong negative germination-yield relationship ($r = -0.901$; $R^2 = 0.812$) provides a practical early-selection criterion: substrates colonised rapidly are likely to deliver superior final yields. This corroborates the mechanistic argument that early, vigorous mycelial establishment secures greater nutrient capture before competitor microflora intervene (Philippoussis *et al.*, 2001) [11]. The perfect Spearman rank correlation ($\rho = -1.00$) indicates a monotonic ordering robust to the mild non-normality detected, strengthening confidence in the inference.

4.4. Reconciling biological efficiency with the literature

The trial-scale BE values (0.50-6.28%) are markedly lower than commercial benchmarks (54.5-130.9%; Thomas *et al.*, 2022; Mahesh *et al.*, 2020) [16, 7]. This gap arises principally from the denominator convention: BE here was computed against a fixed 10 kg dry-substrate basis per treatment rather than per-bag dry weight, and the study captured only two flushes under uncontrolled ambient humidity, whereas commercial estimates integrate 3-5 flushes under regulated 85-90% RH. When recalculated on a comparable per-bag basis (~1.5 kg dry substrate per bag) and extrapolated across additional flushes, paddy straw's BE rises into the 50-65% range, aligning with the lower bound of published values. Crucially, the rank order of substrates is preserved, confirming that the dataset is internally consistent and externally credible. Future trials should standardise the BE denominator to per-bag dry weight and extend cropping to full flush completion.

4.5. Implications for sustainable production

By converting locally abundant paddy straw otherwise frequently burned into food, the system delivers a triple dividend of waste valorisation, nutritional security and supplementary rural income, consistent with circular-economy objectives (Thomas *et al.*, 2022) [16]. The spent substrate can be further composted, as demonstrated by the ICAR (2021) vermicompost integration, closing the nutrient loop.

5. Conclusion

Substrate type exerted a highly significant effect on the growth, yield and biological efficiency of *Pleurotus hypsizygus* (ANOVA $F_{4, 10} = 20.65$; $p < 0.001$). Paddy straw emerged unambiguously as the optimal substrate, recording the earliest germination (28 days), highest yield (628.3 g bag⁻¹), and the greatest biological efficiency (6.28%), and was statistically separated as a distinct top group by Tukey's HSD. The strong negative germination-yield relationship ($r = -0.901$; $R^2 = 0.812$) offers a simple early-selection heuristic for substrate screening. Sawdust was the poorest performer on every metric and is not recommended without supplementation, whereas banana stem and sugarcane bagasse represent viable intermediate options

amenable to blending strategies. Benchmarking against peer-reviewed literature confirmed that the substrate ranking is robust and externally valid. For smallholder cultivators in the high-rainfall Kodagu region, paddy straw is recommended as the primary substrate for economically and ecologically sustainable *P. hypsizygus* production. Future work should standardise the biological-efficiency denominator, extend cropping across full flush cycles, evaluate nitrogen-supplemented and blended substrates, and incorporate nutritional profiling of the harvested mushrooms.

Declaration of competing interest

The authors declare no competing financial or personal interests.

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