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Standardization of grain substrate for spawn production and straw substrate for bed preparation in milky mushroom (*Calocybe indica*) cultivation

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Abstract
Spawn quality and bed substrate selection are the two most decisive factors governing the commercial viability of milky mushroom (*Calocybe indica*) cultivation. Eight grain substrates were evaluated for spawn production and seven lignocellulosic straw substrates were evaluated for bed preparation, each in a Completely Randomized Design with three replications. Among the grains, wheat supported the fastest and most uniform mycelial colonization, achieving complete (100%) spawn run in 8.67 days, followed by sorghum (9.33 days) and maize (10.33 days), while the pulses rajma, tur and gram were consistently the poorest substrates. Among the straw substrates, paddy straw produced the shortest spawn run (20.33 days) and pinhead initiation period (11.33 days after casing), the highest cumulative yield (1056.88 g per bed), the highest biological efficiency (105.68%) and the best economic return (net benefit Rs. 201 per bed; benefit–cost ratio 2.73:1), followed by maize straw (942.08 g per bed; 94.21% B.E.; 2.51:1). Sawdust was the poorest substrate for every parameter recorded (359.62 g per bed; 35.96% B.E.; 1.01:1). Wheat grain for spawn multiplication and paddy straw for bed preparation are accordingly recommended as the most suitable and remunerative combination for commercial cultivation of *C. indica* under Marathwada conditions.

Keywords: *Calocybe indica*, grain substrate, spawn run, straw substrate, biological efficiency

Introduction
Calocybe indica has emerged as the third most important cultivated mushroom in India, prized for its bold white sporophores, firm texture, comparatively long shelf life of 5–7 days and high biological efficiency, often exceeding 100–140 per cent (Subbiah and Balan, 2015) [1]. Because it fruits well at 28–35°C, C. indica is uniquely placed to sustain mushroom production through the hot summer months, when button mushroom cultivation is not feasible and paddy straw and oyster mushrooms also perform poorly (Krishnamoorthy, 2003) [2]. Commercial success however centers on two upstream inputs that are rarely optimized together for a given agro-climatic setting: the grain substrate used to raise vigorous spawn, and the lignocellulosic straw substrate used to prepare the fruiting bed.

Spawn quality directly determines the speed and uniformity of bed colonisation, while bed substrate governs nutrient availability, moisture retention and, ultimately, yield and biological efficiency. Several workers have evaluated cereal and pulse grains for C. indica spawn multiplication, generally identifying wheat and sorghum as the two leading substrates, though their relative ranking differs between reports and appears to be influenced by strain, grain moisture content and supplementation (Singh *et al*., 2017; Krishnamoorthy and Balan, 2015; Senthilnambi *et al*., 2011; Kumar, 2015; Renganathan *et al*., 2018) [3, 4, 5, 6, 7]. Straw substrates prepared from locally available crop residues have likewise been shown to influence pinhead initiation, flush pattern and cumulative yield, but recommendations again vary with region and the raw material at hand.

Marathwada is a major agricultural tract producing cotton, sorghum, maize and sugarcane, generating large volumes of straw and other lignocellulosic residues that are potentially suitable, low-cost substrates for both spawn and bed preparation. No substrate-standardization trial for *C. indica* appears, however, to have been conducted using the specific grains and straws available in this region, and without such data growers have no locally validated basis for substrate choice, nor comparative information on the resulting economics of production.

The present study was therefore designed to evaluate eight grain substrates for spawn production and seven straw substrates for bed preparation of *C. indica* under Marathwada conditions, with the objective of identifying the substrate combination that gives the fastest colonisation, the highest yield and biological efficiency, and the most favourable economic return, so as to provide growers in this region with a standardized, evidence-based cultivation protocol.

Materials and Methods
Study site and fungal material
The experiments were conducted at the Department of Plant Pathology, VNMKV, Parbhani, using the locally isolated strain of *Calocybe indica* characterized in the accompanying identification study. Stock culture was maintained on PDA

slants at 4°C and periodically sub-cultured for use as inoculum.

Preparation of master and commercial spawn

Master spawn was raised on wheat grain. Grains were cleaned, washed three times, soaked in clean water for 6–8 h, boiled for 10 min, rested (covered) for 5–10 min, drained and surface-dried on blotting sheets for about 2 h. The dried grain was amended with 2.5% (w/w) calcium carbonate to buffer pH between 6.5 and 7.5 and prevent clumping; 250–300 g aliquots were filled into 500-ml conical flasks, plugged, and autoclaved at 20 lb psi (126.5°C) for 2 h. After cooling, flasks were exposed to UV light in a laminar air-flow cabinet for 15–20 min, inoculated with actively growing mycelium of the test isolate, and incubated at 30°C for 20–25 days.

Commercial spawn was similarly prepared: cleaned, boiled wheat grain (surface-dried for 30–45 min) was mixed with 2.5% $CaCO_3$, and 500 g was filled into double-layered polypropylene bags, plugged with 1-inch PVC rings and non-absorbent cotton, covered with paper and secured with rubber bands. Bags were autoclaved (20 lb psi, 126.5°C, 2 h), cooled, UV-treated (15–20 min) and inoculated with master spawn. Bags showing uniform, white, contamination-free mycelial growth were retained for the experiments.

Evaluation of grain substrates for spawn production

The experiment was laid out in a Completely Randomized Design (CRD) with three replications to evaluate eight grain substrate treatments: sorghum, wheat, maize, bajra, paddy (rice), rajma, gram, and tur (Arhar). Clean, bold, healthy grains of each type were soaked and cooked for 10–15 min until soft without rupturing the seed coat, drained, and spread on muslin cloth to remove surface moisture. After cooling, gypsum (calcium sulphate) @ 12 g/kg and calcium carbonate @ 6 g/kg of grain were mixed in thoroughly. Aliquots of 100 g were filled into 250-ml conical flasks, plugged with non-absorbent cotton, and autoclaved at 20 lb psi for 2 h. After cooling, flasks were inoculated with actively growing mycelium of the test isolate and incubated at 30°C until the mycelium had colonised the grain surface.

Evaluation of straw substrates for bed preparation

The experiment to evaluate straw substrates for bed preparation was laid out in a Completely Randomized Design (CRD) with three replications and seven straw substrate treatments: soybean straw, paddy straw, sugarcane trash, maize straw, cotton waste, sorghum straw, and sawdust. Well-dried straw was chopped into 3–4 cm pieces. Batches of 10–12 kg were immersed for 16–18 h in 100 L of water containing 125 ml formaldehyde and 7 g carbendazim, then drained and spread on a sloping cemented floor until the moisture content reached 65–70%. Formaldehyde was sprinkled on the floor before spawning, and spawn (the best-performing grain substrate identified in Experiment 1) was mixed into the substrate by the layering method at 10% on a dry-weight basis. Spawned substrate was packed into polypropylene bags (18″ × 27″, 150 gauge), the mouths tied with cotton tags, and 8–10 holes made per bag with a sterile needle for aeration. Bags were held in a growing room at 25–35°C and 80–90% relative humidity, maintained by periodic sprinkling of the walls and floor, until spawn run was complete.

After spawn run, bag mouths were opened and a 3-cm-thick layer of casing material, pre-sterilized with 5% formalin for 48 h, was applied over the spawned substrate. Cased bags were transferred to the cropping room and watered two to three times daily on the casing surface. Pinheads appeared 8–10 days after casing and matured over a further 6–8 days; mature sporophores, harvested just before the pileus margin began to dry or yellow, were picked by gentle twisting and pulling. Three flushes were obtained per bed at intervals of 10–15 days.

Observations recorded: For both experiments: days to 10/25/50/75/100% mycelial (spawn) run; days from casing to pinhead initiation; number of pinheads per bed; number of mature fruiting bodies per bed; stipe length and diameter (cm); pileus diameter (cm); individual sporophore weight (g); flush-wise and cumulative fresh yield per bed (g) and biological efficiency, calculated as:

BE (%) = (Fresh weight of mushroom / Dry weight of substrate used) × 100

For Experiment 2, input costs (substrate, spawn, labour and consumables) and sale returns were additionally recorded for each straw substrate to compute the cost of cultivation, gross and net returns, and benefit–cost ratio.

Statistical analysis

Data from both experiments were analysed following the Completely Randomized Design; treatment means were compared using the critical difference (CD) at the 1% level of probability.

Results

Effect of grain substrate on spawn run

Grain substrate had a highly significant effect on the rate of mycelial colonization at every stage assessed (Table 1). Wheat (T2) attained complete colonization in the shortest time (8.67 days), followed by sorghum (T1; 9.33 days) and maize (T3; 10.33 days); bajra (T4) required 11.67 days. Paddy (T5) and gram (T7) each required 13.67 days, tur (T8) required 15.67 days, and rajma (T6) was the slowest substrate at every stage, requiring 17.67 days for complete colonization.

Table 1: Effect of different grains on spawn development of *C. indica*

Tr. No.	Grains	Days required for mycelial coverage (%)				
		10%	25%	50%	75%	100%
T1	Sorghum	3.33	5.67	7.33	7.67	9.33
T2	Wheat	2.67	4.67	6.33	6.67	8.67
T3	Maize	3.67	6.33	7.67	8.00	10.33
T4	Bajra	3.00	6.67	9.67	10.33	11.67
T5	Paddy	3.67	8.67	9.67	10.67	13.67
T6	Rajma	4.67	10.67	13.67	15.67	17.67
T7	Gram	3.67	8.33	12.67	12.67	13.67
T8	Tur	5.33	8.00	10.67	14.33	15.67
	SEm (±)	0.31	0.37	0.33	0.74	0.33
	CD at 1%	1.28	1.53	1.37	3.07	1.37

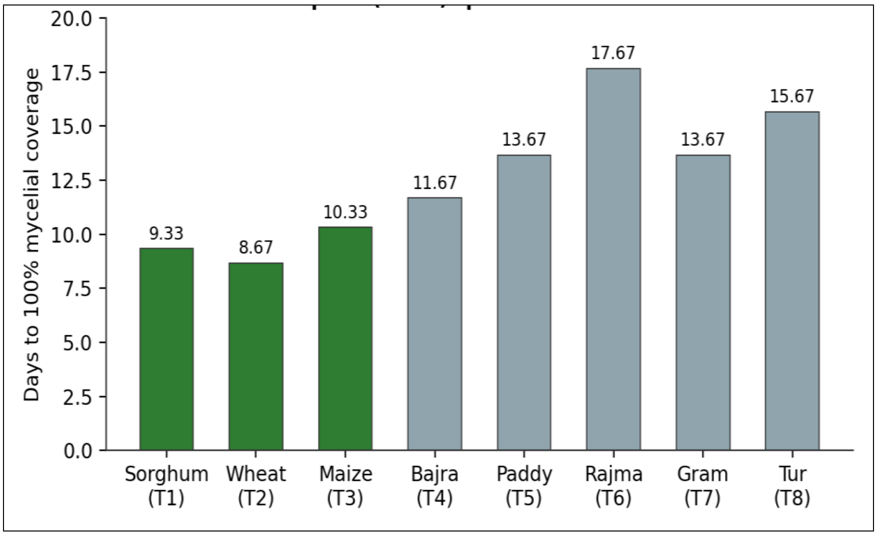

Fig 1: Effect of grain substrate on days required for complete spawn run of *C. indica*

Effect of straw substrate on spawn run and pinhead initiation

Straw substrate significantly affected the duration of spawn run at every stage of colonization and the subsequent time to pinhead initiation (Table 2). Paddy straw (T2) supported the fastest colonization throughout, reaching complete spawn run in 20.33 days and initiating pinheads within 11.33 days of casing, followed by maize straw (T4; 21.67 and 12.00 days, respectively) and sorghum straw (T6; 22.00 and 12.67 days). Sawdust (T7) was consistently the slowest, requiring 28.33 days for complete spawn run and 17.67 days to pinhead initiation an eight-day disadvantage relative to paddy straw that directly extends the cropping-room turnaround time.

Table 2: Effect of different straw substrates on spawn run and pinhead initiation of *C. indica*

Tr. No.	Substrate	Days required for spawn run (mycelial coverage, %)					Days to pinhead initiation after casing
		10%	25%	50%	75%	100%	
T1	Soybean straw	7.00	11.33	16.00	19.67	23.67	13.33
T2	Paddy straw	5.33	9.33	13.00	17.33	20.33	11.33
T3	Sugarcane trash	7.67	12.67	17.67	20.67	24.33	15.00
T4	Maize straw	5.67	10.00	13.67	18.33	21.67	12.00
T5	Cotton waste	7.33	12.67	16.33	20.00	23.00	14.33
T6	Sorghum straw	6.67	10.67	14.67	19.33	22.00	12.67
T7	Sawdust	9.33	14.67	19.00	24.67	28.33	17.67
	SEm (±)	0.31	0.31	0.40	0.38	0.28	0.35
	CD at 1%	0.95	0.95	1.20	1.16	0.86	1.09

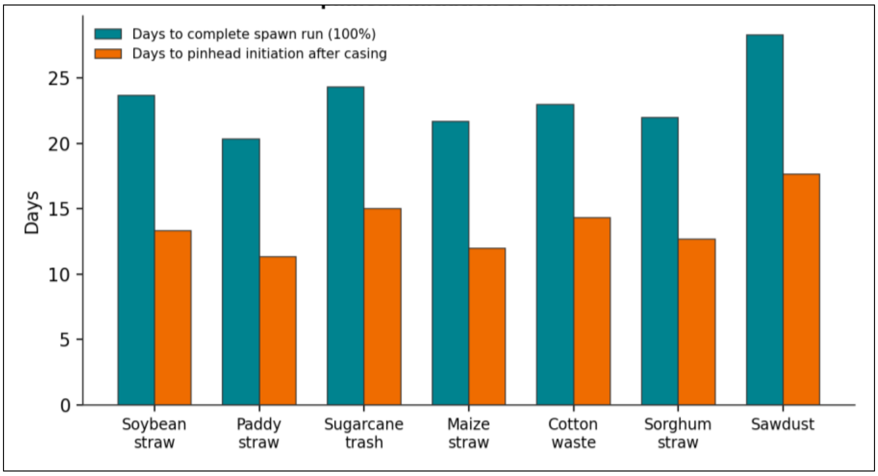

Fig 2: Effect of straw substrate on spawn run and pinhead initiation of *C. indica*

Growth and yield attributes across three successive harvests

Yield attributes were recorded separately for the three flushes obtained from each bed (Tables 3–5). Paddy straw was superior for every parameter at every harvest. In the first flush it produced the greatest number of pinheads (28.00 per bed), the most mature fruiting bodies (4.33), the heaviest sporophores (92.20 g) and the highest yield (453.85 g per bed), together with the largest pileus (11.50 cm) and longest stipe (10.31 cm), while sawdust was lowest on every count (202.35 g per bed; Table 3)

Table 3: Effect of different straw substrates on growth and yield attributes of *C. indica* during the first harvest

Tr. No.	Substrate	No. of pinheads	Mature fruiting bodies	Avg. sporophore wt. (g)	Yield per bed (g)	Stipe length (cm)	Stipe diameter (cm)	Pileus diameter (cm)
T1	Soybean straw	24.00	2.67	86.05	341.00	8.89	3.97	9.77
T2	Paddy straw	28.00	4.33	92.20	453.85	10.31	5.35	11.50
T3	Sugarcane trash	24.67	3.00	83.23	301.78	7.61	3.25	7.79
T4	Maize straw	26.00	3.33	89.63	413.77	9.55	4.91	10.87
T5	Cotton waste	22.67	2.33	86.76	361.02	8.65	4.03	8.96
T6	Sorghum straw	25.00	3.33	89.20	391.50	9.47	4.55	10.46
T7	Sawdust	13.00	1.00	78.20	202.35	5.83	2.58	5.07
	SEm (±)	0.39	0.21	0.46	6.25	0.11	0.07	0.10
	CD at 1%	1.62	0.88	1.89	25.73	0.49	0.30	0.42

Yield declined on every substrate in the second flush but the extent of decline differed: paddy straw retained 76.5 per cent of its first-harvest yield (347.12 g against 453.85 g; Table 4) while sugarcane trash and sawdust retained only 55.0 and 51.6 per cent, respectively, indicating that the superior substrates carry a larger reserve of degradable material rather than merely supporting a more vigorous initial flush.

Table 4: Effect of different straw substrates on growth and yield attributes of *C. indica* during the second harvest

Tr. No.	Substrate	No. of pinheads	Mature fruiting bodies	Avg. sporophore wt. (g)	Yield per bed (g)	Stipe length (cm)	Stipe diameter (cm)	Pileus diameter (cm)
T1	Soybean straw	13.33	2.33	70.49	258.18	8.31	3.36	9.07
T2	Paddy straw	16.33	3.67	75.56	347.12	10.09	4.81	11.79
T3	Sugarcane trash	9.67	2.00	68.25	165.90	6.95	2.71	6.95
T4	Maize straw	16.00	3.33	73.50	319.85	9.22	4.26	10.38
T5	Cotton waste	11.00	2.33	71.57	286.15	7.81	3.03	7.91
T6	Sorghum straw	15.00	2.67	73.11	292.56	9.40	3.83	8.83
T7	Sawdust	8.00	0.67	64.54	104.32	5.37	2.07	3.99
	SEm (±)	0.38	0.20	0.47	5.14	0.03	0.06	0.10
	CD at 1%	1.57	0.84	1.94	21.20	0.15	0.26	0.40

The third flush followed the same order at a further reduced level (Table 5), paddy straw yielding 255.91 g per bed against 52.95 g on sawdust. Expressed as a proportion of cumulative yield, the third harvest contributed 24.2 per cent to paddy straw but only 14.1–14.7 per cent to soybean straw, sugarcane trash and sawdust, confirming that the superior substrates not only yield more but remain productive for longer.

Table 5: Effect of different straw substrates on growth and yield attributes of *C. indica* during the third harvest

Tr. No.	Substrate	No. of pinheads	Mature fruiting bodies	Avg. sporophore wt. (g)	Yield per bed (g)	Stipe length (cm)	Stipe diameter (cm)	Pileus diameter (cm)
T1	Soybean straw	11.33	2.00	57.69	98.61	8.22	2.91	7.62
T2	Paddy straw	13.67	3.33	61.93	255.91	10.07	4.40	11.20
T3	Sugarcane trash	9.33	2.00	55.95	77.90	6.59	2.57	6.79
T4	Maize straw	12.33	3.00	60.27	208.46	9.49	4.17	10.09
T5	Cotton waste	10.67	2.33	58.53	158.68	7.73	3.13	7.62
T6	Sorghum straw	12.00	2.67	59.99	180.98	8.12	3.41	9.07
T7	Sawdust	6.67	0.67	52.91	52.95	5.25	2.04	3.94
	SEm (±)	0.34	0.20	0.42	5.21	0.10	0.05	0.08
	CD at 1%	1.41	0.82	1.73	21.50	0.46	0.21	0.34

Cumulative yield and biological efficiency

Cumulative biological efficiency ranged from 35.96 to 105.68 per cent (Table 6). Paddy straw gave the highest biological efficiency (105.68%), followed by maize straw (94.21%) and sorghum straw (86.50%); sawdust gave the lowest (35.96%).

Table 6: Effect of different straw substrates on total yield and biological efficiency (B.E.) of *C. indica*

Tr. No.	Substrate	Total yield/bed (g)	Biological efficiency (%)
T1	Soybean straw	697.79	69.77
T2	Paddy straw	1056.88	105.68
T3	Sugarcane trash	545.58	54.55
T4	Maize straw	942.08	94.21
T5	Cotton waste	805.85	80.58
T6	Sorghum straw	865.04	86.50
T7	Sawdust	359.62	35.96

Note: Total yield = sum of the first, second and third harvests (Tables 3–5).

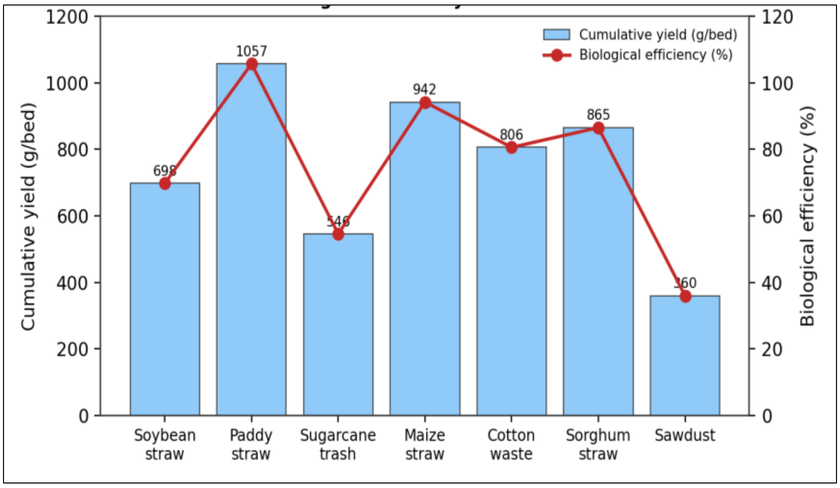

Fig 3: Effect of straw substrate on cumulative yield and biological efficiency of C. indica

Economics of mushroom production

Gross income was computed at the prevailing market price of Rs. 300 per kg of fresh mushroom and the benefit–cost ratio as gross income divided by total input cost (Table 7). Paddy straw was the most remunerative substrate, recording the highest yield (1056.88 g per bed), the maximum net benefit (Rs. 201 per bed) and the highest benefit–cost ratio (2.73:1); despite carrying the highest raw-material cost among the seven substrates, the yield advantage clearly outweighed the additional input cost. Maize straw ranked second (2.51:1), followed by sorghum straw (2.40:1) and cotton waste (2.23:1). Sawdust returned only a marginal ratio of 1.01:1, while sugarcane trash, the cheapest substrate tested, likewise returned a poor ratio (1.56:1).

Table 7: Economics of fresh mushroom production per bed of *C. indica* using different straw substrates

Tr. No.	Substrate	Total inputs (Rs.)	Yield per bed (g)	Gross income (Rs.)	Net benefit (Rs.)	Benefit–cost ratio
T1	Soybean straw	108	697.79	209	101	1.93: 1
T2	Paddy straw	116	1056.88	317	201	2.73: 1
T3	Sugarcane trash	104	545.58	163	59	1.56: 1
T4	Maize straw	112	942.08	282	170	2.51: 1
T5	Cotton waste	108	805.85	241	133	2.23: 1
T6	Sorghum straw	108	865.04	259	151	2.40: 1
T7	Sawdust	106	359.62	107	1	1.01: 1

Note: Input costs — soybean straw @ Rs. 4/kg, paddy straw @ Rs. 6/kg, sugarcane trash @ Rs. 3/kg, maize straw @ Rs. 5/kg, cotton waste @ Rs. 4/kg, sorghum straw @ Rs. 4/kg, sawdust @ Rs. 3.5/kg, casing material @ Rs. 15/kg, wheat-grain spawn @ Rs. 15/bag, formalin @ Rs. 35, polythene bag @ Rs. 5/bag, miscellaneous @ Rs. 5/bag. Selling price: Rs. 300/kg fresh mushroom.

Discussion

Grain substrate and spawn run

Wheat proved the most suitable spawn substrate in agreement with Singh et al. (2017) [3] and Krishnamoorthy and Balan (2015) [4], and with Awais et al. (2025) [8] and Sahu and Gupta (2024) [9], who likewise recorded the fastest colonization on wheat among the cereals tested. A second group of studies has ranked sorghum ahead of wheat (Senthilnambi et al., 2011 ; Lakshmipathy et al., 2012; Kumar, 2015 ; Renganathan et al., 2018 ; Kumari, 2013 ; Khanagouder and Mallesha, 2017; Kudada et al., 2017; Kerketta et al., 2017) [5, 6, 7, 10, 11, 12, 13, 14, 31], but in every case the two substrates occupy the first two positions, differing only in order and generally by a narrow margin; the 0.66 day gap recorded here between wheat and sorghum is consistent with that pattern and suggests that the choice between them is governed more by strain, grain moisture and supplementation (Kumar, 2014; Maury et al., 2019) [15, 16] than by any intrinsic superiority of either grain. Bajra occupied an intermediate position, in contrast to Devia and Sumbali (2021) [17], underlining that grain preference is species and strain specific. The three pulses (rajma, tur, gram) were consistently the poorest substrates, most reasonably because their high protein and low starch content causes softening and clumping on boiling, reducing inter-grain aeration and increasing susceptibility to bacterial contamination, whereas cereal grains retain a discrete structure that provides a more favourable physical and nutritional matrix for mycelial advance.

Straw substrate, biological efficiency and economics

The 105.68 per cent biological efficiency recorded for paddy straw lies within the 71.20–196.12 per cent range reported for this substrate by Pani (2010) [17], Chiwan and Sumbali (2016) [19], Patel and Trivedi (2016) [18] and Chakraborty *et al.* (2016) [19] and agrees with the general superiority of paddy straw noted by Selvaraju *et al.* (2015) [20] and the high fruiting body and yield correspondence reported by Mohit *et al.* (2018) [21] and Hasan *et al.* (2020) [22]. Sugarcane trash performed poorly, consistent with Pushparani *et al.* (2025) [23], and sawdust returned the lowest efficiency, corroborating Chakraborty *et al.* (2016) [19] and Dollison (2023) [24]; this is attributable to its high lignin content, low nitrogen availability and dense structure, which restrict aeration and slow enzymatic degradation, although Eakaphun *et al.* (2026) [25] note that selected wood-based materials may become viable if suitably pre-treated or supplemented.

A substantial body of literature has nonetheless favoured wheat straw over paddy straw (Singh *et al.*, 2017; Hasan *et al.*, 2020; Chaubey *et al.*, 2010; Rawal and Doshi, 2014; Kerketta, 2016; Kerketta *et al.*, 2018; Chinara *et al.*, 2020; Qasim *et al.*, 2025) [3, 22, 26, 27, 28, 29, 30, 31]. Wheat straw was not included among the treatments of the present trial, which constitutes a limitation of this study, and a direct comparison of wheat and paddy straw under local conditions is recommended. Combined substrates that have repeatedly outperformed sole substrates elsewhere an equal wheat–paddy mixture (Kumar *et al*., 2018) [34], a 2:1 wheat–paddy combination (Rawal and Doshi, 2014) [29] and sugarcane leaves with wheat straw at 2:1 (Mohit *et al*., 2018) [21] remain untested here and represent an obvious avenue for improving further on the 105.68 per cent biological efficiency already achieved.

The economic analysis confirms that substrate selection for commercial cultivation should be governed by yield performance rather than raw purchase price: paddy straw, despite being the costliest raw material among the seven substrates tested, returned the highest net benefit and benefit–cost ratio because its yield advantage more than offset the higher input cost, whereas sugarcane trash, the cheapest substrate, returned one of the poorest ratios.

Conclusion

Wheat grain was the most suitable substrate for spawn multiplication of *C. indica*, supporting complete mycelial colonization in the shortest time (8.67 days), followed closely by sorghum and maize, while the pulse grains rajma, tur and gram were consistently unsuitable. Among the straw substrates evaluated for bed preparation, paddy straw was unambiguously superior for spawn run, pinhead initiation, all yield attributes across three successive harvests, cumulative biological efficiency (105.68%) and economic return (benefit–cost ratio 2.73:1), followed by maize straw as a creditable second choice; sawdust was unsuitable on every count. Wheat grain spawn combined with paddy straw beds is therefore recommended as the most productive and remunerative combination for commercial cultivation of *C. indica* under Marathwada conditions, although a direct comparison with wheat straw and with combined substrates is recommended as a priority for future work, since these have outperformed paddy straw alone in several other studies.

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