Int.J.Curr.Microbiol.App.Sci (2026) *15*(1): 213-220

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 15 Number 1 (2026)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2026.1501.026

Evaluation of different substrates on production of *Schizophyllum commune*

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Keywords

Schizophyllum commune, Wheat straw substrate, Paddy straw substrate, Saw dust substrate

Article Info

Received: 24 November 2025
Accepted: 28 December 2025
Available Online: 10 January 2026

A B S T R A C T

Present study was carried out to study spawn production of different *Schizophyllum commune* strains using wheat, maize and paddy grain. Wheat grain was found to be most effective medium for complete spawn development, requiring the shortest duration (9.66 days) by strains SC-06p and SC-01, followed by paddy grain (11.33 days) by strain SC-06p. Among substrates, wheat straw was superior showing the shortest spawn run period, earliest pinhead formation and reduced time to first harvest, followed by Paddy straw. Saw dust required the longest spawn run days by strain SC-02 and SC-03 (19.33 days). The highest yield was recorded on wheat straw substrate by strain Sc-06p (18.30 kg per 100 kg wet substrate), followed by Paddy straw substrate by strain SC-06p (17.66 kg per 100kg wet substrate).

Introduction

Schizophyllum commune is a widely distributed basidiomycetes fungus commonly found on dead and decaying wood. It belongs to the phylum Basidiomycota, order Agaricales, and is characterized by the production of a macroscopic fruiting body (basidiocarp) bearing basidiospores on club-shaped basidia. The mushroom has a small, fan-shaped cap measuring 1-4 cm in width, with a tough, spongy texture and creamy white to pale yellow coloration (Davis *et al.*, 2012). Its most distinctive feature is the presence of longitudinally split gills, which give rise to the common name "split-gill mushroom' (Kuo, M. *et al.*, 2003). The fungus grows in shelf-like clusters without a stalk and can survive prolonged drying, resuming growth upon rehydration.

Although traditionally regarded as a single species with a global distribution, molecular studies suggest that S. *commune* may represent a complex of cryptic species (Taylor *et al.*, 2006). The species is distributed worldwide except Antarctica and commonly grows on rotting trees during the rainy season (Guarro *et al.*, 1999). It is edible and nutritionally rich, containing high levels of protein, vitamins, dietary fiber, and essential minerals such as phosphorus, magnesium, potassium, and selenium (Adejoye *et al*., 2007; Ghorai *et al*., 2009). The mushroom is widely consumed in tropical regions for food and medicinal purposes and is known by local names such as *Kanglayen* in Manipur and *pasi* in Mizoram. S. *commune* also possesses significant medicinal properties, including antibacterial, antifungal, antioxidant, and anticancer activities. Its bioactive compounds, particularly the polysaccharide schizophyllan, have shown effectiveness in cancer therapy and immune modulation without notable side effects, highlighting its potential as a functional food and therapeutic agent.

Materials and Methods

The trails were carried out at Dr. Rajendra Prasad Central Agricultural University's Advance Centre of Mushroom Research, CBS&H, Department of Microbiology, Pusa using six strains of S. *commune*. Wheat grain, Maize grain and Paddy grain were tested for their efficiency in producing spawn for the *Schizophyllum commune* fungus.

Production of spawn

Wheat, maize, and paddy grains were cleaned, rinsed, soaked for one hour in water and the excess water was drained off, and half-cooked (soft but not broken). Calcium carbonate and calcium sulphate were added at 1% and 2% of dry weight basis, respectively, prior to cooling to room temperature. The grains were sanitized, packed into 2/3 capacity of 500ml bottles (empty glucose saline bottles) plugged with non-absorbent cotton wrapped in butter paper, filled, and sterilized in an autoclave at 15 lbs psi pressure for 2 hours.

Preparation of Mother spawn

After sterilization and cooling, bottles containing various substrates were shaken to eliminate clumps. They were inoculated with pure mushroom culture grown on PDA media under laminar airflow. The bits were placed on the top of the substrates and incubated at 25±2ºC. This type of spawn is called as mother spawn. A ruler was used to measure the downward linear extension in spawn bottles containing substrate grain on regular basis.

Preparation of planting spawn

Planting spawn was prepared from mother spawn (strains Sc-01, SC-02, SC-03, SC-04, SC-05, SC-06p). In the present study, wheat grain was used as substrate for preparation of spawn and are filled in polypropylene bags (0.5 or 1kg). Bags were sterilized, inoculated under laminar flow, and incubated at 25±2ºC. Contaminated bags were discarded; mycelial run and spawn run was monitored daily until full colonization.

Screening of different substrates in cultivation of *Schizophyllum commune*

Substrates used during *Schizophyllum commune* cultivation included: wheat straw, paddy straw, maize straw, saw dust, litchi leaf, tea waste. The substrates were soaked overnight and drained over a cloth filter to eliminate surplus water, with soaking and boiling for 30-35 min at 60ºC. The moist substrate (63-64%) grown was filled into polythene bags and autoclaved.

Spawning

After cooling, sterilized substrate bags were inoculated with 2% (w/w) planting spawn under laminar air flow to prevent contamination. The spawned bags were incubated in a mushroom growing room at 15-20ºC and 80-90% relative humidity. Post incubation, bags were performed with sterilized blades for light, spraying, and ventilation; yield of *Schizophyllum commune* (Kg/100Kg substrate) was documented.

Crop Management

Sterilize the cropping room with 2% formalin before spawn bags were moved; walls were covered with gunny bags, and were watered twice daily (6 a.m. and 2p.m.) to regulate temperature and moisture levels.

Harvesting and yield

At the end of the incubation period the crop was harvested in subsequent flushes and observations were recorded on spawn run days, pin head formation days and first harvest days. The cumulative yield was calculated by adding the fresh weight at different harvestings in each replication.

Biological efficiency and Benefit-cost Ratio (B:C Ratio)

The yield was expressed in biological efficiency and calculated by using the following formula

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

B:C ratio can be calculated by the following

$$\text{Benefit-Cost Ratio} = \frac{\text{present value of benefits}}{\text{present value of costs}}$$

Results and Discussion

Effect of different grain substrates for spawn production was studied, out of three grain substrates i.e., Wheat, Maize and Paddy grain, Wheat grain was observed most suitable substrate for spawn production and yield potential of *Schizophyllum commune* presented in Table 1. The present results are related to the reporting of Guler *et al.,* (2007), who reported that wheat grain substrate best for *Agaricus bitorquis* spawn production.

Spawn production of *Schizophyllum commune*

The performance of different grain substrates for spawn production of *Schizophyllum commune* strains was evaluated using wheat, maize, and paddy grains. Wheat grain supported the fastest and most uniform mycelial colonization, with strain SC-06p and SC-01 completing colonization in the shortest period (9.66 days), followed by SC-05 (11.00 days), SC-04 (11.66 days), SC-02 (12.33 days), and SC-03 (13.33 days). On maize grain, colonization required a longer duration, with SC-06p and SC-05 completing growth in 14.33 days, while SC-02 showed the slowest growth (17.66 days). Paddy grain showed intermediate performance, where SC-06p achieved complete colonization in 11.33 days, followed by SC-01 and SC-04 (12.33 days), and SC-02 requiring the longest period (14.00 days). Overall, wheat grain was found to be the most suitable substrate for rapid spawn production of *S. commune*.

Yield potential of *Schizophyllum commune* strains

Screening of different substrate viz.., SDS (saw dust substrate), WSS (wheat straw substrate), PSS (paddy straw substrate), MCS (Maize cob substrate), LLS (litchi leaf substrate) and TWS (Tea waste substrate) against different strains of *Schizophyllum commune* was done.

Spawn run period

From the data in Table 2 shows the spawn run period of different strains S. *commune* in different substrate formulae ranged from 12.66-19.33 days. Saw dust substrate have the longest spawn run time followed by Tea waste substrate, Litchi leaf substrate and Maize cob substrate. In contrast, wheat straw substrate and paddy straw substrate had the shortest spawn run time. Paddy straw substrate generally resulted in shorter spawn run period compared to Maize cob substrate and Litchi leaf substrate, while tea waste substrate and Saw dust substrate, showed the long spawn run time.

Table.1 Screening of different grain substrate for spawn production of *Schizophyllum commune* strains.

GRAIN	Complete spawn development by strains (days)*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WHEAT	9.66	12.33	13.33	11.66	11.00	9.66	**11.28**
PADDY	12.33	14.00	13.66	12.33	13.00	11.33	**12.78**
MAIZE	15.00	17.66	16.33	15.66	14.33	14.33	**15.56**
MEAN	12.33	14.67	14.44	13.22	12.78	11.78	

Factors	C.D. (5%)	SE(d)	SE(m)
Grain	0.368	0.180	**0.128**
Strains	0.521	0.255	**0.180**
Grain × Strains	**0.902**	**0.442**	**0.313**

(*) – Average of three replications

Table.2 Effect of different substrate for spawn run period (days)

SUBSTRATE	Days for spawn run*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WSS	14.00	14.66	13.66	13.33	13.33	12.667	**13.61**
PSS	13.33	15.33	14.33	14.66	14.66	13.333	**14.28**
MCS	14.33	16.33	15.33	15.33	15.66	15.000	**15.33**
LLS	16.66	17.66	16.66	16.66	17.00	15.333	**16.67**
SDS	18.00	19.33	19.33	19.00	18.00	17.333	**18.50**
TWS	17.66	19.00	18.66	17.66	17.66	16.333	**17.83**
MEAN	15.67	17.06	16.33	16.11	16.06	15.000	

Factors	C.D. (5%)	SE(d)	SE(m)
Substrate	0.361	0.181	**0.128**
Strains	0.361	0.181	**0.128**
Substrate × Strains	**0.884**	**0.442**	**0.313**

Table.3 Effect of different substrate for pin head formation (days)

SUBSTRATE	Days for pin head formation*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WSS	20.33	22.00	21.33	22.00	20.66	20.33	**21.11**
PSS	20.66	22.66	21.66	21.33	21.66	20.66	**21.44**
MCS	21.66	22.33	22.33	23.00	22.66	22.00	**22.33**
LLS	22.33	23.33	23.33	22.66	23.00	21.33	**22.67**
SDS	23.00	24.66	25.00	24.00	24.33	22.66	**23.94**
TWS	22.33	24.33	24.00	23.33	23.33	22.00	**23.22**
MEAN	21.72	23.22	22.94	22.72	22.61	21.50	

Factors	C.D. (5%)	SE(d)	SE(m)
Substrate	0.358	0.179	**0.127**
Strains	0.358	0.179	**0.127**
Substrate × Strains	**0.877**	**0.439**	**0.310**

Table.4 Days for first harvest

SUBSTRATE	Days for first harvest*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WSS	27.66	32.33	32.00	31.66	31.33	27.33	**30.39**
PSS	29.66	33.00	32.66	32.00	31.66	29.33	**31.39**
MCS	30.33	34.33	34.00	33.33	33.00	30.00	**32.50**
LLS	32.66	35.33	34.33	34.00	33.33	31.66	**33.56**
SDS	36.00	37.33	37.00	36.66	36.33	35.33	**36.44**
TWS	35.00	36.66	36.33	36.00	35.33	33.67	**35.50**
MEAN	31.89	34.83	34.39	33.94	33.50	31.22	

Factors	C.D. (5%)	SE(d)	SE(m)
Substrate	0.382	0.191	**0.135**
Strains	0.382	0.191	**0.135**
Substrate × Strains	**0.936**	**0.468**	**0.331**

Table.5 Screening of different substrate for yield potential of *Schizophyllum commune* (g/1kg) substrate

SUBSTRATE	Yield g/1kg substrate*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WSS	171.00	137.00	110.66	164.00	151.00	183.00	**152.78**
PSS	158.67	127.33	103.33	149.33	138.67	176.67	**142.33**
MCS	143.33	122.00	95.00	131.33	134.33	152.00	**129.67**
LLS	131.33	110.67	86.00	118.67	127.00	136.67	**118.39**
SDS	99.00	88.00	75.00	91.67	95.00	102.33	**91.83**
TWS	108.67	92.33	80.33	98.67	105.33	111.00	**99.39**
MEAN	135.33	112.89	91.72	125.61	125.22	143.61	

Factors	C.D. (5%)	SE(d)	SE(m)
Substrate	1.759	0.880	**0.622**
Strains	1.759	0.880	**0.622**
Substrate × Strains	**4.309**	**2.156**	**1.524**

Table.6 Screening of different substrate for yield potential of *Schizophyllum commune* (1kg/100kg) substrate.

SUBSTRATE	Yield kg/100 kg substrate*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WSS	17.10	13.70	11.06	16.40	15.10	18.30	**15.28**
PSS	15.87	12.73	10.33	14.93	13.86	17.66	**14.23**
MCS	14.33	12.20	9.50	13.13	13.43	15.20	**12.97**
LLS	13.13	11.06	8.60	11.86	12.70	13.66	**11.84**
SDS	9.90	8.80	7.50	9.16	9.50	10.23	**9.18**
TWS	10.87	9.23	8.03	9.86	10.53	11.10	**9.94**
MEAN	13.53	11.29	9.172	12.56	12.52	14.36	

Factors	C.D. (5%)	SE(d)	SE(m)
Substrate	0.176	0.088	**0.062**
Strains	0.176	0.088	**0.062**
Substrate × Strains	**0.431**	**0.216**	**0.153**

Table.7 Biological efficiency of *Schizophyllum commune* strains on different substrates

SUBSTRATE	Biological efficiency (%) *						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06p	
WSS	29.92	23.97	19.36	28.69	26.42	32.02	**26.73**
PSS	27.76	22.28	18.08	26.13	24.26	30.91	**24.90**
MCS	25.08	21.35	16.62	22.98	23.50	26.59	**22.68**
LLS	22.98	19.36	15.04	20.76	22.22	23.91	**20.71**
SDS	17.32	15.39	13.12	16.04	16.62	17.90	**16.06**
TWS	19.02	16.15	14.05	17.26	18.43	19.42	**17.39**
MEAN	23.68	19.75	16.04	21.97	21.91	25.12	

Factors	C.D. (5%)	SE(d)	SE(m)
Substrate	0.308	0.154	**0.109**
Strains	0.308	0.154	**0.109**
Substrate × Strains	**0.754**	**0.377**	**0.267**

Table.8 Benefit-cost ratio of *Schizophyllum commune* strains on different substrates.

SUBSTRATE	Benefit-cost ratio*						MEAN
	SC-01	SC-02	SC-03	SC-04	SC-05	SC-06P	
WSS	2.42	1.74	1.21	2.28	2.02	2.66	**2.05**
PSS	2.17	1.54	1.06	1.98	1.77	2.53	**1.84**
MCS	1.86	1.44	0.90	1.62	1.68	2.04	**1.59**
LLS	1.62	1.21	0.72	1.37	1.54	1.73	**1.36**
SDS	0.98	0.76	0.47	0.83	0.90	1.04	**0.83**
TWS	1.17	0.84	0.60	0.97	1.10	1.22	**0.98**
MEAN	1.70	1.25	0.83	1.51	1.50	1.87	
Factors	**C.D. (5%)**		**SE(d)**		**SE(m)**		
Substrate	0.036		0.018		**0.013**		
Strains	0.036		0.018		**0.013**		
Substrate × Strains	**0.088**		**0.044**		**0.031**		

Figure.1 Fruiting bodies of different strains of *Schizophyllum commune*.

Pinhead formation

Wheat straw substrate recorded the minimum number of days for pinhead formation followed by paddy straw, maize cob and litchi leaf substrates. While Tea waste and Saw dust substrates required the maximum number of days for pinhead initiation as presented in Table 3.

Days for first harvest

Wheat straw substrate required the minimum number of days to first harvest followed by paddy straw substrate, maize cob substrate and litchi leaf substrate, whereas tea waste substrate and saw dust substrates took the maximum time to reach first table, as, shown in Table 4.

Yield and Biological efficiency

The yield of *Schizophyllum commune* varied significantly across the tested substrates and strains. Maximum yield was recorded on wheat straw substrate (WSS) by strain SC-06p, producing 183.0 g per kg substrate (18.3 kg per 100 kg) with a biological efficiency (BE) of 32.02%. In contrast, the lowest yield was obtained on sawdust substrate (SDS) by strain SC-03, yielding 75.0 g per kg substrate (7.5 kg per 100 kg). These results are presented in Tables 5 and Table 6.

Among the strains grown on wheat straw substrate, strain SC-06p exhibited the highest yield and BE, followed by SC-01 (17.1 kg/100 kg; 29.92% BE), SC-04 (16.4 kg/100 kg; 28.69% BE), and SC-05 (15.1 kg/100 kg; 26.42% BE). Lower yields were observed in strains SC-02 (13.7 kg/100 kg; 23.97% BE) and SC-03 (11.06 kg/100 kg; 19.36% BE). Overall, biological efficiency was highest in strains cultivated on wheat straw substrate compared to other substrates, as shown in Table 7. The present results are comparable with the findings of Singh *et al*. (2021), who evaluated six substrate combinations for *Schizophyllum commune*. They reported yields ranging from 60–90 g/kg of wet substrate with biological efficiency of 12.15–18.33%. The highest yield and biological efficiency were obtained with paddy straw + wheat bran, followed by wheat straw + wheat bran, while sawdust substrate produced the lowest values.

Benefit-cost ratio

The highest benefit–cost (B:C) ratio was recorded on wheat straw substrate (WSS) by strain SC-06p (2.66), followed by SC-01 (2.42), SC-04 (2.28), and SC-05 (2.02). Lower B:C ratios were observed in strains SC-02 (1.74) and SC-03 (1.21). Overall, strains cultivated on wheat straw substrate exhibited higher benefit–cost ratios compared to those grown on other substrates, as presented in Table 8.

Data availability

The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Author Contributions

Panchagiri Akhil: Investigation, analysis, writing original draft, Dayaram: Methodology, writing-reviewing, Birudukota Monika: Conceptualization, methodology, writing,

Declarations

Ethical Approval Not applicable.

Consent to Participate Not applicable.

Consent to Publish Not applicable.

Conflict of Interest The authors declare no competing interests.

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How to cite this article:

Panchagiri Akhil, Dayaram and Birudukota Monika. 2026. Evaluation of different substrates on production of *Schizophyllum commune*. *Int.J.Curr.Microbiol.App.Sci.* 15(1): 213-220.
doi: https://doi.org/10.20546/ijcmas.2026.1501.026