



Evaluation of Different Substrates for Spawn Preparation of *Calocybe indica* and its Impact on Yield and Biological Efficiency

Dibakar Panda, Mohan Kumar Biswas, Bhola Nath¹

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ABSTRACT

Background: Popularities of mushrooms is because of attractive texture and delicious taste, among the cultivated mushrooms milky mushroom has ability to grow in higher temperature and is also considered to be alternative of non-veg foods for vegetarians. In India some part of the country holds good promise towards the cultivation of milky mushroom, for fast growth of mycelium or easy cultivation. This experiment is mainly focused on evaluation of yield and yield contributing characteristic of milky mushroom (*Calocybe indica*).

Methods: The spawn production process was carried out at the Experiential Learning Unit, Mushroom Laboratory, Department of Plant Pathology, Institute of Agriculture, Palli Sikha Bhavana, Visva Bharati in the year 2018-2019. Pure culture of milky mushroom procured from Tropical Mushroom Research Centre, OUAT, Bhubaneswar and was maintained on PDA medium. Different food grains for preparation of spawn viz., wheat grain, paddy grain, bajra grain and sorghum grain were used.

Result: In the present work, we have observed that the four easily available food grains can efficiently be used as substrate for preparation of spawn. This study enables us to take decision about selection of food grains for quality spawn production.

Key words: *Calocybe indica*, Mushroom, Mycelium, Spawn substrates, Yield.

INTRODUCTION

The existence of mushroom is of million years ago and it is known for its food value including nutrition, texture and flavor. The productivity of mushroom is much higher so it can lower the malnutrition in many countries. Cultivation of mushroom is economical in which agricultural wastes are convert into valuable food (Yella *et al.*, 2021).

Spawn consists of mushroom mycelium which provides support for better growth and nutrition to the mushroom mycelium. The fully grown mushroom mycelium use as planting material for preparation of beds by the mushroom farmers regarded as spawn. This spawn is treated as equivalent as seed of higher plant (Pathak *et al.*, 2000). During the cultivation of mushroom spawn is known as inoculum and the medium cover with mycelium prepared from pure culture of particular mushrooms strain. Producing of spawn is carried under controlled environment in which mycelium of mushroom grown by increasing over sterilized grains *i.e.*, wheat grain, bajra grain, paddy grain and sorghum, grain *etc.* (Jain and Vyas, 2005).

Calocybe indica has the quality to cultivate in tropical and subtropical region, so milky mushroom has ability to grow on temperature more than 30°C where no other mushrooms like oyster, paddy straw and button can grow better in summer season due to their temperature sensibility (Subbiah and Balan, 2015). This mushroom (*C. indica*) has the alternating option at this temperature and milky mushroom has the high biological efficiency *i.e.*, more than 90 per cent with whitish fruiting body and long shelf-life. Due to the exceptional character the present research work targets to prepare spawn by using different cereal grains as

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substrate for spawn preparation and to observe the effects of the same on growth attributing characters of milky mushroom.

MATERIALS AND METHODS

The spawn production process was carried out at the Experiential Learning Unit, Mushroom Laboratory, Dept. of Plant Pathology, Institute of Agriculture, Palli Sikha Bhavana, Visva Bharati in the year 2018-2019. Pure culture of milky mushroom procured from Tropical Mushroom Research Centre, OUAT, Bhubaneswar and was maintained on Potato Dextrose Agar (PDA) medium. Same medium was used by Thulasi *et al.* (2010) for maintaining the pure cultures.

Dehariya and Vyas (2015) used wheat, jowar and sorghum grains for the preparation of spawn. In the present work, four cereal grains like wheat grain, paddy grain, bajra grain and sorghum grains are used as spawn substrate

(Table 1). The inert matters in these grains were removed manually and washed thoroughly. These grain substrates were boiled in hot water for 30 minutes, then allowed for cooling for a while and drained out excess water. Then allowed to dry by spreading over polythene sheet. After drying the grains, calcium carbonate was added @ 2% for neutralizing the substrates. The sterilized (Hot air oven 160°C for 2 hours) glass bottles were used for filling of substrates. After filling 3/4th volume of these bottles, mouth of the bottles were plugged by using non-absorbent cotton. Then wrapping of the neck of the bottles are done by using paper and rubber bands. Following this process, the bottles were kept in autoclave for sterilization where the temperature was maintained at 121.6°C for 2 hours. After sterilization, the bottles were removed from the autoclave and placed in a room for cooling. After cooling, the bottles were placed into laminar air flow chamber for inoculation. Before inoculation, the laminar air flow chamber was sterilized by UV rays and floor were sterilized by alcohol. These bottles were inoculated aseptically by using mother culture and placed into BOD incubation chamber for growth. In BOD, the temperature was maintained at 27-30°C. After some days the mycelium were colonized in those bottles, then it is used for preparation of mushroom beds.

All the bed substrates were chemically sterilized with 500ppm formalin and 75 ppm carbendazim solution. Mixture of soil and sand (2:1) were used as casing material and those casing materials were sterilized in autoclave for 2 hours at 121.6°C. After following all the necessary processes, the yield and various morphological characters were recorded.

RESULTS AND DISCUSSION

As per the analysis is concerned all the treatment and interactions were significant (at 5% level of significance) to highly significant (at 1% level of significance) that further suggests the post-hoc comparison of all of them to get the

best treatment. To do so we have used least significant difference (LSD) Method of treatment comparison and obtained critical difference (CD) value separately for all the treatments and interactions of all the parameters. By arranging treatments in their descending order and taking

Table 1: List of treatment combinations.

Treatment	Combination
A1B1	Wheat grain + paddy straw
A1B2	Paddy grain + paddy straw
A1B3	Bajra grain + paddy straw
A1B4	Sorghum grain + paddy straw
A2B1	Wheat grain + paddy straw and garden waste
A2B2	Paddy grain + paddy straw and garden waste
A2B3	Bajra grain + paddy straw and garden waste
A2B4	Sorghum grain + paddy straw and garden waste

Table 2: Days required for growth of mycelium on spawn substrates.

CD (5%)	Treatments	Difference	Significance
1.79	T4-T2 (17.60-16.40)	1.20	NS
	T2-T3 (16.40-13.80)	2.60	S
	T3-T1 (13.80-12.40)	1.40	NS

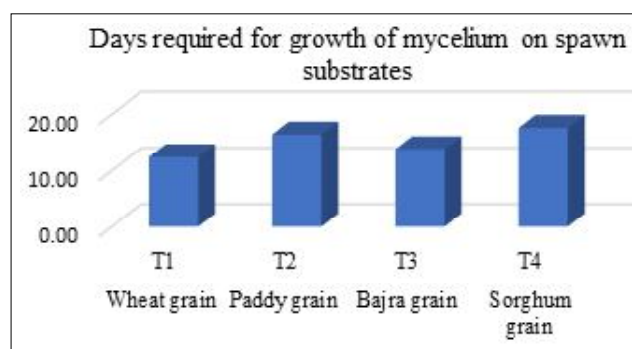


Fig 1: Days required for growth of mycelium on spawn substrates.

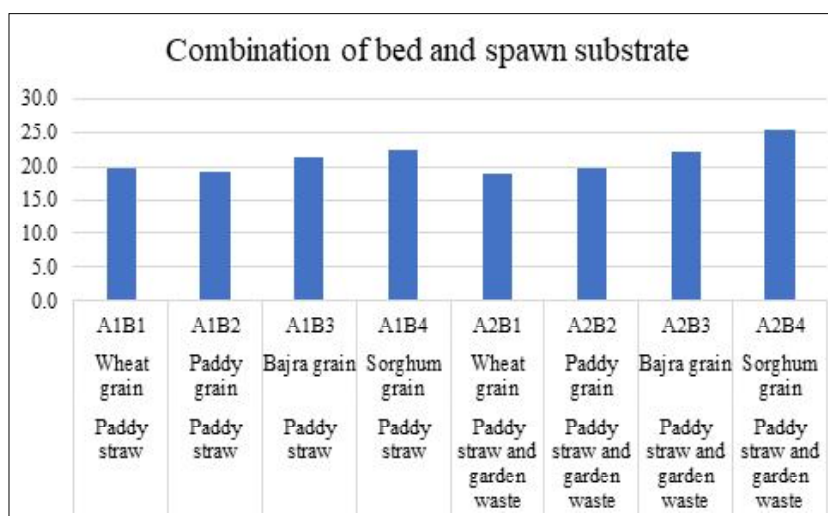


Fig 2: Average number of days for spawn run.

the pairwise difference of them, we have chosen the best treatments of A, B and AB separately with the help of CD for all the biological parameters.

Fig 1 shows the minimum number of days required for the growth of mycelium on spawn substrates and it is observed in T3 (13.80 days) which was resulted in the

analysis of observed data (Table 2). In interaction of both bed substrate and spawn substrate the combination of A1B4 i.e., paddy straw added with spawn prepared from sorghum grain (Fig 2) gives the spawn run in the minimum number of days (22.30 days) the same is evident in (Table 3) similar finding was reported by Senthilnambi *et al.* (2011). Fig 3

Table 3: Days required for spawn run.

Name	CD (5%)	Treatments	Difference	Significance
A	0.810	A2-A1 (21.50-20.67)*	0.83	S
B	1.145	B4-B3 (23.83-21.67)*	2.16	S
		B3-B2 (21.67-19.50)*	2.17	S
		B2-B1 (19.50-19.33)	0.17	NS
AB	1.619	A2B4-A1B4 (25.30-22.30)*	3.00	S
		A1B4-A2B3 (22.30-22.00)	0.30	NS
		A2B3-A1B3 (22.00-21.30)	0.70	NS
		A1B3-A1B1 (21.30-19.70)	1.60	NS
		A1B1-A2B2 (19.70-19.70)	0.00	NS
		A2B2-A1B2 (19.70-19.30)	0.40	NS
		A1B2-A2B1 (19.30-19.00)	0.30	NS

Note: Figures in the parentheses are the average number of days to spawn run substrates for treatment respective combinations.

Table 4: Days to pin head formation.

Name	CD (5%)	Treatments	Difference	Significance
A	0.656	A2-A1 (21.83-20.68)*	1.15	S
B	0.928	B3-B4 (22.65-22.50)*	0.15	NS
		B4-B2 (22.50-20.20)	2.30	S
		B2-B1 (20.20-19.65)	0.55	NS
AB	1.313	A1B4-A1B3 (23.70-23.00)	0.70	NS
		A1B3-A2B3 (23.00-22.30)	0.70	NS
		A2B3-A2B1 (22.30-22.00)	0.30	NS
		A2B1-A2B2 (22.00-21.70)	0.30	NS
		A2B2-A2B4 (21.70-21.30)	0.40	NS
		A2B4-A1B2 (21.30-18.70)*	2.60	S
		A1B2-A1B1 (18.70-17.30)*	1.40	S

Note: Figures in the parentheses are the average number of days to Pin head formation substrates for treatment respective combinations.

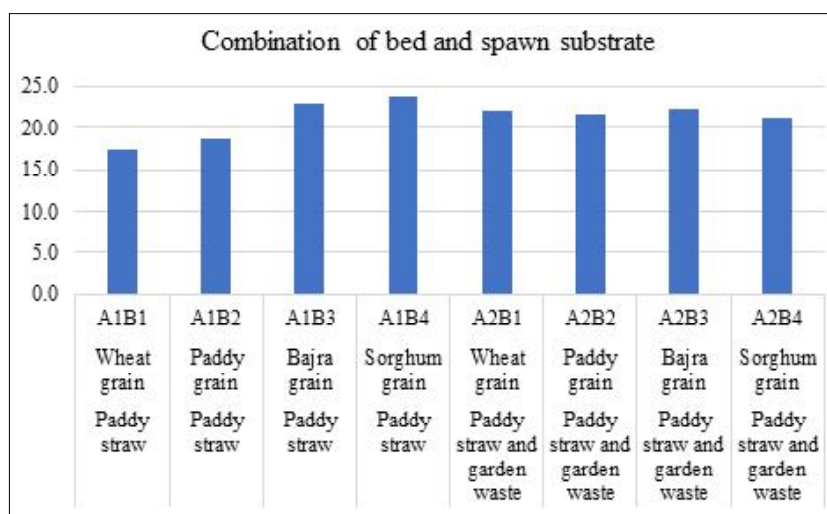
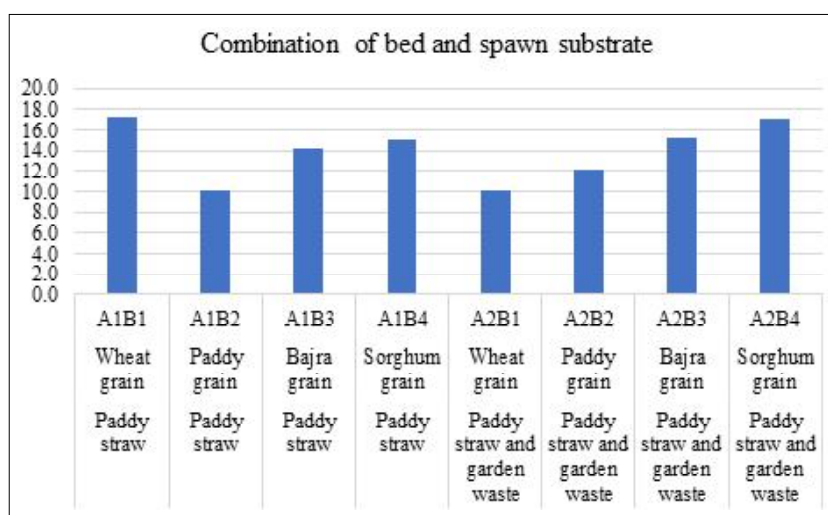
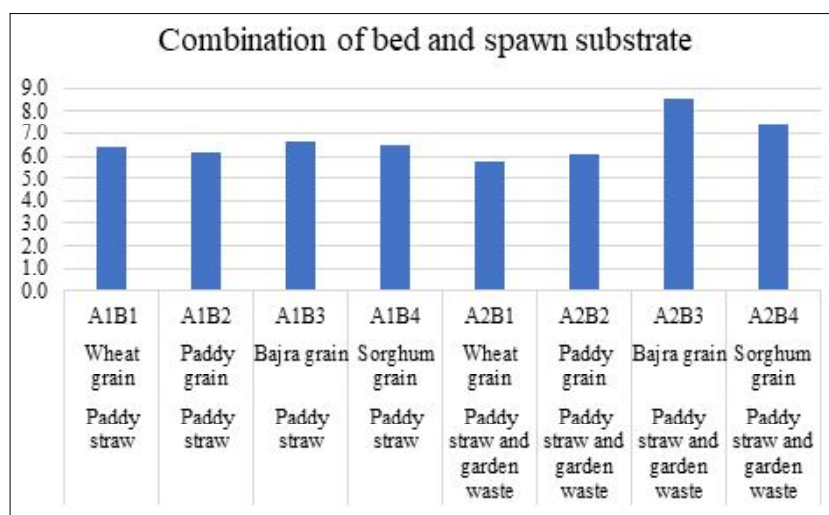


Fig 3: Average number of days for pin head formation.

Table 5: Average stalk length.

Name	CD (5%)	Treatments	Difference	Significance
A	0.305	A1-A2 (14.15-13.67)*	0.48	S
B	0.431	B4-B3 (16.06-14.79)*	1.27	NS
		B3-B1 (14.79-13.74)	1.05	S
		B1-B2 (13.74-11.04)	2.70	S
AB	0.610	A1B1-A2B4 (17.30-17.10)*	0.20	NS
		A2B4-A2B3 (17.10-15.30)	1.80	S
		A2B3-A1B4 (15.30-15.00)	0.30	NS
		A1B4-A1B3 (15.00-14.20)	0.80	NS
		A1B3-A2B2 (14.20-12.10)*	2.10	S
		A2B2-A2B1 (12.10-10.10)*	2.00	S
		A2B1-A1B2 (10.10-10.00)	0.10	NS

Note: Figures in the parentheses are the average stalk length of mushrooms on substrates for treatment respective combinations.

**Fig 4:** Average stalk length of mushrooms.**Fig 5:** Average pileus diameter of mushrooms.

shows paddy straw added with spawn prepared from wheat grain *i.e.*, A1B1 gives the pin head formation in the minimum number of days (17.30 days) data was analysed and tabulated in (Table 4) similar results were reported by Purkayastha and Nayak (1981). Fig 4 shows paddy straw added with spawn prepared from wheat grain *i.e.*, A1B1 gives

the maximum average of stalk length (17.10 cm) the same is evident in (Table 5). The combination of paddy straw and garden waste substrates added with spawn prepared from bajra grain (Fig 5) *i.e.*, A2B3 gives the maximum average of pileus diameter (8.5 cm) data was analysed and tabulated in (Table 6). The combination of paddy straw and garden

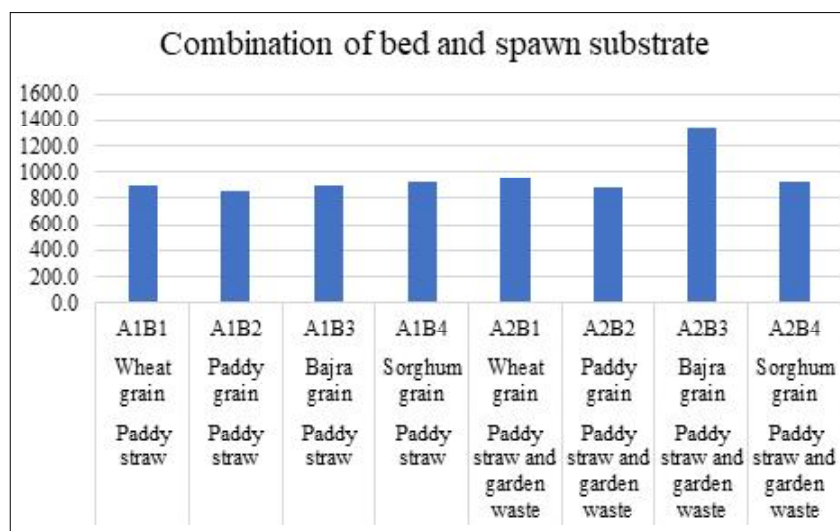


Fig 6: Average yield for different treatment combinations.

Table 6: Pileus diameter.

Name	CD (5%)	Treatments	Difference	Significance
A	0.314	A2-A1 (6.96-6.44)*	0.52	S
B	0.444	B3-B4 (7.56-6.96)*	0.60	S
		B4-B2 (6.96-6.17)	0.79	S
		B2-B1 (6.17-6.09)	0.08	NS
AB	0.628	A2B3-A2B4 (8.5-7.4)*	1.10	S
		A2B4-A1B3 (7.40-6.60)	0.80	S
		A1B3-A1B4 (6.60-6.50)	0.10	NS
		A1B4-A1B1 (6.50-6.40)	0.10	NS
		A1B1-A1B2 (6.40-6.20)	0.20	NS
		A1B2-A2B2 (6.20-6.10)	0.10	NS
		A2B2-A2B1 (2.8-2.5)	0.30	S

Note: Figures in the parentheses are the average Pileus diameter of mushrooms on substrates for treatment respective combinations.

Table 7: Yield (g).

Name	CD (5%)	Treatments	Difference	Significance
A	78.793	A2-A1 (1027.50-897.50)*	130.00	S
B	111.430	B3-B1 (1121.67-929.17)*	192.50	S
		B1-B4 (929.17-927.50)	1.67	NS
		B4-B2 (927.50-871.67)	55.83	NS
AB	157.586	A2B3-A2B1 (1340-955)*	385.00	S
		A2B1-A2B4 (955-931.7)	23.30	NS
		A2B4-A1B4 (931.70-923.30)	8.40	NS
		A1B4-A1B1 (923.30-903.30)	20.00	NS
		A1B1-A1B3 (903.30-903.30)	0.00	NS
		A1B3-A2B2 (903.30-883.30)	20.00	NS
		A2B2-A1B2 (883.30-860.00)	23.30	NS

Note: Figures in the parentheses are the average yield of mushrooms on substrates for treatment respective combinations.

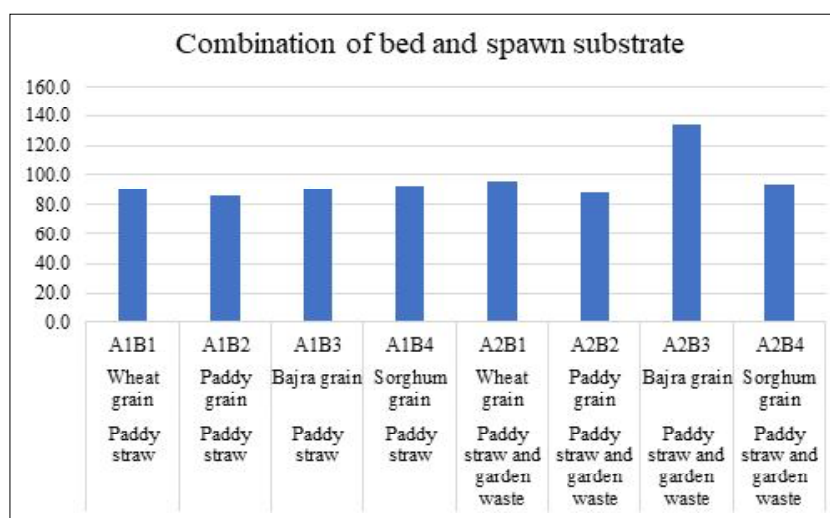


Fig 7: Biological efficiency of different treatment combinations.

Table 8: Biological efficiency.

Name	CD (5%)	Treatments	Difference	Significance
A	7.904	A2-A1 (102.58-89.75)*	12.83	S
B	11.178	B3-B1 (112.17-92.83)*	19.34	S
		B1-B4 (92.83-92.67)	0.16	NS
		B4-B2 (92.67-87.00)	5.67	NS
AB	15.809	A2B3-A2B1 (134.00-95.50)*	38.50	S
		A2B1-A2B4 (95.50-93.00)	2.50	NS
		A2B4-A1B4 (93.00-92.30)	0.70	NS
		A1B4-A1B1 (92.30-90.30)	2.00	NS
		A1B1-A1B3 (90.30-90.30)	0.00	NS
		A1B3-A2B2 (90.30-88.00)	2.30	NS
		A2B2-A1B2 (88.00-86.00)	2.00	NS

Note: Figures in the parentheses are the average biological efficiency of mushrooms on substrates for treatment respective combinations.

waste substrates added with spawn prepared from bajra grain (Fig 6) i.e., A2B3 gives the maximum yield (1340 kg) as well as biological efficiency (134.0%) the similar result is evident in the study of Vijaykumar *et al.* (2014) (Table 7 and 8). Minimum days (13.80 days) required for growth of mycelium on spawn substrates recorded from bajra grain, maximum biological efficiency was recorded from spawn prepared from bajra grain (Fig 7) followed by the spawn prepared from wheat grain (95.50%). Maximum yield was recorded from spawn prepared from bajra grain (1340 g) followed by the spawn prepared from wheat grain (955.0 g), primordia initiation occurred prior in spawn prepared from wheat grain (17.30 days) and later in spawn prepared from paddy grain (18.70 days). The maximum stalk length observed with spawn prepared from wheat grain (17.30 cm). The maximum pileus diameter observed with spawn prepared from bajra grain (8.50 cm). The earliest spawn run observed in spawn prepared from sorghum grain (22.30 days). In the present study, it is found that bajra grain is the most efficient spawn

substrate for commercial cultivation of *C. indica*, which is similar to the findings of Maurya *et al.* (2019).

CONCLUSION

Yield contributing factors of *C. indica* were evaluated on the basis of spawn prepared by using grain substrates viz., wheat, paddy, bajra, sorghum grains.

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