

Suitability of grain substrates and cereal bran additives for spawn production of *Lentinula edodes* (Berk Pegler.)

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Mushrooms are fleshy fungi that develop spore-bearing reproductive structures on organic substrates. For thousands of years, extracts from medicinal mushrooms have been esteemed as crucial remedies for preventing and treating numerous diseases (Wasser and Weis, 1999; Kidd, 2000; Israilides and Philippoussis, 2003; Joshi and Sagar, 2019). Mushrooms rely on substrates for nutrition, which typically consist of lignocellulosic material that supports their growth, development and fruiting. Shiitake is the most significant culinary and medicinal fungus, ranking first in global mushroom production (Sharma *et al.*, 2024). Shiitake mushrooms, scientifically known as *Lentinula edodes* (Berk.) Pegler, are a type of basidiomycetous fungus renowned as a Japanese specialty. The name “Shiitake” derives from two components: “Shii,” representing the oak tree species (*Castanopsis cuspidata*) and “take” denoting mushroom. Additionally, this fungus is known by other names, including Sawtooth oak mushroom, Black forest mushroom, golden oak mushroom and oakwood mushroom. The mushroom can be found growing naturally in Papua New Guinea, China, Taiwan, the Himalayan Mountains, Borneo, and Japan (Annepu *et al.*, 2019). Its popularity continues to rise due to its exotic flavour, nutritional richness, and diverse medicinal benefits make them integral to human diets and health (Etich *et al.*, 2013; Tarushi *et al.*, 2020). A unique advantage of shiitake mushrooms is their

ability to be sold dried, offering an extended shelf life compared to most other mushrooms, which are typically marketed fresh (Royse, 2004; Chauhan and Jaswal, 2015).

Mushroom cultivation begins with spawn production (Stanley and Awi-Waadu, 2010; Thakur *et al.*, 2023). The production of spawn is a critical stage in the cultivation process of any mushroom, as the quality of the spawn directly correlates with the robustness of basidiocarp yield. Acting as the inoculant, spawn is pivotal for fostering the vegetative growth of the mushroom. The production of shiitake spawn depends on the selection of substrate type. Various materials, predominantly agricultural products, can be used in the preparation of mushroom spawn. Commonly used substrates for spawn production include wheat, rye, millet, sorghum, rice and maize (Stanley, 2010). Healthy spawn leads to the production of healthy fruit bodies and higher profits. Utilizing various agricultural and industrial by-products or wastes as economic growth substrates has emerged as an attractive solution for mushroom production (Kuforiji and Fasidi, 2008). The addition of supplements to the basal substrate is a common practice aimed at enhancing the yield, nutritional, and medicinal value of mushrooms. This is achieved by supplying the precise nutrients needed for the growth of the mycelium, which is a critical component of mushrooms (Singh *et al.*, 2017a, b).

Given the importance and popularity of growing shiitake mushrooms in both rural and urban areas of the country, an experiment was conducted to study the effect of different organic additives (wheat bran, rice bran and corn cob powder) on various cereal grains for the spawn growth of *Lentinula edodes*.

The pure culture of Shiitake mushroom (*Lentinula edodes*) strain DMRO-327 was obtained from the Directorate of Mushroom Research Chambaghat, Solan and maintained on potato dextrose agar medium through successive sub-culturing after 15-20 days. The mother spawn of shiitake mushroom was prepared on different grains *i.e.* wheat, pearl millet, barley, maize and soybean. Effect of different cereal bran (Wheat, Rice and Corncob powder) additives were also evaluated at three different concentrations (2, 4 and 6 %). Spawn was produced following standard procedure. After boiling and cooling for 24 h, the grains were mixed with calcium carbonate @ 0.1 per cent to maintain pH at 7.5 and organic additives on wet basis at 2, 4 and 6 per cent concentration on wet weight basis of grains. Spawn bottles were autoclaved at 121°C for 2 hours under 1.5 Kg/cm², cooled down and inoculated with pure culture. The linear growth of mycelium on each substrate was measured at 10-, 20-, 30- and 40- days' interval until complete colonization.

Mycelial Run Rate (MRR) was calculated as downward mycelial growth was recorded with the help linear scale. The grains were inoculated with master spawn on the top surface of the grain substrate and downward linear growth was measured in mm.

$$\text{MRR} = L / N \text{ (mm / day)}$$

{Where, L = Length of the mycelium in mm; N = Number of days}

The laboratory experiments were conducted with 3 replications and results were statistically analysed

by using Completely Randomized Design (CRD) and online software OPSTAT.

Data in Table 1 depicts the mycelial run rate of *L. edodes* starting from 10 to 60 days of incubation. It was observed that various grain substrates had varied effect on mycelial run rate of *L. edodes*. Mycelial run rate ranged from 0.50 mm/day to 1.35 mm/day. Mean maximum mycelial run rate was observed in barley grains (1.27 mm/day) followed by pearl millet grains (1.15 mm/day). However, mycelial run rate on pearl millet was at par to wheat grains (1.14 mm/day) and lowest mean mycelial run rate was observed in soybean (0.63 mm/day). During the study, it was also observed that mean maximum mycelial run rate was found maximum after 50 days of incubation (1.15 mm/day).

The table 1 and Fig. 1 also revealed that the maximum mycelial run rate was observed in barley grains (1.35 mm/day) after 50 days of incubation followed by wheat grains (1.29 mm/day) and pearl millet (1.28 mm/day). Minimum mycelial run rate was observed in soybean grains (0.50 mm/day) after 60 days of incubation. It was concluded from the data that up to 50 days of incubation the mycelial run rate in wheat and barley grains increased and after that started to decline, whereas, in pearl millet, maize and soybean, mycelial run rate increased continuously. The results indicated that increase and decrease of MRR in different substrates varied may be due their physical texture and nutrient release.

It was observed that fortification with wheat bran on various grain substrates had varied effect on mycelial run rate of *L. edodes* (Table 2). Mean maximum mycelial run rate was observed maximum in wheat grains (1.95 mm/day) followed by pearl millet (1.92 mm/day) with 6 per cent wheat bran supplementation. The mean maximum mycelial growth rate was observed after 10 days of incubation (1.61 mm/day) followed by 60 days (1.60 mm/day). The

Table 1. Effect of different grain substrates on mycelial run rate of *Lentinula edodes*

Substrate	Mycelial run rate (mm/day)						Mean
	10	20	30	40	50	60	
Wheat	0.88	1.05	1.18	1.22	1.29	1.24	1.14
Pearl millet	0.80	1.12	1.21	1.19	1.28	1.28	1.15
Barley	1.27	1.25	1.19	1.31	1.35	1.23	1.27
Soybean	0.58	0.60	0.77	0.71	0.60	0.50	0.63
Maize	0.95	0.95	1.14	1.21	1.23	1.29	1.13
Mean	0.90	0.99	1.10	1.13	1.15	1.11	
Factors	C.D.	S.E.(d)					
Factor(A)	0.03	0.01					
Factor(B)	0.03	0.02					
Factor(A X B)	0.07	0.04					

Table 2. Effect of wheat bran supplementation on mycelial run rate of *Lentinula edodes* on different grain substrates

Substrate	Concentration (%)	Mycelial run rate (mm/day)						Mean
		10	20	30	40	50	60	
Wheat	2	1.73	1.27	1.44	1.56	1.73	1.79	1.59
	4	1.93	1.63	1.66	1.84	1.92	1.94	1.82
	6	2.20	1.78	1.83	1.97	1.94	1.99	1.95
Pearl millet	2	1.63	1.35	1.42	1.51	1.67	1.75	1.55
	4	1.87	1.62	1.56	1.81	1.92	1.92	1.78
	6	2.07	1.82	1.79	1.92	1.91	1.98	1.92
Barley	2	1.57	1.20	1.35	1.44	1.55	1.76	1.48
	4	1.73	1.38	1.47	1.60	1.72	1.96	1.64
	6	1.93	1.58	1.59	1.72	1.93	1.98	1.79
Soybean	2	1.03	0.93	0.72	0.60	0.53	0.44	0.71
	4	1.43	0.98	0.74	0.60	0.53	0.46	0.79
	6	1.60	1.15	0.94	0.71	0.62	0.52	0.92
Maize	2	0.97	0.77	0.65	1.06	1.29	1.57	1.05
	4	1.10	0.83	0.71	1.51	1.68	1.93	1.29
	6	1.37	0.93	0.92	1.63	1.79	1.98	1.44
Mean		1.61	1.28	1.25	1.43	1.52	1.60	
Factors		C.D.	S.E.(d)					
Factor A		0.05	0.03					
Factor B		0.03	0.02					
Interaction (A×B)		0.13	0.06					

maximum mycelial run rate was observed in 6% wheat bran supplemented wheat grains (2.20 mm/day) after 10 days of incubation. It was concluded from

the data that up to 10 days of incubation the mycelial run rate in all the treatments showed increasing trends at all concentration and after 20 days started to

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decline. However, in case of soybean grains, the maximum mycelial run rate was observed after 10 days of incubation and after that the mycelial run rate started to declines continuously up to 60 days of incubation. Interaction of additives with wheat grains had a positive impact on mycelial run rate of shiitake mushroom.

It was observed that fortification with rice bran on various cereal substrates had varied effect on mycelial run rate of *L. edodes* (Table 3). The maximum mean mycelial run rate was observed in wheat grains (1.84 mm/day) followed by pearl millet (1.83 mm/day) at 6% concentration. Irrespective of the concentrations of organic additives, the maximum mean mycelial growth was observed after 10 days of incubation (1.76 mm/day) followed by 60 days (1.33 mm/day). The maximum mycelial run rate was observed in supplemented wheat grains (2.33 mm/day)

and pearl millet grains (2.23 mm/day) at 6 per cent rice bran concentration after 10 days of incubation. It could be inferred from the data that mycelial run rate increased after 10 days of incubation in all the treatments at all concentration and after 20 days started to decline, thereafter 30 days of incubation run rate again started to increase.

In corncob powder supplemented substrate, maximum mean mycelial run rate was observed in wheat grains (2.05 mm/day) followed by maize grains (2.03 mm/day) at 6 per cent concentration irrespective of days of incubation. The mean maximum mycelial growth was observed after 10 days of incubation (2.02 mm/day) followed by 20 days (1.66 mm/day). The table 4 revealed that the maximum mycelial run rate was observed in maize grains (2.77 mm/day) at 6 per cent concentration followed by pearl millet grains (2.70 mm/day).

Table 3. Effect of rice bran supplementation on mycelial run rate of *Lentinula edodes* on different grain substrates

Substrate	Concentration (%)	Mycelial run rate (mm/day)						Mean
		10	20	30	40	50	60	
Wheat	2	1.87	1.08	1.44	1.54	1.50	1.53	1.49
	4	2.13	1.18	1.66	1.63	1.77	1.87	1.71
	6	2.33	1.27	1.83	1.81	1.88	1.93	1.84
Pearlmillet	2	1.93	1.08	1.42	1.56	1.46	1.47	1.49
	4	2.13	1.20	1.56	1.62	1.65	1.82	1.66
	6	2.23	1.33	1.79	1.86	1.83	1.96	1.83
Barley	2	1.67	1.13	1.35	1.43	1.45	1.44	1.41
	4	1.87	1.22	1.47	1.53	1.57	1.76	1.57
	6	1.93	1.28	1.59	1.61	1.65	1.79	1.64
Soybean	2	1.17	0.88	0.72	0.47	0.41	0.36	0.67
	4	1.53	1.00	0.74	0.51	0.41	0.35	0.76
	6	1.73	1.03	0.91	0.60	0.49	0.42	0.86
Maize	2	1.07	0.92	0.62	0.81	0.93	1.00	0.89
	4	1.30	1.15	0.71	0.94	0.97	0.99	1.01
	6	1.47	1.20	0.92	1.06	1.27	1.23	1.19
Mean		1.76	1.13	1.25	1.26	1.28	1.33	
Factors		C.D.	S.E.(d)					
Factor A		0.05	0.02					
Factor B		0.03	0.01					
Interaction(A×B)		0.12	0.06					

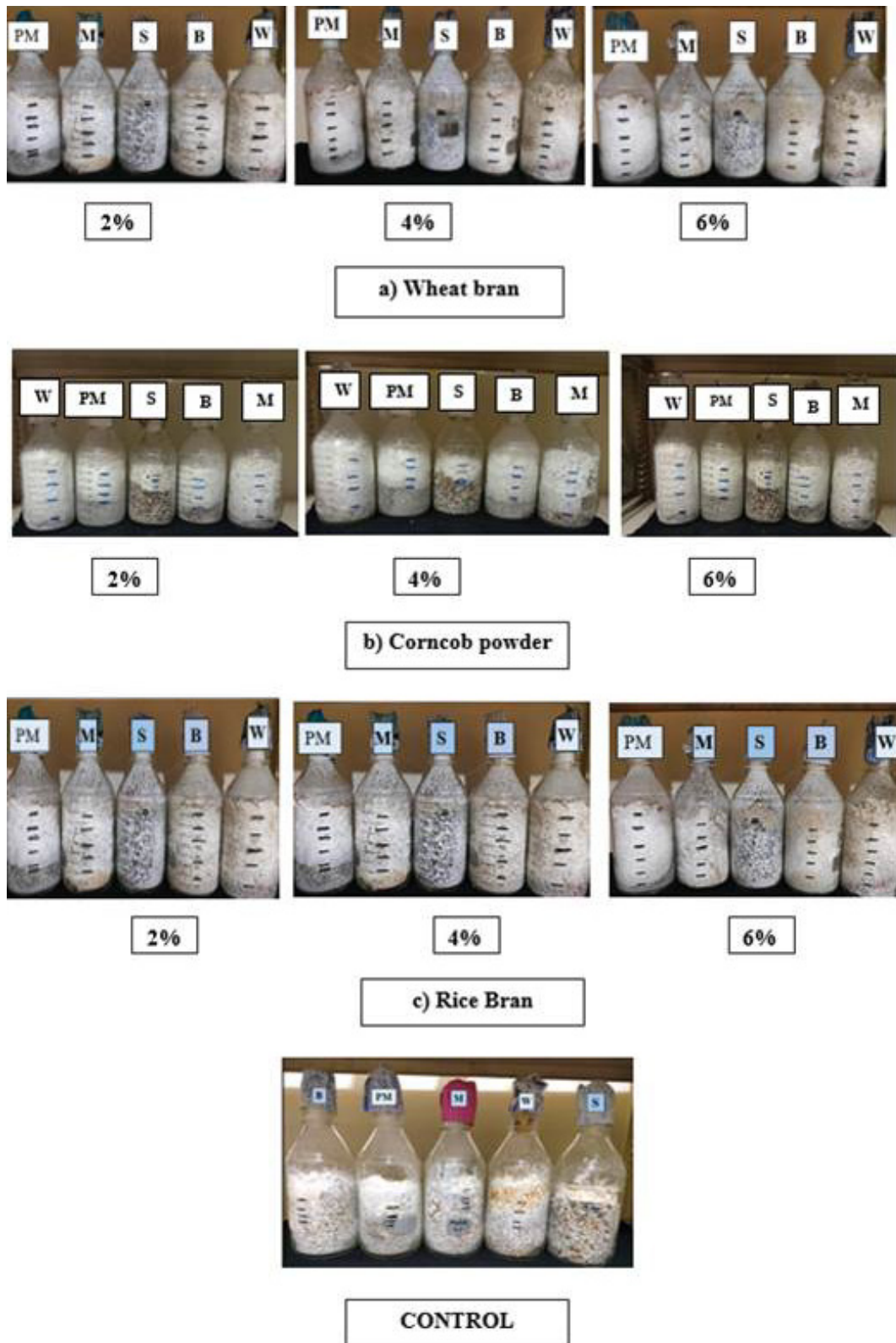


Fig. 1. Effect of different concentrations of cereal bran on the linear mycelial growth of *Lentinula edodes* spawn on various grain substrates, whereas, PM: Pearl millet, W: Wheat, M: Maize, B: Barley, S: Soybean

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Table 4. Effect of corncob powder supplementation on mycelial run rate of *Lentinula edodes* on different grain substrates

Substrate	Concentration (%)	Mycelial run rate (mm/day)						Mean
		10	20	30	40	50	60	
Wheat	2	2.33	1.78	1.58	1.61	1.63	1.56	1.75
	4	2.47	1.92	1.89	1.68	1.70	1.68	1.89
	6	2.70	1.95	2.11	1.85	1.82	1.88	2.05
Pearl millet	2	1.33	1.27	1.39	1.26	1.30	1.12	1.28
	4	1.80	1.55	1.45	1.37	1.35	1.27	1.47
	6	1.93	1.68	1.59	1.46	1.37	1.23	1.54
Barley	2	1.73	1.35	1.39	1.32	1.31	1.36	1.41
	4	1.83	1.67	1.56	1.44	1.35	1.39	1.54
	6	2.13	1.82	1.81	1.61	1.43	1.60	1.73
Soybean	2	1.13	1.17	0.97	0.89	0.85	0.71	0.95
	4	1.37	1.50	1.13	1.02	0.83	0.69	1.09
	6	1.87	1.75	1.24	1.12	0.91	0.77	1.28
Maize	2	2.27	1.67	1.56	1.58	1.47	1.54	1.68
	4	2.57	1.85	1.72	1.62	1.67	1.66	1.85
	6	2.77	1.97	1.94	1.81	1.82	1.87	2.03
Mean		2.02	1.66	1.56	1.44	1.39	1.36	
Factors		C.D.	S.E.(d)					
Factor A		0.06	0.03					
Factor B		0.04	0.02					
Interaction(A×B)		0.14	0.07					

Overall results showed that among all the tested substrates, barley and wheat grains were found to be the best for *Lentinula edodes* spawn production without supplementation. Among all tested substrate after cereal bran supplementation, wheat and pearl millet were observed to be best for spawn production with corncob powder and wheat bran supplementation at 6 per cent concentration. The present results align with findings from earlier reports (Rai, 2003; Dadwal and Jamaluddin, 2004) regarding the common use of wheat grain for spawn production of *Ganoderma lucidum*. Kumar *et al.* (2012) found that the maximum growth was achieved for strain CI-4 in wheat, bajra and maize grains in milky mushroom. Kumar *et al.* (2019) observed that for strain of milky mushroom, CI-17-08 gave maximum spawn growth on wheat grains with rice powder at 1 per cent followed by maize powder at same concentration with

the minimum growth in control. Singh *et al.* (2021) reported maximum spawn growth in maize flour with a growth rate of 9.92 mm/day which was significantly similar to barley flour with a growth rate of 9.81 mm/day as compare to control in *Cordyceps militaris*. The study concluded that the incorporation of cereal brans as an additive significantly facilitated rapid mycelial run rate in *L. edodes*. Additionally, supplementation was identified as the optimal approach for expediting spawn production.

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