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Short Communication

Response of shock treatment on fructification of shiitake mushroom using synthetic logs of different substrates

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Shiitake mushroom [*Lentinula edodes* (Berk.)Pegler] is a palatable fungus which derived its name from two words: "Shii" which means oak tree and "take" means mushroom. This mushroom contains important polysaccharide "lentinan" which exhibits many medicinal properties. The synthetic log method for the cultivation of shiitake mushroom was developed in the Fujian Province of China in 1986. With the development of this method, China was able to dominate the market and is currently known as the largest producer of *Lentinula edodes* (Singh *et. al*., 2020). The present investigations were planned to produce synthetic logs of different substrates and their combinations to evaluate the effect of shock treatment with ice cold water on them.

The pure culture of Shiitake mushroom (*Lentinula edodes*) was procured from Directorate of Mushroom Research, Solan. The pure culture was maintained on Potato Dextrose Agar (PDA) medium slants by sub culturing for further studies. The mother spawn of Shiitake mushroom was prepared on wheat grains by using standard procedure.

For the preparation of the sawdust spawn of Shiitake, five locally available sawdust substrates, Eucalyptus, Mango, Mulberry, Poplar and Tooni were evaluated. The sawdust of different substrate was mixed with wheat bran in the ratio 2:1. Calcium carbonate @ 0.2% of the mixture was added. The moisture level of the mixture was maintained at 65% with tap water. Spawn bottles (18 X 40 cm) were filled with 100 g of the mixture and sterilized in an autoclave at 121 °C under 1.5 kg/cm^2 pressure and allowed to cool down for 24 hrs and transferred to Laminar air flow chamber. The substrate filled bottles were opened under aseptic conditions and a hole was made in the center of the bottle with the help of sterilized glass rod and the hole was filled with grain spawn. Each treatment was replicated four times. The inoculated spawn bottles were incubated at 23 ± 2°C. After 10 days of incubation data on mycelial run rate (MRR) and number of days to complete the mycelial growth on each substrate was recorded.

Preparation of synthetic logs: Various substrates mentioned above were used to prepare synthetic logs for the cultivation of the shiitake according to the procedure given by Directorate of Mushroom Research, Chambaghat, Solan (Sharma, *et. al.*, 2011). The sawdust of five substrates viz., Eucalyptus (E), Mango (M), Mulberry (Mu), Poplar (P), Tooni (T) and their combinations in the ratio of 1:1 were prepared. The combinations of five substrates made were E + M, E + Mu, E + P, E + T, M + Mu, M + P, M + T, P + Mu, P + T, Mu + T. The individual substrates and their combinations was soaked for 12 hours in a plastic bucket. Next day, wheat bran was soaked for 2 to 3 hours prior to the filling of the bags. The sawdust and wheat bran were mixed in the ratio 2:1. The moisture of the mixture was maintained @ 60-70 % using tap water. For the preparation of the 4 bags of each substrate, the total mixture of 1600 g (wet weight basis) was prepared which included 800

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g of sawdust (dry weight basis) and 400g of the wheat bran (dry weight basis). Here the saw dust used was having 25% water content while wheat bran contained 10% water before wetting. The bag size was kept 400 g wet weight per bag. The bags were compressed tightly with hand so that synthetic log prepared would be compact in nature. They should not easily break after autoclaving. The filled bags were autoclaved for 2 hrs at 121°**C** under pressure of 1.5 kg/cm^2 and were allowed to cool down at room temperature for 7- 8 hours.

The synthetic logs of five sawdust substrates and their combinations were evaluated to record the minimum number of days required to complete the incubation process. The effect of cold water shock treatment on the primordial initiation of the Shiitake mushroom was also recorded.

The best substrate on which all the stages were completed in minimum number of days was eucalyptus + poplar combination i.e. 66 days followed by eucalyptus, mulberry, poplar and tooni alone (72 days). Maximum number of days i.e. 88 days was recorded on the combination of Mango + poplar sawdust. The incubation process includes four stages which took different number of days to be completed on various substrates and their combination as shown in Table 1. The different stages have been shown in Fig. 1.

Data recorded on the growth of shiitake on various sawdust substrates and their combinations during the different stages of incubation revealed that there were significant differences in all the stages (Table 1). The substrate combination of eucalyptus + poplar took least number of days for completion of

Table 1. Stages of *Lentinula edodes* during incubation on different substrates and their combinations

S. No.	Substrate	Days to Mycelial Run	Mycelial Coat hardening	Mycelial Bump Formation	Pigmentation	Total incubation days
1.	Eucalyptus	29.3	10.8	22.3	9.5	72
2.	Mango	29.8	11.8	28.5	10.0	80
3.	Mulberry	29.0	10.0	24.3	8.8	72
4.	Poplar	28.0	11.5	22.5	9.5	72
5.	Tooni	27.0	12.0	21.8	10.8	72
6.	Eucalyptus + Mango	30.5	11.8	24.5	10.0	77
7.	Eucalyptus + Mulberry	28.0	10.3	24.0	12.5	75
8.	Eucalyptus + Poplar	23.3	10.3	21.0	11.8	66
9.	Eucalyptus + Tooni	31.0	11.5	24.5	11.8	79
10.	Mango +Mulberry	31.8	12.3	29.0	12.3	85
11.	Mango + Poplar	33.8	13.5	27.5	13.3	88
12.	Mango + Tooni	29.8	10.5	24.8	10.5	76
13.	Poplar + Mulberry	30.0	12.0	21.8	9.8	74
14.	Poplar + Tooni	30.8	11.8	25.5	10.8	79
15.	Mulberry + Tooni	30.3	10.8	23.8	11.5	76
	CD (0.05)	4.2	1.9	2.7	2.1	

Fig. 1. Incubation stages of Shiitake mushroom (*Lentinula edodes*) mycelium on various sawdust substrates A) Mycelium running stage B) Coat Hardening stage C) Mycelial bump formation stage D) Pigmentation

mycelial run stage (23 days), mycelial coat hardening phase (10 days) and mycelial bump formation stage (21 days) while mulberry took least number of days to complete the pigmentation phase. The maximum number of days required for mycelial run (33.8 days), mycelial coat hardening phase (13.5 days) and pigmentation stage (13.0 days) was recorded in mango + poplar sawdust substrate whereas, maximum number of days i.e. 29 days were required for mycelial bump formation stage in case of mango + mulberry combination of substrates. The mulberry, eucalyptus and poplar took minimum number of days to completely turn the white mycelial bumps into brown colored bumps. Among all the substrates and their combinations, the data recorded on during incubation process revealed that combination of sawdust mango + poplar took maximum number of days i.e. 88 days to complete the life cycle of shiitake mushroom while minimum number of days required to complete all the stages was observed on eucalyptus + poplar substrate (66 days) followed by eucalyptus, mulberry, poplar and tooni alone (72 days).

Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Palomo *et al*., 1998; Grodzinskaya *et al*., 2003). Various species of trees have been used for its cultivation, but most production is reported on saw dust of the species of oak (Quercus spp.) (Stamets and Chilton, 1983; Przybylowicz and Donoghue, 1988; Baktemur *et al*., 2022). Regardless of the saw dust used, starchy supplements such as wheat bran, rice bran, millet, rye or corn, can be added at 10 to 40% of dry weight of saw dust (Ivan *et al*., 2003; Royse, 1996). Different tree saw dust have been evaluated by several workers for growth of shiitake mushroom such as babla, champa, garzon, ipil-ipil, jackfruit, mango, segun, shimul, shisoo, rain tree, sawdust mixtures and rice straw (Ashrafuzzaman *et al*., 2009) and reported jackfruit saw dust for faster mycelial growth, pinhead (primordium) and fruiting body.

Temperature shock treatments are necessary to initiate the process of fructification. Data was recorded on the days to primordia initiation and first harvest to study the influence of shock treatment and presented in table 2 and Fig. 2.

To end the vegetative phase of *Lentinula edodes* mycelium, the synthetic logs were exposed to external environment after 90 days of incubation. One lot of synthetic logs of 15 treatments was observed for primordial initiation without giving shock treatment and other lot was given the shock treatment. The shock treatment was given to synthetic logs with ice cold water at 4 to 5 °C for 4 to 5 hours. The primordial initiation was observed only on synthetic logs of eucalyptus, mango, eucalyptus + mango, eucalyptus + mulberry and mango + poplar without giving ice cold water treatment (Table 2).

Table 2. Effect of shock treatment on primordial initiation in *Lentinula edodes*

S. No.	Substrate	Days to Primordia initiation after 90 days of incubation		Days to first harvest	
		Without shock treatment	With shock treatment	Without shock treatment	With shock treatment
1.	Eucalyptus	6	3	15	10
2.	Mango	4	1	10	7
3.	Mulberry	No fruiting	4	-	12
4.	Poplar	No fruiting	2	-	11
5.	Tooni	No fruiting	3	-	9
6.	Eucalyptus + Mango	3	2	13	10
7.	Eucalyptus+ Mulberry	3	3	13	10
8.	Eucalyptus + Poplar	No fruiting	3	-	11
9.	Eucalyptus + Tooni	No fruiting	2	-	10
10.	Mango + Mulberry	No fruiting	1	-	8
11.	Mango + Poplar	5	1	10	8
12.	Mango + Tooni	No fruiting	1	-	10
13.	Poplar + Mulberry	No fruiting	2	-	10
14.	Poplar + Tooni	No fruiting	1	-	10
15.	Mulberry +Tooni	No fruiting	4	-	12

Fig. 2. Primordia initiation with shock treatment

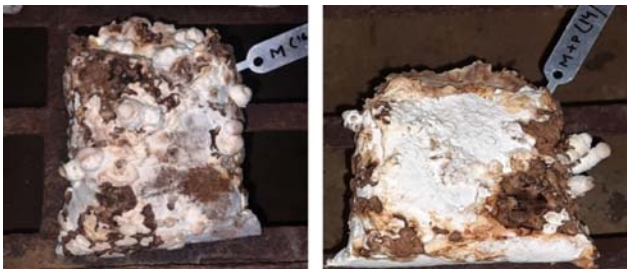

Fig. 3. Primordia initiation without shock treatment

Data recorded on the initiation of primordia with or without shock treatment is presented in Table 2, showing no fruiting on mulberry, poplar, tooni and their combinations without shock treatments except mango + poplar and eucalyptus + mulberry. After ice cold water shock treatment to the synthetic logs of shiitake mushroom for 5 to 6 hours, primordia "baby shiitake" were observed in all the treatments. Data recorded on primordia initiation after the shock treatment revealed that minimum number of days were required for primordia initiation in sawdust substrates of mango and its combinations with other four substrates (1 day) followed by eucalyptus + mango, eucalyptus + tooni, poplar (2 days). Maximum number of days was required by mulberry and its combination with tooni (4 days). Days to first harvest were also affected by shock treatment. It was observed that 7 to 12 days were required for the harvesting of first flush of shiitake mushroom with shock treatment while first harvesting was delayed if the shock treatment was

not given to the synthetic logs. Minimum days required for baby shiitake to become fully grown mushroom were 10 days in case of mango and its combination with poplar substrate and maximum days to first harvest were required by eucalyptus sawdust substrate i.e. 15 days.

Earlier studies have reported that most pin heads are induced in Shiitake mushroom by immersing logs in water at a temperature between 5°C and 20°C (Przybylowicz and Donoghue, 1988; Song *et. al.* 1991; Tokimoto, 2005), for a time between 8 and 72 hours (Stamets and Chilton, 1983). Longer immersion times results with greater diameters or densities of fruiting bodies (Tokimoto, 2005). This mechanical shock results in strong impacts to logs after they are removed from the immersion tank. Its use dates back to eleventh century in China when the mushroom was cultivated under natural conditions (Chen, 2005). This practice was widely used years ago, however, without experimental data (Teixeira and Machado, 1997). The temperature shock treatments were necessary for fructification and it was verified by Tokimoto and Komatsu (1982) who reported that prolonged freezing temperature is not suitable for emerging baby shiitake mushrooms whereas, they can tolerate low temperature treatment if given in shifts. Shiomi et al. (2007) also studied the effect of thermal and mechanical shocks on the first flush of production of *Lentinula edodes* on *Eucalyptus saligna* logs. They concluded that with the increase in the immersion time in water, there is decrease in the exponential behavior of sporophore fresh mass per log, number of sporophores per log, and fresh mass per sporophore on eucalyptus logs.

From the above experiments, it could be conducted that in vegetative growth phases of *Lentinula edodes,* Eucalyptus + Poplar saw dust supported the mycelia growth, coat hardening, bump formation and pigmentation while mango saw dust and its combination with other substrates could prove better in fruit body initiation.

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