DOI: 10.36036/MR.29.2.2020.116258
***Mushroom Research* 29 (2) : 195-201, 2020**

Evaluation of different sawdust substrates for spawn production of shiitake mushroom [*Lentinula edodes* (Berk.)]

[1]Tarushi, [2]Deepika Sud and [3]Arun Sud

[1,2]Department of Plant Pathology, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. 176061, (HP), India
[3]Directorate of Extension Education, CSK Himachal Pradesh Krishi Vishvavidyalaya, Palampur. 176061, (HP), India
[*]Corresponding author, E-mail: attritarushi947@gmail.com

ABSTRACT

Shiitake mushroom is one of the most widely cultivated specialty mushrooms in the world. It is considered as one of the delicious, edible mushrooms which has medicinal properties. Quality spawn production is the key factor in its successful cultivation. More vigorous the spawn, better will be the fruit production. Sawdust is the most popular basic ingredient used for producing spawn for shiitake. However, there is need to evaluate the best sawdust substrate for synthetic log cultivation of shiitake mushroom. In the present study, five locally available sawdust substrates of broad-leaved trees, *viz*., eucalyptus, mango, mulberry, poplar and tooni were evaluated to produce shiitake spawn following standard procedure. The growth of the spawn was measured in terms of MRR [Mycelial Run Rate (cm day^{-1})]. Observations revealed that the MRR on eucalyptus substrate formulation was fastest among all other formulations. Shiitake mushroom required 44 days to complete mycelial growth on eucalyptus followed by 54 days on tooni while all other substrates took more than 60 days for complete growth. The MRR on different substrates ranged from 0.16 to 0.30 cm day^{-1}. The highest MRR was observed in eucalyptus sawdust (0.30 cm day^{-1}) followed by tooni (0.25 cm day^{-1}). There was no significant difference in the MRR of mulberry and poplar when used as sawdust substrates. The lowest MRR was recorded on mango sawdust i.e. 0.16 cm day^{-1}. The higher rate of mycelial running in eucalyptus sawdust may be due to the presence of right proportion of alpha-cellulose, hemi-cellulose, pectin, lignin and suitable C: N ratio.

Keywords: Mycelial run rate, sawdust, spawn, substrate

Mushrooms are macro fungi which are ubiquitous in nature. In today's world, mushrooms are inescapable because of their nutritional benefits. Nowadays, people are more aware and concerned about their health. Therefore, mushrooms are considered as the better option to meet their nutritional requirements easily. *Lentinula edodes* (Berk.), commonly known as the ''shiitake mushroom", is the second most popular edible mushroom in the global market which is attributed not only to its nutritional value but also to possible potential for therapeutic applications (Bisen *et al*., 2010). Its nutritional components include bio-active polysaccharides such as b-D-glucan, heteroglucan, xylomannan, lentinan and eritadenine; free sugars including arabinose, arabitol, mannose, mannitol, tre-halose and glycerol; vitamins (B2, B12, D2) and dietary fibre (Hobbs, 2000; Sharma and Annepu, 2018).

http://epubs.icar.org.in/ejournal/index.php/MR/article/view/116258

India is a largely agricultural country and producing a huge quantity of agro-wastes every year approximately 620 million tons (Singh and Sidhu, 2014). Mushrooms depend on substrates for nutrition and the substrate is normally a source of lingo-cellulosic material which supports growth, development and fruiting of mushroom (Chang and Miles, 2004). In India, successful cultivation of shiitake mushroom on industrial scale has been reported on sawdust, wheat straw, rice bran, wheat bran, hazel nut or their combinations etc. (Shukla, 1995; Kaur and Lakhanpal, 1995; Sharma *et al*., 2006; Puri, 2011; Sharma *et al*., 2011; Annepu *et al.,* 2018). This mushroom is cultivated on both natu-ral and artificial logs since this white rot fungus is able to colonize different types of agricultural wastes as growth substrates, although exploitation of the substrate varies with the species, strain, and cultivation technology (Annepu *et al.,* 2019). In particular, the use of sawdust-based cultivation to replace natural logs has contributed to expanding the production and consumption of shiitake mushroom (Vladimar *et al.,* 2015). As per the information, shiitake mushroom is usually cultivated on the 'shii' trees [*Castanopsis cuspidata* (Thunb.) Schott] or wood logs in Japan. Hence, for the successful cultivation of the shiitake mushroom in subtropical areas of Himachal Pradesh, there is a need to assess best indigenous sawdust substrate for the quality spawn production of this mushroom. Therefore, in present studies different indigenous sawdust substrates were compared for the successful colonization of shiitake mycelium for the best spawn production.

MATERIALS AND METHODS

Culture and spawn preparation

The pure culture of shiitake mushroom (*Lentinula edodes*) was procured from the ICAR-Directorate of Mushroom Research, Solan and maintained on Potato Dextrose Agar (PDA) medium slants for further studies. The growth of the mycelium was observed on the PDA slants after 7-10 days. The sub-culturing of the culture was done by transferring the culture on fresh media subsequently after 15 to 20 days. The mother spawn of shiitake mushroom on wheat grains was prepared by using standard procedure. For preparation of spawn on sawdust substrates five locally available sawdust substrates *viz.,* eucalyptus, mango, mulberry, poplar and tooni were evaluated and the details regarding these substrates are given in table 1. 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 × 40 cm) were filled with 100g of the mixture. The bottles were sterilized in an autoclave at 121°C under 1.5 Kg/cm^2 pressure and allowed to cooled down for 24 hrs and transferred to Laminar air flow chamber (Ashrafuzzaman *et al*., 2009).

Under aseptic conditions, the substrate filled bottles were opened and a hole was made in the center of the bottle with the help of sterilized glass rod. The grain spawn prepared containing shiitake mushroom mycelium was placed in the opening of the spawn bottles such a way that spawn fall into the hole

Table 1. Broad –leaved tree sources of sawdust used as substrate base for spawn production of shiitake Mushroom

Sr. No.	Substrate	Common name	Scientific name	Family
1.	Eucalyptus	Safeda	*Eucalyptus spp.*	Myrtaceae
2.	Mango	Aam	*Mangifera indica*	Anacardiaceae
3.	Mulberry	Shehtoot	*Morus alba*	Moraceae
4.	Poplar	Poplar	*Populus spp.*	Salicaceae
5.	Tooni	Red Cedar	*Tuna cilliata*	Meliaceae

made with glass rod to ensure uniform growth of mycelium. Each treatment was replicated quadruple times. The inoculated spawn bottles were incubated at 23 ± 2°C. After 10 days of incubation data on MRR was calculated on each substrate and number of days to complete the mycelial growth on each substrate was recorded.

Mycelial run rate (MRR)

Mycelial run rate is a measurable tool used to find the growth of mycelium of *L. edodes*. It is calculated by using the following formula:

$$\text{Mycelial run rate (cm day}^{-1}\text{)} = \frac{\text{Length of the mycelium running (cm)}}{\text{Number of days}}$$

RESULTS AND DISCUSSION

Different indigenous sawdust substrates were studied for the days required for spawn development and to compute the rate at which mycelium was running per day. The data regarding the substrates used and days required for spawn development was presented in table 2. Significant difference was observed in the spawn development of shiitake mushroom on different sawdust substrates. Out of five sawdust substrates, eucalyptus substrate required minimum period (44 days) to complete the spawn development in glass bottle followed by tooni, poplar and mulberry. However, mango substrate took maximum days more than 60 days for the complete mycelial growth. The details regarding the observations on the growth characteristics of the shiitake mushroom on different substrates are presented in table 3 and Fig.1. The MRR of the shiitake mushroom on the different sawdust substrates were presented in table 4. The observations revealed that, highest MRR was recorded on the eucalyptus substrate *i.e.* 0.30 cm /day or 3.0 mm/day and lowest MRR observed on mango substrate i.e. 0.16 cm /day or 1.6 mm/day. It was observed that sawdust substrates eucalyptus, mango, mulberry and tooni were showing maximum mycelial growth after 30 days of incubation except poplar. The poplar sawdust was showing maximum growth after 20 days of incubation. After 30 days of mycelial growth the rate of mycelial run became slow and it started declining. The percentage increase in the mycelial growth of shiitake mushroom was observed maximum in eucalyptus. However, there is no significant percentage increase was seen in case of mango, mulberry and poplar. These were showing the same percentage increase in the mycelial growth of shiitake mushroom (Fig. 2). Hence, we concluded that eucalyptus sawdust as the best substrate for the spawn production of the shiitake mushroom in the Kangra district of Himachal Pradesh.

Production of quality spawn is the most imperative factor for the successful cultivation of any mushroom. Sawdust substrate comprises of different nutrients

Table 2. Days required for complete mycelial growth of *Lentinula edodes* on various substrates

Sr. No.	Sawdust substrate	Spawn development (days)
1.	Eucalyptus	44
2.	Mango	65
3.	Mulberry	60
4.	Poplar	60
5.	Tooni	54

Table 3. Growth characteristics of shiitake mycelium on sawdust of different substrates

Sr. No.	Substrates	Growth characteristics
1.	Eucalyptus	Thick, cottony and long white thread like mycelium
2.	Mango	Thick, fluffy and creamish thread like mycelium
3.	Mulberry	Thin, cottony and small thread like mycelium
4.	Poplar	Thin, cottony and small thread like mycelium
5.	Tooni	Thick, fluffy and white thread like mycelium

Fig. 1. Growth of shiitake mushroom (*Lentinula edodes*) mycelium on different sawdust substrates A) Eucalyptus B) Mango C) Mulberry D) Poplar E) Tooni

Table 4. Effect of various sawdust substrates on the MRR of the shiitake mushroom

Sr. No.	Substrate	MRR at different time interval (days)					
		10	**20**	**30**	**40**	**50**	**60**
1.	Eucalyptus	0.24	0.28	0.30	0.25	Co	Co
2.	Mango	0.18	0.18	0.22	0.20	0.16	0.16
3.	Mulberry	0.18	0.18	0.21	0.20	0.18	0.17
4.	Poplar	0.18	0.21	0.19	0.19	0.19	0.18
5.	Tooni	0.22	0.21	0.25	0.22	0.20	Co
	CD(0.05)	0.438	0.955	1.568	0.829	0.989	0.813

which are needed by fungus for its growth. It acts as container of many carbohydrates, hemi-cellulose, tannins, pectins, lignins and appropriate C:N ratio. The vigour of mycelium for the growth depends on the C:N ratio. Spawn, frequently called "inoculum," is the vegetative tissue of the fungus and consists of a medium which has been permeated by mycelium.

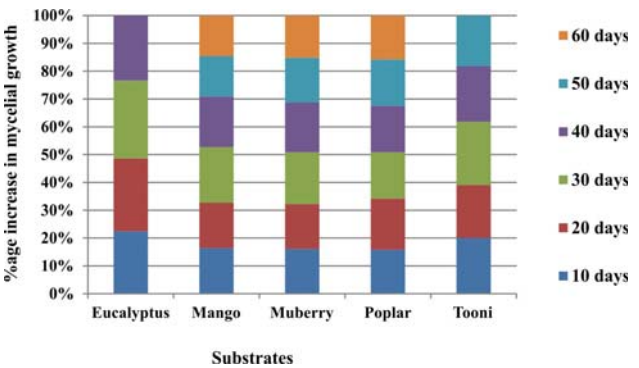

Fig. 2. Percentage increase in the mycelial growth of shiitake mushroom after 10 days of intervals from incubation to complete mycelial growth

Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Miller and Jong, 1987; Palomo *et al.*, 1998; Grodzinskaya *et al*., 2003; Annepu *et al.,* 2019). The other basal ingredients are straw and corn cobs, or their mixes. Irrespective of the main ingredient used for the spawn production, supplements rich in starch such as wheat bran, rice bran, millet, rye or corn, can be added at 10 to 40% of dry weight to the main ingredient (Ivan *et al*., 2003; Royse *et al.,* 1990; Royse, 1996). Shiitake spawn commercially prepared on wooden pegs is known as "plugs," on supplemented sawdust called "sawdust spawn" (Przybylowics and Donoghue, 1988; Kozak and Krawczyk, 1989), and recently also produced on cereal grains (Mata *et al*., 1998). Wheat straw is an abundant and relatively inexpensive raw material that successfully has been used to produce shiitake in France (Delpech and Olivier, 1991) and in India (Annepu *et al.,* 2018). Previously millet seeds have been used as substrate to produce spawn (Mata *et al.*, 2001), and also to increase the yield of shiitake by millet supplementation of wood chip substrate. For the production of healthy fruit bodies of any mushroom, one needs to ensure that we produce healthy and vigorous spawn. Therefore, we need to utilize those substrates which are rich in cellulose, hemi-cellulose, proteins and having high C:N ratio. These substrates are helpful in providing proper nutrients to the fungus for its mycelial run. More vigorous is the mycelium, more spawn produce in short time span. There are many substrates which are used for the production of the shiitake mushroom such as grains substrates and sawdust substrates.

CONCLUSION

From the above experimental findings, we concluded that the eucalyptus sawdust is the best substrate for the spawn production of the shiitake mushroom followed by tooni. The sawdust of poplar and tooni are easily available in Himachal Pradesh and

mushroom growers' can make use of it for the cultivation of this edible and medicinal mushroom to earn huge profits. Spawn production of this mushroom can also be a profitable venture as many growers' of the state are shifting from button and oyster mushroom cultivation to shiitake cultivation as it is highly profitable and can be consumed fresh as well as in dried form.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to the Head of the Department, Department of Plant Pathology, CSKHPKV, Palampur for providing suitable laboratory facilities.

REFERENCES

1. Annepu, S.K., V.P. Sharma, S. Kumar, A. Barh, S. Banyal and S. Kamal. 2018. Enzyme profile of shiitake mushroom strains grown on wheat straw. *Indian Journal of Horticulture* **75(3):** 475-481.

2. Annepu, S.K., V.P. Sharma, A. Barh, S. Kumar, M. Shirur and S. Kamal. 2019. Effects of genotype and growing substrate on bio-efficiency of gourmet and medicinal mushroom, *Lentinula edodes* (Berk.) Pegler. *Bangladesh Journal of Botany* **48(1):** 129-138.

3. Ashrafuzzaman, M., A.K.M. Kamruzzaman, M.R. Ismail, S.M. Shahidullah and S.A. Fakir. 2009a. Substrate affects the growth and yield of shiitake mushroom. *African Journal of Biotechnology* **8(13):** 2999-3006.

4. Ashrafuzzaman, M., A.K.M. Kamruzzaman, M.R. Ismail and S.M. Shahidullah. 2009b. Comparative studies on the growth and yield of shiitake mushroom (*L.edodes*) on different substrates. *Advances in Environmental Biology* **3(2):** 195-203.

5. Bisen, R.K. B.S. Baghel, G.S. Sanodiya and G.B.K.S Prasad. 2010. *Lentinus edodes*: A Macrofungus with Pharmacological Activities. *Current Medicinal Chemistry* **17:** 2419-2430.

6. Chang, S.T. and P.G. Miles. 2004. Mushrooms: cultivation, nutritional value, medicinal effect, and environmental impact, 2nd ed., CRC Press, Boca Raton, FL P.S. 5.

7. Delpech, P. and J.M. Olivier. 1991. Cultivation of shiitake on straw based pasteurized substrates. *In:* Maher, J. (ed) Science and Cultivation of Edible Fungi. Balkema, Rotterdam, pp 523-528.

8. Donoghue, J.D., A.K. Somonson and W.C. Denison. 1996. Spawning techniques for sawdust based shiitake production: past, present and future. *Mushroom News* **44(7):** 6-17.

9. Elisashvili, V., E. Kachlishvili and M. Asatiani. 2015. Shiitake Medicinal Mushroom, *Lentinus edodes* (Higher Basidiomycetes) Productivity and Lignocellulolytic Enzyme Profiles during Wheat Straw and Tree Leaf Bioconversion. *International Journal of Medicinal Mushrooms* **17(1):** 77-86.

10. Grodzinskaya, A.A., H.D. Infante and N.M. Piven. 2003. Cultivation of edible mushrooms using agricultural and industrial wastes. *Agronomia Tropical (Maracay)* **52:** 427-447.

11. Hobbs, Ch. 1995. Medicinal Mushrooms: An Exploration of Tradition, Healing, and Culture, 2nd Ed.; Botanica Press, Inc.: Santa Cruz, CA, USA.

12. Ivan, H.R., A.C. Monteiro, J.O. Machado and J.L. Andrioli. 2003. Shiitake (*Lentinula edodes*) production on a sterilized bagasse substrate enriched with rice bran and sugarcane

molasses. *Brazilian Journal of microbiology* **34(1):** https://doi.org/10.1590/S1517-83822003000100014

13. Joshi, M. and A. Sagar. 2016. Evaluation of various substrates for spawn production and cultivation of shiitake mushroom using corn cobs. *Mushroom Research* **25(2):** 119-124.

14. Kaur, M.J. and T.N. Lakhanpal. 1995. Cultivation of Japanese mushroom shiitake (*L.edodes*) in India. *Indian J Microbiol* **35:** 339-342

15. Kozak, M.E. and J. Krawczyk. 1989. Growing Shiitake Mushrooms in a Continental Climate. ABC Printers, Marinette.

16. Mata, G., P. Delpech and J.M. Savoie. 2001. Selection of strains of *Lentinula edodes* and *Lentinula boryana* adapted for efficient mycelial growth on wheat straw. *Revista Iberoamericana de Micología* **18:** 118-122.

17. Miller, M.W. and S.C. Jong. 1987. Commercial cultivation of shiitake in sawdust filled plastic bags. *Dev Crop Sci* **10:** 421-426.

18. Palomo, A., C. Door and L. Mattos. 1998. Comparative study of different substrates for the growth and production of *Lentinus edodes* Berk ("Shiitake"). *Fitopatologia* **33:** 71-75.

19. Przybylowicz, P. and J. Donoghue. 1988. Shiitake Growers Handbook: the Art and Science of Mushroom Cultivation. Dubuque, Iowa: Kendall/Hunt Publishing Company.

20. Puri, S. 2011. Agricultural wastes as substrate for spawn production and their effect on shiitake mushroom cultivation. *International Journal of Science and Nature* **2(4):** 733-736.

21. Savoie, J.M., P. Delpech, C. Billete and G. Mata. 2000. Inoculum adaptation changes the outcome of competition between *Lentinula edodes* and *Trichoderma* spp. during shiitake cultivation on pasteurized wheat straw. *Mush Sci* **15:** 667-674.

22. Sharma, V.P. and S.K. Annepu. 2018. Advancement in medicinal mushroom research. *In*: Singh B., Peter K. (eds) *New Age Herbals*. Springer, Singapore. https://doi.org/10.1007/978-981-10-8291-7-8.

23. Sharma, S.R., S. Kumar and V.P. Sharma. 2006. Physiological requirement for cultivation of Malaysian strain of shiitake mushroom. *Journal of Mycology and Plant Pathology* **36:** 149-152.

24. Sharma, V.P., S. Kumar and S.R. Sharma. 2011. Cultivation of shiitake (*Lentinula edodes*). *In*: Mushrooms Cultivation, Marketing and Consumption (Singh, M., B. Vijay, S. Kamal and G.C. Wakchaure eds.) p. 207-14, Directorate of Mushroom Research (ICAR), Solan, (HP), India.

25. Shukla, A.N. 1995. Effect of hormones on the production of shiitake, *Lentinus edodes* (Berk.) Sing. *Mushroom Res* **4:** 39-42.

26. Singh,Y. and H.S. Sidhu. 2014. Management of cereal crop residues for sustainable rice-wheat production system in the Indo-Gangetic Plains of India. *Proceedings of the Indian Academy of Sciences.* **80(1)**: 95-114.