J. Mycopathol. Res. **59(4)** : 403-406, 2021; (ISSN 0971-3719)

Crop residues as the substrate for the cultivation of medicinal mushroom *Lentinula edodes*

AMOGHAVARSHA CHITTARAGI[1] AND ASHWANI KUMAR[2]

[1]*Department of Plant Pathology, University of Agricultural and Horticultural Sciences, Shivamogga, Karnataka- 5772041,*

[2]*Chaudhary Charan Singh Haryana Agricultural University, Hisar, Haryana, India- 1250042*

Received : 30.06.2021 Accepted : 28.09.2021 Published : 27.12.2021

Crop residue management is the most significant task in agriculture. Majority of the farmers burn the crop-residue to clean the filed for next crop. Such crop residue can be used effectively in many ways, and one among them is mushroom cultivation. In the current study, we have used the crop residues such as wheat and pearl millet straw for the cultivation of shiitake mushroom *Lentinula edodes*. The study found the fastest spawn run on the pearl millet (38 days) and wheat straw (42 days) followed by sawdust (44 days). Sawdust, wheat, and pearl millet straw took 92, 84 and 83 days respectively for browning and 98, 87 and 86 days for fruiting. The number of fruiting bodies per bag was highest on wheat straw (13.5/ bag) followed by sawdust formulation (12.6/ bag), but it was least on pearl millet straw (5.6/ bag).

Key words: Crop residue, *Lentinula edodes*, medicinal mushroom, Shiitake

INTRODUCTION

India is known for the broad cropping diversity with a vast variety of crops grown every year leading to the enormous production of agro wastes like crop residues, tree wastes, and weeds of land and aquatic system which make the potential renewable resources (Puri, 2011). The agro residue, previously considered as a burden for disposing of, is now considered as a useful resource (Grimm and Wosten, 2018). Numerous techniques are available for the better exploitation of agro wastes in which one among them being mushroom cultivation (Chittaragi et al., 2018a). Mushroom cultivation is an efficient biological process for recovering the food protein and nutrients from the agricultural wastes (Philippoussis, 2009). Mushroom represents 63 billion US dollars market consisting of cultivated edible (54%), medicinal mushrooms (38%) and wild (8%) mushrooms (Royse *et al*. 2017). The most important mushrooms under cultivation are *Lentinula* (Shiitake mushrooms), *Pleurotus* (Oyster mushrooms), and *Auricularia* (Wood ear mushrooms). The button mushroom (*Agaricusbisporus*) though is the most popular edible mushroom, yet it is at fourth position (Grimm and Wosten, 2018). Shiitake mushroom [*Lentinula edodes* (Berk.) Pegler] is the edible and medicinal mushroom which has originated from East Asia and cultivated and consumed in numerous Asian nations (Halpren, 2007). The shiitake cultivation has gained importance because of its medicinal properties, nutritional value, and biodegradation abilities (Li et al., 2018).

The shiitake mushroom is a primary decomposer and degrades cellulose, hemicellulose, lignin, and other plant components (Atila, 2019). The fungus produces different enzymes that can degrade the lignocellulosic residues, thereby using it for their growth and reproduction (Cai *et al*., 2017). Shiitake mushrooms grow on rotting wood of hardwood trees and generally cultivated on short length cut logs. The commercial production of shiitake mushroom involves the use of sawdust and wooden logs. The use of locally available agro-residues provides a way to minimize the cost of cultivation and also helps to reduce the environmental pollution that occurs due to the burning of various agro-residues in the field after the harvest. With this background, we have used different cereal crop residues such as

Correspondence: amoghchittaragi@gmail.com

pearl millet and wheat straw for the evaluation of the bioefficacy shiitake mushroom on these substrates.

MATERIALS AND METHODS

Preparation of pure culture

The Shiitake strain OE-388S obtained from ICAR-Directorate of Mushroom Research, Solan, India, is used in the present investigation. The pure culture of Lentinula edodes was maintained by regular subculturing on Potato Dextrose Agar (PDA).

Preparation of mother culture

Mother culture was prepared by using wheat grain. Calcium carbonate was added at 0.2 per cent, and the substrate was maintained with 65 per cent moisture. Polypropylene bags with size 18 x 25 cm were filled tightly with 200 g of the substrate (wheat grain) and packed. The neck of the bag was fitted with heat resistant PVC (polyvinyl chloride) tube. A hole was made at the middle of the bag with a sharp end stick to place the inoculum. The neck of the bag was plugged firmly with cotton. The bag packets were sterilized in an autoclave for about 90 min at 121 □ with 1.5 kg/cm2 pressure. After the sterilization, the packets were allowed to cool for 24 hours and later transferred into a laminar chamber. A disc of mycelia obtained from the pure culture medium of shiitake mushroom was transferred aseptically into the hole of mother culture packet and again plugged with cotton wool and incubated in the growth chamber at 25± 1°C for two weeks with intermittent mixing.

Preparation of substrates

The straw of Pearl millet and the wheat crop was cut into small pieces (2-3 cm). The substrates like Wheat, Pearl millet and sawdust was used at 85 per cent separately and supplemented with 15 per cent wheat bran and 0.2 per cent calcium carbonate. An equal volume of water was added and mixed thoroughly.

Preparation of spawn packet

The spawn packets were prepared separately for each type of substrate. Polypropylene bags (18 x 25 cm) were filled with 350 g of mixed substrates and packed tightly. A hole was made with a sharp end stick at the centre for the inoculation. The neck of the bag was fitted with heat resistant PVC (polyvinyl chloride) pipe of 1.5 cm length, and the mouth of the bag was plugged with cotton. The bags were sterilized in an autoclave for 90 min at 121 °C with 1.5 kg per cm2 pressure. The bags were allowed to cool for 24 hours. Later two teaspoonful of spawn from mother culture was placed aseptically through the hole of each packet. Inoculated packets were again plugged with cotton and kept in an iron rack in an incubation room at 20 to 23 ? temperature and >80 per cent relative humidity. The data on different parameters recorded periodically.

Fructification

Homogenous production of mushrooms requires a thermal shock. For this purpose, the culture blocks were dipped in chilled water for 15 to 30 min. Then the blocks were transferred to the fructification room. During fruiting, the temperature of 15 to 20 °C and 80 to 90 per cent RH was maintained in total darkness. The water used in humidification contains sodium hypochlorite (5%).

Data Collection

The parameters such as time taken for spawn run, mycelial coat formation, mycelial bump formation, browning/ pigmentation, and fruiting was noted at regular intervals. The number of fruit bodies per bag and average weight of fruit body was also calculated. Biological efficiency (B.E.) was deduced using the formula (Chang and Miles, 2004).

$$\frac{\text{B.E. (\%) = Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

RESULTS AND DISCUSSION

Lentinula edodes is a white-rot fungus that produces a set of lignocellulolytic enzymes, allowing it to grow on lignocellulosic substrates (Elisashvili*et al.*2009). It has the potential to convert cheap lignocellulosic substrates into valuable protein at a low cost (Chittaragi *et al.*, 2018b). Several lignocellulolytic enzymes that are released by it play a significant role in the biodegradation process, and the production of these enzymes depends on the composition of substrates. The present work focused on the use of such locally available high lignocellulose agro residues such as wheat and pearl millet straw for the cultivation of shiitake mushroom.

The spawn run was lengthy in sawdust combination (44 days) as compared to pearl millet (38 days) and wheat straw (42 days). The earliest span of spawn run was in the pearl millet straw formulation which showed significant difference with both sawdust and wheat straw substrates. The time for mycelial coat formation showed no significant difference concerning the substrate formulation used in the study. In case of mycelial bump formation, there was no significant difference between the wheat and pearl millet straw which took 63 and 60 days, respectively but significant difference was observed for pearl millet straw and sawdust formulation (69 days). The days required for browning and fruiting showed the significant difference between sawdust and other formulations, but pearl millet and wheat straw were at par with each other. Sawdust, wheat and pearl millet straw took 92, 84 and 83 days, respectively for browning and 98, 87 and 86 days for fruiting (Table 1). In the present study, Wheat straw encouraged the early fructification in comparison to pearl millet straw and sawdust (Fig. 1). Nitrogen content and C: N ratio of substrates have a role in mushroom production. Early fructification is positively related to nitrogen content. Substrate mixtures with lower C: N favours earlier sporophore induction (Philippoussis *et al*. 2007). Sawdust substrates in bag cultivation showed the fastest fructification compared with traditional log wood method (Moraise*t al*. 2000). Even in our study, the use of agro-residues reduced the days required for the fructification, and it may be due to the early decomposition of the agriculture residues in comparison to sawdust, that exhausted the nutrients for further mycelial growth of the fungus and imposed the kind of stress which made it complete the browning and fruiting earlier in comparison to sawdust.

Table 1: Effect of substrates on Shiitake growth

Growth phase Substrate	Spawn run (days)	Mycelial coat formation (days)	Bump formation (days)	Browning (days)	Fruiting (days)
Saw dust	44	54	69	92	98
Pearl millet straw	38	49	60	83	86
Wheat straw	42	51	63	84	87
CD @ 5 %	3.0	N/S	6.4	3.3	4.7
SE (m)	1.0	1.4	2.1	1.1	1.6

*Values are the mean of three replications.

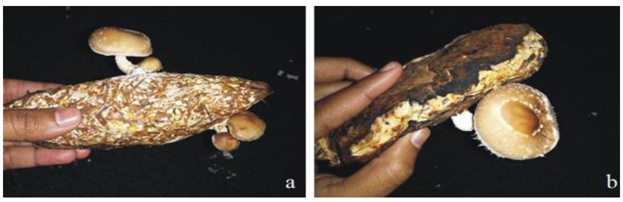

Fig. 1 : Shiitake mushroom production on crop residue (100 g). a) Wheat straw, b) Pearl millet straw.

Fig. 2. Shiitake harvested from wheat straw (a) and pearl millet straw (b) (350 g substrate).

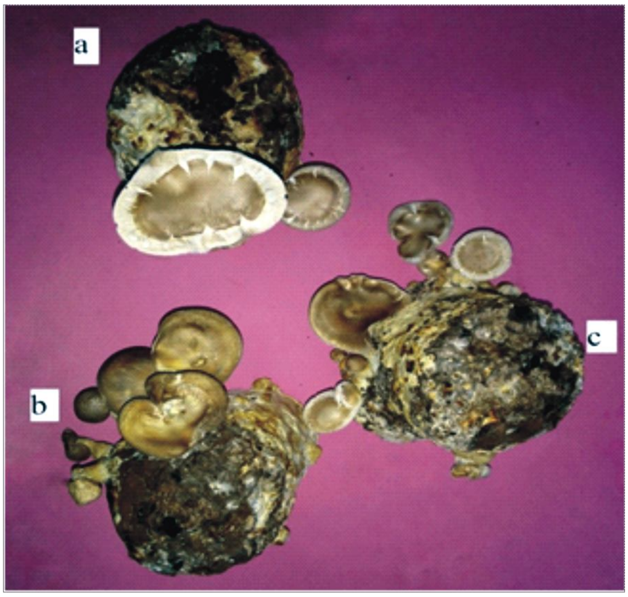

Fig. 3. Shiitake growth on different substrates. a) Pearl millet straw, b) Wheat straw and d) Sawdust.

Table 2: Effect of substrates on Shiitake yield

Substrate	Attribute: No of fruit bodies (per bag)	Fruit body (g/bag)	Biological efficiency (Percent)
Saw dust	12.6	104.2	29.7
Pearl millet straw	5.6	41.7	11.8
Wheat straw	13.5	117.7	33.5
C.D @ 5%	2.0	11.5	3.3

*Values are the mean of three replications

The effect of pearl millet straw, sawdust and wheat straw on various yield parameters viz., number of fruiting bodies, average fruit body weight and biological efficiency were compared per bag basis (350 gram of substrate) (Fig. 3). The number of fruiting bodies per bag were highest on wheat straw (13.5/ bag) which was at par with sawdust formulation (12.6/ bag) but both of them showed significant difference with pearl millet straw (5.6/ bag) which showed the lowest number of fruiting bodies per bag (Fig 2). A similar trend was also observed in the average weight of fruiting bodies per bag which showed highest in wheat straw (117.7 g/ bag) followed by sawdust formulation (104.2 g/ bag) (Table 2). The biological efficiency was highest for the wheat straw (33.5 %) followed by the sawdust (29.7 %) and least by the pearl millet straw (11.8 %).

The present study has found the successful cultivation of shiitake mushroom on the crop residues which encourages the farmers to take up the cultivation by using the locally available residues.

ACKNOWLEDGEMENTS

The first author (AC) acknowledges the Indian Council of Agriculture Research (ICAR) for granting ICAR-PG fellowship.

REFERENCES

Atila, F. 2019. Compositional changes in lignocellulosic content of some agro-wastes during the production cycle of shiitake mushroom. *Sci. Hortic.***245**:263-268.

Cai, Y., Gong, Y., Liu, W., Hu, Y., Chen, L., Yan, L., Zhou, Y., Bian, Y. 2017. Comparative secretomic analysis of lignocellulose degradation by Lentinula edodes grown on microcrystalline cellulose, lignosulfonate and glucose. *J. Proteome.*163:92-101.

Chang, S.T., Miles, P.G. 2004. Mushrooms: Cultivation, Nutritional Value, Medicinal Effect and Environmental Impact, 2nd edition. CRC Press, Boca Raton, FL. pp. 241.

Chittaragi, A., Kumar, A., Muniraju, K. M. 2018a, Evaluation of Various Lignocellulosic Products for the Cultivation of Shiitake Mushroom [*Lentinula edodes* (Berk.) Pegler]. Int. J. *Curr. Microbiol. App. Sci.* **7**: 2199-2203.

Chittaragi, A., Kumar, A., Singh, S. 2018b. Effect of different media on mycelial growth of *Lentinula edodes.Int. J. Curr. Microbiol. App. Sci.* **7**: 2193-2198.

Elisashvili, V., Kachlishvili, E., Tsiklauri, N., Metreveli, E., Khardziani, T.Agathos, S.N. 2009. Lignocellulose-degrading enzyme production by white-rot Basidiomycetes isolated from the forests of Georgia. *World J. Microbiol. Biotechnol.* **25**:331-339.

Grimm, D., Wösten, H.A. 2018. Mushroom cultivation in the circular economy. *Appl.Microbiol.Biotechnol.***102** :7795-7803.

Halpren. G.M. 2007. Healing Mushrooms. Square One Publishers, pp. 48.

Li, S., Wang, A., Liu, L., Tian, G., Wei, S., Xu, F. 2018. Evaluation of nutritional values of shiitake mushroom (Lentinus edodes) stipes. J. *Food Meas. Charact.***12**:2012-2019.

Morais, M.H., Ramos, A.C., Matos, N. 2000. Production of shiitake mushroom (Lentinus edodes) on lignocellulosic residues. *Food Sci. Technol. Int.***6:** 123-128.

Philippoussis A. 2009.Production of mushrooms using agro-industrial residues as substrates. In: *Biotechnology for Agro-industrial Residues Processing*(Eds.P. Sing Nigam and A. Pandey) Springer, pp. 163-196.

Philippoussis, A., Diamantopoulou, P. ,srailides, C. 2007. Productivity of agricultural residues used for the cultivation of the medicinal fungus *Lentinula edodes*. *Int. Biodeter. Biodeg.***59**: 216–219.

Puri, S. 2011. Agricultural wastes as substrate for spawn production and their effect on shiitake mushroom cultivation. *Int. J. Sci. Nat.*, **2**: 733-736.

Royse, D.J., Baars, J., Tan, Q. 2017. Current overview of mushroom production in the world. In: *Edible and medicinal mushrooms: technology and applications*. (Eds. D.C.JohnZied DC, and A.Pardo-Giminez) Wiley & Sons LtD, Hoboken, pp 5–13.