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CULTIVATION OF *LENTINULA EDODES* (BERK.) PEGLER ON SAWDUST SUBSTRATES AND AGRICULTURAL WASTES

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ABSTRACT
Two different strains of *Lentinula. edodes* fungus (L1 and L2) were cultivated on different saw dusts and agricultural wastes viz., wheat straw, coir pith, poplar saw dust, teak saw dust and Sal sawdust etc. alone and in combinations with one another and their yield, biological efficiency, numbers and size of sporocarps and spawn runtime were recorded. Substrate of wheat-straw gave significantly higher yield (80.4g) with 45.9% biological efficiency for strain L1. Ten percent supplementation of wheat bran was the best among all the supplements tried. The wheat straw substrate produced the heaviest and beautiful brown sporocarps with maximum number of fruiting bodies. No satisfactory yield was obtained from coir pith and Sal saw dust either alone or in combination. The minimum time for colonization (55 days) was recorded in the mixture of wheat straw and poplar saw dust and maximum (85 days) in the mixture of Sal and coir pith.

KEYWORDS: *Shiitake* mushroom, Agricultural wastes, Yield, Fruiting body

INTRODUCTION

Lentinula edodes (Berk.) Pegler also known as Shiitake is a white rot wood decay fungus, which produces flavorful brown sporocarps with medicinal properties. Its fungal mycelia has high content of proteins, fibers, vitamins, minerals and low content of lipid specifically cholesterol (Yang et al, 2002). For a long time, this mushroom has been valued for its unique taste and flavor and as a medicinal tonic (Sugui et al, 2003). According to a Chinese folkfare, it was capable of generating stamina, curing colds, improving the circulation and lowering blood pressure (Silva et al, 2007). Recent studies showed that *Lentinula* is effective in lowering serum cholesterol levels and possessing antitumor and antiviral activities (Chang and Miles, 2004). It was also believed to prevent premature aging. Cultivation of this mushroom not only generates huge income but is also helpful in managing agricultural wastes. Cultivation of Shiitake on natural logs is an established industry, especially in Japan and China (San Antonio, 1981). The Shiitake mushroom is the second most important edible mushroom in the world from the standpoint of production just after *Agaricus bisporus*. Its share in total world production of edible mushroom was 25.4 per cent in 1997 (Chang, 1999). In an attempt to develop a more efficient and dependable method for the production of Shiitake mushrooms researchers have focused on the cultivation of *L. edodes* on synthetic sawdust substrates and agriculture wastes. *L. edodes* belongs to a separate group of non-green organisms lacking chlorophyll, called fungi. It can grow saprophytically or parasitically on other organisms and derive their food from complex organic materials found in dead or living tissues of plants and animals. Growing mushrooms on agricultural waste is an example of a transaction between plant and fungal kingdoms. Because of their ability to grow naturally on some organic substrates, mostly waste materials from farms, plantations or factories, any waste that is produced in agriculture system can be recycled or used to produce this value-added product. Currently, millions of tons of agricultural wastes are discarded, burned and neglected. In the process of mushroom growing, however, environmental pollution from such practices may be reduced. Therefore, mushroom cultivation is a complicated procedure, involving a number of operations, which include the preparation of a fruiting culture, spawn and compost and the most significant aspect of mushroom cultivation, if managed properly, is to create zero emissions (no waste) with generation of exciting returns for the growers. Examples of such agro-wastes are straw, corncobs, grass, sawdust, sugarcane bagasse, cotton waste, oil palm waste, coffee pulp and water hyacinth plants, coconut husks, tree leaves, branches and logs (Ashrafuzzaman et al, 2009). These all lignocellulolytic wastes can be used alone or in combination to create edible mushroom growing substrate (Kalmi and Kalyoncu, 2006). Therefore, this study was set up to assess the suitability of saw dusts obtained from different hard wood trees and agricultural wastes like coir pith and wheat straw, supplemented with various levels of different supplements for the production of Shiitake mushroom.

MATERIALS AND METHODS

Culture and spawn preparation -Two isolates of *Lentinula edodes* (Berk.) Singer namely L1 and L2 were obtained from the Mushroom Research and Training Centre (MRTC), Pantnagar. They were maintained on PDA at 25°C. The spawn was prepared on wheat grains.

Substrate preparation- The saw dusts of shagaun or teak (*Tectona grandis*), Sal (*Shorea robusta*) and poplar

(*Populus alba*) trees and agricultural wastes like wheat straw, coir pith were screened. They were used individually as well as in following mixtures (wheat straw and poplar saw dust; 1:1, w/w), wheat straw and coir pith (1:1, w/w), wheat straw and teak sawdust (1:1, w/w), wheat straw and Sal sawdust (1:1, w/w), poplar sawdust and coir pith (1:1, w/w), poplar sawdust and teak sawdust (1:1, w/w), poplar sawdust and Sal sawdust (1:1, w/w), coir pith and teak sawdust (1:1, w/w), coir pith and Sal sawdust (1:1, w/w), teak sawdust and Sal sawdust (1:1, w/w); all enriched with 10 per cent wheat bran. All the substrates were mixed thoroughly. The substrate mixture was filled only 3/4 the capacity, in 2 kg capacity polypropylene bags. The neck of the bags were plugged with non-absorbent cotton and sterilized at 22 lbs pressure (121°C) for 90 minutes. After cooling, the bags were inoculated with the two strains of the fungus.

Different supplements of wheat bran, rice bran and sugarcane bagasse were added in substrate with highest yield at @ 5 and 10 % and their yields were recorded. To compare effect of supplements, wheat straw substrate without any supplement was kept as check.

Cultivation- The bags were kept in the crop room at relative humidity of 80-85 %, at 25°C temperature in the dark for 60-70 days for complete spawn run. After the spawn run, slitting was done and relative humidity of 80-90 % was maintained by sprinkling water. Pinhead initiation started after 20-25 days after slitting.

Yield and biological efficiency- The time taken for pinning after spawn run as well as the number and weight of sporocarps were recorded. Fruit bodies were harvested after maturity. Biological efficiency was calculated using the following formula:

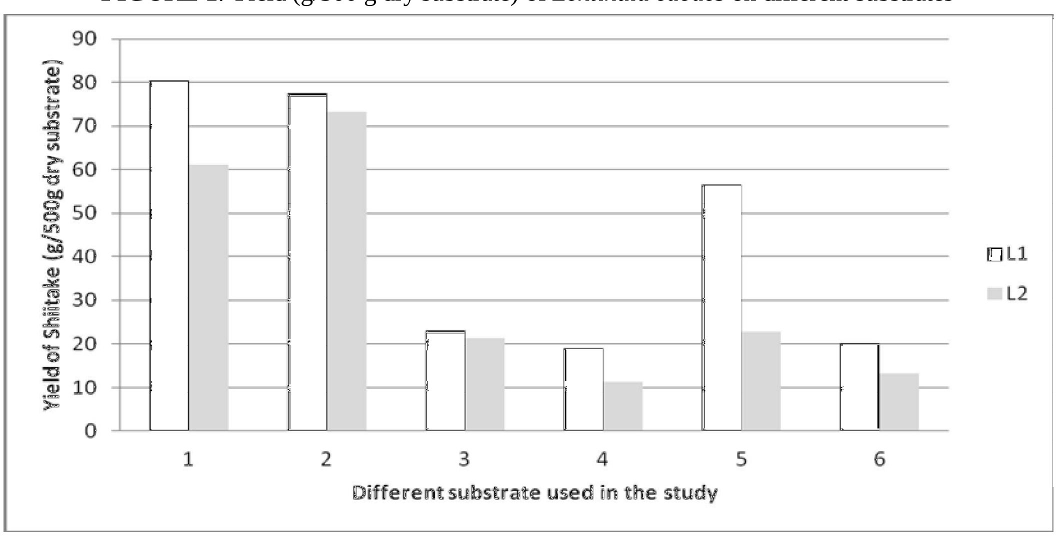
$$\text{Biological efficiency (\%)} = \frac{\text{Fresh weight of fruit body}}{\text{Dry weight of substrate}} \times 100$$

RESULTS AND DISCUSSION

A variety of agricultural crops are grown in India. There are enormous potential of agro wastes in India like crop residues, tree wastes, aquatic weeds etc. They form the potential renewable resources. Several methods have been adopted for the better exploitation of agro wastes. Mushroom cultivation is an eco-friendly method of solid waste management. It is obvious that mushroom cultivation opens the dead lock in the biological degradation of natural resources. Mushrooms are protein rich eco-friendly food and it is cultivable initially as an empirical process. But the scientific understanding of mushroom cultivation will help in improving the cultivation technology.

The results of present experiments reveal the yield, B.E. and runtime of *L.edodes* strains L1 and L2 on different agro-wastes alone or in combination. All the substrates showed significant difference to one another in terms of yield. Wheat straw gave significantly higher yield among all the substrates evaluated. The yield of L1 ranged from 19.0 to 80.4 g/500 g dry substrate whereas the yield of L2 ranged from 11.4 to 61.1 g/500 g dry substrate on wheat straw substrate. Minimum yield was observed in popular sawdust (Figure 1).

FIGURE 1. Yield (g/500 g dry substrate) of *Lentinula edodes* on different substrates



#In Fig 1. Substrates of Wheat straw + coirpith (WS+CP), Wheat straw + Sal sawdust (WS+SSD), Poplar sawdust + coirpith (PSD+CP), Poplar sawdust + Sal sawdust (PSD+SSD), Teak sawdust + coirpith (TSD+CP), Teak sawdust + Sal sawdust (TSD+SSD),Coirpith (CP), Coirpith + Sal sawdust (CP+SSD), Sal sawdust (SSD) produced no fruiting body and hence, are not shown.

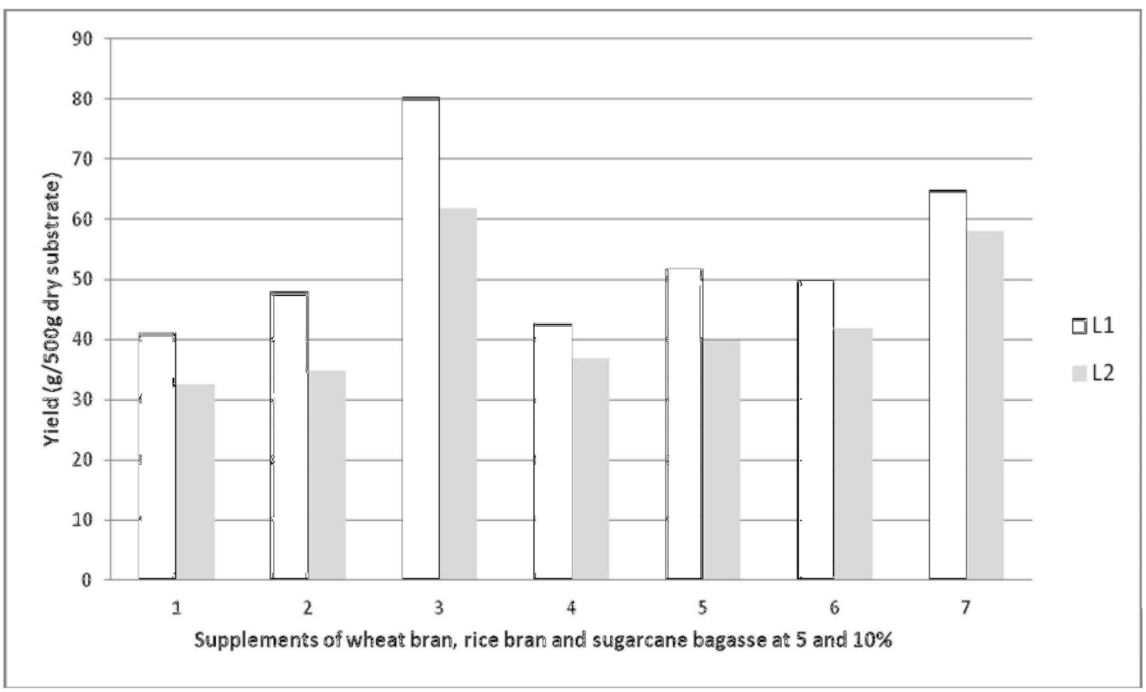
##Numbers from 1 to 6 depict six different substrates, Wheat straw + 10% wheat bran (WS), Wheat straw + poplar sawdust + 10% wheat bran (WS+PSD), Wheat straw + teak sawdust + 10% wheat bran (WS+TSD), Poplar sawdust + 10% wheat bran (PSD), Poplar sawdust + teak sawdust + 10% wheat bran (PSD+TSD), Teak sawdust + 10% wheat bran (TSD) respectively.

TABLE 1. Screening of substrates for production of *Lentinula edodes* strains (L1 and L2)

S. No.	*Substrates*	L_1 Numbers	L_2 Numbers	L_1 Biological efficiency (%)	L_2 Biological efficiency (%)
1.	Wheat straw + 10% wheat bran (WS)	11	11	45.9	40.7
2.	Wheat straw + poplar sawdust + 10% wheat bran (WS+PSD)	9	9	33.2	31.2
3.	Wheat straw + teak sawdust + 10% wheat bran (WS+TSD)	3	3	9.9	9.2
4.	Poplar sawdust + 10% wheat bran (PSD)	5	3	8.14	4.9
5.	Poplar sawdust + teak sawdust + 10% wheat bran (PSD+TSD)	7	3	24.2	9.8
6.	Teak sawdust + 10% wheat bran (TSD)	3	2	5.8	3.8
	CD ($P \leq 0.05$)			10.3	

#Substrates of Wheat straw + coirpith + 10% wheat bran (WS+CP), Wheat straw + Sal sawdust + 10% wheat bran (WS+SSD), Poplar sawdust + coirpith + 10% wheat bran (PSD+CP), Poplar sawdust + Sal sawdust + 10% wheat bran (PSD+SSD), Teak sawdust + coirpith + 10% wheat bran (TSD+CP), Teak sawdust + Sal sawdust + 10% wheat bran (TSD+SSD),Coirpith + 10% wheat bran (CP), Coirpith + Sal sawdust + 10% wheat bran (CP+SSD), Sal sawdust + 10% wheat bran (SSD) did not produce fruiting bodies and hence, are not shown in Table 1.

FIGURE 2. Effect of supplementation of wheat bran on wheat straw substrate on the yield of *L. edodes* strains



#In Fig. 2 numbers show different treatments namely, Wheat straw alone, Wheat straw and wheat bran @ 5 %, Wheat straw and wheat bran @ 10 %, Wheat straw and Rice bran @ 5 %, Wheat straw and Rice bran @ 10%, Wheat straw and Sugarcane baggases @ 5 %, Wheat straw and Sugarcane baggases @ 10 %, respectively.

The wheat straw substrate gave largest heaviest sporocarps with pileus diameter and fruit body weight 75-90 mm and 60-85 g, respectively. However, the substrate of poplar sawdust and teak sawdust individually, produced smallest fruiting bodies (pileus diameter- 20-35 mm and fruit body weight- 18-24 g). The frequent digestion of wheat straw by the fungus suggests that cellulosic and hemicellulosic components of wheat straw cell wall favor the mycelial growth of the fungus. *L. edodes* produces hydrolytic and oxidative enzymes responsible for degradation of organic substrates and the activity of these enzymes depends on the substrate composition and environmental conditions. In the case of wheat straw and poplar saw dust the fungus utilized the organic materials of the substrates for mycelial growth and convert it into sporocarps.

Highest biological efficiency was recorded for L1 on wheat straw substrate 45.9% followed by L2 (40.7%) (Table 1). On comparing sawdust substrate with agriculture wastes, it was found that sawdust substrates gave biological efficiency ranged from 3.8-24.2 % for all the sawdusts which was lower than that of recorded for agricultural wastes (9.2 to 45.9%). Supplementation of wheat bran @ 10 per cent gave significantly higher yield (80.4 g/500 g dry substrate) than other supplements (Figure 2).

In the mixture of wheat straw and coirpith, poplar sawdust and coirpith, teak sawdust and coirpith, Sal sawdust and coirpith, alone, the mycelium completely colonized the above substrates and a few primordia also appeared but they failed to develop into fruiting bodies. In the mixture of wheat straw and Sal sawdust; poplar sawdust and Sal sawdust, the mycelium colonized the substrate after a much longer time but produced no primordia even under favorable conditions. The sawdust of Sal and mixture of Sal and teak sawdust was not colonized completely with the mycelial growth and stopping after a few days and producing no primordial. Several fungi viz. *Trichoderma* spp. , *Penicillium*, *Aspergillus* were found to contaminate these bags during spawn run and hence, inhibiting complete colonization of these substrates by *L.edodes*. Several other workers had also reported *Trichoderma* spp. and *Penicillium* spp. within seven days after the first harvest of mushrooms (Laixuthai *et al*., 1987, Ito, 1987).

The colonization of different sawdust substrates was achieved at durations varying from 55-85 days. The minimum time for colonization (55 days) was recorded in the mixture of wheat straw and poplar sawdust and maximum (85 days) in the mixture of Sal and coirpith. In fact, the sawdust of Sal was never colonized by the fungus completely, the growth stopping after sometime. The wheat straw and sawdust of poplar, when used individually were colonized in a much longer period than when they were used in mixture. The first important stage in the cultivation of a mushroom on a solid substrate is the speed of the hyphal colonization. Once the mycelium has spatially occupied the substrate it will also utilized the nutrients it encounters. This may explain why coirpith and Sal sawdust alone and in combination also gave no satisfactory yield. As in the case of Sal sawdust the substrate is never colonized by the fungus completely and the growth stopped after sometime. The extent of colonization of the substrates is of direct economic importance as the substrate not utilized by *L. edodes* are available to contaminating fungal and bacterial infections resulting in reduced yields.

Reports on cultivation of the shiitake mushroom on similar by-products have manifested variable levels of B.E. These variations are mainly related to spawn rate, fungal species used and supplement added to the substrate (Mane et al., 2007). Rani et al. (2008) recorded biological efficiency of 55–65% for *Pleurotus eous* and *Lentinus connotus* in paddy straw followed by sorghum stalk (45%) and banana pseudostem (33%), respectively. Chou (1984) reported maximum yield (569 g/kg) with 56.9 per cent biological efficiency on tawa sawdust supplemented with barley, whereas the yield obtained from the pure *P. radiata,* poplar and beach had been reported to be negligible. Kaur and Lakhanpal (1995) used *Populus* sawdust alone obtained a very low yield with 6 per cent biological efficiency in comparison to the sawdust mixture. Kovacsne and Kovacs (2000) and Zervakis *et al.* (2001) also reported wheat straw as a most suitable substrate for production *L. edodes*. Variable ranges in biological efficiencies of Shiitake have been reported by different workers with different saw dusts. Philippoussis *et al*. (2003) reported the production of heavier mushrooms on wheat straw and oakwood sawdust substrates with biological efficiency 54.17 per cent whereas, Gaitan *et al.* (2004) reported biological efficiency wheat straw substrate ranged from 24.8 to 55.6 per cent. Nikitina *et al.* (2007) and Silva *et al.* (2005) also found that eucalyptus residues supplemented with cereal brans supported fast growth of *L. edodes* indicating that mycelium extension is related to the bioavailability of nitrogen. The type and concentration of nutrient supplement has a considerable effect both on substrate colonization and on the type of hydrolytic and oxidative enzymes produced. These characteristics may be useful for mushroom growing. Ten per cent formulations with wheat brans help in increasing the mycelial growth of *L. edodes* on all the substrates, as this provides high amount of nitrogen to the growing fungus. It is generally believed that the low amount of available nitrogen (N) in the lignocellulosic substrate is a limiting step in the utilization of wood components. Therefore, the nitrogen added to the substrate serves as nutrients to provide an optimum growth medium. Royse (1985) cultivated Shiitake on the mixture of maple and birch (60:40) sawdust substrate with 10 per cent spring wheat bran and 10 per cent millet and found it was the best formula for nutritional components. Royse *et al.* (1990) advocated that regardless of the main ingredient used, starch based supplements such as wheat bran (10-40% dry weight) serve as nutrients to provide an optimum growing medium.

CONCLUSION

Commercial production of Shiitake mushrooms is largely determined by the availability and utilization of cheap materials of which agricultural lingo-cellulosic waste represents the ideal and most promising substrates for cultivation. We have recorded the yield of *L. edodes* using teak sawdust, wheat straw and poplar sawdust, wheat straw and teak sawdust and poplar and teak sawdust as substrate. The present study thus explored the possibilities for *the* cultivation of *L. edodes* using largely available agro-industrial wastes. This in turn would help to meet the growing demand of the protein need. The substrates used in this study can be considered practical and economically feasible due to their availability throughout the year at little or no cost in large quantities. Utilization of these agro-wastes for the production of Shiitake mushrooms could be more economically and ecologically practical.

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