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EFFECT OF SUPPLEMENTATION OF WHEAT BRAN ON THE PRODUCTION OF SHIITAKE (*LENTINUS EDODES* (BERK) PEGLAR) USING WHEAT STRAW AND SAW DUST SUBSTRATES

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ABSTRACT
Lentinus edodes (Berk.) Peglar, the shiitake mushroom, is worldwide one of the most widely cultivated mushrooms. The cultivation of edible mushrooms is a biotechnological process that uses various residues to produce food of high nutritional value which can be a solution to problems of global importance, such as the lack of protein in developing countries and the possibility of environmental management. Five different strains of *Lentinus edodes* (LeS, OE-38, OE-142, OE-329 and OE-388) were cultivated on wheat straw and saw dust and spawn run time, weight of fruit bodies and biological efficiency were recorded. Wheat straw substrate produced maximum biological efficiency in OE-388 (66.8%) and OE-329 (46.2%) strains. Ten percent supplementation of wheat bran was the best among all the supplements tried. However, in case of sawdust substrate, maximum biological efficiency was obtained at 5 per cent level of wheat bran supplementation in LeS, OE-142, OE- 388 strains. The days for spawn run were also less in wheat straw substrate as compared to saw dust substrate.

INTRODUCTION

Lentinus edodes (Shiitake) is the second most important cultivated mushroom in the World after *Agaricus bisporus* accounting for about 26 per cent of the total world production of cultivated mushrooms. Shiitake is known to contain proteins, lipids (primarily linoleic acid), carbohydrates, fibres, minerals, vitamins B1, B2 and C and ergosterol, the D provitamin, vitamin E and selenium. Shiitake is a rich source of D, C and A vitamins. It is also reported as a good source of antioxidants, such as vitamin E. Shiitake is a white rot fungus capable of decomposing all the structural components of wood, including both cellulose and lignin (Jong, 1989).

Today, shiitake cultivation is widely practiced, not only in Southeast Asia (China, Taiwan, Japan, Korea, Singapore, the Philippines, Sri Lanka and Thailand) but also in North America (The United States and Canada), Europe (France, Germany, the Netherlands, Spain, Italy, England, Switzerland, Belgium, Finland, Sweden), Australia and New Zealand (Oei, 1996; Romanens, 2001) making its cultivation a global industry. In India, cultivation of this mushroom was first reported at Solan in Himachal Pradesh (Shukla, 1994). Worldwide, the most popular basal ingredient used in synthetic formulation of substrate for the commercial production of *L. edodes* is hardwood sawdust which is not a substrate of choice that would find commercial acceptance in regions where sawdust is not easily available. The relative abundance of other agricultural residues could be exploited for commercial production of this mushroom. Examples of such agrowastes 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). All these lignocellulolytic wastes can be used alone or in combination to create edible mushroom growing substrate (Kalmi and Kalyoncu, 2006).

Addition of organic and inorganic supplements to the substrate during cultivation is known to influence the yield of various species of mushroom (Marimuthu and Krishnamoorthy, 1991; Fasidi and Kadiri, 1993). These supplements contain a mixture of protein, carbohydrate and fat, where the protein is the main source of nitrogen. They contain minerals and vitamins that also influence the growth of the fungus. The addition of these supplements aims mainly to increase the levels of nitrogen and carbohydrates available (Han *et al.,* 1981; Royse and Schisler, 1986; Przybylowicz and Donoghue, 1990). Starch based supplements such as wheat bran, rice bran, millet, rye or corn can be added at 10 to 40 per cent of dry weight to the main ingredient for shiitake cultivation (Royse *et al.,* 1990; Royse, 1996; Ivan *et al.,* 2003). The biological efficiency, mean number of mushrooms and mushroom quality of shiitake were evaluated by Rossi *et al*. (2003) and they found that the substrate supplemented with 25 and 30 per cent rice bran yielded higher (98.42 and 99.84%, respectively) as compared to the supplementation levels of 15 and 20 per cent rice bran. Any amount of rice bran added to the bagasse improved mushroom quality with the best being 15 per cent rice bran addition. Kapoor *et al*. (2009) supplemented wheat straw with five organic supplements namely cotton seed meal (CSM), peanut meal (PM), wheat bran (WB), rice bran (RB) and soybean

meal (SBM) @ 10 per cent and 20 per cent (on dry weight basis) and obtained improved mycelial extension rate with 10 per cent rice bran supplementation and with 20 per cent wheat bran supplementation.

Thus, mushroom cultivation is an economical strategy, requiring low resources and area, can be grown throughout the world and all over the year from low-cost starting materials. There is tremendous potential and appeal for growing a highly nutritious food with excellent taste from substrates that are plentiful and not very expensive. Also, it is very environmental friendly, capable of converting the lignocellulosic waste materials into food, feed and fertilizers (Hadar *et al.*, 1992; Jaradat, 2010). Since hard wood saw- dust is not readily available in various regions, the use of wheat straw as a cheap and readily available agri-residue is now being promoted for cultivation of shiitake mushroom. There is, however, a need for evaluating *L. edodes* strains capable of giving high biological efficiency on this substrate and optimizing the rate of supplementation of the substrate. The present study was, therefore, carried out to evaluate the yield of five strains of *L. edodes* on wheat straw and saw dust supplemented with wheat bran @ 5, 10, 15 and 20 percent on dry weight basis.

MATERIALS AND METHODS

Culture and Spawn Preparation

Five strains of *Lentinus edodes* (LeS, OE-38, OE-142, OE-329 and OE-388) were procured from germplasm collection bank of Directorate of Mushroom Research (DMR), Solan. These cultures were maintained on Potato dextrose agar (PDA) slants. The spawn was prepared on wheat grains.

Substrate Preparation

Wheat straw and sawdust were used as a substrate for the fruit body production of *L. edodes* (Agarwal, 2007). The straw was spread on a cemented floor and soaked with water overnight so as to have a final moisture content of 65-70 per cent. The substrate was supplemented with varying amounts of wheat bran i.e. @ 5-20 per cent (w/w) on dry weight basis and calcium sulphate and calcium carbonate were mixed @ 2 per cent and 5 per cent w/w, to give a pH of about 5. The wet substrate and supplements were mixed well manually and then filled in polypropylene bags (40 × 25 cm) @ 1 kg substrate per bag on wet weight basis. By using a test tube, a hole was made in the centre of the bag which was used later on for inoculation. After putting a plastic ring and cotton plug, the bags were sterilized by autoclaving at 20 psi for 90 minutes. After cooling, the bags were inoculated aseptically with wheat grain spawn @ 2-3 per cent on wet weight basis.

Cultivation and Opening of bags

The bags were kept in the crop room at relative humidity of 80-85 per cent, at 25 ± 2°C temperature in the dark for 60-70 days for complete spawn run. After completion of spawn run, the bags were allowed to turn brown and the opening of bags was done after browning and bump formation had taken place in the bags. At this stage, the polypropylene bags were removed with the help of blade/knife and cold shock treatment was given to the blocks of substrate impregnated with mycelium by dipping them in chilled water (4-5ºC) for about 5-10 minutes. These were then incubated at 18 ± 2ºC and 12-16 hr light/ day with 85 ± 5 per cent relative humidity.

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 (Chang and Miles, 2004) was calculated using the following formula:

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

RESULTS AND DISCUSSION

Yield evaluation of *L. edodes* strains on wheat straw

The effect of supplementation of wheat bran revealed that maximum biological efficiency was obtained at 10 per cent level in all the strains. Moreover, 10 per cent wheat bran supplementation resulted in more fruiting bodies and mycelial spread was also fast in all the strains. A significant reduction in yield at higher rate of supplementation of wheat bran (15% and 20 %) was observed. Mycelial rate was fastest with 10 per cent supplementation in all the strains except OE-388 strain.

Two strains, namely, OE-329 and OE-388 were found to give

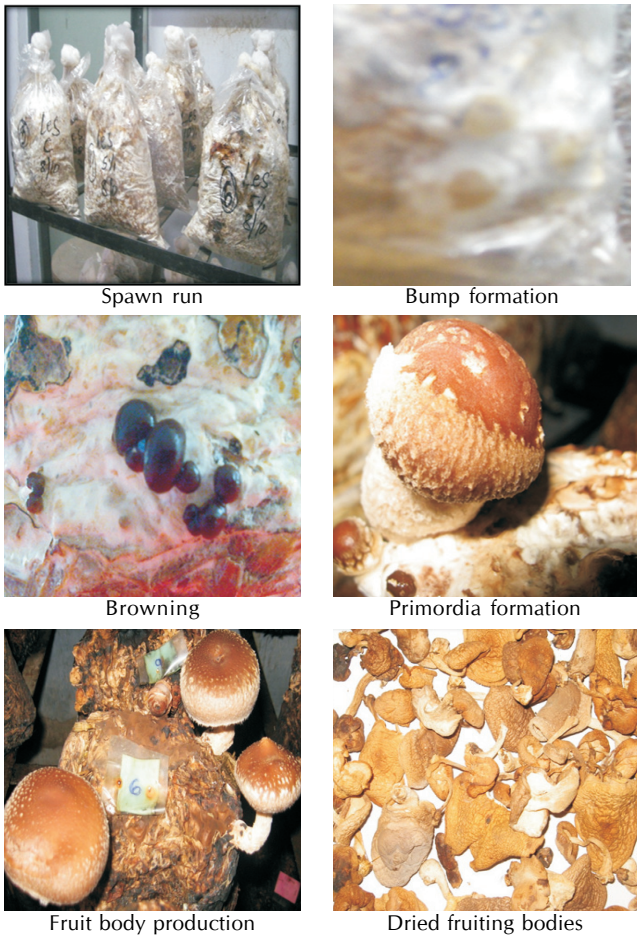

Plate 1: Different stages of fruit body production of *L. edodes* strains

significantly higher yield (46.2 % and 66.8% respectively) as compared to other strains with 10 per cent wheat bran supplementation (Plate 1). OE-142 was the least productive with a maximum biological efficiency of 26.7 per cent in substrate supplemented with 10 per cent wheat bran. However, supplementation of wheat bran @ 20 per cent gave significantly less biological efficiency and yield in strains (OE-38, OE-142 and OE-388). LeS and OE-329 strains showed less weight of fruit bodies per bag and biological efficiency in unsupplemented substrate (without wheat bran). The days for spawn run was minimum in OE-388 strain (52 days) in 5 per cent wheat bran supplementation and maximum was in OE-142 strain (75 days) in 20 per cent wheat bran supplementation. The weight of fruit bodies varied from 41.3 g bag^{-1} to 233.8 g bag^{-1} for all the strains. The highest weight of fruit bodies was observed in OE-388 strain (233.8 g bag^{-1}) supplemented with 10 per cent wheat bran while minimum was observed in OE-142 strain supplemented with 20 per cent wheat bran supplementation (Table 1). Thus, in none of the strains, increase in supplementation supported higher productivity. Variations in biological efficiencies of shiitake have been reported by different workers with different substrates. The production of heavier mushrooms on wheat straw and oakwood sawdust substrates with biological efficiency of 54.17 per cent were reported by Philippoussis *et al*. (2003) whereas, Gaitan and Mata (2004) reported biological efficiency on wheat straw substrate ranging from 24.8 to 55.6 per cent. Agarwal (2007), Lalitesh (2009) and Kaur (2010) also reported improved mycelial extension rates and higher biological efficiencies of *L. edodes* strain Le-S on supplementation of wheat straw with wheat bran.

Yield Evaluation of *L. edodes* Strains on Saw dust

Wheat straw gave significantly higher yield as compared to saw dust as substrate in all the strains except OE-388 strain. Moreover, days for spawn run were also less in wheat straw substrate as compared to saw dust substrate. In case of saw dust, the effect of supplementation of wheat bran revealed that maximum biological efficiency was obtained at 5 per cent level in LeS, OE-142 and OE-388 strains. Moreover, 5 per cent wheat bran supplementation resulted in more fruiting bodies and mycelial spread was also fast in these strains (Table 2). However, supplementation of wheat bran @ 10 per cent gave significantly higher yield in case of OE-38 (392 g bag^{-1}). A significant reduction in yield at higher rate of supplementation of wheat bran (15 % and 20 %) was also observed in saw dust substrate. Out of these strains, OE-329 strain was the least productive. It was also found that sawdust substrates gave biological efficiency ranged from 7.20-81.0 per cent in all the strains.

Reports on cultivation of the shiitake mushroom 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). According to Fasidi and Kadiri (1993), the increased productivity of *L. subnudus* on a straw based substrate was attributed to the carbohydrates, amino acids and minerals present in the supplements used. Worral and Yang (1992) reported very sparse mycelial growth on sawdust alone but obtained fast growing dense mycelium when sawdust was supplemented with apple pomace. They also reported low yield on sawdust alone but improved yields were obtained on apple pomace supplementation. 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 the most suitable substrate for production *L. edodes*. Silva *et al*. (2005); Nikitina *et al*. (2007) 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

Table 1: Effect of wheat bran supplementation on the yield of *Lentinus edodes* strains on wheat straw substrate

Supplement level (%)	Strains LeS			OE-38			OE-142			OE-329			OE-388		
	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)
Control	67	85.5	24.4	67	98.3	28.1	72	42.5	12.1	63	47.8	13.7	65	175.0	50.0
5	69	154.2	44.1	60	68.1	19.5	67	81.3	23.2	59	158.5	45.3	52	207.1	59.2
10	60	157.2	44.9	60	108.8	31.1	65	93.6	26.7	55	161.6	46.2	55	233.8	66.8
15	65	104.9	30.0	65	76.2	21.8	70	54.6	15.6	62	152.6	43.6	60	181.8	52.0
20	65	99.3	28.3	70	43.3	12.4	75	41.3	11.8	64	133.0	38.0	59	87.03	24.9
CD at 5%			2.42			3.16			0.76			3.62			0.84

DSR- Days for spawn run, Dry weight of substrate/bag- 350g, WFB- Weight of fruiting bodies, BE- Biological efficiency

Table 2: Effect of wheat bran supplementation on the yield of *Lentinus edodes* strains on saw dust substrate

Supplement level (%)	Strains LeS			OE-38			OE-142			OE-329			OE-388		
	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)	DSR	WFB(g) Bag^{-1}	BE (%)
Control	69	195	26.0	75	276	36.8	76	75	10.0	65	72	9.60	58	355	47.3
5	67	206	27.5	69	340	45.3	163	192	25.6	75	76	10.1	56	608	81.0
10	68	187	24.9	67	392	52.3	70	107	14.3	73	92	12.3	57	463	61.7
15	68	181	24.1	72	265	35.3	74	105	14.0	79	55	7.33	65	314	41.8
20	70	130	17.3	80	208	27.7	72	70	9.33	80	54	7.20	68	205	27.3
CD at 5%			1.12			1.34			1.11			0.90			1.01

DSR- Days for spawn run, Dry weight of substrate/bag- 350g, WFB- Weight of fruiting bodies, BE- Biological efficiency

with wheat bran help in increasing the mycelial growth of *L. edodes* on all the substrates, as this provides high amount of nitrogen to the growing fungus. Two different strains of *L. edodes* (L1 and L2) were cultivated by (Puri *et al*., 2011) 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. They observed that wheat straw gave significantly higher yield with 45.9 per cent biological efficiency for strain L1. Ten percent supplementation of wheat bran was the best among all the supplements tried. Moreover, wheat straw substrate produced the heaviest and beautiful brown sporocarps with maximum number of fruiting bodies.

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