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AGRICULTURAL WASTES AS SUBSTRATE FOR SPAWN PRODUCTION AND THEIR EFFECT ON SHIITAKE MUSHROOM CULTIVATION

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ABSTRACT
Lentinula edodes or Shiitake is a white rot wood decay fungus that produces flavourful brown sporocarps with medicinal properties. According to a Chinese folk fare, this mushroom is an "elixir of life", capable of generating stamina, curing colds, improving circulation, and preventing premature aging. Many countries have developed production technology of this mushroom but detailed accounts are not available in literature. Therefore, present investigations were undertaken to use locally available poplar and teak sawdust, sorghum and wheat grains in combination with chalk powder and gypsum for selection of an ideal material for Shiitake spawn production. *Lentinula edodes* had maximum and faster linear growth rate in test tubes having sorghum and its combinations. It colonized sorghum substrate rapidly without any contamination and the spawn prepared with sorghum had maximum yield and biological efficiency @ 6-8% spawn doses. The results showed that sorghum is an ideal material for Shiitake spawn production.

KEYWORDS: *Lentinula edodes*, yield, sorghum, sawdust, black oak mushroom

INTRODUCTION
Lentinula edodes (Berk.) Pegler**,** commonly known as the Shiitake or Black Oak Mushroom, is one of the most widely grown species of mushrooms and a very efficient biodegrader (Royse, 1985). It is well known, not only for its delicious flavour and nutritional value, but also for potential use in medicinal applications. Owing to these qualities, large-scale production of mycelium and cultivation of sporocarps of *L. edodes* is very important in medicinal, chemical and fermentation industries (Veena and Pandey, 2006). In India, successful cultivation of Shiitake mushroom has been reported on sawdusts, on wheat straw and rice or wheat bran etc (Thakur and Sharma, 1992; Shukla, 1995; Sharma *et al*, 2006). For mushroom seed or spawn production, generally sterilized grains, sawdust, dowels or wood chips etc (Stamets, 2005) are used. In Korea, China and Japan, sawdust is used as a substrate for spawn production of *Lentinula* and *Ganoderma* spp. These are the countries where the artificial cultivation of the mushrooms started. Spawn production using sawdust is a very popular and economical method. Sawdust is one of the wood-sawmilling wastes which may reach 15 % of the total volume and gives more robust mushrooms that are less prone to contamination (Stamets, 2000). However, wheat (Dadwal and Jamaluddin, 2004), sorghum (Veena and Pandey, 2006) and other grains are the materials used for spawn production of Shiitake in India. The quality and quantity of the spawn used in the cultivation of mushrooms directly influenced their quality and yield. Studies were therefore, made to select an ideal material, which is economical and produces higher yields in a shorter period and with less contamination problem for commercial cultivation and spawn production in *L.edodes*.

MATERIALS AND METHODS
Evaluation of substrates for spawn production- Wheat and sorghum grains alone and in combination with chalk powder ($CaCO_3$-5%), gypsum ($CaSO_4$-5%), sawdust and wood chips alone and in combination with wheat bran, chalk powder or gypsum were evaluated. These appropriately moistened (40-45%) substrates were filled uniformly in test tubes. The tubes were levelled and plugged with non absorbent cotton and sterilized for 1.5 hours at 121 °C (Pandey and Tewari, 1990). After cooling, the tubes were inoculated with three agar plugs cut from a growing colony in a Potato Dextrose Agar (PDA) containing petri dishes. Tubes were incubated at 25 °C and five replications for each treatment were kept. Linear growth was measured at an interval of 24 hours.

Mass production of spawn- For the mass production of spawn, the best material was selected according to the preliminary evaluation of substrates and 250g of it was filled in polypropylene bags and plugged with non-absorbent cotton and sterilized. Each bag containing 250 g substrate was inoculated by 20 g of *Lentinula edodes* and incubated at 25°C. The number of days taken to cover the substrate and contamination (if any) were recorded. Mother spawn was raised in bottles.

Spawn dose standardization- Different doses of spawn were test inoculated @ 2,4,6,8 and 10% of wet weight of substrate. Days taken for complete spawn run and biological efficiency were recorded.

Substrate preparation- Bag system was adopted for cultivation. 65% moisture level was adopted for substrate (wheat straw and 10% wheat bran) and maintained by adding appropriate quantity of water. The substrate mixture was filled only 3/4 the capacity, in 2 kg capacity polypropylene bags. The necks 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 fungus.

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

Yield and biological efficiency- The time taken for pinning after spawn run was recorded. Fruiting bodies were harvested after maturity. Biological efficiency and yield were 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

Fourteen different combinations of substrates were evaluated for measuring the linear growth of *Lentinula edodes* per day. Among these substrates, the fungus had maximum linear growth on sorghum and its combination with chalk powder and gypsum, followed by poplar sawdust and its combinations (Table 1). Linear growth was significantly low in wheat and teak sawdust combinations. Even though, wheat is a very common material used for spawn production (Rai, 2003; Dadwal and Jamaluddin, 2004), the linear growth was not promising on wheat grains.

TABLE 1. Growth rate of *Lentinula edodes* mycelium on different substrates

Substrates	Linear growth of mycelium (mm/day)
Sorghum grain	11.2
Sorghum and chalk powder (5%)	11.4
Sorghum and gypsum (5%)	12.4
Wheat grain	5.9
Wheat and chalk powder (5%)	6.9
Wheat and gypsum (5%)	6.0
Poplar sawdust	9.0
Poplar sawdust and chalk powder (5%)	8.2
Poplar sawdust and gypsum (5%)	10.1
Poplar sawdust with wheat bran (10%) and gypsum (5%)	8.8
Teak sawdust	5.7
Teak sawdust and chalk powder (5%)	7.6
Teak sawdust and gypsum (5%)	5.5
Teak sawdust with wheat bran (10%) and gypsum (5%)	5.8
CD (P=0.01)	1.4

TABLE 2. Time requirement of *Lentinula edodes* to colonize various substrates for spawn production

Substrates (250g) for spawn production	Days
Sorghum grain	11.3
Sorghum and chalk powder (5%)	8.5
Sorghum and gypsum (5%)	10.0
Poplar sawdust	10.5
Poplar sawdust and chalk powder (5%)	13.0
Poplar sawdust and gypsum (5%)	12.2
Poplar sawdust with wheat bran (10%) and gypsum (5%)	9.5
Poplar sawdust with wheat bran (10%)	9.8
Poplar sawdust with wheat bran (10%) and chalk powder (5%)	12.5
CD (P=0.01)	1.9

On the basis of preliminary screening of substrates in test tubes, sorghum and poplar sawdust were used as base materials for spawn production. Sorghum grains mixed with chalk powder had maximum fungal growth and it took only 8.5 days to cover the 250g substrate in polypropylene bags (Table 2). In general, *Lentinula edodes* colonized the substrates of sorghum and its combinations faster than other substrates. No contamination was observed in any bags utilized for producing commercial spawn on different treatments.

The vigour of the spawn is the major deciding factor for success in mushroom cultivation. When spawn made from sorghum, poplar sawdust and their combinations were used for cultivation of Shiitake mushroom, the spawn run period was significantly shorter for sorghum and its combinations. The shortest spawn run period was however, for sorghum added with gypsum at 5% (Table 3). Poplar sawdust and its combinations took extra week for complete spawn run in the bags. However, combination of sawdust 80%, rice bran 10% and wheat bran 10% also supported good spawn run with more C:B ratio and considered as the cheap and best spawn base in term of spawn production (Thiribhuvanamala *et al*, 2005) . Some other combinations of sawdust, sucrose with potassium nitrate and calcium carbonate; combination of sawdust, sucrose, wheat bran and calcium carbonate were used for *L.edodes cultivation* (Chang and Miles, 1993). Moreover, all the bags inoculated with spawn prepared with sorghum grains and their combinations showed uniform growth of the fungus whereas in 11 to 33 per cent bags having spawn made up of poplar sawdust and its combinations improper or poor growth of the fungus was recorded.

TABLE 3. Effect of different substrates on spawn run and yield of *Lentinula edodes*

Substrates	Spawn run period (days)	Bags with non uniform growth (%)	Bags with contamination (%)	Total loss of bags (%)	Yield (g/kg wet substrate)	Biological efficiency (%)
Sorghum grain	30.1	0	0	0	82.0	23.4
Sorghum and chalk powder (5%)	28.6	0	0	0	87.5	24.9
Sorghum and gypsum (5%)	22.7	0	7.1	7.1	82.4	23.5
Poplar sawdust	38.4	11.1	11.1	22.2	41.2	11.7
Poplar sawdust and chalk powder (5%)	36.0	11.1	55.5	66.6	27.9	7.9
Poplar sawdust and gypsum (5%)	37.6	22.2	22.2	44.4	13.7	3.9
Poplar sawdust with wheat bran (10%) and gypsum (5%)	35.0	33.3	55.5	88.8	20.9	5.9
Poplar sawdust with wheat bran (10%)	36.4	11.1	33.3	44.4	NIL	-
Poplar sawdust with wheat bran (10%) and chalk powder (5%)	33.6	33.3	44.4	77.7	33.3	13.3
CD (P=0.01)	8.9				23.4	

After complete spawn run, the bags were kept for fructification. Those bags having spawn run blocks made from sawdust spawn showed high incidence of contamination (37%) and led to crop loss. Poplar sawdust and rice bran (10%) did not produce any sporocarps showed complete yield loss and no biological efficiency due to contamination with competitor moulds like *Trichoderma* spp. and *Aspergillus* etc. Thiribhuvanamala *et al* (2005) recorded lowest yield of Shiitake in sawdust and sugarcane bagasse and wheat bran (4%) which could be due to competitor moulds such as *Aspergilus niger* and *Trichoderma* spp. which probably inhibited the growth and development of basidiocarps. Similarly, Bhandal and Mehta (1986) also faced contamination problems with *T. viride* and *T. koningii* in wheat straw substrates however, obtained significantly higher yields. Very low incidence (2.3%) of contamination was recorded in bags having spawn run block of sorghum spawn. No significant difference was found in biological efficiency among three sorghum combinations. The biological efficiency of sawdust spawn was lower than sorghum spawn. Stamets (2000) found that out of many cereal grains used for making spawn, rye grains was the most suitable. Virtually, all the grains can be used for spawn preparation and its selection depends on the availability of raw materials, yield and experience of the spawn makers. He also found that sawdust obtained from furniture manufactures is either very fine or mixed with wood shavings. Thus, results in an inconsistently compromised substrate which is difficult to use, difficult to replicate and poor in quality. Acquiring sawdust from a single kind of tree and of uniform particle size is a difficult task in India (Veena and Pandey, 2006). In this study also poor quality of the sawdust may be a reason of its poor performance. But in the case of sorghum spawn, the grains acted as a carrier for evenly distributing the mycelium and also served as a nutritional supplement. Thus, it results in fast and uniform mycelial growth. In addition to this, yield of Shiitake was recorded higher with sorghum spawn than Poplar sawdust spawn. Though wheat grains are the most popular and widely used substrate for spawn production, but considering the highest growth rate, short spawn run period, less or no contamination and higher biological efficiency, sorghum emerged as an ideal material under Indian condition for the production of Shiitake spawn. Chen (2002) pointed that choice of the spawn should be based on the lowest possibility for contamination and high rate of spawn run.

As evident from the data presented in Table 4, the shortest spawn run period was recorded with 10 % spawn. However, there was no significant difference between 8 and 10 percent spawn dose. Although the highest biological efficiency was obtained with 8 percent and there was no significant difference in yield with 6, 8 and 10 percent spawn doses. The increased rate of spawning accelerated colonization, narrowed the gap of opportunity for competitor invasion, and significantly boosted yields (Stamets, 2000). Increased rate of spawn dose increased the cost of production, which is not desirable. Considering spawn cost and performance shown by different doses, 6 to 8 percent is ideal dose for the cultivation of Shiitake mushroom.

TABLE 4. Effect of different doses of spawn on yield of *Lentinula edodes*

Spawn dose (wet weight basis)	Spawn run (days)	Yield (g/kg wet substrate)	Biological efficiency (%)
2%	39.1	60.9	15.2
4%	31.8	59.0	14.7
6%	18.1	99.8	24.9
8%	15.6	118.5	29.6
10%	11.4	117.9	29.4
CD (P=0.01)	4.7	33.9	

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