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Substrate standardization for spawn production technology of Shiitake (*Lentinula edodes*) mushroom in Sikkim

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ABSTRACT

Shiitake mushrooms have become a popular food in the last few years and its consumption among the recent generation poses a great demand on its availability. Therefore, in the present study, the substrate for spawn production of shiitake mushroom was standardized on easily available grain substrates and agri-residues. Six different substrates were used in the study namely sorghum, maize, finger millet, paddy grain, paddy husk and sawdust supplemented with 5% gypsum and chalk powder among which the promising substrate was found to be sorghum grain, followed by paddy and maize respectively. Generally, all kind of grains are used for spawn production but the selection of substrate greatly depends on the availability, economics, and efficiency of substrate and yield of spawn on the grain. This investigation aims to develop a cost effective and sustainable technology for spawn production of shiitake mushroom in Sikkim and the study proves sorghum grains to a promising substrate in terms of mycelial extension, time taken for mycelia run and mycelia weight.

1. Introduction

The black oak mushroom, *Lentinula edodes* (Berk.) Pegler is ranked the most important mushroom produced and consumed across the world. It is commonly known as Shiitake mushroom as called by Japanese and Xiang-gu in China (Chen 2005). This species of mushroom has become the highest cultivated mushroom worldwide with 22% of the total production accounting to about 7.48 x 10^6 tons, displacing the white button mushroom (*Agaricus bisporus*) that has been relegated to the fourth position with 15% of the total production (Royse et al. 2017). Currently, China is the world's leading producer of shiitake with more than 95% of the total production. The shiitake mushroom is estimated to have been cultivated for the first time in China between 1,000- 1,100 AD on wood logs where it is an integral part of gastronomy, art and culture, which is why there are often diverse manifestations alluding to this species (Chang 1993). The great emphasis on commercial cultivation of shiitake mushroom is due to its unique flavor, nutritive value and medicinal properties (Sugui *et al.*, 2003; Silva *et al.*, 2005) because of which the medicinal, fermentation and chemical industries promote the mycelium and sporocarp production on large scale. Thus, cultivation of shiitake is potentially a lucrative enterprise. It is cultivated on logs of broad leaved trees especially tree trunk of Shia trees (Quercus sp.) or Oak. In recent times, successful cultivation of shiitake has been reported on saw dust, paddy, wheat straw and bran. The mushroom seed or spawn is generally produced on sterilized grains, sawdust, dowels or wood chips etc (Stamets, 2005). 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). Spawn production using sawdust is a very popular and economical method. The countries like Korea, China and Japan where the artificial cultivation of the mushrooms started, use of sawdust as a substrate for spawn production of

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Lentinula and *Ganoderma* spp. is of common practice. Shiitake is globally a well known cultivated species, yet to find a place in Indian market. Grains of wheat (Dadwal and Jamaluddin, 2004), sorghum (Veena and Pandey, 2006) and other crops are mostly 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. There have been sporadic research efforts to standardize the cultivation technology but could not reach the commercial level. A few growers from North Eastern states like Manipur, Mizoram and Sikkim have initiated cultivation trials of shiitake mushroom based on Japanese log system with limited success. The lack of cultivation technology on locally available substrates and temperature suitable strains are the reason for its consistent unavailability in India. This study has therefore been conducted to select a low cost efficient grain substrate which is easily available in North Eastern states especially Sikkim and produces higher yields in a shorter period and with less contamination problem for commercial spawn production in *L. edodes*.

2. Material and Methods

Collection of mushroom and development of pure culture:

The *Lentinula edodes* mushroom species in this study was collected from Pakyong, East Sikkim, Sikkim, India. The fresh fruiting bodies of mushroom were used to obtain pure culture by placing a small piece of inner tissue from the mushroom of 2×2 mm size. The pieces of mushroom were surface sterilized in 5% Sodium hypochlorite (NaClO) followed by three washings in sterile distilled water and then placed on freshly prepared Potato Dextrose Agar (PDA) medium both on test tube slants and on Petri plates and sealed using stretch tape to avoid contamination (de Leon et al., 2013). They were then incubated at temperatures around 22-25ºC. The growing cultures were sub-cultured several times so as to obtain pure culture of the mushroom. The pure culture was also deposited in the culture collection of ICAR-Directorate of Mushroom Research, Solan, Himachal Pradesh, India accession number DMRO 1183. The mycelial stocks were stored at 25ºC and the viability of the fungus was ensured by sub-culturing at regular intervals.

Evaluation of substrates for spawn production

A total of six substrates were selected among which four were grains and two were lignocellulosic wastes. Paddy, maize, finger millet, sorghum grains, rice husk and saw dust in combination with chalk powder ($CaCO_3$-5%), gypsum ($CaSO_4$-5%) were evaluated for optimization of substrate for spawn production of shiitake. About 1 kg of well sieved and dried sorghum, paddy, maize, finger millet grains were washed in clean water three times to remove chaff, dust and other particles. These substrates were then soaked in water overnight for maximum absorption of moisture. The following day, soaked substrates were again washed in cold water, cooked in hot water for 25-30 minutes. The cooked grains were air-dried in shade and then mixed with 5% calcium carbonate ($CaCO_3$) and 5% gypsum ($CaSO_4$) to maintain moisture (60-65%), pH (7.0-7.8) and to keep the seeds friable. Substrates weighing 250 g of each *viz*., paddy, maize, sorghum, finger millet, paddy husk and sawdust were filled into polypropylene bags, sterilized for 1.5 hours at 121ºC and incubated (Pandey and Tewari, 1990). The materials upon steam sterilzation were cooled and the actively growing mycelium was transferred aseptically upon the sterilized substrates inside the spawn covers and re-plugged to avoid contamination. They were incubated at 22-25°C for spawn run for ease of colonization. Best spawn run was recorded on 6th, 12th and 18th day. Each treatment was replicated thrice in randomized block design.

3. Results and Discussion

Spawn is the basic input and most important component of mushroom cultivation. The vigour of the spawn is the major deciding factor for mushroom cultivation. Although, the genetic constitution of the strain determines the potential of spawn, the substrate material also plays an important role. In the present study, six different substrates were examined under Sikkim conditions. The growth was calculated in terms of mycelia coverage and number of days taken for coverage. The monitoring was done on 6th, 12th and 18th day. Among these different base materials, sorghum grain promoted best growth followed by paddy and maize grains (Fig. 2). The mycelia growth of *L. edodes* was initiated on 4th day in the sorghum seeds and in 5 days on paddy grains. In saw dust alone no mycelial growth was noticed. The extensive growth of mycelia on sorghum and paddy grain is because of favorable temperature, water holding capacity and sufficient surface for growth for mycelia on substrate, similar to the establishment of Tinoco *et al.,* 2001 that larger surface area and pore of substrates can hold more mycelium growth rate. The combination calcium carbonate and calcium sulphate with grain spawn sorghum, paddy, millets or wheat has been well studied and reported (Upadhyay and Rai, 1999; Thiribhuvanamala *et al*., 2005; Kumar and Kumar, 2017). However, very slight growth rate was observed on rice husk and no growth on saw dust substrate (Fig. 2), due to lack of moisture holding ability and less surface space for mycelia growth. Foremost spawn run were observed in rice husk after 10 days (Figure 1). In finger millet, mycelia growth initiates at 10th day after inoculation (Figure 1), related results were obtained by Kumar and Kumar 2017 in *P. grammocephalus.* Accordingly, it took 14 days on sorghum grain, 18 days on

Paddy grain and 20 days in maize for spawn run (Figure 1). In this experiment, sorghum paddy and maize grain functioned really well as substrate by providing nutritional additives especially starch and supporting equal disperse of the mycelium (Figure 2.), which is in coherence with the reports of Tinoco *et al.,* 2001, Stanley and Awi 2010, in which maize variety (Bende Local) was found to be most suitable for mycelial extension and mycelial fresh weight of *P. tuber*– regium and *P. pulmonarius*. In case of finger millet and rice husk rapid mycelia run was observed at the initial 10-15 days after inoculation, which gradually paused after a few days. However, no spawn run was observed in saw dust alone, which could be due to less surface area for mycelia growth, less availability of nutrient needed for mycelia growth and lack of water holding capacity. Similar observation has been made by Kumar and Kumar, 2017 where finger millet and saw dust exhibited poor spawn run on *P. grammocehalus*.

Thus, sorghum and paddy grain have proven to be successful substrate for spawn production of *L. edodes*. Beside from result discuss above *L.edodes* is observed to be fast growing if all the requirements like; temperature, substrate and nutrient supply are properly maintained.

4. Conclusion

Mycelia growth of *L.edodes* was faster in all the substrate except on saw dust. The best substrate for spawn production was sorghum and paddy grain; hence it is proven to be promising substrate for the production of *L.edodes*. The substrates are also economical and easily assessable for mushroom producers in Sikkim. Hence, these substrates can be standardized for commercial *L. edodes* in the state production and fill the lacuna of nutritive food in different places especially rural population of India, who are prone to malnutrition, it is reported that scheduled tribe children have the poorest nutritional status in the nation (Debnath, and Bhattacharjee 2014). Collectively, standardization of substrate for *L.edodes* will not only provide food security but it can play important role in supplying many nutritive properties needed for proper functional of the human body and also encourage sustainable development of the nation.

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6. References

Chang ST (1993) Mushroom and mushroom biology. In: Chang ST, Buswell JA, Miles PG (eds) Genetics and breeding of edible mushroom. Gordon and Breach Science Publishers, Amsterdam. Pp. 1-13.

Chang ST, and Wasser SP (2012). The role of culinary-medicinal mushrooms on human welfare with a pyramid model for human health. *International Journal of Medicinal Mushrooms,* 14(2): 95-134.

Chen AW (2005) What is shiitake. In: Shiitake Cultivation, Mushroom Growers' Handbook 2. MushWorld, Seul. Pp. 3-28.

Dadwal VS, and Jamaluddin (2004). Cultivation of *Ganoderma lucidum* (Fr.) Karst. Indian Forester, 130(4): 435-440.

Debnath A, and Bhattacharjee N (2014). Factors associated with malnutrition among tribal children in India: a non-parametric approach. *Journal of Tropical Pediatrics,* 60(3): 211-215.

De Leon AM, Reyes RG, and Dela Cruz TEE (2013). *Lentinus squarrosulus* and *Polyporus grammocephalus*: Newly Domesticated, Wild Edible Macrofungi from the Philippines. Philippine Agricultural Scientist, 96: 411– 418.

Kumar M, and Kumar SS (2017). Standardization of substrate for Spawn Production of Wild Common Edible Mushroom *Polyporus grammocephalus*. *Journal of Academia and Industrial Research,* 6(1): 2218-5213.

Pandey M, and Tewari RP (1990). Antagonism of *Pleurotus sajor-caju* by some weed fungi. Mushroom Journal Tropics, 10:52-58.

Royse DJ, Baars J, Tan Q (2017). Current overview of mushroom production in the world. In: Zied DC, PardoGimenez A (eds) Edible and medicinal mushrooms: Technology and applications. John Wiley and Sons, NY. Pp. 5-12.

Silva EM, Machuca A, and Milagres AMF (2005). Evaluating the growth and enzyme production from *Lentinula edodes* strains. Process Biochemistry, 40: 161- 164.

Stamets P (2000). Growing gourmet and medicinal mushrooms, 3rd Edition. In: Ten Speed Press, USA ISBN: 0-89815-608-4. Pp: 574.

Stamets P (2005). Mycelium Running, How Mushroom can Save the World? Ten Speed Press, Berkeley, CA ISBN: 1580085792, pp: 339.

Stanley HO, and Awi-Waadu GD (2010). Effect of substrates of spawn production on mycelial growth of oyster mushroom species. *Agriculture and Biology Journal of North America*, 1(5): 817-820.

Sugui MM, De Lima PA, Delmanto RD, Da Eira AF, Salvadori DM, Ribeiro LR (2003). Antimutagenic effect of Lentinula edodes (BERK.) Pegler mushroom and possible variation among lineages. Food and Chemical Toxicology, 41(4):555-60.

Thirubhuvanamala G, Krishnamurthy AS, Shanthi K, and Marimuthu T (2005). Development of suitable substrates for spawn and mushroom production of *Lentinula edodes* and *Auricularia polytricha*. Madras Agricultural Journal, 92 (4-6) : 344-348.

TinoCo R, Pickard MA and Duhalt RV (2001). Kinetic differences of purified laccases from six pleurotus ostreatus strains. *Letters in Applied Microbiology*, 32:331-335.

Upadhyay RC, and Rai RD (1999). Cultivation and nutritive value of *Lentinus squarrosulus*. Mushroom Research. 8: 35-38.

Veena SS, and Pandey M (2006). Evaluation of the locally available substrates for the cultivation of indigenous *Ganoderma* isolates. Journal of Mycology and Plant Pathology, 36(3): 434-438.

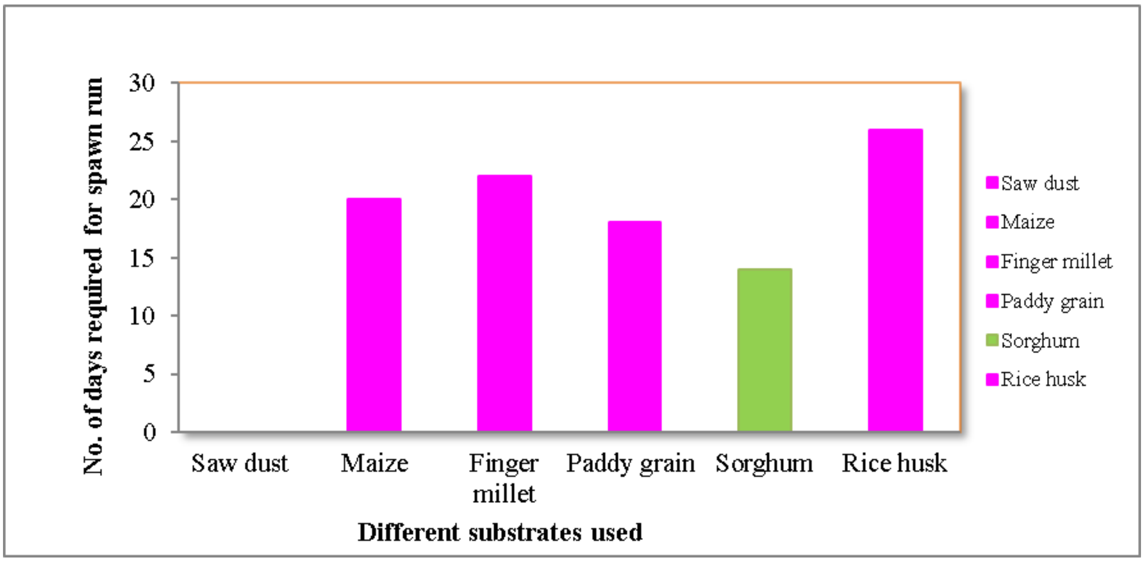
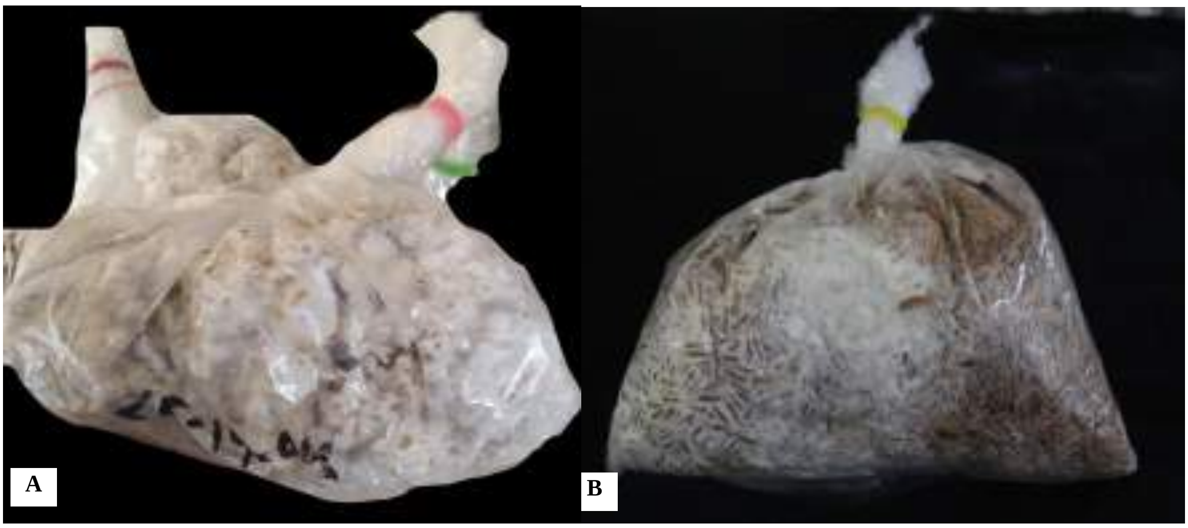
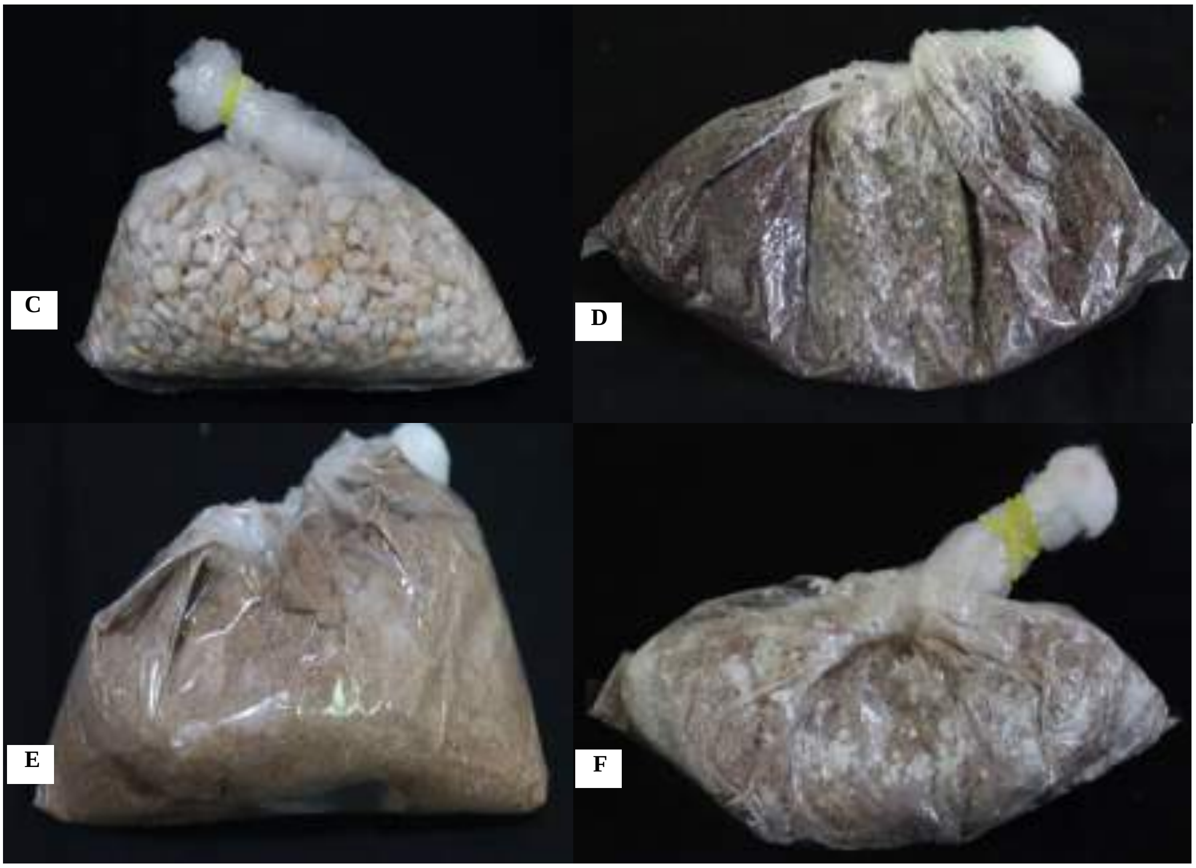


Figure 1: Time duration for complete spawn run by *L. edodes* on different substrates





A: sorghum B: paddy C: maize D: finger millet E: saw dust F: rice husk