International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 07-08-2021, Accepted: 22-08-2021, Published: 06-09-2021
Volume 6, Issue 5, 2021, Page No. 262-265

Studies on vegetative growth, spawn and sporophores production of *Lentinula edodes* (Berk.) Pegler on varied substrates

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Abstract
Lentinula edodes, is one of the important edible mushrooms and is popularly known as Shiitake. The present studies were carried out on the comparison mycelial growth, spawn production and cultivation of *Lentinula edodes*. The mycelium grows faster on PDA media in comparison to MEA. For the *Lentinula* spawn production, the grains of wheat, maize, and bajra were used as substrate. It was found that 8hrs of soaking/60 minutes of boiling and addition of $CaSO_4$ and $CaCO_3$ (3%:0.75%) gave the best result in the case of wheat grains; 20hrs soaking and 120 minutes of boiling and the addition of 3%:0.75% of gypsum and chalk in case of maize grains; and 12 hrs soaking and 25 minutes boiling gave quality spawn with bajra. Wheat grain spawn was used as inoculum on sawdust substrate of eucalyptus, fir and the combination of powdered paddy straw and rice bran, powdered paddy straw alone were also tried for cultivation under controlled conditions. From the above said four substrates it was found that the best fruiting bodies were found on eucalyptus (hard wood). It was enough good on fir (soft wood), then on powdered paddy straw (agro-waste) mixed with fir (1:1) supplemented with rice bran. Deformed fruit-bodies with short stipe and stalk were reported on powdered paddy straw alone. So, eucalyptus and fir found to be suitable for commercial production of *Lentinula*.

Keywords: *Lentinula edodes*, cultivation, spawn, vegetative growth

Introduction

Out of the 2000 species of edible mushroom, as many as 283 are reported to be available in India (Purkayastha & Chandra 1985) [8]. Many species of mushrooms have been cultivated successfully in various parts of the world, but only *Agaricus* L., *Lentinula* Fr., *Pleurotous* Fr. (nom. cons.), *Auricularia* Bull. *ex* Juss., *Flammulina* P. Karst., *Volvariella* Spegazzini have hit the international market and their production has been steadily increasing since world war II. *Lentinula edodes* (Berk.) Pegler is an edible mushroom and is popularly known for its therapeutic value. In terms of production, it stood second after Button Mushroom. The present work was the foremost attempt on the following parameters viz. growth of vegetative mycelium, spawn production, and trial on standardizing cultivation methodology in Chandigarh, Punjab.

The earlier record regarding *Lentinula* appears to be cited by Singer (1961) [13], wherein he mentions 'that historical documents in Japan recorded that Emperor Chuai in the year 199 A.D. praised the Shittake given to him by the natives of Kyushu." In English, its common name is Black forest mushroom. It is known as 'Shiitake' in Japan and 'Shiang-gu' or 'Hong-ho' in China. It is accepted as a genus of the family- Omphalotaceae of order- Agaricales of the subclass- Agaricomycetidae, of class- Agaricomycetes, of the subdivision- Agaricomycotina belonging to division- Basidiomycota.

Chang & Miles (1989) [1] have been investigated its cultivation in China. It grows naturally in the wood logs in different climatic conditions. According to Mori *et al.* (1976) [6], in wild it is reported from broad- leaved tree species throughout the Asian continent (from China, Japan, Korea, Thailand, Burma, Nepal, North Borneo, Philippines, Papua & New Guinea). In 1994 Kaur [3] attempted the cultivation of Shiitake in Himachal Pradesh University, Shimla and Shukla [12] in Mushroom Research Centre, Solan in India. The sporophores are most often eccentric, sometimes centrally stiptate; characterized by its whitish lamellae which do not darken with age (Purkayastha & Chandra 1985) [8].

Materials and Methods

In the present study, the pure culture was raised from fruit bodies and was maintained on Potato Dextrose Agar (PDA). The relative growth of the vegetative phase was observed on Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) by pouring 15ml media in Petri dishes and incubating at 21±1ºC (Fig. 2). The optimum pH of MEA was 5.4±0.2 and PDA 6.6 adjusted with 0.1N NaOH and 0.1N HCL. Radial growth was estimated by measuring the diameter of the mycelia along the two perpendicular axes from the center marked on the backside of the Petri dish.

The spawn was raised on the grains of wheat, maize, and bajra. Calcium carbonate was added to adjust the pH and gypsum was used to prevent the grains from sticking together (Stoller, 1962) [14] in 1:4 ratios on a wet weight basis. Glass bottles and polypropylene bags were used for spawn production at 21±1ºC. The medium was sterilized by autoclaving at a temperature of 121ºC and a 15 lb/sq^2 inch pressure for 25 minutes. The pure culture of *Lentinula* was used as inoculums.

Biological efficiency and yield were studied on sawdust of *Eucalyptus* sp. (hardwood) and *Abies* sp. (Fir –as softwood), and powdered paddy straw (*Oryza sativa)* alone and in combinations of Fir & powder paddy straw in ratio of 1:1 supplemented with rice bran and $CaCO_3$. The bag size 15x30cm and 15x20cm used for the cultivation of *Lentinula*. The substrate (sawdust) mixtures were filled only ¾ the capacity. The neck was plugged with non-absorbent cotton and autoclaved at 121ºC & 15lb pressure for 60 mins. After cooling, the bag was spawned by adding 2% spawn on a dry weight basis of sawdust used under aseptic conditions. The inoculated substrate was then incubated at 25± 1ºC in the culture room for 90-100 days (Fig. 6). When the substrate was completely colonized by the mycelia then the bags logs were given cold water treatment at 10ºC overnight. The bag logs were then shifted to 14± 1ºC temperature and relative humidity around 85-90% was maintained by sprinkling the bag logs with water. The fully mature mushrooms were harvested and oven-dried at 35ºC-45ºC (overnight) and packed in polythene bags for future use.

Observations and Discussion

The growth of mycelium in Petri dishes and test tube slants containing PDA was sparse but much rapid than that on MEA (Fig. 2 & 3). A significant mycelial growth i.e. 6mm on PDA was observed on the 11th and 12th day (Table 1 & Fig. 1). These results are following the findings of Khan *et al*., 1991 [4].

The grains of wheat, maize, and bajra were used as a substrate for spawn production. The results revealed that 8hrs of soaking/60 minutes of boiling and addition of $CaSO_4$ and $CaCO_3$ in the ratio of 3%:0.75% gave the best result in the case of wheat grains. These results are similar to the findings of Rawat (1996) [9] for *Pleurotus* spawn. Stoller (1962) [14] used 6gm gypsum and 1.5gm chalk for one lb of grain to avoid clumping in preparation of rye grain spawn. To standardize the production of spawn on maize and bajra grains different soaking and boiling periods were tried. In the case of maize grains 20hrs soaking and 120 minutes of boiling and the addition of 3%:0.75% of gypsum and chalk gave the best result. Likewise in the case of bajra grains 12 hrs soaking and 25 minutes boiling gave quality spawn (Fig. 4). These basic findings are inconsistent with earlier research reports (Kumar *et al*., 1975; Royse, 1985) [5, 10].

Table 1: Comparative growth rate of *Lentinula edodes* on MEA* and PDA**

Days	Average growth rate of mycelia of *L. edodes* in mm on	
	PDA	MEA
1st- 2nd	0	0
3rd-4th	2	0
4th -5th	2	2
5th-6th	4	4
6th -7th	4	2
7th -8th	4	4
8th -9th	4	2
9th -10th	4	4
10th -11th	4	4
11th -12th	6	4

*Malt Extract Agar, ** Potato Dextrose Agar

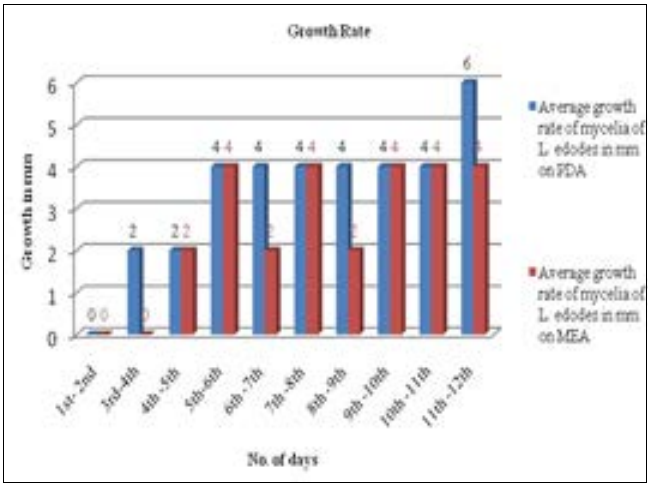

Fig 1: Graph showing 'Growth Rate" of mycelium on PDA and MEA

For the cultivation trial sawdust of (i) eucalyptus (ii) fir (iii) powdered paddy straw and (iv) mixture of fir & rice bran (powdered) agro-waste, and calcium carbonate was used as substrate. Fungal mycelium took 42-45 days for complete colonization (Fig. 5). For the initiating of fruiting bodies, the bags logs were treated with cold water treatment at 10ºC for 24hrs. Then the bog's logs were shifted at 14± 1ºC temperature and relative humidity around 85-90% by the sprinkling of water. After 15-20 days pin-heads started appearing and in the next 7-10 days, pin-heads completely matured to form fully opened fruit bodies (Fig. 6). Sphorophores change their shape gradually from the primordial to the over mature stage. The primordia were grayish-brown in color. Margins were whitish-brown or yellow-brown during the button stage. Pileus starts separating from the stipe and color changed to chocolate-brown at the initial stage. The stalk becomes elongated, and the unexpanded pileal stage is reached. The pileus opened up but was not fully expanded, at first convex, then depressed; light brownish at the center and whitish-brown at the margins, scales darker in the center, lighter towards the margin. Stipe color was white, shiny at button stage, and changed to whitish-brown, with cream-colored scales at maturity. It was soft at the primordial stage, the hard and stiff at the mature stage, and the surface became scaly. Gills crowded, at first whitish, later whitish brown, usually get separated from the stipe and becoming free as the stipe elongated. Flesh generally white, brownish under the surface of the pileus, soft, fleshy when tender, tough and coracious when old, odour mild and taste slightly acidic and not pleasant. Fruit bodies can be dried and stored for use in the future (Fig. 7).

Out of the four substrates, it was found that Eucalyptus (as hardwood) supported the best growth and the substrate was completely colonized within 38-40 days. Many earlier reports show a similar result with hardwood. In the case of fir the fruit bodies were enough good, then on fir sawdust & powdered paddy straw with additional supplements. Completely deformed fruit-bodies having short stipe and stalk was reported on powdered paddy straw alone. Similarly, Tan and Chang (1989) [15] reported the best growth of *L. edodes* on the sawdust enriched with used tea leaves, wheat bran and $CaCO_3$, and these substrate was completely colonized within 35 days, whereas Diehle & Royse (1986) [2] reported that the mixture of maple and

birch sawdust substrate was completely colonized in 40 days. Thus all the findings are following many earlier reports (Puri, 2012; Sharma *et al*., 2013) [7, 11].

Fig 2: Completely colonized Petri dish

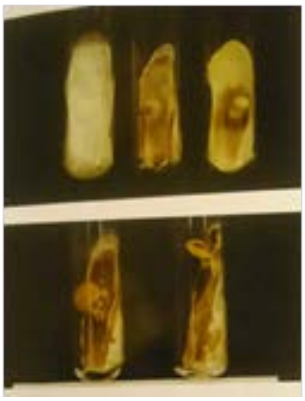

Fig 3: Colonized culture tube with fruit bodies

Fig 4: Spawn prepared on Bajra grains

Fig 5: Mycelia running phase in poly bag

Fig 6: Fruit bodies on sawdust logs

Fig 7: Dried fruit bodies of Shiitake

It is clear from all these reports that complete colonization of the substrate, initiation of primordial and formation of fruit bodies in *L. edodes* took almost the same time with different substrates tried except powdered paddy straw. Thus use of paddy straw although available in abundant in Punjab found not suitable for the cultivation of Shiitake.

Keeping in view its medicinal importance, the cultivation trial of this mushroom must be practiced in different climatic conditions with locally available agro-waste with additional cheap supplements so that its demand and supply can be managed. The cultivation of *Lentinula* in addition to *Agaricus* and other mushrooms will certainly cater to the market need and augment the income of growers.

Conclusions

In the present investigation, the vegetative growth of *Lentinula edodes* was found to be faster on PDA media in comparison to MEA. Spawn produced on wheat grains with 8hrs of soaking/60 minutes of boiling and addition of $CaSO_4$ and $CaCO_3$ (3%:0.75%) gave the best result and preferred because of better consistency over maize (produced 20hrs soaking and 120 minutes of boiling and the addition of 3%:0.75% of gypsum and chalk); and bajra grains (12 hrs soaking and 25 minutes boiling). Inoculation with spawn (produced on wheat) on four different combination of sawdust substrate of eucalyptus, fir singly and the mixture of fir with powdered paddy straw (1:1) and rice bran, powdered paddy straw alone were also tried for cultivation under controlled conditions. From the above said four substrates it was found that the best sporocarp production was found on eucalyptus. Enough good sporocarp on fir sawdust, then on fir & powdered paddy straw with rice bran as additional supplements ($CaSO_4$ and $CaCO_3$), and distorted fruit-bodies with tiny stipe and stalk was reported on powdered paddy straw. It shows that powdered paddy straw although show good mycelia growth but completely exhausted before sporocarp forming stage, thus not recommended. It is further suggested to explore cultivation

trial of this mushroom on varied agro-waste locally available. There is ample scope for carrying out commercial cultivation of Shiitake on eucalyptus sawdust. The cultivation of *Lentinula* in addition to other mushrooms will surely cater to the market need and enhance the income of growers.

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