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VEGETATIVE GROWTH AND FRUITING INDUCTION OF *LENTINULA EDODES* STRAINS ON DIFFERENT SUBSTRATES

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ABSTRACT
Lentinula edodes or Shiitake is an edible and medicinal mushroom, widely used for preparation of dietary supplements and other delicacies. Rich diversity of mushrooms is found in Uttarakhand state of India, besides other flora fauna. Two strains of *L. edodes* fungus were collected from Uttarakhand hills. To study the physiological and yield attribute, mycelial growth and yield of *L.edodes* isolates werecompared on different media, temperature, pH and agricultural wastes like sugarcane bagasse, paddy straw, poplar sawdust, coirpith, teak and sal sawdust alone and in combinations of 1:1. Potato dextrose agar (PDA) medium was found to be superior in terms of radial growth. The fungus showed maximum radial growth at 25ºC and acidic pH. The cultivation of mycelium was successful at all the substrates tested. On the basis of morphological, cultural and yield characteristics, strain L_1 was superior to L_2.

INTRODUCTION

Lentinula edodes (Berk.) (Pegler, 2003) also known as Shiitake or black oak mushroom is a white rot wood decay fungus that produces flavorful brown sporophores with medicinal properties. After the button mushroom (*Agaricus bisporus*), Shiitake is the most cultivated mushroom in the world (Chang, 1999). The fungus is saprophytic and grows on dead material (Chang and Miles, 2004). Various species of trees have been used for its cultivation, but most production is on species of oak (*Quercus* spp.) (Harris, 1986). Many biologically active substances, in particular polysaccharides have been isolated from *L. edodes,* which are particularly effective in retarding the progress of various cancers and other diseases through immune stimulating effects (Mizuno, 1995). A number of products prepared from *L. edodes* are sold throughout the world as dietary supplements (DS). The worldwide production and market value of mushroom DS products was more than 1,564,000 MT and US $ 6 billion per year, respectively (Chang and Miles, 2004). India is a big market for these products and coming into our country through multi-level marketing system (MLM). The growing global consciousness regarding the use of natural health products had created a challenge to scientists to develop new technologies for the cultivation of medicinal mushroom. In India, cultivation trials of Shiitake were done and mushrooms have been developed successfully (Sohi and Upadhyay, 1988; Thakur and Sharma, 1992). Shiitake mushroom was artificially cultivated in India on wood logs, artificial medium and saw dusts and corn cobs supplemented with wheat and rice bran (Dhar, 1976; Suman and Seth, 1982). Shukla (1994) firstly reported the cultivation of Shiitake mushroom in India on oak logs. Uttarakhand state of India is highly rich in biodiversity, also in mushrooms. It has enormous mushroom diversity which needs to be identified and conserved. As *Lentinula* spp. favor low temperature, the prevalent climatic conditions in this state favor its development and growth. It was therefore felt essential to evaluate media and substrates which favour maximum vegetative growth and higher production of mycelial biomass and fruiting body under both laboratory and field conditions. Understanding the impact of substrate on mycelial growth and shiitake yield will be invaluable for the effective bioconversion of locally available agricultural wastes and for mushroom crop diversification in India.

MATERIALS AND METHODS

Collection and isolation of fungus

Two strains of *Lentinula edodes,* namely L1 and L2 were collected from Munsyari town (Pithoragarh *District) and were maintained on Potato dextrose agar (PDA)*. The experiments were conducted in the Mushroom Research and Training Centre (MRTC) laboratory, Pantnagar.

Radial growth on different media

Culture media employed were Malt Extract Agar (MEA), Potato Dextrose Agar (PDA), Yeast Extract Agar (YEA), Water Agar (WA), Shagaun or Teak Sawdust Agar (Teak sawdust extract in PDA) and Poplar Sawdust Agar (Poplar sawdust extract in PDA) (PODA) at room temperature. Petri plates containing 25 mL of the medium were inoculated at the center with 7 mm diameter disc of actively growing mycelium under aseptic

conditions in three replications, for each strain.

Mycelial development assessment at different temperatures

The effect of different temperature ranges viz., 15, 20, 25, 30 and 35ºC was also studied on the growth of *L.edodes* by incubating the plates in incubators set at such temperatures.

Assessment of radial growth at different pH

The optimum pH for the growth was studied on different pH levels viz., 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 by adjusting pH with 0.1 N NaOH or 0.1 N HCl. Radial growth was estimated by measuring the diameter of the mycelia along two perpendicular axes.

Screening of different substrates

Biological efficiency and yield attributes (number and weight of fruiting bodies) were studied using paddy straw, poplar sawdust, coirpith, teak and sal sawdust alone and in combinations of 1:1 as substrates supplemented with wheat bran (10% w/w) For each treatment, five replications were maintained. The substrate mixture was filled only 3/4 the capacity, in 2kg capacity polypropylene bags. The neck of the bags were plugged with non-absorbent cotton and sterilized at 22 lbs pressure (121ºC) for 90 minutes. After cooling, the bags were inoculated with the two strains of the fungus. The grains spawn of *Lentinula edodes* were mix thoroughly @ 5 per cent and the bags were kept in the crop room at relative humidity of 80-85 per cent, at 25ºC temperature in the dark for 60-70 days for complete spawn run. Fruit bodies were harvested after maturity.

Morphological characteristics of fruiting bodies

Morphological differences among the fruiting bodies of different strains were studied selecting 5 fruit bodies per strain randomly per days starting from the first flush of the crop till the termination of the crop. Morphological variations among fruit bodies of different strains were studied by measuring the diameter of the pileus and stipe, thickness of the pileus and length of the stipe, length of the fruit bodies and weight per fruit body. Color variations between two strains (if any) were also recorded.

Data analysis

To compare the morphological variations among the fruit bodies of different strains simple CRD (Completely Randomized Design) was used. In order to compare the growth rates of different strains on different media, pH, temperature and to compare yield variations among different strains simple CRD and two factorial CRD was used.

RESULTS AND DISCUSSION

Radial growth on solid media

Among the different media tested, the fungus showed the highest growth rate (8.7 mm/day) on Potato dextrose agar (PDA) (Table 1). Least growth was observed in yeast extract agar (1.04 mm/day). Strain L_1 gave significantly highest radial growth on all the different media tested on the 8th day of inoculation. These findings are in accordance with Furlan *et al*. (1997) and Rossi *et al*. (2000) who also reported that PDA medium provided the highest speed, vigour and estimated biomass values for *L. edodes*. Addition of calcium carbonate and other organic additives in PDA increased the biomass and lignocellulolytic enzyme production of *L. edodes* (Ramkumar *et al.,* 2011).

Radial growth on different temperatures

of the five temperatures tested, both the strain (L1 and L2) showed maximum growth rate (10.0 and 8.8 mm/day, respectively) at temperature 25ºC (Fig. 1). Decrease in average growth rate was observed the by decreasing the temperature below 15ºC and increasing the temperature up to 35ºC. These results are in accordance with the findings of Khan *et al*. (1991). They also reported maximum radial growth of *L. edodes* at 25ºC, whereas, Furlan *et al.,* (1997) observed maximum growth rates at 30ºC than at 20 or 25ºC on wheat dextrose agar (WDA) medium. Maki *et al*. (2001) reported ideal temperature for mycelial cultivation ranged between 25 to 28ºC. In practice, the best strains for best quality seem to be those which require lower temperatures to reach the fructification phase (8-16ºC). Ability of tested strains to grow on a wide range of temperature is beneficial for the growers. Mata *et al.,* (2001) compared mycelial growth of *L. edodes* and *L.boryana* to select the better strain for cultivation. For cultivation of these strains in Indian conditions, ability to produce fruiting bodies at high temperatures is a prerequisite, to meet the needs of the mushroom market in country with a tropical climate. As mycelial growth and fructification are different stages of the shiitake life cycle, the ideal temperature for one is not always the ideal for the other. Thus, the temperature effect on the two development stages should be considered, and strains that can grow under various temperatures should be selected to meet the grower needs (Chang and Miles, 2004).

Effect of pH on radial growth

The fungus grew on all the pH tested, but as we go on the alkaline side there was a decrease in growth. Maximum radial growth was observed at pH 5.0 (Fig. 2). Khan *et al*. (1991) and Balazs *et al*.(1996) also reported the mycelial growth of *L. edodes* at acidic pH and observed that the most suitable pH for growth of the fungus was 5.0. Affinity of *L.edodes* towards acidic media is explained by Campbell (1930 and 1932) who worked on the chemical aspect of wood rots and led to the conclusion that acid production and enzyme action are closely related. He suggested that acids might be formed by the action of an oxidase on lignin and pentosans and then these react together with the oxidase to bring about the later stages of decay in which cellulose is decomposed. That's why acidic environment is needed for better mycelial growth of Shiitake.

Yield and biological efficiency on different substrates

Lentinula edodes is a white rot fungus that produces a set of lignocellulolytic enzymes, which allow it to grow on lignocellulosic substrates rich in lignin (Leatham, 1986). Thus, it is having the potential to convert cheap lingo-cellulosic substrates into valuable protein at a low cost. Several lignocellulolytic enzymes that are released by it play major role in the biodegradation process and the production of these enzymes depends on the composition of substrates. Combinations of different saw dusts and paddy straw and coir pith substrates had very good mycelial growth, highest numbers of pin head formed and numbers of fruiting bodies

Table 1: Radial growth of *Lentinula edodes* (in mm) strains on different media at room temperature

Media	SEDA			MEA			PDA			PODA			YEA		
Strains Day	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean	L_1	L_2	Mean
2	0.93	0.5	0.72	2.7	1.3	2.0	3.3	2.3	2.8	3.3	2.0	2.7	0.0	0.0	0.0
4	3.7	2.0	2.8	19.3	16.7	18.0	18.7	19.5	19.1	18.7	16.7	17.7	0.23	0.2	0.2
6	10.3	3.5	6.9	37.7	35.3	36.5	39.0	38.9	39.0	34.0	28.6	31.3	10.2	2.7	6.5
8	22.0	18.0	20.0	69.7	68.0	68.8	69.0	69.3	69.1	58.7	50.7	54.7	12.3	4.7	8.5
Growth rate (mm/day)	2.8	2.3	2.5	8.5	8.2	8.4	8.6	8.7	8.7	7.3	6.3	6.8	1.5	0.6	1.0
CD at 5%	1.21	2.32	3.51	1.64	1.30										

Abbreviations: SEDA = PDA + extract of Sal sawdust, MEA = Malt extract agar, PDA = Patato dextrose agar, PODA = PDA + extract of Poplar sawdust, YEA = Yeast extract agar

Table 2: Effect of substrates on the growth and biological efficiency of different strains of *Lentinula edodes* (L_1 and L_2)

Substrates	Mycelial colonization		Pinning		Number of fruiting bodies		Biological efficiency	
	L_1	L_2	L_1	L_2	L_1	L_2	L_1	L_2
PAS	Very Good	Very Good	12	15	11	14	45.9	40.7
PAS + PS	Very Good	Very Good	20	17	19	17	33.2	31.2
PAS + TS	Very Good	Very Good	22	19	21	13	40	35
PAS + CP	Very Good	Very Good	25	24	25	23	50.14	49.9
PS + SS	Good	Good	15	10	14	10	33.3	28.2
PS	Poor	Poor	8	5	7	4	24.2	19.8
PS + TS	Good	Good	16	12	15	12	29.0	25.1
PS + CP	Very Good	Very Good	27	25	26	25	52.8	50.7
PS + SS	Good	Good	15	12	15	12	19	14
TS	Poor	Poor	6	7	4	3	5.8	3.8
TS + CP	Very Good	Very Good	27	24	25	22	42	39.6
TS + SS	Good	Good	22	20	21	20	42.3	38.2
CP	Poor	Poor	15	13	11	10	34	32
CP + SS	Very Good	Very Good	28	29	25	26	52.3	50.4
SS	Poor	Poor	10	8	7	5	25.2	18

Abbreviations: PAS = Paddy straw, CP = Coirpith, SS = Sal sawdust, PD = Poplar sawdust, TS = Teak sawdust

developed with maximum biological efficiency (Table 2). Alone these substrates were not so effective. The yield of mushrooms was affected by different substrates. Of the fifteen different combinations, poplar sawdust and coirpith (PS + CP) substrate gave highest yield followed by paddy straw + coirpith combination (Fig. 3). Ashrafuzzaman *et al*. (2009) found that no primordia produced on paddy straw substrate, it gave no yield and took maximum time in completion of mycelium running. It can be stated that paddy straw give good results when used in combination with coirpith and/or poplar sawdust. Anastazia *et al*. (1982) observed that legumes rich in nitrogen gave a higher yield in combination with paddy or wheat straw or corncobs. All of these substrates were enriched with ten percent wheat bran. Again in terms of yield also combinations of different agricultural wastes as substrates were more productive than individual substrates. The strain L_1 was best and gave highest yield and biological efficiency (Fig. 3 and Table 2).

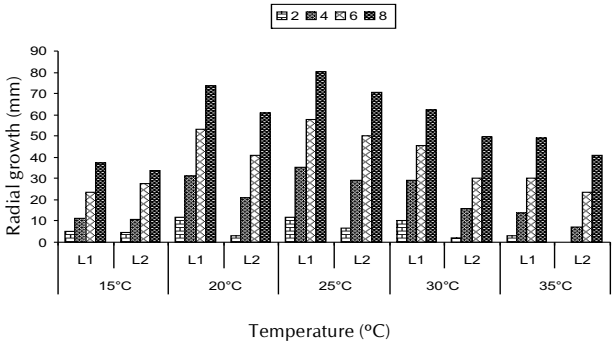

Figure1: Radial growth of *Lentinula edodes* strains (in mm) on Potato Dextrose Agar at different temperature

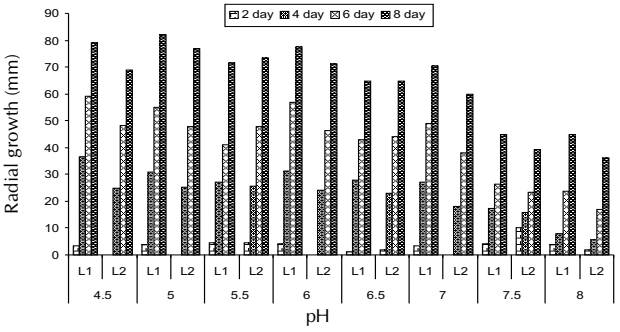

Figure 2: Effect of pH on radial growth (in mm) of *Lentinula edodes* strains on Potato Dextrose Agar medium

Morphological characteristics

The thickness of pileus of the two strains did not vary significantly. As evident from the results, L_1 strain had thick pileus (20.6 mm) and long stipe (39.0 mm) with fruit body longer than L_2. Maximum and minimum diameter for stipe was found in strain L1 (9.9 mm) and L2 (8.2 mm), respectively (Table 3). Purkayastha and Chandra (1985) recorded the pileus diameter and stipe length of Shiitake mushroom up to 11.0 cm and 3.0-4.0 × 0.8-1.3 cm long, respectively. Pegler (2003) recorded the diameter of pileus 5-15 cm across and Gaitan and Mata (2004) recorded the pileus diameter ranging from 5 to 20 cm. A slight variation was also recorded in the color of the fruiting bodies. L_1 was of brown color and L_2 was of dark brown color. On the basis of morphological, cultural and yield

Table 3: Morphological measurements of fruiting bodies of *Lentinula edodes* strains L_1 and L_2

Strains	L_1	L_2	CD at 5%
Pileus Diameter (mm)	87.9	77.4	6.2
Pileus Thickness (mm)	20.6	16.6	4.1
Stipe Length (mm)	39.0	33.0	1.6
Stipe Diameter (mm)	9.9	8.2	1.2
Fruit body length (mm)	92	76	1.4
Fruit body weight (g)	376	300	58.4

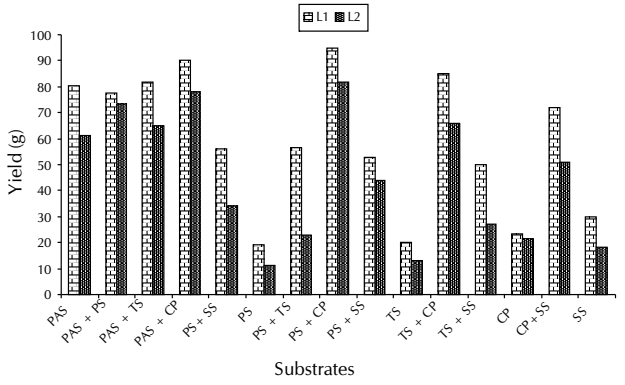

Figure 3: Yield of *Lentinula edodes* strains (L1 and L2) on different substrates

Abbreviations: PAS = Paddy straw, CP = Coirpith, SS = Sal sawdust, PD = Poplar sawdust, TS = Teak sawdust

characteristics, strain L_1 was superior to L_2. It can be grown on any of the tested substrates with 10 percent wheat bran supplementation. The biological conversion of different agriculture wastes by *L.edodes* into high protein diet not only reduces waste disposal problem but also utilizes them into the ecology.

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