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Appraisement of growth behaviour and yield performance of *Lentinula edodes* (Berk.) Pegler using different substrates

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Shiitake mushroom, *Lentinula edodes*is one of the most important medicinal and edible mushroom cultivated throughout the world. In the present investigation cultivation of *Lentinula edodes* on different substrates was done and observationswererecorded at various phases during incubation, different growth parameter, yield and biological efficiency were evaluated. Five different substrates *viz*., Teak, Poplar, Mango, Eucalyptus, Wheat straw and their combinations were used. The minimum days for spawn run (24.00 days), mycelial coat formation (8.66 days), mycelial bump formation (21.33 days) was recorded in the combination of Eucalyptus+ Poplar+ Rice bran+Wheat straw while browning (9.00 days) was observed minimum in Teak + Rice bran+Wheat bran. Appearance of pinhead (67.33 days), fruiting body formation (76.66 days) were observed minimum in Eucalyptus+ Poplar+ Rice bran+ Wheat bran. The maximum pileus diameter (13.93 cm), stipe length (6.99 cm), stipe diameter (4.30 cm) was observed in Poplar + Mango + Wheat straw+ Rice bran. Highest total yield (666.03 g) and biological efficiency (66.63 %) was recorded in the combination of Poplar + Mango + Rice bran +Wheat bran.

Keywords : Biological efficiency, *Lentinula edodes,*mycelial, Shiitake mushroom, substrates, yield

INTRODUCTION

Shiitake mushroom, *Lentinula edodes* (Berk.) Pegler is a traditionally well-known medicinal and palatable mushroom which derives its name from a Japanese word “Shii” means *Castanopsis cuspidate* “Take” means mushroom that means mushroom from “Shii tree.” It is a wood-rotting mushroom but potentially useful fungus that belongs to the class basidiomycetes.The button mushroom *Agaricusbisporus*, Shiitake is the most cultivated mushroom in the world (Chang and Miles, 2004). It is saprophytic in nature and grows on dead material. Shiitake mushrooms relays on different substrates for nutrition and the substrate is basically a sawdust which contains a good source of lignocellulose.

High cellulose content in wood results in enhanced cellulose enzyme production and increased yield of mushroom material which supports growth, development and fruiting of mushroom Sawdust is the most popular basal ingredient used in substrates to produce shiitake (Grodzinskaya *et al*. 2003). This mushroom is a native to Japan, China and other East Asia countries. Shiitake mushroom, also known by the names Japanese wood mushroom or Chinese black mushroom or golden oak mushroom or oriental black mushroom is scientifically called as *Lentinula edodes.* At present China accounts for more than 95% of the total shiitake mushroom produced in the world which is about 7.6 billion kg (Royse, 2014). Mushroom production is 258 thousand tons in 2021 with a 6 per cent increase from the previous year production (242 thousand tons). The production of mushrooms in Uttarakhand is about 12.40 thousand tons in the year 2021 (Anonymous, 2021).Shiitake mushroom has been found to contain 90% water, 7% carbohydrates, 2% protein, less than 1% fat, vitamins B and minerals and Vitamin D when exposed or dried under sunlight or artificial light (Ko *et al*. 2008; Cardwell *et al*. 2018).This mushroom contains important polysaccharide “lentinan” which exhibits many medicinal properties. Its nutritional components include bio-active polysaccharides such as b-D- glucan,

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heteroglucan, xylomannan, lentinan and eritadenine; (B_2, B_{12} and D_2) and dietary fibre (Hobbs, 2002). The Shiitake mushroom is also used to treat respiratory diseases, liver trouble, poor blood circulation, weakness, exhaustion and premature ageing. It has the capacity to increase the stamina, curing of colds and chronic diseases like cancer, AIDS and heart diseases (Dewangan, 2005). Lentinan, a bioactive chemical isolated from the fruit bodies of shiitake, has been reported to have multiple anti-tumor activities and has therapeutic implications in several cancer treatments (Bisen *et al*. 2010). Due to these attributes, its mass scale production is crucial for chemical, pharmaceutical and fermentation industries (Hermandez *et al*. 2006). This mushroom is cultivated on both natural and artificial logs since this white rot fungus can colonize different types of agricultural wastes as growth substrates, although exploitation of the substrate varies with the species, strain, and cultivation technology (Annepu *et al*. 2019). The present investigation has been carried out to determine the effects of various sawdust on the growth and development of *Lentinula edodes*.

MATERIALS AND METHODS

The present investigations were carried out at College of Horticulture, VCSG UUHF, Bharsar (Pauri Garhwal) Uttarakhand during 2022-2023. The experiments were conducted in the Department of Plant Pathology, Mushroom Research Unit, NAHEP Central Instrumental Laboratory. Four indigenous broad-leaved sawdust and wheat straw substrates were used for conducting the experiments and selected based on their low cost and easy availability. The substrates included were Eucalyptus, Mango, Poplar, Teakand wheat straw and the combination of (Teak + Poplar, Teak+ Mango, Poplar + Mango, Mango+ Eucalyptus, Eucalyptus + Poplar).They were evaluated for growth and yield basis performance. Observations had recorded for spawn run period (days), mycelial coat formation (days), mycelial bump formation (days), browning (days), appearance of pin head (days), fruiting bodies formation (days), stipe length (cm), cap diameter (cm), stipe diameter (cm), flush wise yield (g), total yield (g) and biological efficiency (%). The pure culture of Shiitake mushroom (*Lentinula edodes*) Strain DMRO-327 was procured from the Directorate of Mushroom Research Chambaghat, Solan. The maintenance of the culture was done by subsequent sub culturing method. The inoculated test tubes were incubated at 22 ± 2ºC. The wheat grains were used as a substrate for spawn preparation and additives such as chalk powder ($CaCO_3$) and gypsum ($CaSO_4$) were added in the ratio of 1:3. The grain filled into polypropylene bags and bags were sealed using cotton plugged and autoclaved at 121°C, 15 lbs psi, for 2 hrs. Then bags were inoculated with mycelial culture of *Lentinula edodes* and kept into the incubator at the temperature of 25°C till the grains were fully impregnated with the mycelium. The mushroom bags were prepared from the sawdust of five individual substrates *viz.,* Eucalyptus, Mango, Poplar, Teak, Wheat straw and their combinations in the ratio of 1:1 were prepared. The sawdust of substrates and their combinations were soaked for 12 hours in a plastic bucket. Next day, wheat bran and rice bran were soaked for 2 to 3 hrs prior to the filling of the bags. The sawdust, rice bran and wheat bran were mixed manually. The moisture of the mixture was maintained @ 60-65 %. For the preparation of the bags of each substrate, the total mixture of 1000g (wet weight basis) were prepared in varying quantity of 800 g of sawdust + 200g of the wheat bran and rice bran + 10g of calcium carbonate. The filled bags were autoclaved for 30 min at 121ºC under pressure of 1.5 kg/cm^2. After that, Autoclaved bags were allowed to cool down at room temperature for 7- 8 hr. The spawn was added @ 3% on wet weight basis (*i.e.,* 30 gm per each bag log weighing 1kg) (Tarushi and Sud, 2022). The bags were transferred to cropping room, temperature and relative humidity of cropping room were maintained between 15-25^{0}C and 70-90%.

The biological efficiency was determined by the following formula :

$$B.E = \frac{\text{Total Weight of Fresh Mushroom}}{\text{Total Dry Weight of Substrate}} \times 100$$

The Statistical analysis was done by using OPSTAT and the differences exhibited by treatments were tested for their significance by employing the Completely Randomized Block

Table.1: Effect of substrates on different growth stages of *Lentinula edodes* during incubation

Treatments	Spawn run periods (days)±SE(m)	Mycelial coat formation (days)±SE(m)	Mycelial bump formation (days) ±SE(m)	Browning (days) ±SE(m)	Total incubation days
Teak +Ricebran +Wheat bran	30.00*± 0.57	11.33*±0.88	24.33*±0.33	9.00±0.577	75
Poplar +Ricebran +Wheat bran	28.66*±0.66	12.66*±1.20	23.00±0.57	9.66±0.333	73
Mango +Ricebran +Wheat bran	28.33*±0.33	12.00*±0.57	29.33*±0.88	10.33±0.882	80
Eucalyptus +Ricebran +Wheat bran	29.66*±0.88	12.33*±0.33	23.33±1.33	10.33±1.202	76
Teak + Poplar +Ricebran +Wheat bran	30.66*±0.88	13.33*±0.88	26.33*±0.88	11.33±1.453	82
Teak + Mango +Ricebran +Wheat bran	32.33*±0.33	14.66*±0.66	30.66*±1.20	12.33*±0.333	90
Poplar + Mango +Ricebran +Wheat bran	34.00*±0.57	13.00*±0.57	26.66*±0.88	14.33*±0.882	88
Mango + Eucalyptus+Ricebran +Wheat bran	31.66*±0.33	11.66*±0.88	27.00*±1.15	11.00±1.528	81
Eucalyptus + Poplar +Ricebran +Wheat bran	24.00±0.00	8.66±0.33	21.33±0.33	9.66±0.333	63
Wheat straw +Ricebran +Wheat bran	35.33*±0.88	14.66*±0.33	30.66*±0.88	14.66*±1.45	95
SE (d)	0.86	1.02	1.28	1.43	-
C.D. $_{(0.05)}$	1.82	2.14	2.69	3.00	-

*Significant at 5% level of significance as compared with minimum days (desirable)

Design (CRD)

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RESULTS AND DISCUSSION

The analysis of growth and yield of *Lentinula edodes* on different substrates gave more or less significant results. The data have been presented in (Tables1, 2, 3 and Fig.1). While determining the effect of different substrates on *Lentinula edodes* for spawn run period (days), mycelial coat formation (days), mycelial bump formation (days), browning (days), total incubation (days), it was observed that the during incubation the minimum days required for spawn run period (24 days), mycelial coat formation (8.66 days), mycelial bump formation (21.33 days) were observed in the combination of Eucalyptus + Poplar + Rice bran + Wheat bran but the minimum number of days required for browning was observed in Teak+ Rice bran + Wheat bran (9.00 days) Whereas maximum days required for spawn run period (35.33days), mycelial coat formation (14.66 days), mycelial bump formation (30.66 days) and for browning (14.66 days) were observed in wheat straw + Rice bran + Wheat bran, so the total incubation days were minimum for Eucalyptus + Poplar + Rice bran + Wheat bran (63 days) while maximum for wheat straw + Rice bran + Wheat bran (95 days).Chittaragi*et al*. (2018) reported that wheat straw took forty days for spawn run. Tarushi and Sud (2022) found the similar for the spawn run, mycelial coat formation and mycelial

Table 2: Effect of different substrates on growth parameters of *Lentinula edodes*

Treatments	Appearance of pinhead (days)±SE(m)	Fruiting body formation (days)±SE(m)	Pileus diameter (cm)±SE(m)	Stipe length (cm)±SE(m)	Stipe diameter (cm)±SE(m)
Teak +Ricebran +Wheat bran	78.00*±0.57	91.00*±0.57	8.89*±0.15	4.16*±0.50	1.64*±0.23
Poplar +Ricebran +Wheat bran	75.33*±1.20	86.33*±0.33	11.31*±0.61	5.30*±0.19	3.44*±0.08
Mango +Ricebran +Wheat bran	81.33*±0.33	88.33*±0.88	9.39*±0.51	5.83*±0.31	1.80*±0.09
Eucalyptus +Ricebran +Wheat bran	78.00*±1.15	90.00*±1.52	12.39*±0.44	5.14*±0.14	3.54*±0.13
Teak + Poplar +Ricebran +Wheat bran	84.66*±1.20	93.66*±1.20	7.83*±0.21	6.29±0.04	2.02*±0.06
Teak + Mango +Ricebran +Wheat bran	94.00*±1.15	98.66*±0.66	8.76*±0.43	4.92*±0.04	1.14*±0.13
Poplar + Mango +Ricebran +Wheat bran	89.33*±0.88	97.33*±0.88	13.93±0.32	6.99±0.06	4.30±0.13
Mango + Eucalyptus+Ricebran +Wheat bran	82.66*±1.45	91.00*±0.57	8.14*±0.15	6.14*±0.08	2.68*±0.17
Eucalyptus + Poplar +Ricebran +Wheat bran	67.33*±0.88	76.66±0.33	10.81*±0.52	5.61*±0.38	3.65*±0.29
Wheat straw +Ricebran +Wheat bran	97.33*±0.88	99.33*±1.45	7.33±*0.54	3.91*±0.24	1.10*±0.31
SE (d)	1.44	1.32	0.60	0.35	0.26
C.D. $_{(0.05)}$	3.03	2.78	1.26	0.74	0.5

* Significant at 5% level of significance as compared with best result (minimum for days and maximum for Pileus diameter, stipe length or Stipe diameter.

bump formation in *L. edodes*. Kim *et al*. (2009) have shown that intensity of browning can vary according to light, aeration and temperature, the process is accelerated by oxygen and light exposition. The difference in time period for the browning was reported by many investigators. Philippoussis *et al*. (2007) and Hernandez *et al*. (2006).

Effect of different substrates on growth parameters of *Lentinula edodes* was observed in the cropping room after incubation. The minimum days required for pinhead formation (67.33 days), fruiting body formation (76.66 days) were observed in the combination of Eucalyptus + Poplar + Rice bran + Wheat bran, whereas maximum days required for pinhead formation (97.33 days), fruiting body formation (99.33 days) were observed in wheat straw + Rice bran + Wheat bran. The maximum pileus diameter (13.93 cm), stipe length (6.99 cm), stipe diameter (4.30 cm) was recorded in the combination of Poplar + Mango + Rice bran + Wheat bran while the minimum pileus diameter (7.33 cm), stipe length (3.91 cm), stipe diameter (1.10 cm) was recorded in wheat straw + Rice bran + Wheat bran. Tarushi and Sud (2022) observed that (7-12 days) were required for the fruiting body

T_1 (Teak + Rice bran + Wheat bran)

T_2 (Poplar+ Rice bran + Wheat bran)

T_3 (Mango+ Rice bran + Wheat bran)

T_4 (Eucalyptus + Rice bran + Wheat bran)

T_5 (Teak +Poplar+ Rice bran + Wheat bran)

T_6 (Teak +Mango+Rice bran + Wheat bran)

T_7 (Poplar+Mango + Rice bran + Wheat bran)

T_8 (Mango+Eucalyptus + Rice bran + Wheat bran)

T_9 (Eucalyptus+Poplar+ Rice bran + Wheat bran)

T_{10} (Wheat straw + Rice bran + Wheat bran)

Fig.1: Yield performance of *Lentinula edodes* on different substrates

Table 3: Biological efficiency and yield performance of *Lentinula edodes* on differentsubstrates

Treatments	Yield (g)/1000g substrates				
	1st flush (g) ±SE (m)	2nd flush (g) ±SE (m)	3rd flush (g) ±SE (m)	Total yield (g) ±SE (m)	Biological efficiency (%)
Teak +Ricebran +Wheat bran	108.06*±0.14	75.67*±0.47	58.15*±0.52	241.89*±43.86	24.18
Poplar +Ricebran +Wheat bran	136.13*±0.46	95.20*±1.62	71.53*±1.24	302.86*±56.60	30.28
Mango +Ricebran +Wheat bran	188.63*±1.25	134.30*±1.36	88.73*±0.38	411.66*±86.62	41.16
Eucalyptus +Ricebran +Wheat bran	153.03*±1.15	103.86*±1.33	84.03*±0.70	340.93*±61.52	34.09
Teak + Poplar +Ricebran +Wheat bran	196.16*±2.08	143.56±*2.28	94.23*±1.21	433.96*±88.29	43.39
Teak + Mango +Ricebran +Wheat bran	112.73*±0.93	78.26±*0.58	68.60*±1.79	259.60*±40.18	25.96
Poplar + Mango +Ricebran +Wheat bran	270.06±0.90	215.00±2.03	180.96±0.26	666.03±77.87	66.63
Mango + Eucalyptus+Ricebran +Wheat bran	247.00*±0.87	195.30*±2.64	130.36*±1.21	572.66±101.22	57.26
Eucalyptus + Poplar +Ricebran +Wheat bran	231.79*±2.24	180.88*±0.38	120.46*±1.15	533.14±96.53	53.31
Wheat straw +Ricebran +Wheat bran	85.51*±1.70	65.81*±0.75	45.39*±1.64	196.71*±34.75	19.67
SE (d)	1.89	2.18	1.59	102.63	-
C.D. (0.05)	3.97	4.59	3.35	215.59	-

* Significant at 5% level of significance as compared with maximum yield

formation. Ashrafuzzaman *et al*. (2009 a,b) Annepu *et al*. (2019), Chittaragi *et al*. (2018) also reported the related findings for pileus diameter, stipe length and stipe diameter.

Flush wise yield (g) per 1000 (g) substrate, was recorded maximum in Poplar + Mango + Rice bran + Wheat bran *i.e*., 1st, 2nd and 3rd (270.06g, 215.00g and 180.96g) and minimum yield was found in wheat straw + Rice bran + Wheat bran 1st, 2nd and 3rd (85.51g, 65.81g and 45.39 g). Maximum average total yield and biological efficiency (B.E) was found in the combination of Poplar + Mango + Rice bran + Wheat bran (666.03g) and (66.63%) respectively while the lowest total yield and biological efficiency (B.E) was recorded in wheat straw + Rice bran + Wheat bran (196.71 g) and (19.67%) respectively. Sharma *et al*. (2017) reported that 10% supplementation of wheat bran and rice bran enhances the yield. Tarushi and Sud (2022) recorded the maximum yield in Mango and Poplar combination (720g). Annepu *et al*. (2019) found that the saw dust substrate gave higher yield over the wheat straw but the fruiting was earliest on wheat straw. Therefore, there is possibility of variations in production of enzymes to degrade phenolic compounds, lignin, hemicelluloses etc. and hence, resulted in variations in growth characteristics. Ashrafuzzaman *et al*. (2009) found that the product of cellulolytic action is simple and soluble carbohydrates and the end products being glucose was absorbed by the fungal mycelium for growth and energy. Therefore, cellulose rich organic substrates are good for the cultivation of mushroom.

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DECLARATIONS

Conflict of interest: Authors declare no conflict of interest.

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