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Assessment of different media and grain extract on mycelia growth of Shiitake mushroom *Lentinula edodes* (Berk.) Pegler

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Abstract
Shiitake mushroom (*Lentinula edodes*) is a temperate mushroom which is rich in flavor, taste aroma and medicinal potential. Regarding medicinal potentials it contains, polysaccharides like "lentinan" which has medicinal value. Shiitake mushroom has been proved to be having anti-HIV, anti-cancer, anti-diabetic properties and ability to promote the immune system. In present investigation six different media and five grain extract were evaluated for mycelia growth of *Lentinula edodes.* In observations of experiment of different media, maximum mycelial growth (90.00 mm) was observed on Sweet potato extract agar media (SPEA) followed by Malt extract agar media (MEA). Among different grain extract media, fastest radial growth was observed on Oat extract agar media (OEA) followed by Black gram extract media (BGEA).

Keywords: Shiitake, mushroom, media, cereal extract, mycelial

Introduction
Mushroom include well over 14,000 species of achlorophyllus, saprophytic, macro fungus that can grow seasonally all over the world with different shape, size, color, appearance and edibility. According to Chang and Miles, 1993 [3] "Mushroom is a macro fungus with a distinctive fruiting body which can be either epigeous (aboveground) or hypogeous (underground) and large enough to be seen with naked eyes and to be picked by hands." Most of the mushrooms has low calorific value and probably contain every mineral including phosphorous and potassium, low amounts of iron and calcium. Mushroom is an excellent source of vitamins *viz.* riboflavin (B2), thiamine (B1), biotin, niacin, and Vitamin C. Vitamins A and D are rare although several species contain detectable amounts of β-carotine and ergosterol which transformed into active vitamin D when exposed to ultraviolet radiation and contains all the essential classes of lipid compounds including free fatty acids, mono-, di and triglycerides, sterols, sterol esters and phospholipids, levels are generally low, around 2-8% of dry weight (Breene, 1990) [2].
The word 'shiitake' was orginated from two Japanese words: shii which means oak and take which means mushroom, reflecting the significance of oak wood as the natural host of the fungus (Davis 1993; Royse 2001) [7, 17]. In China, different forms of shiitake are known byarious names such as means 'Xiang-gu' (or Siang-gu) "The fragrant mushroom", 'Donggu' "The winter mushroom", and 'Hua-gu', "The flower mushroom" or "The variegated mushroom" (Chen, 2001) [6]. Shiitake usually have stalks attached to the center of umbrella shaped mushroom caps that are light to dark brown and 5-25cm in diameter. It is a saprophytic wood rotting mushroom (Stamets 1993) [24] that grows on dead material (Chang and Miles, 2004) [4]. It improves bone strength, promote skin health, fight obesity, increase probiotic activity in intestine and stomach, have antitumor as well as anticancer property. In both China and Japan, it is recognized to be an "elixir of life" and is widely consumed.
Singh *et al.* (2020) [21] collected data from several sources and concluded that "world mushroom production in 2018-19 was 43 million tonne (MT) with *Lentinula edodes* (shiitake) contributing 26% (most cultivated mushroom)", While in India, mushrooms like shiitake, Kirajari, Reishi mushroom, etc. combined account for only one percent (Sharma *et al.*, 2017) [18]. Although shiitake is globally well known mushroom but it is yet to find a place in market of countries like India. This is mainly because of little information on the cultivation, consumption or medicinal properties of *Lentinula edodes* in the India.

Thus, the present study can be important to elucidate the potential of *Lentinula edodes* mushrooms as functional & medicinal in the country.

Experimental Site

The experiment were conducted in Mushroom Research and Training Center, Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut -250110 (Uttar Pradesh) India. Located between 29°01' N latitude and 77°45'E longitude at an elevation of 237 meters above mean sea level.

Material & Methods:

Establishment of pure culture

The culture of Shiitake mushroom (*Lentinula edodes*) was obtained from Directorate of Mushroom Research, Solan. The culture of Shiitake mushroom (*Lentinula edodes*) was multiplied in sterilized Petri plate on Potato dextrose agar (PDA) for 12-15 days. Single branched hyphae from the periphery of growing colony were marked under low power (10x) in compound microscope and transferred to PDA slants and petri-plates for maintenance. These culture tubes and petri-plates were incubated at 25±2 °C in BOD incubator. Again subculture on PDA and then stored in refrigerator at 10-12 °C for further use.

Effect of different media on mycelial growth of *Lentinula edodes*

For the present investigation, different media such as Malt extract agar media (MEA) (20 gm malt extract and 20 gm Agar in 1 litre distilled water), Yeast extract agar(YEA) (3gm yeast extract, 5gm peptone and 20 gm Agar in 1 litre distilled water), Sweet potato extract agar (SPEA) (200 gm peeled sweet potato, 20 gm dextrose and 20 gm Agar in 1 litre distilled water), Carrot extract agar (CEA) (200 gm carrot, 20 gm dextrose and 20 gm Agar in 1 litre distilled water) and sugar beet extract agar (SBEA) (200 gm Sugarbeet, 20 gm dextrose and 20 gm Agar in 1 litre distilled water) media were used.

Effect of different Cereal extract on mycelial growth of *Lentinula edodes*

For the present investigation, different media such as Wheat extract agar (SPEA) (200 gm Wheat grain, 20 gm dextrose and 20 gm Agar in 1 litre distilled water), Oat extract agar (CEA) (200 gm Oat grain, 20 gm dextrose and 20 gm Agar in 1 litre distilled water), Bangal Gram extract agar (BGEA)(200 gm Bangal gram, 20 gm dextrose and 20 gm Agar in 1 litre distilled water), Green Gram (GGEA) (200 gm Bangal gram, 20 gm dextrose and 20 gm Agar in 1 litre distilled water) and Cowpea extract agar (200 gm Cowpea, 20 gm dextrose and 20 gm Agar in 1 litre distilled water) media were used.

Pouring, Incubation and Data collection

Then these media were dispensed in 500 ml flask with care that it made upto 2/3 of flask volume or in culture tube 8-10 ml media in each tube. The conical flask or culture tubes were tightly plugged with the help of non-absorbent cotton plugs cover with butter paper that was secured with rubber bands. Then conical flasks and culture tubes containing media were autoclaved at 121 °C (15 psi) for 15-20 minutes. After sterilization the media were poured into the sterilized Petri plates of 90 mm @ 25 ml/plate of each treatment in the three replication under aseptic condition inside laminar air flow (LAF). The plate after cooling, were inoculated centrally with a 9 mm disc of 7 days old culture in laminar air flow and incubated at 25±1 °C in B.O.D. The observations of radial growth in were taken at each 48 hrs till the colony covered the first full plate.

Statistical analysis

In this study, all the experiments were conducted in CRD (completely randomized design) in 3 replications and the data thus obtained were analyzed statistically. The analysis of variance (ANOVA) technique and critical difference (CD) was calculated at 5 percent level of significance for comparison with other treatments.

Result and Discussion

Effect of different media on mycelial growth of *Lentinula edodes*

The culture of *Lentinula edodes* was grown on different media *viz.* Malt extract agar media (MEA), Sweet potato extract agar media (SPEA), Carrot extract agar media (CEA), Yeast extract agar media (YEA), Sugarbeet extract agar media (SBEA), Potato dextrose agar media (PDA) as shown in Table 1 and Fig 1.

The observations were recorded on 3rd, 6th, 9th, 12th and 15th day of inoculation in Petri plates. The result revealed that on 15th day of inoculation in petri plates, in strain IVTL-06 maximum mycelial radial growth (90.00 mm) with growth rate 6.00 mm per day was found on Sweet potato extract agar media (SPEA), followed by Malt extract agar media (MEA) with radial growth 88.66 mm and growth rate 5.91 mm per day which are statistically higher than Potato dextrose agar (PDA) which had radial growth 83.00 mm with 5.53 mm per day growth rate. The minimum radial mycelia growth of *Lentinula edodes* was observed on Sugarbeet extract agar media (SBEA) i.e. 73.77 mm with 4.88 mm per day growth rate followed by Carrot extract agar media (CEA) i.e. 82.33 mm growth rate 5.48 mm per day.

The results are in accordance with Puri *et al.* (2006) [16] who tested media such as malt extract agar, yeast extract agar, potato dextrose agar (PDA), teak sawdust agar and poplar sawdust agar. They reported that the strain LI of shiitake showed the highest growth rate i.e. 8.7 mm/day on PDA and least growth was observed in yeast extract agar i.e. 1.04 mm/day on the 8th day of inoculation. While Bilay *et al*., (2000) [1] used four commercially available media (malt extract agar, yeast malt extract agar, wort agar and experimental agar medium) to evaluate mycelial growth and found that potato dextrose agar medium (PDA) as the most suitable medium for the growth of edible fungi. Dewangan (2005) [8] as observed that the radial growth of two stains of *Lentinula edodes* was higher in potato dextrose agar i.e. 87.50 mm followed by malt extract agar medium i.e. 83.25 mm.

Effect of different cereal extract on mycelial growth of *Lentinula edodes*

IVTL-06 and IVTL-10 strains of shiitake mushroom (*Lentinula edodes*) were grown on different grain extract media *viz*. Wheat extract agar media (WEA), Oat extract agar media (OEA), Cow pea extract agar media (CPEA), Black gram extract agar media (BGEA), Green gram extract agar media (GGEA), Potato dextrose agar media (PDA) as shown in Table 2 and Fig 2.

The observations were recorded on 3rd, 6th, 9th, 12th and 15th day of inoculation in Petri plates. In this investigation on 15th day, maximum radial growth of *Lentinula edodes* was found on Oat extract agar media (90 mm with growth rate 6.00 mm/day) followed by Black gram extract agar media (BGEA) (86.33 mm with growth rates 5.75 mm/day, Green gram extract agar media (83.33 mm with growth rates 5.55 mm/day), Potato dextrose Agar (PDA) (83.00 mm with growth rates 5.53 mm/day), and least radial growth of both strains were found on Cowpea extract agar media (CPEA) (17.66 mm with growth rates 1.17 mm/day) followed by Wheat extract agar media (WEAM) (51.66 mm with growth rates 3.44 mm/day).

The results were found in accordance with the finding of Kannan and Eswaran (2010) [10] who carried out a study to evaluate the suitable medium for the culture of *L. edodes*. Five media *viz.*, malt extract agar, Czapeck dox agar, malt yeast extract agar, oat meal agar and potato dextrose agar were tested. The fastest mycelial growth was recorded on oat meal agar i.e. 90 mm/9 days followed by potato dextrose agar. Sowmya and Chandra (2020) [23] observed the maximum mycelium growth of *L. edodes* on PDA medium was observed in the strain (89.4 mm) on the 20th day after inoculation, while minimal mycelium growth was observed in the wheat extract medium (72.50 mm). Result were almost similar with our findings.

Table 1: Effect of different media on mycelial growth of *Lentinula edodes*

Treatment	3rd day	6th day	9th day	12th day	15th day	Growth per day (mm/day)
Malt Extract media	14.00	29.33	46.66	67.66	88.66	5.91
Yeast Extract media	12.33	20.66	28.66	39.33	49.33	3.28
Sweet potato Extract media	11.66	27.00	42.33	66.33	90.00	6.00
Carrot Extract media	12.0	26.33	40.66	61.66	82.33	5.48
Sugarbeet Extract media	12.33	24.33	36.00	54.66	73.33	4.88
PDA	12.33	26.33	40.33	61.66	83.00	5.53
CD	1.122	2.435	2.508	1.642	1.529	-
S.E(m)	0.36	0.782	0.805	0.527	0.491	-

Average of Three Replication

Table 2: Effect of different cereal extract on mycelial growth of *Lentinula edodes*

Treatment	3rd day	6th day	9th day	12th day	15th day	Growth per day (mm/day)
Wheat extract media	11.33	17.00	22.66	37.33	51.66	3.44
Oat Extract media	11.66	28.66	45.66	68.00	90.00	6.00
Cowpea Extract media	9.66	11.33	12.00	14.33	17.66	1.17
Black gram Extract media	11.66	27.66	43.33	65.00	86.33	5.75
Green gram Extract media	11.66	27.33	42.66	63.33	83.33	5.55
PDA	12.33	26.33	40.33	61.66	83.00	5.53
CD	1.122	2.162	1.586	1.149	1.341	-
S.E(m)	0.360	0.694	0.509	0.360	0.430	-

Average of Three Replication

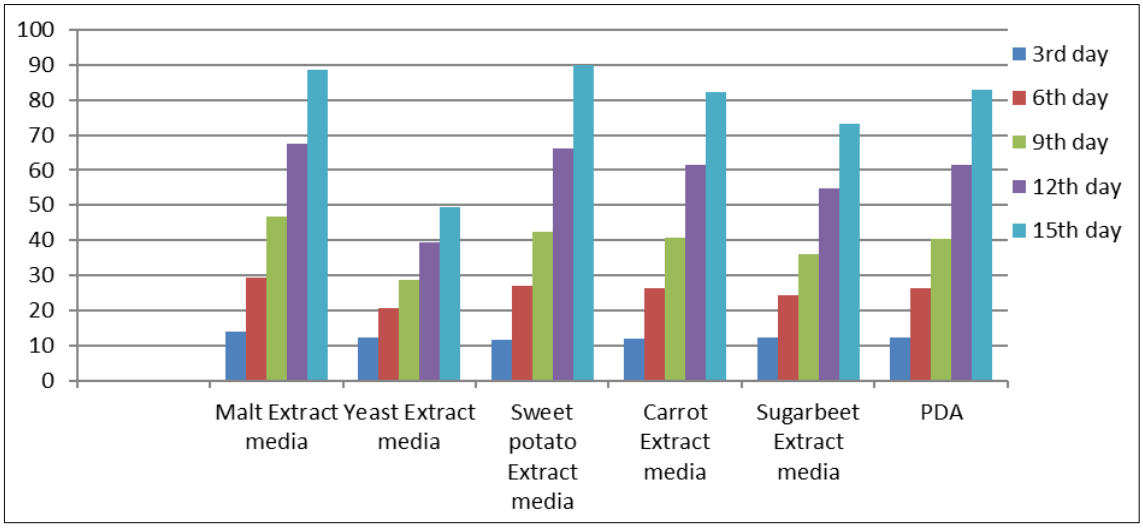

Fig 1: Effect of different media on mycelial growth of *Lentinula edodes*

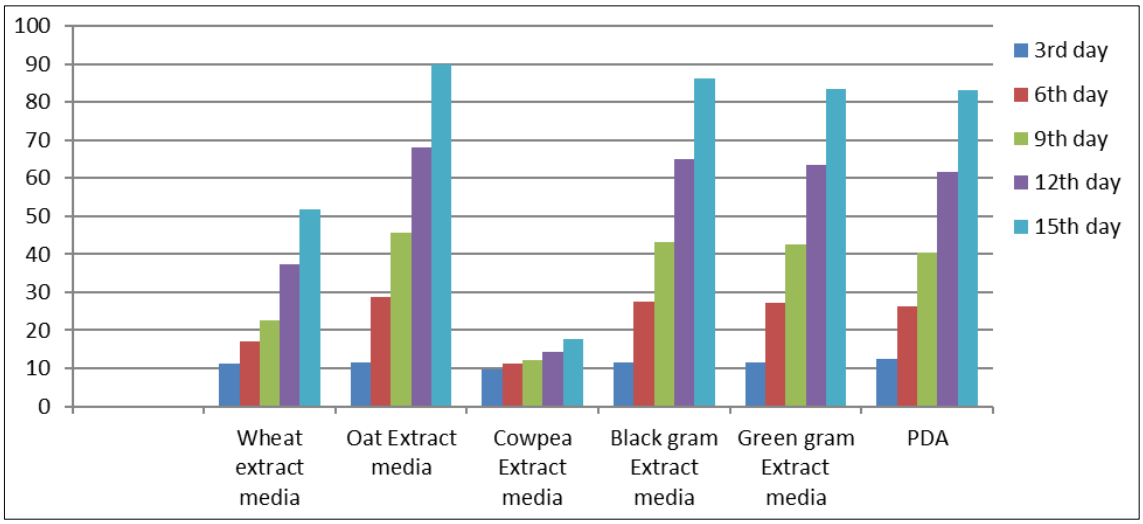

Fig 2: Effect of different cereal extract on mycelial growth of *Lentinula edodes*

Conclusion

Among six different media *viz.* includes Malt extract agar media (MEA), Yeast extract agar media (YEA), Sweet potato extract agar media (SPEA), Carrot extract agar media (CEA), Sugarbeet extract agar media (SBEA), Potato dextrose agar media (PDA) evaluated for thr mycelia growth of shiitake mushroom (*Lentinula edodes*), maximum radial growth was observed on Sweet potato extract agar media (SPEA) and least mycelial growth was observed on Yeast extract media (YEA).

Among different grain extract media *viz.* Wheat extract agar media (WEA), Oat extract agar media (OEA), Cow pea extract agar media (CPEA), Black gram extract agar media (BGEA), Green gram extract agar media (GGEA) and Potato dextrose agar media (PDA) evaluated for best mycelia growth of shiitake mushroom (*Lentinula edodes*), maximum radial growth was found on Oat extract agar media (OEA) (90 mm) and least radial growth was found on Cowpea extract agar media (CPEA).

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