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Effect of different media, temperature and pH on radial mycelial growth of *Lentinula edodes* strain Le-17-04

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Abstract
Lentinula edodes (Berk.) Pegler is one of the lignicolous mushrooms that produces brown sporocarps. Presently standardized cultural conditions for shiitake mushroom is unavailable. So the present study has been conducted for better understanding of cultural conditions for shiitake mushroom. Under *in-vitro* study the vegetative growth of Le-17-04 was also evaluated on culture media, pH and temperature. It was found that *Lentinula edodes* Le-17-04 was well grew best on PDA medium at 24ºC and at both pH 5.0 and pH 6.0 with maximum 90 mm, 83.33 mm and 90 mm radial growth at 15, 12 and 12 days after inoculation, respectively.

Keywords: *Lentinula edodes*, Le-17-04, mycelial

Introduction
Fungi are known all around the world more than 400 million years, they have a wide diversity including mushrooms which have been used by people for food and medicinal purposes. Out of 1.5 million species of fungi, 14,000 to 15,000 species are considered as mushrooms and about 2000 species are edible. Among the total edible species, about 80 species are grown experimentally and 20 are cultivated commercially. However, *Agaricus, Pleurotus, Flammulina, Lentinula* and *Auricularia* species are adopted at industrial scale worldwide (Royse, 2014) [3]. *Lentinula edodes* (Berk.) Pegler. also known as shiitake or oak mushroom. It is an important edible mushroom native of East Asia, which is consumed and cultivated in many Asian countries since long back. Shiitake has traditionally been cultivated on hardwood logs, mainly oak. However, due to the dwindling supply of hardwoods and the long spawn-run period of up to a year before the first harvest, an alternative technique of cultivation on artificial logs consisting of supplemented sawdust, wood chips or other lignocellulosic wastes was developed. This technique was first reported in the late seventies in Japan (Fuzisawa *et al*., 1978; Mee, 1978) [1, 2] and has gained importance. The period of long spawn run is the main problem of the shiitake cultivation. The time period of spawn run can be minimized with organic and inorganic supplements along with the use of the different lignocellulosic substrate. The pH and temperature of the substrate should be such that it should promote better growth of shiitake mushroom. Any change in temperature and pH may cause fruiting bodies to fall. The high pH may result in poor or even no spawn run without fruiting. High temperature may lead to fast dehydration of mushroom, so we must have knowledge about the standard growing condition for shiitake mushroom. Keeping the above problems in consideration and the importance of shiitake mushroom, substrate, and supplements for its cultivation, the present investigation was conducted to study the effect of media, pH, temperature and on radial mycelial growth of Le-17-04.

Material and Method
Mushroom Culture and its Maintenance
All research experiments of the present study were carried out in Mushroom Research and Training Centre, G.B. Pant University of Agriculture and Technology, Pantnagar. During the present study a strain of *Lentinula edodes* symbolized as Le-17-04 was obtained from above centre and subsequently maintained in form of pure culture on potato dextrose agar medium. The full grown pure culture as prepared in test tubes and petri plates were kept in refrigerator for further use.

Media
In the beginning of study five media *viz*, potato dextrose agar (PDA), malt extract agar (MEA), oat meal agar (OMA),

Czapek's dox medium and V 8 juice agar medium have been prepared in conical flask and poured into petri plates @ 20ml/petri plates. The poured petri plates then inoculated at the center with 5 mm diameter disc of actively growing mycelium of *Lentinula edodes* Le-17-04 under aseptic conditions. Three replications of each medium (potato dextrose agar, malt extract agar, oat meal agar, czapek's dox and V 8 juice agar medium) were prepared and potato dextrose agar medium was taken as check to make the comparison in between. After inoculation, the inoculated plates were incubated at 24ºC. The petri plates were analysed at every three days interval to measure radial growth of mushroom mycelium in mm till it covered the entire surface of medium. The secondary data on growth rate (mm/day) and per cent increase/decrease in mycelium growth over PDA were also calculated.

Temperature

The present study was further proceeded after selecting the PDA medium among the five media tested on radial mycelial growth of *Lentinula edodes* Le-17-04. The PDA was prepared as per the standard procedure in sufficient amount and poured into sterilized petri plates @ 20ml/petri plate to test the mycelial growth of mushroom at five temperatures 22ºC, 24ºC, 26ºC, 28ºC and 30ºC. The poured petri plates were kept in aseptic environment to be solidified and then inoculated with 5 mm diameter disc of actively growing mycelium at the centre of the medium. All five treatments of temperature were replicated thrice. The observations on the mycelial growth of mushroom was recorded at every three days interval until the mycelium did not cover the entire surface of medium. Further, per cent decrease in the growth of mushroom mycelium was also calculated to observe the growth trend among the treatments.

pH

Above experiments on media and temperature confirms maximum radial grow of *Lentinula edodes* Le-17-04 on PDA medium at 24ºC. Therefore, these two were opted for the experiment of pH. In which the growth behaviour of *Lentinula edodes* Le-17-04 was studied on pH 4.0, pH 5.0, pH 6.0, pH 7.0 and pH 8.0. In this experiment potato dextrose broth was prepared, autoclaved at 121ºC for 20 minutes and allowed it to cool. After cooling at desired temperature, it was poured into five conical flasks assigned each for different pH. Now, the five pH level as stated above were adjusted with different potato dextrose broth using concentrated HCl and concentrated NaOH solutions. The pH was tested with the help of pH meter. Now, agar was added into broth slowly and steadily for avoiding clumps formation. Media of different pH was poured into petri plates under aseptic condition. 5-mm disc of freshly active mycelium of *Lentinula edodes* Le-17-04 was transferred in the center of the petri plate. Inoculated petri plates were incubated at 24°C temperature and three replications of each treatment (pH 4.0, pH 5.0, pH 6.0, pH 7.0 and pH 8.0) were maintained. Radial mycelial growth of *Lentinula edodes* was measured in mm at every 3 days interval until the mycelium had not been covered the entire surface of the medium. Further, pH 8.0 was regarded as check and per cent increase over the check at last day was also calculated.

Result and Discussion

Effect of media on radial growth of *Lentinula edodes* Le-17-04

During present experiment, the radial mycelial growth of *Lentinula edodes* Le-17-04 was studied *in vitro* on potato dextrose agar (PDA), V 8 juice agar, malt extract agar (MEA), czapecks dox and oat meal agar (OMA) medium. The results showed that highest 90 mm radial growth was recorded on PDA medium on 15 DAI. Mycelium of the mushroom was spread on PDA @ 6 mm/day. However, MEA, Czapecks dox, V 8 Juice agar and OMA medium were showed statistically gradual decline in radial growth of 79.33 mm, 72.66 mm, 51.33 mm and 12 mm, respectively, against the results as obtained on PDA. The radial growth rate of mushroom was also measured by 5.29 mm/day, 4.84 mm/day, 3.42 mm/day and 0.8 mm/day on the respective media MEA, Czapecks dox, V 8 juice agar and OMA. Percent decrease in radial mycelial growth of *Lentinula edodes* Le-17-04 was further calculated against the growth observed on PDA at 15 DAI. It was found that 86.66%, 42.97%, 19.26% and 11.85% decreased growth was recorded on OMA, V 8 juice agar, Czapecks dox and MEA as computed over the radial mycelial growth of mushroom on PDA. Growth pattern also varied among media used. A very light and fine mycelial growth was observed in Czapecks dox; dense, dull white and fluffy growth was seen on V-8 juice agar; growth pattern in PDA and MEA was nearly same as compact growth and dull white while there was very little constricted growth observed in OMA.

Table 1: Effect of media on the radial growth of *Lentinula edodes* Le-17-04.

Media	Radial growth of *Lentinula edodes* Le-17-04 (mm) 15DAI	% decrease over the check having highest radial growth at 15DAI.	Growth rate (mm/day)
MEA	79.33[b]	11.85	5.29
CZAPECKS DOX	72.66[c]	19.26	4.84
V 8 JUICE AGAR	51.33[d]	42.97	3.42
OAT MEAL AGAR	12.00[e]	86.66	0.8
PDA (check)	90.00[a]	-	6.00
CD at 5%	1.62	-	-
CV	1.46	-	-

Means with the same letters do not significantly differ by the critical difference (CD) test at P≤0.05 with a completely randomized design.

PDA V 8 Juice Agar OMA

MEA Czapeck's Dox

Plate 1: Radial growth of *Lentinula edodes* Le-17-04 on culture media.

Effect of temperatures on radial growth of *Lentinula edodes* Le-17-04

In the previous experiment of media, PDA was found best in view to support fast mycelium growth of *Lentinula edodes* Le-17-04 with its excellent vigour. Hence, it was selected as a growing medium to test the mycelium growth of the fungi against the effect of temperature and pH of preceeding experiment mentioned in Table 2 and Table 3, respectively. Five temperatures 22^{O}C, 24^{O}C, 26^{O}C, 28^{O}C and 30^{O}C have evaluated to the best support of mushroom mycelium as shown in Table 4.3. The results showed that highest 83.33 mm radial mycelial growth was recorded at 24^{O}C at 12 DAI. It grew on PDA @ 6.94 mm/day. However, the growth of *Lentinula edodes* Le-17-04 was declined gradually with decrease and increase in temperature from 24^{O}C. At 22^{O}C, 26^{O}C, 28^{O}C and 30^{O}C radial growth was observed of 78.66 mm, 77.33 mm, 63.33 mm and 56.66 mm with respect of the growth at 24^{O}C (Plate 2). Though, the effect of 22^{O}C and 26^{O}C on the growth was statistically *at par*. Percent decrease in radial mycelial growth at 12 DAI was observed 31.99%, 24 %, 7.20 % and 5.60% at temperature 30^{O}C, 28^{O}C, 26^{O}C and 22^{O}C, respectively, as compared to radial growth at 24^{O}C

Table 2: Effect of temperature on the radial growth of *Lentinula edodes* Le-17-04.

Temperature	Radial growth of *Lentinula edodes* Le-17-04 (mm) at 12DAI	% decrease over the check having highest radial growth at 12 DAI	Growth rate (mm/day)
22^{O}C	78.66^{b}	5.60	6.55
26^{O}C	77.33^{b}	7.20	6.44
28^{O}C	63.33^{c}	24.00	5.27
30^{O}C	56.66^{d}	31.99	4.72
24^{O}C (check)	83.33^{a}	-	6.94
CD at 5%	3.11	-	-
CV	2.38	-	-

Means with the same letters do not significantly differ by the critical difference (CD) test at P≤0.05 with a completely randomized design.

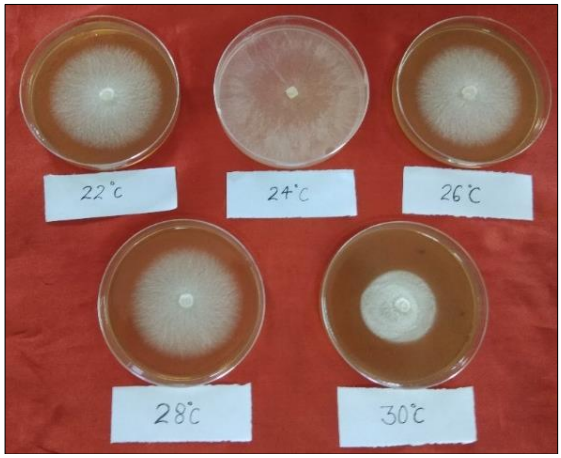

Plate 2: Radial growth of *Lentinula edodes* Le-17-04 on different temperatures.

Effect of pH on radial growth of *Lentinula edodes* Le-17-04

In this experiment, the effect of pH was studied on the fungal growth *in-vitro*. The data revealed that out of pH 4.0, 5.0, 6.0, 7.0 and 8.0, highest vegetative growth of *Lentinula edodes* Le-17-04 was recorded by 90 mm in the medium adjusted with pH 5.0 and pH 6.0 at 12 DAI, with about same growth rate of 8.18 mm/day of mushroom mycelium. However, the remaining pH were more or less unfavourable to the vegetative growth of the mushroom in which 85.33 mm (7.11 mm/day), 79.33 mm (6.61 mm/day) and 51.33 mm (4.28 mm/day) growth was measured at pH 7.0, pH 4.0 and pH 8.0, respectively. Further growth was measured at all the pH to be increased by 54.54%, 66.23%, 75.33% and 75.33% at pH 4.0, pH 7.0, pH 5.0 and pH 8.0 respectively, compared with least growth as observed at the pH8.0 at 12 DAI in which 51.33 mm was recorded. Mycelial growth for pH 4.0 and pH 7.0 was appeared statistically *at par* at 3 and 6 DAI but with slight change in the trend of growth pattern of the mushroom, pH 5.0 and pH 6.0 were also had almost equally effect on the growth at 12 DAI. It was well evident from this experiment that acidic pH favours the growth of *Lentinula edodes* while, increasing pH towards alkalinity decreases mycelial growth of the mushroom.

Table 3: Effect of pH on the radial growth of *Lentinula edodes* Le-17-04.

Ph	Radial growth of *Lentinula edodes* Le-17-04 (mm) at12DAI	% increase over the check having highest radial growth at 12 DAI	Growth rate (mm/day)
pH 4.0	79.33[c]	54.54	6.61
pH 6.0	90.00[a]	75.33	7.5
pH 7.0	85.33[b]	66.23	7.11
pH 8.0(Check)	51.33[d]	-	4.28
pH 5.0	90.00[a]	75.33	8.18
CD at 5%	1.62	-	-
CV	1.12	-	-

Means with the same letters do not significantly differ by the critical difference (CD) test at P≤0.05 with a completely randomized design.

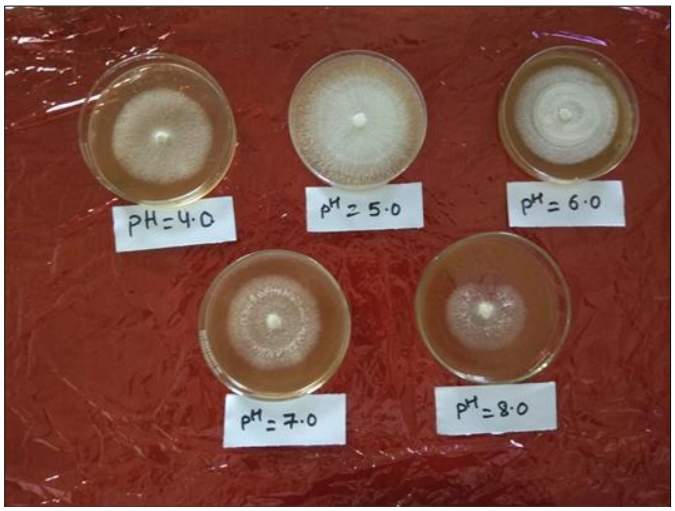

Plate 3: Radial growth of *Lentinula edodes* Le-17-04 at different pH.

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