

Effect of Culture Conditions on Radial Growth, Submerged Biomass and Moisture Content of *Lentinus Tuberregium*, A New Edible Mushroom

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ABSTRACT

The growth performance of mushroom culture *Lentinus tuberregium* was studied using both cultural and environmental factors. Development of cellular biomass was analysed in 10 different media along with effect of inoculum size, incubation period, pH and temperature. Different media showed different growth characteristics with more or less impact to the mycelium development. Among the 10 different media used, Yeast malt extract medium (YME) was found to be the best medium, however malt extract broth, glucose yeast extract peptone and potato dextrose broth media was also found to be good towards cellular biomass production. No supportive effect of inoculum size was observed on cellular growth development. Among various pH and temperature range, 6.0 and 25 °C impact best condition for the growth of mushroom culture.

Keywords: Mushroom, mycelium, chemosynthetic media, cellular biomass

INTRODUCTION

Mushrooms are a means of attraction for research studies as they are the treasure house of multifunctional biologically active compounds that have wide application in cosmetics, agricultural and pharmaceutical sectors and used extensively by the local people as food items (Kuforiji et al., 2003; Okhuoya et al., 2005; Chang, 2007). They play key role as food in our day today life as have applications in treating diseases like cancer, diabetics, immunity related problems, hyper-lipidemia, bacterial and viral infections etc (Wasser and Weis, 1999). However, cultivation of mushroom is restricted to seasonal variation along with time, labour, space and purity in normal environmental conditions and its yield is essential as per its applications in different developmental fields. Hence, its productivity must be increased to such an extent so that its bioactive compounds can be extracted in large amount. For which submerged culture cultivation is found to be best. Now a days most researchers followed submerged fermentation technique for growing mushroom mycelium in a defined medium and proved as a rapid and alternative method to obtained fungal biomass of good quality (Tang and Zhong, 2002). This technique offers

more mycelial production in shorter time period in limited space with least/ no possibility of contamination (Yang and Liao, 1998). Therefore, it is essential to optimize the fermentation conditions in order to produce biomass and a high rate of compounds with biological and pharmacological activities.

Lentinus is a genus of fungi in the family Polyporaceae, have widespread distribution especially in subtropical regions. The genus name *Lentinus* is derived from the Latin Lent, meaning "Pliable" and inus meaning "resembling". It's a wild variety of mushroom with high nutritional value which is cultivated least worldwide. As a good protein source, this mushroom can be used as alternative to meat and fish. It has very good curing activity against tuberculosis (Stamets, 2002) and found to be effective against many other pathogenic bacteria, yeast & fungi (Manjunathan and Kaviyaran, 2011).

This mushroom has been collected from wild by the local people and consume as food. In spite of importance of this mushroom, it has never been attempted to grow in large scale. However few reports are available for mycelial development and growth under

Received date: Jul 28, 2020; Accepted date: August 24, 2021; Published date: September 4, 2021

Citation: Dash S (2021) Effect of culture conditions on radial growth, submerged biomass and moisture content of *Lentinus tuberregium*, a new edible mushroom. *J Appl Microbiol Open Access*. 7:7 P492

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different nutritional conditions (Manjunathan and Kaviyarasan, 2011).

Materials and Methods

Microorganism and culture media

The stock culture of *Lentinus tuberregium* was maintained on slant and petridishes with malt agar medium incubated at 28 °C ± 2 °C and stored at 4 °C. 10 different media were used to evaluate the optimum development of cellular biomass by *Lentinus tuberregium*. The 10 different media includes: GYEP (Glucose yeast extract peptone), MCM (Mushroom complete medium), TAK (Tien and Kirk medium), YME (Yeast malt extract medium), ME (Malt extract medium), Starch medium, PK (Pikovskaya's medium), PD (Potato dextrose medium), SD (Sabouraud dextrose medium) and CZ (Czapekdox medium) were used in both solid and broth form and sterilized at 121 °C for 15 minutes. All experiments were performed in triplicates and in static condition.

Solid plates

All 10 media are prepared, sterilized at 121 °C, 15 lbs pressure, for 15 minutes. Inoculation of the seed culture was done by punching 6 mm agar disc and transferred into the prepared media plates. The plates were incubated at 28 °C for 7 and 14 days. The radial diameter was measured at 7 and 14 days of incubation.

Liquid submerged fermentation

All 10 media are prepared, sterilized at 121 °C, 15 lbs pressure, for 15 minutes. Inoculation of the seed culture was done by punching 6 mm agar disc and transferred into the prepared liquid broth media 50 ml each in 250 ml conical flasks. The flasks were incubated at 28 °C for 14 days under static condition. The growth performance was analysed and fresh and dry weight of the mycelium in each medium was recorded after 14 days of incubation period.

Inoculum size

Different inoculum size of *L. tuberregium* (6 mm, 12 mm, 18 mm, 24 mm and 30 mm) were punched out and inoculated in the prepared and sterilized YME broth medium and incubated for 14 days for biomass development. After 14 days of incubation period fresh and dry biomass was recorded.

Incubation period

Different incubation periods (5, 10, 15 and 20 days) were followed to get best mycelial growth of *L. tuberregium* in YME broth medium. Growth was analysed according to fresh and dry weight of the mycelium extracted.

PH

As pH affects growth and metabolism of mycelium, four different pH was maintained in the medium (YME) and Growth performance of *L. tuberregium* was performed at 4.0, 5.0, 6.0 and 7.0 pH. The mycelial growth was observed at 14 days of incubation period and the cellular biomass was extracted, both fresh and dry weight was recorded.

Temperature

Development of cellular biomass of *L. tuberregium* was performed at different temperature (25 °C, 30 °C, 32 °C and room temperature) in liquid medium of YME. Here also growth was analysed according to the biomass (both fresh and dry weight).

RESULTS AND DISCUSSION

Macrofungus are highly important as nutritional and medicinal purpose, hence their production in high rate is the need of the present hour. Large scale production of these mushroom can fulfill the need of the population with respect to nutraceutical and pharmaceutical fields. *L. tuberregium* grown similarly in both solid and liquid media. Measurement of radial growth of mycelial biomass has been recorded at 7 days and 14 days of growth period and % enhancement of growth was calculated (Table - 1).

Lentinus tuberregium grows more densely in YME agar and broth medium with radial diameter of 5.47±0.25 cms and 8.5±0.61cms at 7 and 14 days of incubation period along with fresh weight and dry weight of 4.40±0.40 gms and 0.33±0.07 gms at 14 days of incubation period which was the highest among all the media used (Table - 2). Growth in Czapek dox agar medium was the least however its radial diameter was measured to be 6.27±0.25 cms and 8.6±0.53 (7 and 14 days of incubation period).

It has been evident from table - 1 that % of enhancement in growth is high in starch agar medium (108%) followed Sabouraud dextrose agar and Mushroom complete agar medium (95.41% and 81.82%) respectively. The fungus did not prefer other media for biomass development than the above.

In liquid broth culture, other than YME medium, Malt extract broth, Glucose yeast extract peptone and Potato broth medium supports good growth (1.43±0.87, 1.09±0.36 and 1.03±0.65 gms of fresh weight) with negligible growth in starch broth medium (Table - 2). % of moisture content in the cellular biomass of *Lentinus tuberregium* cultured in Starch, YME, TAK and GYEP broth medium exhibited from 85.32% to 99.90%.

The impact of inoculum size on growth and development of fungal mycelium was not exhibited as fungus showed good growth in similar way irrespective of inoculum size (Table - 3).

Effect of incubation period on biomass development of this fungi exhibited in table - 4. According to the experiment performed 5 days incubation period shows the least growth with fresh biomass of 0.31±0.16 gms and 0.04±0.02 gms dry weight. Gradually increase in biomass was observed with increasing incubation period and revealed best incubation period to be 20 days (3.74±0.29 gms fresh weight and 0.36±0.05 gms dry weight).

Biomass production of *Lentinus tuberregium* was studied in different pH in YME medium. The fungus preferred 6.0 pH for better growth in YME medium at liquid culture and static condition. However, other *Lentinus* sp., *Lentinus citrinus* and

Lentinus squarrosulus preferred well in neutral and little acidic pH, respectively (Kirsch et al., 2011; Ahmad et al., 2013)

Finally, effect of temperature was evaluated which revealed that 25 °C to be the best temperature for growing culture with highest biomass yield i.e 0.57 ±0.34 gms (fresh weight) and higher temperature generally reduce the growth rate which is in accordance with Hassegawa et al., 2005.

From experiment performed, it was observed that different media, inoculum size, incubation period, pH and temperature do have specific effect on growth of mushroom mycelium. *Lentinus tuberregium* shows good growth potential in YME medium with varying growth in different inoculum size. Best pH and temperature for good cellular biomass was found to be 6.0 and 25 °C. However improved biomass may be produced by modifying the nutrient component, growth elicitors like vitamins, hormones, amino acids and other micronutrients (Manjunathan and Kaviyaran, 2011).

Under different cultural condition, moisture content was also calculated along with fungal fresh and dry biomass and presented in terms of %. It is very interesting to note that *Lentinus tuberregium* have moisture retaining capacity which is effected by nutritional media, the environmental conditions like incubation period, temperature, pH. Inoculum size did not have much impact in this regard. The fungus showed more or less similar quantity of moisture content indirectly exhibited moisture retention capacity.

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