



# PHYSICOCHEMICAL STUDIES ON *LENTINUS SQUARROSULUS*

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## Abstract

Morphological characteristics of the mycelia growth of wild strain of *Lentinus squarrosulus* collected from the region of North Tamilnadu was studied. *Lentinus squarrosulus* edible higher fungus is valued for its high nutritional composition. Among the different media tried, Mushroom Complete Agar was found to be the best. We observed the effects of temperature, pH, illumination followed by different concentration of Carbon and Nitrogen showed significant increment on biomass of mycelium with the amendment of dextrose and yeast extract. optimum growth at 25°C, pH-6.5. The fungus was obtained from dead wood and maintained on potato dextrose agar. We observed the effects of temperature, pH, illumination followed by different ratio of Carbon and Nitrogen showed significant increment on biomass of mycelium with the amendment of dextrose, yeast extract.

## Keywords:

*Lentinus squarrosulus*, radial growth, carbon, nitrogen, temperature, pH, illumination, Physicochemical.

## Introduction

*Lentinus squarrosulus* is an edible macrofungi commonly found in the study area belonging to the family Polyporaceae. The genus *Lentinus* belongs to class Agaricomycetes, family Polyporaceae and contains forty species (Kirk PM, Cannon PF, 2008). There are a total of 20 valid species of genus *Lentinus* encountered from India (Sharma SK and Atri NS, 2015).

*L. squarrosulus* is a paleotropical species showing wide distribution extending throughout equatorial Africa, Southeast Asia, the Pacific islands, and Australasia (Pegler DN, 1983). *L. squarrosulus* is a white rot saprophytic fungus. Morphologically, the basidiocarp is characterized by either whitish or greyish with notable conspicuous squamules on the surface (Njouonkou AL et.al, 2013).

The macrofungi is usually found on fallen tree trunks, old stumps, and buried or exposed roots of trees. It usually grows in caespitose clusters, consisting of three to six basidiocarps, but sometimes, a tuft of up to thirty basidiocarps may be found (Mortimer PE et.al, 2014).

*Lentinus squarrosulus* is an edible mushroom commonly found in the wild and has not been cultivated on a large scale for the production of fruit bodies. The tough fruit body is rich in proteins, sugars, lipid, amino acids, vitamin B, C, and D, and minerals. It has been reported that liquid fermentation of mushroom produces high amounts of uniform mycelial biomass as a source of bioactive compounds. (Noorlidah Abdullah et.al, 2011).

All edible fungi are seasonal; they are not available all year round. It is with this background knowledge of making the fungus available for consumers all through the year that this study underscore.

## Materials and Methods

### Mushroom Collection

*Lentinus squarrosulus* was collected from the Vandalur forest of Kancheepuram District, Tamil Nadu, India. The fungal culture was maintained through periodic transfer onto potato dextrose agar and PDB (Potato Dextrose Broth) under aseptic conditions and maintained at  $25 \pm 1^\circ\text{C}$  and pH 6.5.

### Media Used

Both solid as well as liquid media were evaluated for the vegetative growth of *L. squarrosulus*. The two solid media, used for evaluation are Potato Dextrose Agar (PDA) and Mushroom Complete Agar (MCA) were used during experimentation for making comparative observation on vegetative growth of *L. squarrosulus*. To measure the growth rate of mycelium in solid media, the diameter of mycelial colonies was measured in cm scale and the average growth rate of mycelium was calculated.

So as to measure the mycelial growth rate in various liquid media, the mycelia mat from each flask was harvested, washed and dried at  $65^\circ\text{C}$  for 24 hours. The dry weight of mycelium was recorded for two subsequent days and an average of the two was taken as the actual weight.

### Physical Requirements of Mycelial Growth

#### Temperature

The inoculated plate was placed in different temperature condition such as  $23^\circ\text{C}$  and  $32^\circ\text{C}$ . The temperature was monitored using a thermometer. All the inoculated plates (in triplicates) were incubated to allow full ramification of its mycelia.

#### pH Level

The Mushroom Complete Agar medium was adjusted to different pH levels: 5, 7 and 9 using 0.1 M NaOH and 0.1 M HCl. The culture medium with different pH levels was aseptically inoculated with fungal culture to allow the ramification of mycelia.

#### Illumination

The inoculated plates that are incubated in a normal light, partial light and dark condition. All treatments were incubated to allow the full ramification of mycelia.

### Effect of carbon compounds

The carbon source of dextrose was amended in the Mushroom Complete Agar medium having the pH of 6.5. The fungal mycelia was aseptically inoculated in the flasks including the control flask i.e. Mushroom Complete Agar medium without any carbon source. All the flasks were kept undisturbed for 28 days of incubation. After the incubation period the mycelia mat collected and recorded.

### Effect of nitrogen compounds

The nitrogen source of yeast extract was added in the Mushroom Complete Agar medium. Sterilization, inoculation and assessment of dry weight were carried out as described for the carbon sources above. Mushroom Complete Agar medium alone was used as control.

## Results and Discussion

During evaluation of media, liquid media used for evaluation of vegetative growth, significantly higher vegetative growth was recorded in Mushroom Complete Agar medium (3.9g) followed by Potato Dextrose Agar medium(3.2g). The result obtained in the present study is similar with the result of De Leon et al. (2013b) reported that the mycelial growth of both *L. squarrosulus* and *P. grammacephalus* was significantly highest in CWG with mean mycelial growth of 80 mm in diameter and with thick mycelial density on the 8th day of incubation. The same result was obtain by Dulay et al. (2012a) on *Lentinus tigrinus* wherein the mycelia grew best on coconut water gulaman (local crude agar) as solid medium.

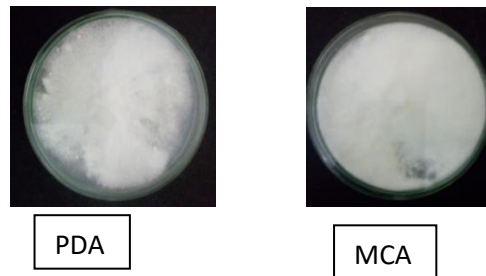


Fig.1-Mycelial growth of *Lentinus squarrosulus* on different culture media: PDA-Potato Dextrose Agar, MCA – Mushroom Complete Agar

**Table 1-** *Lentinus squarrosulus* on various nutritional and physical requirements for mycelial growth

	Dry Weight (mg)	Mycelial Density
<b>Culture media</b>		
PDB medium	3.9	+++
MCM medium	4.6	++++
<b>Temperature</b>		
23°C	2.8	+++
32°C	2.1	+++
<b>pH</b>		
5	0.8	++
7	2.7	++++
9	1.9	+++
<b>Illumination</b>		
Light	2.9	+++
Dark	2.4	++
Light and dark	3.5	+++
<b>Carbon Source(Dextrose)</b>		
High concentration	2.9	+++
Low concentration	3.3	++++
<b>Nitrogen Source (Yeast extract)</b>		
High concentration	2.8	+++
Low concentration	3.1	++

Note: Mycelial density were evaluated as (+) very thin, (++) thin, (+++) thick, (++++) very thick and (-) no growth

The result obtained in this study revealed that the best radial mycelia extension (4.1 cm) was observed at 23°C (air-conditioned) among the temperature tested (Table 1). This is the optimum temperature for the growth of *L.squarrolus*. There was considerable growth at 32°C (room temperature), respectively. Temperature is found to be an important environmental factor that controls the growth of most microorganisms.

The optimum pH of growth of *L.squarrolus* was found to be 7 where the highest vegetative growth was observed (3.2 g). The growth of (2.8 g), which was the second best, was recorded in the mushroom complete medium of 9 while the least growth (1.8 g) was observed in the acidic medium of 5 (Table 1).

Most cultivated fungi are exposed to alternating periods of daylight and darkness (Chang and Miles 2004). The influence of light conditions on mycelial growth of *Lentinus squarrosulus* is shown in (Table 1). Among the three light conditions, cultures exposed to alternating light and dark condition significantly recorded the largest mycelial growth (3.5 g) and thick mycelial density while those exposed to lighted condition registered significantly smallest mycelial growth (2.9 g) and very thin mycelial growth after 28 days of incubation. Chang and Miles (2004) reported that the growth of most fungi is not sensitive to light.

Positive response to darkness of this mushroom is comparable to the other basidiomycetes like *V. volvacea* (Reyes et al. 1998), *C. comatus* (Lopez et al. 2009), *Agaricus blazei* (Galangam et al. 2009) and *Lentinus tigrinus* (Dulay et al. 2012a). However, this results contradicts the report of Dulay et al. (2012) on basidiospore germination of *Lentinus tigrinus*.

This shows that *L.squarrosulus* produces enzymes that utilize dextrose better than any other carbon source. Ofofu-Asiedu A.O et al also reported that *Volvariella volvacea* utilizes glucose and starch better than other carbon sources. Kadiri et al obtained more growth of *V. volvacea* with glucose than starch. Luo et al also reported that fructose, glucose and maltose were the most suitable carbon sources for *Auricularia auricular*. Kadiri et al reported that the best utilizable carbon sources for *Lentinus subnudus* were fructose, maltose, dextrin and glucose. This study showed that *L.squarrosulus* utilizes dextrose better in lower concentration and glucose has been good respiratory substrate. *L.squarrosulus* utilizes nitrogen better and found to be considerable mycelia growth. This result may provide a sustainable means of adding value to *L.squarrosulus* cultivation which will result in increasing human protein.

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