

Study on Mycelial Growth Pattern of Five Wild *Lentinus* Species

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Abstract: In the present paper five wild fungal species namely *Lentinus sajor - caju*, *Lentinus connatus*, *Lentinus torulosus*, *Lentinus cladopus*, *Lentinus squarrosulus* have been collected, identified and isolated from different regions of North West India to study the behavior of culture on solid medium (Potato Dextrose Agar). On the daily basis observations, all species exhibited different growth characteristics with respect to their growth rate, odor and colour. It was observed that among the five cultures the fastest average growth was observed in *Lentinus squarrosulus* (1.8 cm/day) followed by *Lentinus cladopus* (1.5 cm/day), *L. sajor – caju* (1.3 cm/day) and *L. connatus* (0.8 cm/day). Whereas, the slowest for the same was recorded for *L. torulosus* (0.5 cm/day). It was also observed that at initial stages the color of mycelium in all the species was found white but towards maturity it changed according to the species. In *L. sajor – caju* the color changed from white to light brown and then to yellowish brown. In *L. connatus* the color changed from white to blackish brown, while in *L. torulosus* the color remained white throughout maturity. In *L. cladopus* white colour changed to blackish brown. In *L. squarrosulus* the pattern of change in colour occurred in the form of appearance of light brown patch on the mycelium later becoming dark brown. Among the cultures no specific odor was smelled. Mycelial mat was observed to thin at initial stages but later on changing to thick. In all the species the hyphal construction was found to be dimitic having generative hyphae with prominent clamps and skeletal hyphae with thick walls.

Key words: Wild species • *Lentinus* • Mycelial Growth

INTRODUCTION

Mushrooms are the valuable source of food used by the human being from ancient time. Wild edible and medicinal properties of mushrooms were known to many ancient civilizations. Some mushrooms have been reported as therapeutic foods, useful in preventing diseases such as hypertension, hypercholesterolemia, atherosclerosis and cancer [1-4]. These functional characteristics are mainly due to their chemical composition [5]. Genus *Lentinus* belongs to the family Polyporaceae and order Polyporales. Forty species of this genus has been reported worldwide [6]. All the studied species are edible and important because of their culinary credentials. Most of the edible mushrooms belong to Ascomycotina and Basidiomycotina [7]. Some mushrooms, such as truffles and morels are Ascomycetes, but most of others are Basidiomycetes [8]. Study of mycelial

behavior is also important in studying the life cycle and other cultivation aspects of medicinally important mushrooms. There are several reports on such kind of work on morels has led to understand the cultivation aspects of these important mushrooms [9-16] which play a direct role in understanding the life cycle of these [17-19]. It becomes quite important to study the culture behavior of mushrooms as it is directly linked with the cultivation as well as pharmaceutical aspects. Present paper deals with the study of the mycelial behavior on the Potato Dextrose Agar medium so as to notice the different phases during the growth of mycelium.

MATERIALS AND METHODS

Collection: All the species were collected from the different localities of North West India varying in altitude and host (Table 1 and Figure 1).

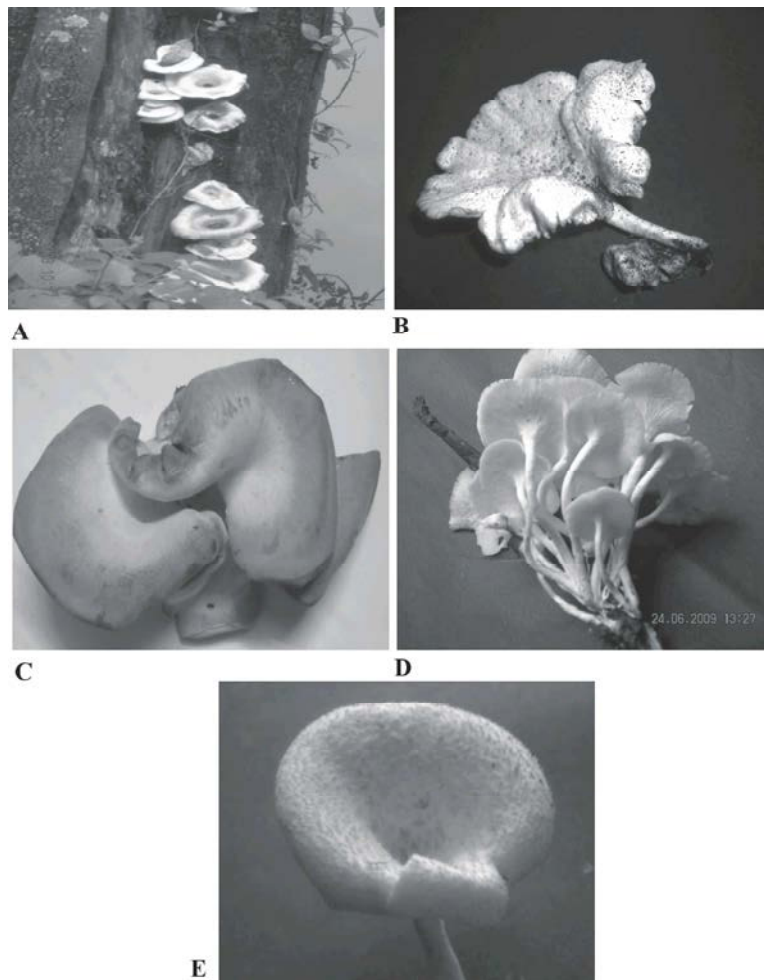


Fig. 1: A. *Lentinus sajor – caju* B. *Lentinus connatus*. C. *Lentinus torulosus* D. *Lentinus cladopus* and E. *Lentinus squarrosulus*.

Table 1: Showing associated natural host and location with altitude and forest type

| Species | Host | Location | Altitude (m) | Type of forest |
|------------------------------|---------------------------|------------------|--------------|----------------|
| <i>Lentinus sajor-caju</i> | <i>Bauhinia variegata</i> | Sirmour (H.P) | 672 | Mixed |
| <i>Lentinus connatus</i> | <i>Mangifera indica</i> | Chandigarh (Pb.) | 200 | Plains |
| <i>Lentinus torulosus</i> | <i>Pinus roxburghii</i> | Palampur (H.P) | 850 | Pine forest |
| <i>Lentinus cladopus</i> | <i>Albizia chinensis</i> | Palampur (H.P) | 1200 | Mixed |
| <i>Lentinus squarrosulus</i> | <i>Albizia chinensis</i> | Palampur (H.P) | 1200 | Mixed |

Isolation: Pure culture of collected wild species was raised from the pileus portion of the mushroom where the lamellae join the stipe. For raising the culture a small piece of mushroom tissue was cut and removed with the help of sterilized scalpel under aseptic conditions and subsequently sterilized by dipping in 0.02% mercuric chloride solution and inoculated into the pre-prepared sterilized PDA slants. The inoculated PDA slants were stoppered with the cotton plugs and incubated at $27\pm 1^\circ\text{C}$. The total operation of culturing was done aseptically under laminar flow. After 3 to 4 days (depending upon species), white mycelium started

emerging and spreading on the PDA slants. Purification of the culture for further maintenance and utilization in experiments was done through repeated sub-culturing. Then the equal bits of the mycelium were put in petriplate and the growth was measured on daily basis in triplicates.

Observations: Observations were made on daily basis till the maturity of the culture. Observations related to mycelial growth rate, color, odor and hyphal constructions were taken from day one to maturity of the culture in petriplates.

RESULTS AND DISCUSSION

On the basis of observations, in case of *Lentinus sajor – caju* at the initial stages of the growth the growing margins of the mycelium were found to be irregular, but the overall growth was in concentric manner. The mycelium of this species grew @ 1.3 cm/day on an average basis. In *Lentinus connatus* at the initial stages mycelium growth was quite slow. After two days of inoculation of the tissue in petriplate containing Potato Dextrose Agar (solid Medium), the mycelium grew @ 0.8 cm on an average daily basis, but later on the mycelium grew at faster rate. *Lentinus torulosus* exhibited a unique growth pattern. It showed a slow growing nature as compared to other *Lentinus* species studied. The mycelium of this species has been recorded to grow @ 0.5 cm on an average daily basis with colony showing irregular margin all around. On the sixth day of inoculation of this species mycelium at the center becomes thick and forms small protuberance. As the fungus grows, these buds also grow in size. Usually 3-4 protuberances emerge from one point. On the ninth day the protuberance reaches up to 5 cm in length. Normally out of 3 - 4 protuberances only one reaches up to 5 cm in length and rest are abortive. Overall the mat appears thin with smooth concentric aggregation of hyphae except in the center where the mat is thick and protuberances are formed. Mycelium of this fungus grew at fast rate. Growth of mycelium started on the second day of inoculation of the tissue in petriplate containing Potato Dextrose Agar (solid Medium) and it reached up to 1 cm in diameter on third day. On the fourth day of inoculation the diameter of mycelium reached up to 2 cm the average growth of the mycelium was smooth with irregular margins and concentric orientations. As culture grew older it became denser and its diameter also increased. On the seventh day the colony attained 7 cm diameter. It took nine days for mycelium to cover the entire petriplate. The mycelium was recorded growing @ 1.5 cm on an average daily basis. *L. squarrosulus* growth of mycelium started on the second day of inoculation of the tissue in petriplate contained Potato Dextrose Agar (Solid Medium). To begin

with mycelium color white and grows in concentric manner with irregular margins forming a thin mat. On overall basis the mycelium was observed growing @ 1.8 cm/day on an average daily basis. The growth rate of mycelium depends upon the species [20].

A considerable color change was observed in mycelium in *Lentinus sajor - caju* during 7th - 8th day of growth the colour gradually changes from white to light brown and then to yellowish brown. The change in color was not in a uniform manner, but it occurred in patches and by 11th day of incubation almost entire mycelial mat became yellowish brown scattered whitish patches. In *Lentinus connatus*, initially the color of the mycelium was white but with the passage of time and maturity the color changed from white to blackish brown and at the 6th in the form of patches from center. At the 7th to 8th day patches of thin and thick mycelium were clearly visible on petriplates. The change was not in a uniform manner but it appeared in the form of patches with maturity. Such colour change and patches became apparent after 6th day of inoculation and by 8th day these changes became more prominent. In *Lentinus torulosus* mycelium remain white throughout growth period. In *Lentinus cladopus* initially the color of the mycelium was white which became blackish brown toward the end of 5th day of inoculation. This blackish brown patch increased in size uniformly from center to the periphery. At maturity entire white mycelium changed into blackish brown colour. In *Lentinus squarrosulus* on the sixth day of inoculation noticeable change in color occurred in the form of appearance of light brown patch on the mycelium. This patch extended in a uniform manner from center towards the periphery. As culture grows older its diameter also increases. On the 8th day of inoculation the dark brown patch covers the entire petriplate. No specific odor was smelled from the mycelium of all the species during the entire growth stage. Hyphal construction was found to be dimittic with clamped generative hyphae and thick walled skeletal hyphae in all the species. The mycelial growth rate was found more than some morels [21] (Table 2 and Figure 2).

Table 2: Culture characteristics of five wild *Lentinus* species from North West India

| Species | Growth Rate(cm/day) | Color | Odor |
|------------------------------|---------------------|--|--------------|
| <i>Lentinus sajor - caju</i> | 1.3 | White to light brown and then to yellowish brown | No -specific |
| <i>Lentinus conatus</i> | 0.8 | White to blackish brown | No -specific |
| <i>Lentinus torulosus</i> | 0.5 | White throughout | No -specific |
| <i>Lentinus cladopus</i> | 1.5 | White which become blackish brown | No -specific |
| <i>Lentinus squarrosulus</i> | 1.8 | White to Light brown and later becoming Dark brown | No -specific |

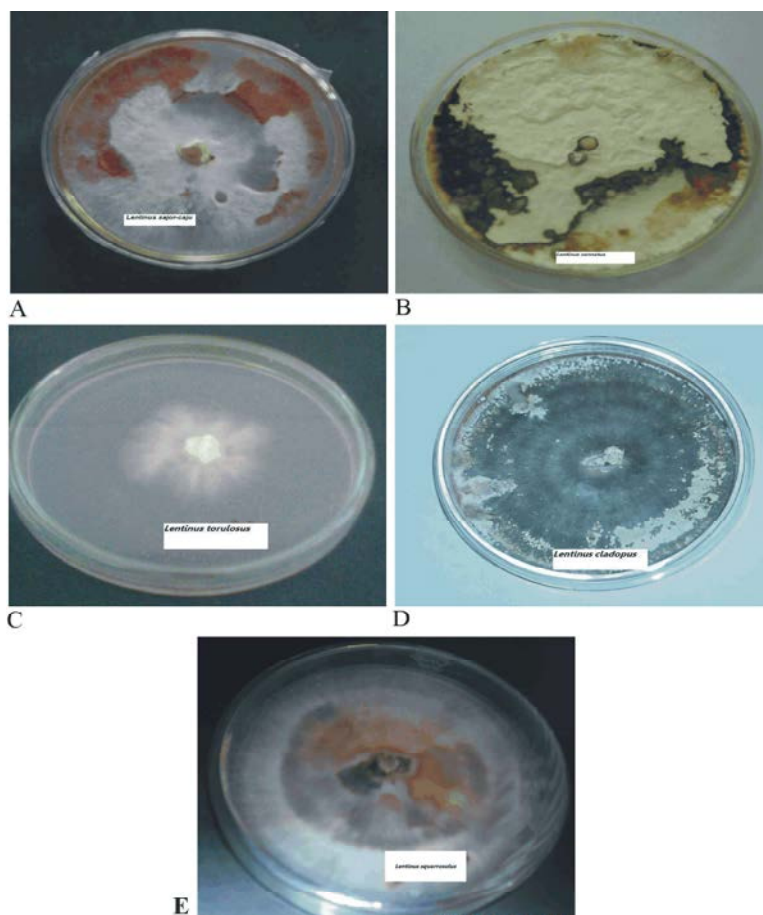


Fig. 2: A Mature culture of *Lentinus sajor – caju* B. Mature culture of *Lentinus connatus* C. Five day's old culture of *Lentinus torulosus* D. Mature culture of *Lentinus cladopus* E. Mature culture of *Lentinus squarrosulus*

CONCLUSION

Among all the species the growth rate varied from 0.5 to 1.8 cm/day. A considerable change in the color was also noticed which was varied according to the species. It is quite evident from the results that species differ in their growth pattern in spite of the same conditions.

ACKNOWLEDGEMENTS

Thanks are due to Head Department of Botany, Punjabi University Patiala for providing laboratory facilities and to U.G.C. for grant under ASIST and DRS (SAP III) Programs.

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