

*Full Length Research Paper*

# **Growth of mycelium of three ectomycorrhizal macrofungi, *Infundibulicybe geotropa*, *Tricholoma anatolicum* and *Lactarius deliciosus* in culture media containing various carbon sources**

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**A comparative evaluation was conducted to assess the effects of different carbon sources on the mycelial growth of three species of ectomycorrhizal macrofungi; *Infundibulicybe geotropa* (Bull.) Harmaja, *Tricholoma anatolicum* H.H. Doğan & Intini and *Lactarius deliciosus* (L.) Gray. All carbon sources were found to be equally beneficial for mycelial growth. However, sucrose, glucose and arabinose were found to be better carbon source in solid culture for *L. deliciosus*, *T. anatolicum* and *I. geotropa* as the maximum biomass values in the liquid medium at the end of 56 days incubation period and were measured as 66.0, 43.0 and 97.0 mg, respectively.**

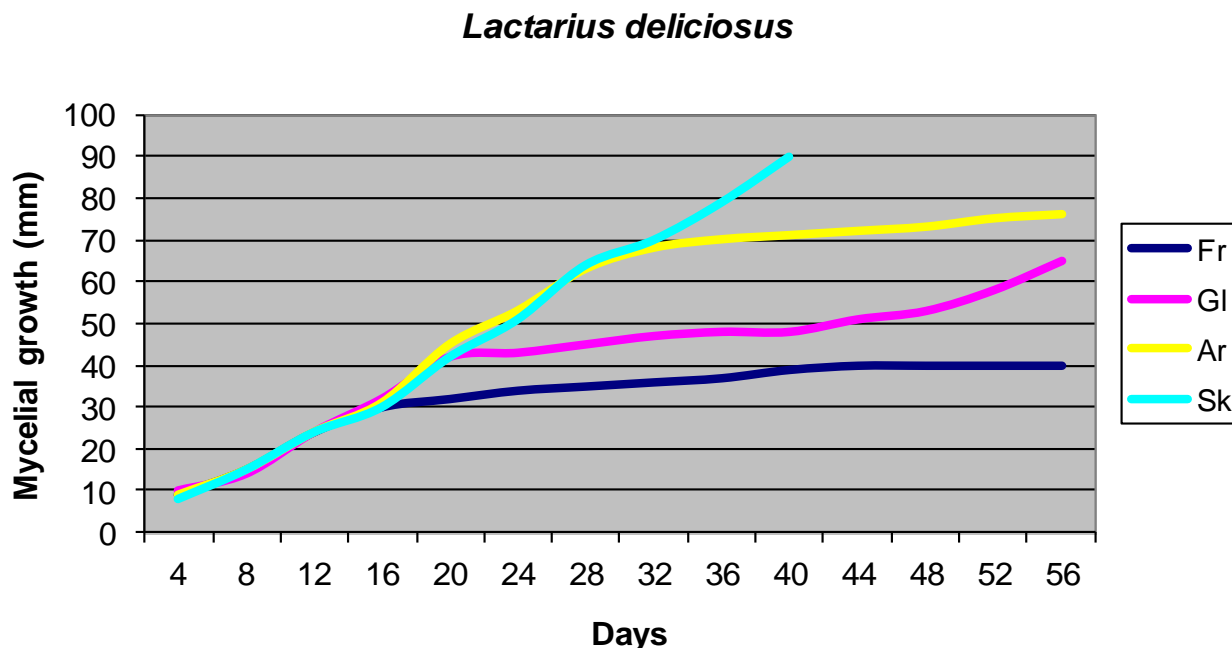
**Key words:** Carbon sources, *Infundibulicybe geotropa*, *Tricholoma anatolicum*, *Lactarius deliciosus*.

## **INTRODUCTION**

The predominant form of macrofungi used for the most experimental studies carried out in the laboratory is the mycelium. The fungal mycelium, a vegetative form of macrofungi, plays crucial role in many industrial processes, such as the production of enzymes, vitamins, pigments and lipids (Zhou et al., 2001; Wessenberg et al., 2003). Mineral and carbon nutrients available in both solid and liquid culture media can be transported to the points supporting growth of the mycelial margin, as the carbon resource acts as the hub for extension of growth (Meskauskas et al., 2004). Growth may also be defined as the orderly increase in mycelium length leading to an increase in biomass. Nevertheless most of our knowledge on interactions between fungal mycelium and substrates is based on studies performed in the laboratory

*Infundibulicybe geotropa*, *Tricholoma anatolicum* and *Lactarius deliciosus* are ectomycorrhizal, non-cultivated edible macrofungi and collected from natural habitats as a food source. All three ectomycorrhizal mushrooms selected for the present study are very popular and highly priced food in western region of Turkey, due to the unique flavor and taste (Solak et al., 2007). Since the mycorrhizal macrofungi are reluctant to grow on micro-biological media due to special nutritional requirement, commercial production of these mushrooms at artificial culture medium has not been established up to now unfortunately. As the environmental and cultural factors such as water availability, temperature and pH and their interactions have significant impacts on basidiomycete colonisation and fruiting potential, many studies have been performed to determine the optimal growth conditions and nutritional requirements of different fungi (Buscot, 1992; Ohta, 1994; Sanchez et al., 2001; Jonathan and Fasidi, 2001; Kalmis and Kalyoncu, 2008).

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**Figure 1.** Mean radial growth (diameter, mm) of *Lactarius deliciosus* cultures, in the presence of different carbon sources.

The aim of this paper is to get knowledge on mycelia growth in media containing various carbon sources of the three mycorrhizal macrofungi spp. The experiment has thus evolved in two directions; firstly, mycelial growth in solid media containing various carbon sources was measured, biomass production in liquid culture determined on dry weight basis was also determined.

## MATERIALS AND METHODS

### Strain and media

The mycelia were isolated from the fruiting bodies of *I. geotropa*, *T. anatolicum* and *L. deliciosus* which were collected from south-west Anatolia, Turkey. Several pieces of tissue were transferred into sterile Petri dishes containing approximately 10 ml of modified liquid Hagem's medium; 4 g malt extract, 1 g yeast extract, 5 g D-Glucose, 0.5 g NH<sub>4</sub>Cl, 0.5 g KH<sub>2</sub>PO<sub>4</sub>, 0.5 g MgSO<sub>4</sub> · 7H<sub>2</sub>O, 0.5 ml FeCl<sub>3</sub> (1% aqueous solution), 100 ml biotin (50 mg/ml aqueous solution), 100 ml thiamine (1 mg/ml aqueous solution) in 1 L dd H<sub>2</sub>O (Gregory and Matthew, 2002). All cultures were incubated in the dark at 25°C. Mycelia obtained from the tissue were transferred on Hagem's medium and maintained in this medium at +4°C.

### Measurement of radial extension

Agar plugs 6 mm in diameter were taken from colonies growing vigorously on Hagem's medium and they were transferred to the center of Petri dishes containing the same media containing 5 g D-glucose, 5 g arabinose, 5 g sucrose and 5 g fructose, respectively. Five replicates for each experiment were prepared and incubated at 25 ± 1°C for 56 days. The radial extension of the mycelium was measured with a caliper gauge along two diameters positioned at

right angles to one another, and the average values were calculated for each plate. The five measurements were then averaged to obtain a mean radial growth per Petri dish (Kalmis and Kalyoncu, 2008).

### Determination of biomass in liquid culture

Inocula consisted of two 6 mm agar plugs of three week-old cultures grown on Hagem's medium at 25 ± 1°C. Inocula were transferred to 250 ml Erlenmeyer flasks containing 50 ml of Hagem's media, which were kept in static culture in an incubator at 25 ± 1°C for at 56 days. During the incubation period, flasks containing the static cultures were shaken gently once a day to avoid fungal mat formation. The dry mass of the mycelia representing the biomass of the samples, which were produced in liquid media was determined at 56th days of incubation. The mycelia were harvested from the cultivation liquid by filtration through filter paper and washed with distilled water, before being dried at 105°C for 1 day.

### Statistical analysis

All statistical analyses were performed using Systat 11 (SPSS Inc., Chicago, IL, USA) on a PC running on Windows XP.

## RESULTS AND DISCUSSION

The mycelium growth of the three mycorrhizal mushroom species obtained in solid culture medium with different carbon sources is shown in Figures 1, 2, and 3. All of the carbon sources used, with the exception of sucrose, mycelium at the beginning of incubation; the mycelium significantly increased radial growth of *L. deliciosus*

### *Tricholoma anatolicum*

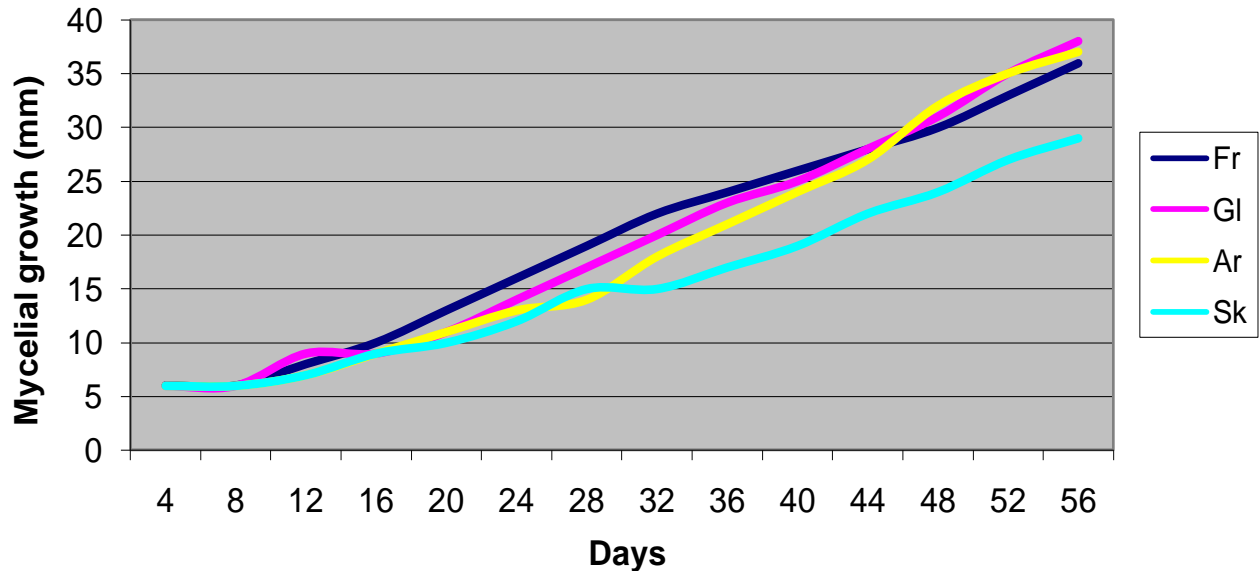


Figure 2. Mean radial growth (diameter, mm) of *Tricholoma anatolicum* cultures, in the presence of different carbon sources.

### *Infundibulicybe geotropa*

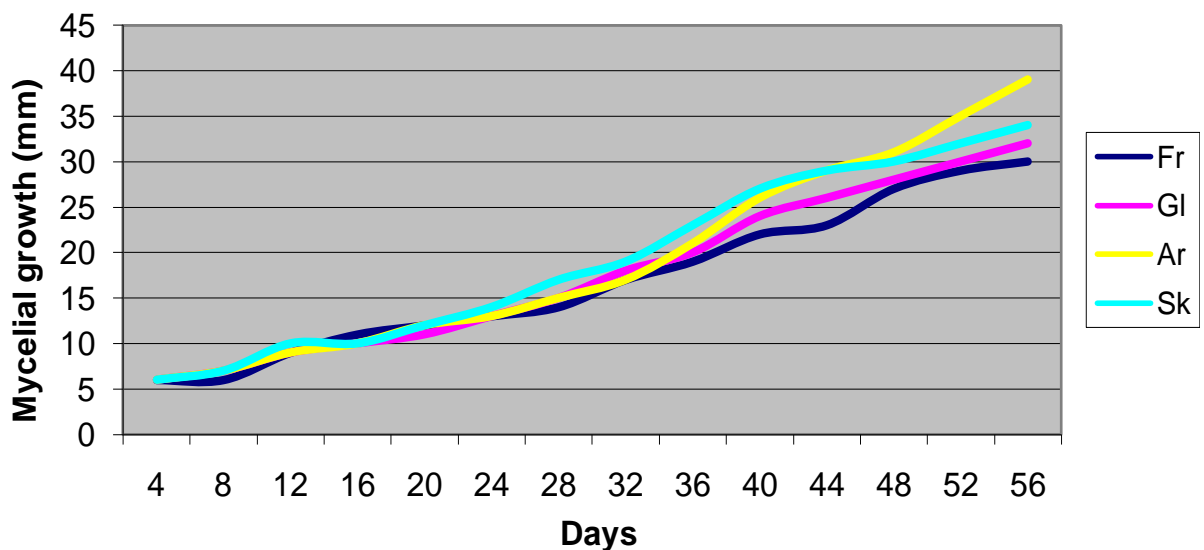


Figure 3. Mean radial growth (diameter, mm) of *I. geotropa* cultures, in the presence of different carbon sources.

growth of *L. deliciosus* in the presence of sucrose significantly increased on the 40th day of incubation. In the presence of arabinose, the mycelium biomass increased significantly until the 28th day, and the increment was negligible until the 56th day of incubation. On

the other hand, mycelium growth rate of this organism in Hagem's medium with glucose and fructose was high during the first 16 days of incubation. After the 44th day of incubation in the medium with fructose, mycelia growth rate was considerably high, as the 40 mm diameter

**Table 1.** Biomass production of organisms obtained from liquid culture (dry weight, mg).

Nutrient	<i>L. deliciosus</i>	<i>T. atanicum</i>	<i>I. geotropa</i>
Fructose	48.0	20.0	97.0
Glucose	66.0	43.0	76.0
Arabinose	54.0	23.0	75.0
Sucrose	21.0	24.0	85.0

measured at the end of incubation. Much higher mycelium growth was observed in solid culture medium containing sucrose, as the mycelia covered the agar plate within the 40 days of incubation.

There were no significant differences in the mean growth rate of the *T. atanicum* mycelium in the media with different carbon sources (Figure 2). The mean growth rate of the mycelium in the media containing different carbon sources was noted and found that the mycelium reached to 36, 37 and 38 mm diameter in the samples containing fructose, arabinose and glucose respectively on the 52th day of incubation. It was interesting to measure 29 mm diameter in sucrose containing medium for this organism in 54 days, moreover mycelium growth has been stopped in this medium between 28th and 32nd days of incubation.

There were no significant differences in the mean growth rates of *I. geotropa* and *T. atanicum* mycelia (Figure 3). The mycelium growth rate of *I. geotropa* in the presence of arabinose, sucrose and glucose was low throughout the 56 days of incubation. Mycelium of this organism reached to 39, 34, 32 and 30 mm diameters in arabinose, sucrose, glucose and fructose containing media, respectively at the end of the incubation; whereas mycelium growth stopped in sucrose containing medium between 9 and 15 days. Same pattern of weak growth was also observed in fructose containing medium.

As shown in Table 1, the biomasses of the organisms studied were significantly different in liquid and solid media. While the highest biomass production was measured in all of the carbon sources used for *I. geotropa*, the lowest biomass yield was recorded in all of the corresponding *T. atanicum* samples. On the contrary, the lowest biomass production by *L. deliciosus* was determined in sucrose containing solid Hagem's medium. Glucose was found to be the best carbon source for mycelium growth of *T. atanicum* in liquid and solid culture samples.

Whereas glucose was the best carbon source for biomass production of *L. deliciosus*, the same carbon source weakly retarded the mycelium growth of *I. geotropa* in liquid culture. Biomass of *T. atanicum* obtained from fructose containing medium was the lowest of all of carbon sources tested. Constant mycelium growth of *T. atanicum* was observed however, in fructose containing solid medium.

It has been reported that as glucose is readily metabolized to produce cellular energy, its utilization may be easy (Morrison and Boyd, 1992; Jonathan and Fasidi, 2001). Glucose was found to be the best sugar for mycelium growth of *T. atanicum* in solid culture and it was also the best carbon source for *L. deliciosus* and *T. atanicum* in liquid media. Submerged cultivation is a promising alternative for the efficient production of mycelia and metabolites (Xiao et al., 2006). Biomass obtained from liquid culture for *I. geotropa* was the highest with its approximately 1.5 to 1.7 g/L<sup>-1</sup> value, which was higher than other two strains.

## Conclusion

In conclusion, the literature data and the results obtained in this experimental study show that the effect of carbon sources on mycelium growth depends on the fungal strain. Comparison of the growth of strains in solid media showed the presence of significant differences and erratic growth patterns between them. For example, while the best carbon source was glucose for biomass production of *L. deliciosus* on the solid medium, fructose was the best carbon source for biomass production of *I. geotropa* at the same conditions. The future of metabolic engineering and physiological researches of fungi is brilliant, but apparently researchers working on mycorrhizal mushrooms will need more basic information on their requirements.

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