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The Effects of Some Environmental Parameters on Mycelial Growth of Six *Morchella* species

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A comparative evaluation was conducted to assess the effects of some environmental parameters such as pH, type of carbon source, salinity and temperature on the mycelial growth of six species of an ectomycorrhizal fungus, *Morchella* spp. (*M. costata*, *M. elata*, *M. esculenta*, *M. hortensis*, *M.intermedia* and *M. rotunda*) collected from different parts of Turkey. All carbon sources were found to be beneficial for mycelial growth. However, glucose, sucrose, maltose and fructose were better sources of carbon for some morels. Maximum mycelial growth in Petri dishes was achieved at 20 and 25^{0}C after three weeks incubation for all *Morchella* species. Growth was reduced significantly below 15^{0}C and above 30^{0}C. Different pH and salinity (NaCI) levels markedly affected the mycelial growth of the fungi.

Key words: Ectomycorrhiza, Environmental parameters, *Morchella* spp., Mycelial growth.

About 14,000 species of mushrooms are now known in the world. Recently, macrofungi have become attractive as functional foods and a source of physiologically beneficial medicine. Among the most desirable edible wild macrofungi, morels are well known around the world[1].

Morels are high gastronomic quality mushrooms distinguished by their textured cap, hollow body and earthy flavor. Medicinal applications have also been described, for example methanolic extracts from mycelia showed antioxidant activity and high scavenging effects on radicals[2]. Many researchers have been investigated into growing them in the liquid media and on solid substrates[3,4]. Despite many attempts, the commercially cultivation of morels has not been exactly possible up to now[5].

During the last two decades, commercially important wild mushrooms, including chanterelles, morels, matsutakes and truffles have been harvested from forests around the world. Nowadays morels are very important non-wood forest product for peasant who is living

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the nearby forest area. *Morchella* spp. appears from the late April until the end of May is widely distributed in the temperate zones of the world. In general, ascocarps of morels occur in a variety of habitats including riverbanks, mountain slopes, pastures, burned-out forests and near plants that have been injured. They emerge in sand, moist soil with abundant organic matter and in mud[6]. Ectomycorrhizal fungi are physiologically capable of absorbing amino acids and small peptides from the soil due to the presence of specific transporter proteins in the plasma membrane[7]. Many studies have determined the optimal growth conditions and nutritional requirements of different fungi[8,9]. The mycelial growth of some ectomycorrhizal fungi has been shown to decline when potassium was limiting but increase when phosphorus was limiting[10,11]. It has been also reported that *Cantharellus cibarius* grew best in well-drained forest soils with low nitrogen content and a pH range of 4.0 -5.5[12].

The aim of this study is firstly to investigate the effects of various environmental conditions such as pH, type of carbon source, salinity and temperature on mycelial growth of six *Morchella* species and secondly to determine optimum growth conditions *in vitro* for these organisms.

MATERIAL AND METHODS

Culture conditions and storage

Fruit bodies of six species of morels (*M. costata* MCC27, *M. elata* MCC01, *M. esculenta* MCC36, *M. hortensis* MCC04, *M.intermedia* MCC30 and *M. rotunda* MCC58) were collected from different regions of Anatolia, Turkey in spring 2006 – 2008. After the completion of the macroscopic examinations both on site and in the laboratory, all fruiting bodies were cleaned in wet paper with sterile water, and several pieces of tissue obtained from the ascocarp of morels were transferred to sterile Petri dishes containing approximately 20 ml of modified Hagem's medium: 4.0 g malt extract, 1.0 g yeast extract, 5.0 g D-glucose, 0.5 g NH_4Cl, 0.5 g KH_2PO_4, 0.5 g $MgSO_4.7H_2O$, 0.5 ml $FeCl_3$ (1% aqueous solution), 100 ml biotin (50 mg / ml aqueous solution), 100 ml thiamine (1 mg / ml aqueous solution) in 1 L dd H_2O[13]. All cultures were incubated in the dark at 25.0°C. Mycelia obtained from tissue were transferred on Potato Dextrose Agar (PDA) and maintained in this medium +4.0°C. All strains were deposited in the Mushroom Culture Collection (MCC) of the Department of Bioengineering, Ege University, Turkey.

Determination of the effects of various environmental conditions

Carbon sources

Fructose, glucose, galactose, sucrose, maltose, lactose, xylose and arabinose were selected as carbon sources and added at a concentration of 1% to Kirk's Basal Medium (KBM)[9]. Carbon sources were filter sterilized separately and concentrated to prevent possible heat damage.

Temperature and pH

The effects of different temperatures (15.0, 20.0, 25.0, 30.0 and 35.0°C) and different pH (5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) on fungal growth were assessed. Media pHs were regulated by using either highly diluted HCl or NaOH[14].

Salinity

KBM amended with different concentrations of NaCI (0.5%, 1.0%, 2.0% and 5.0%) was prepared and autoclaving for 15 min at 121°C for salinity studies[15].

Cultures of morels used in this study firstly were grown on KBM for carbon sources, pH, temperature and salinity experiments. Agar plugs 6.0 mm in diameter were taken from actively growing colonies on KBM and transferred to the center of Petri dishes. Five replicates were prepared for all experiments and incubated for 30 days. The radial extension of the mycelium was measured daily as described by Weitz et al.[16] with a caliper gauge along two diams at right angles to one another and the average for each plate calculated.

Statistical analysis

The data presented are the averages of the results of five replicates with a standard error of less than 5%.

RESULTS AND DISCUSSION

Ectomycorrhizal fungi exploit carbon and other essential organic substances from their host tree and in return assist the host tree absorb

mineral salts, water and metabolites. In this comparative study, six different species of *Morchella* were examined for their mycelial growth in various culture conditions, pH, temperature, salinity and carbon sources.

Effects of carbon sources on mycelial growth

As shown in Table 1, all carbon sources tested in this study were found to beneficial while fructose was the best carbon source for *M. rotunda*, glucose was the best for *M. elata* and *M. esculenta*, maltose was the best for *M. costata* and *M. intermedia* and sucrose was the best for *M. hortensis*. As different as our study, in Winder[17], the two isolates of *M. elata* (DE4 and JB4) growth in sucrose medium faster than glucose medium. Several studies have reported a considerable difference in the utilization of mannose and cellobiose among *Suillus luteus* and *S. grevillei*[18,19]. It was also reported that five different species of ectomycorrhizal mushrooms were unable to utilize both mannose and cellobiose[20]. Bae et al.[21] have reported that maltose for the best carbon source for *Paecilomyces japonica*. Similarly, Shim et al.[22] reported that maltose was the best carbon source known for stimulating mycelial growth of another higher fungus, *Sparassis crispa*. Mao et al.[23] found to be the cells of *Cordyceps militaris* grew best in galactose medium.

Temperature effects

In the temperature experiment, strains *M. esculenta*, *M. elata* and *M. intermedia* were reached maximal growth rate at 20°C while *M. costata*, *M. hortensis* and *M. rotunda* were achieved maximal growth rate at 25°C (Table 2). In a previous study, Winder[17] reported that an

Table 1. Effect of different carbon sources on mycelial growth for *Morchella* species

	Mycelial Growth (mm)					
Carbon Source	MC (12)	ME (14)	MES (14)	MH (16)	MI (15)	MR (12)
Arabinose	60 ± 2.36	65 ± 2.30	78 ± 1.65	75 ± 2.10	70 ± 2.45	65 ± 2.85
Fructose	72 ± 1.95	84 ± 1.25	82 ± 0.25	83 ± 1.60	72 ± 1.20	90 ± 0.21
Galactose	68 ± 2.14	68 ± 2.00	80 ± 1.64	76 ± 2.32	71 ± 2.58	74 ± 2.24
Glucose	85 ± 1.05	90 ± 0.95	90 ± 0.30	81 ± 1.47	83 ± 1.84	78 ± 1.62
Lactose	65 ± 1.64	70 ± 1.55	84 ± 0.58	72 ± 1.25	66 ± 2.55	70 ± 1.15
Maltose	90 ± 1.68	70 ± 1.80	83 ± 0.90	75 ± 1.65	90 ± 0.90	75 ± 1.40
Sucrose	76 ± 0.58	83 ± 1.48	80 ± 1.45	90 ± 1.20	78 ± 2.41	80 ± 1.75
Xylose	63 ± 2.48	60 ± 2.50	74 ± 2.56	70 ± 2.35	55 ± 1.85	63 ± 3.20
NC	32 ± 2.05	35 ± 2.39	41 ± 3.20	30 ± 2.56	39 ± 2.60	39 ± 2.60

MC: *Morchella costata*; ME: *M. elata*; MES: *M. esculenta*; MH: *M. hortensis*; MI: *M. intermedia*; MR: *M. rotunda*; NC: Carbon free; Numbers between parentheses show the day when the maximum growth was measured; Best growth has been indicated with the bold characters in table.

Table 2. Effect of different temperature on mycelial growth for *Morchella* species

	Mycelial Growth (mm)					
Temperature	MC (17)	ME (16)	MES (14)	MH (18)	MI (16)	MR (15)
15.0^{0}C	52 ± 2.48	76 ± 2.10	64 ± 2.60	68 ± 2.50	71 ± 2.47	63 ± 2.10
20.0^{0}C	81 ± 1.58	90 ± 1.54	90 ± 0.85	82 ± 1.68	90 ± 1.85	80 ± 1.80
25.0^{0}C	90 ± 1.36	75 ± 2.95	79 ± 2.45	90 ± 1.06	77 ± 2.35	90 ± 1.23
30.0^{0}C	72 ± 2.15	65 ± 3.65	62 ± 2.90	73 ± 2.26	63 ± 2.41	70 ± 2.65
35.0^{0}C	26 ± 3.20	32 ± 3.25	35 ± 2.58	30 ± 2.75	28 ± 2.60	30 ± 2.40

MC: *Morchella costata*; ME: *M. elata*; MES: *M. esculenta*; MH: *M. hortensis*; MI: *M. intermedia*; MR: *M. rotunda*; Numbers between parentheses show the day when the maximum growth was measured; Best growth has been indicated with the bold characters in table.

isolate of *M. elata* (JB4) reached a maximal growth rate at 16°C, while another isolate (DE4) achieved maximal growth rate at 20°C, agree with the present study. Kim *et al*.[24] obtained an optimal temperature of 25°C for mycelial growth of *Paecilomyces sinclairii*. Bae et al.[21] have reported that temperature for mycelial growth of *P. japonica*, which is another fungus of the ascomycetes, was found to be 25°C.

pH effects

The effect of pH on mycelial growth of six morels was determined at six different pH levels. All *Morchella* species grew over the pH range of 5.5-8.0 with the greatest growth rate at pH 6.0 for *M. intermedia*, pH 6.5 for *M. costata* and *M. hortensis* and pH 7.0 for *M. elata*, *M. esculenta*, and *M. rotunda* (Table 3). Winder[17] reported that DE4 isolate of *M. elata* reached maximal growth at pH 7.0 – 7.5. According to Song et al.[25] , the suitable pH for *Morchella conica* was 6.2. Jonathan and Fasidi[26] reported appreciable growth of *Psathyrella atroumbonata* at pH 6.5. Bae et al.[21] obtained an optimal pH for mycelial growth of *Paecilomyces japonica* was 5.0. However, Kim et al.[24] have reported that an optimal pH for *P. sinclairii* was 6.0. In addition, Xiao *et al.*[27] have found the optimal mycelial growth of *Cordyceps jiangxiensis* JXPJ 0109 was at pH 5.0.

Salinity effects

Vegetative growth studies of six *Morchella* species on selected salt concentrations showed that all strains could tolerate NaCI at different concentrations. The mycelia of the morels grew on all the salt concentrations. The mean days of complete colonization of the substrate was found to increase with increasing levels of concentration except *M. esculenta* (0.5 % of NaCI) and *M. costata* and *M. rotunda* that 1 % salt concentration was the best for these

Table 3. Effect of different pH on mycelial growth for *Morchella* species

pH	Mycelial Growth (mm)					
	MC (18)	ME (14)	MES (16)	MH (16)	MI (15)	MR (14)
5.5	69 ± 2.54	51 ± 2.45	60 ± 2.55	63 ± 2.40	78 ± 1.85	52 ± 2.55
6.0	81 ± 1.25	65 ± 2.15	71 ± 2.25	77 ± 1.90	90 ± 0.55	63 ± 1.85
6.5	90 ± 0.65	80 ± 1.90	82 ± 1.85	90 ± 1.10	84 ± 1.60	78 ± 1.40
7.0	83 ± 1.45	90 ± 0.95	90 ± 1.20	81 ± 1.75	75 ± 1.25	90 ± 0.80
7.5	70 ± 2.10	77 ± 2.20	80 ± 1.57	68 ± 1.65	62 ± 2.10	75 ± 1.60
8.0	58 ± 2.55	65 ± 2.75	68 ± 2.65	51 ± 2.30	48 ± 2.60	62 ± 2.36

MC: *Morchella costata*; ME: *M. elata*; MES: *M. esculenta*; MH: *M. hortensis*; MI: *M. intermedia*; MR: *M. rotunda*; Numbers between parentheses show the day when the maximum growth was measured; Best growth has been indicated with the bold characters in table.

Table 4. Effect of salinity on mycelial growth for *Morchella* species

Salinity %	Mycelial Growth (mm)					
	MC (18)	ME (15)	MES (16)	MH (18)	MI (16)	MR (16)
0.0	81 ± 1.25	90 ± 1.05	84 ± 1.21	90 ± 0.45	90 ± 1.15	78 ± 1.35
0.5	80 ± 1.63	83 ± 1.40	90 ± 0.65	79 ± 2.41	80 ± 1.40	82 ± 1.42
1.0	90 ± 0.90	71 ± 1.80	72 ± 2.10	63 ± 2.35	65 ± 2.60	90 ± 1.02
2.0	68 ± 2.15	56 ± 2.63	54 ± 2.84	48 ± 2.69	52 ± 2.55	65 ± 2.74
5.0	36 ± 2.55	38 ± 2.50	30 ± 2.70	29 ± 2.05	33 ± 2.80	41 ± 2.95

MC: *Morchella costata*; ME: *M. elata*; MES: *M. esculenta*; MH: *M. hortensis*; MI: *M. intermedia*; MR: *M. rotunda*; Numbers between parentheses show the day when the maximum growth was measured; Best growth has been indicated with the bold characters in table.

species (Table 4). Matsuda *et al.*[15], studied effects of NaCI on growth of some ectomycorrhizal fungi. They have reported that the mycelial growth of *Pisolithus tinctorius* at 100mM NaCI was significantly higher than other concentrations. They also found the mycelial growth of *Cenococcum geophilum* and *Suillus luteus* decreased with increasing NaCI concentrations. Ayodele and Ojoghoro[28] reported that *Pleurotus tuberregium* grew best in no salt medium.

CONCLUSIONS

In culture conditions and in nature many macrofungi that are ectomycorrhizal with forest trees, form a sheath of mycelium covering the root and penetrating the cortex and are able to utilize peptides and proteins, as sources of nitrogen and carbon. The fastest and most extensive degradation of complex carbon and nitrogen sources is caused by fungi. Basidiomycetes are most effective, but some ascomycetes and anamorphic fungi are also effective[14].

In this work, the effects of some environmental parameters such as, pH, temperature, type of carbon source and salinity were studied. Optimal pH range was found 6.0 – 7.0 for morels. Glucose, fructose, sucrose and maltose found the best carbon sources for six *Morchella* species. Optimal temperature for studied species was found at 20 and 25^{0}C. Salinity experiments showed that *Morchella* species grew best between 0.0 and 1.0 % NaCl concentration. To our knowledge the present study is the first report on *in vitro* utilization of various environmental parameters by six economically important ectomycorrhizal macrofungi collected from Anatolia. Different levels of carbon utilization and responses to pH, temperature and salinity change may be indicate and underlying genetic diversity of strains that may have potential for future exploitation.

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