

## Cultural Characteristics of *Morchella esculenta* Mycelium on Some Nutrients\*

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**Abstract:** The cultural characteristics of *Morchella esculenta* mycelium were investigated in potato dextrose agar, malt extract agar and complete medium yeast plates prepared with casein (cas), Casamino Acids (caa), peptone (p) and sodium nitrate (NaNO<sub>3</sub>). Aerial and vegetative mycelium types were determined in the agar media with cas, caa and p. The third colonial characteristic was observed in the agar media with NaNO<sub>3</sub>. In this media, a colonization increase in circular forms spreading from the centre to the edges was determined. Mycelium colonization periods were 20-24 days in agar media with NaNO<sub>3</sub>.

**Key Words:** Cultural characteristics, *Morchella esculenta*, mycelia development, nutritious.

### Bazı Besleyiciler Üzerindeki *Morchella esculenta* Misellerinin Kültürel Özellikleri

**Özet:** Kazein (cas), Kazamino asit (caa), pepton (p) ve sodyum nitrat (NaNO<sub>3</sub>) ilavesiyle hazırlanan patates dekstroz agar, malt ekstrakt agar, komplete medium yeast agar üzerinde geliştirilen *Morchella esculenta* misellerinin kültürel özellikleri incelendi. Cas, caa,p ilaveli besiyerlerinde hava ve vegetatif misel tipleri belirlendi. NaNO<sub>3</sub> eklenen besiyerlerinde üçüncü bir koloni misel tipi belirlendi. Bu besiyerlerinde kolonizasyon merkezden kenarlara doğru dairesel gelişme göstererek arttı. NaNO<sub>3</sub>'lı besiyerlerinde misel kolonizasyon süresi 20-24 gündür.

**Anahtar Sözcükler:** Kültürel özellikler, *Morchella esculenta*, misel gelişimi, besleyiciler.

### Introduction

Morels (*Morchella* spp.) are some of the most desirable edible mushrooms known (1). There are numerous studies dealing with the spore germination, culture, cytology, morphology, anatomy and physiology of morels (2-9), but few reports (10-12) dealing with the details of morels' reproduction and life-cycle. Morel spores germinate quickly, and extensive mycelium forms in a relatively short time. However, when mycelium encounters a physical boundary, a non-nutritional zone or competitors, it stops expanding, collapses and forms a subterranean structure called a sclerotium (13). Studies on the sclerotia formation of *Morchella esculenta*, *Morchella crassipes* and *Morchella conica* have been published (4, 10-12, 14-16). Volk and Leonard (1989 b) (11) reported that the Casamino Acids had the most significant effect on

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sclerotium production. Of the two-way interactions, only the interaction of the Casamino Acids with trace elements was significant, although the interaction of Casamino Acids with peptone was nearly significant. Casamino Acids containing all amino acids would be expected to boost both vegetative growth and sclerotium formation. Morel mycelium is the fastest growing of all mushrooms and has the same nutritive content and aroma as its ascocarps (13,17-20). The morphological, cytological and nutritional characteristics of the morel vegetative mycelium are well known (8). In various studies, (17,20-25) *Morchella* mycelia have been grown on agar media with a variety of undefined substrates such as vegetable wastes, sulfite liquor, peat hydrolysates, citrus wastes, cheese whey, pumpkin and carob bean. Dizbay and Karaboz (1986) (17) reported that black olive juice, beet molasses, potato, pumpkin and carob are used as substrates in submerged culture mediums.

This paper reports the mycelial characters of pure cultures of *Morchella esculenta* grown on sixteen different agar media with added casein, Casamino Acids, peptone and sodium nitrate.

### Materials and Methods

*Morchella esculenta* fructifications were collected from İçel-Anamur, Türkiye, in March 1990. Representative ascocarps were placed individually in paper bags and stored in a cooler for transport to the laboratory. They were dried in a sterile inoculation cabin and then refrigerated at 4°C. Stock cultures were grown on potato dextrose agar (PDA) (26), complete medium yeast (CYM) (10,11), 2% malt extract agar (2% MEA) (20) and 3% malt extract agar (3% MEA) .

These media were modified to yield 16 test systems by adding 4 g/l of casein (cas), peptone (p), Casamino Acids (caa) and sodium nitrate ( $\text{NaNO}_3$ ). The pH of the media without amendment was 6.5. The pH in the modified media was between 4.4 and 6.5 (Table 1).

In this research, the ascospores were inoculated and germinated into agar media with the purpose of forming main cultures. The ascospores were collected by shaking dried mature ascocarps. Inoculation and germination were accomplished by the multiple spore method (27).

Mycelial discs with a diameter of 8 mm taken from main cultures were transferred to the center of 70-90 mm diameter petri dishes and incubated at 19 to 23°C with a humidity value of 75% in the dark except during daily checks. Colony diameters were measured daily until the mycelium completely covered the agar. Cultures were then kept in the refrigerator at 4°C.

### Results

Mycelium development and color changes in the main culture had common characteristics in which mycelia and pigmentation spread starting from the center of inoculation. The criteria used in the study of mycelium forms and types concerning culture colonies were circular beamed in mycelium forms and vegetative mycelia growing vertical to the surface in a form identical to lace, wool and thread. Furthermore, the density of the vegetative mycelia, increased on joining the aerial mycelia, which resembled flappy cotton advancing from the surface of the agar plate

Table 1. The pH values of the agar media used during the research.

Agar media pH	PDA 6.5	2 % MEA 6.5	3 % MEA 6.5	CYM 6.5
Agar media pH	PDA+cas 5.6	2 % MEA+cas 5.4	3 % MEA+cas 4.6	CYM+cas 5.6
Agar media pH	PDA+p 5.6	2 % MEA+p 5.6	3 % MEA+p 4.5	CYM+p 5.8
Agar media pH	PDA+caa 5.3	2 % MEA+caa 4.7	3 % MEA+caa 4.5	CYM+caa 5.5
Agar media pH	PDA+ NaNO <sub>3</sub> 5.4	2 % MEA+ NaNO <sub>3</sub> 4.6	3 % MEA+ NaNO <sub>3</sub> 4.4	CYM+ NaNO <sub>3</sub> 5.0

towards the petri cover. The pigmentation in the main culture of mycelium showed a variety of colors: yellow-brown and brown.

### 1. Mycelium development in the agar media of the control groups:

Mycelia that grew in the center of PDA agar media covered the whole area (90 mm) of the petri dish in 5 days. No pigmentation was observed in mycelia that grew in the form of vegetative hyphae. MEA agar media were prepared by adding malt extract of 2% and 3%. In the MEA agar media prepared with 2% malt extract, mycelia development began 24 hours after inoculation, and colonization was complete within 4 days. Mycelia with poor development formed a rather regular vegetative hyphae in linear longitude. Yellow pigmentation was observed. Mycelia in 3% agar media was accomplished and colonization was completed within 7 days. No pigmentation was observed in mycelia that grew in the form of aerial hyphae. The mycelia in the CYM agar media that had a composition like ice crystals and that formed vegetative hyphae was in the CYM agar media and covered the entire area of the petri dish in 5 days. The period of colonization was 5 days (Fig.1, Figs. 2-5).

### 2. Mycelium development in enriched agar media

#### 2.1. The effect of casein (cas) on mycelium development

Mycelia colonization in 3% MEA+cas agar media was complete within a period of 8 days. In this period, while spreading from the center to the surrounding area, mycelia colonized irregularly. It was noted that mycelia that formed in the first half of the colony region increased in density, whereas in the second half the newly formed mycelia were weaker. Mycelia completed colony formation in 5 days in 2% MEA+cas, and in 7 days in CYM+cas and PDA+cas (Fig. 6, Figs. 7-10).

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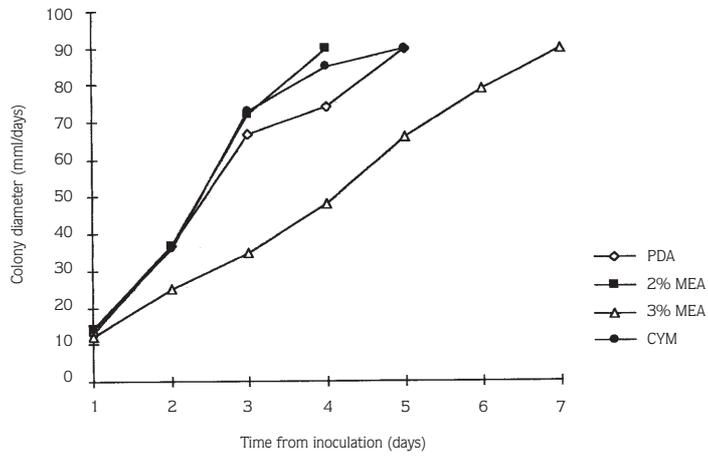
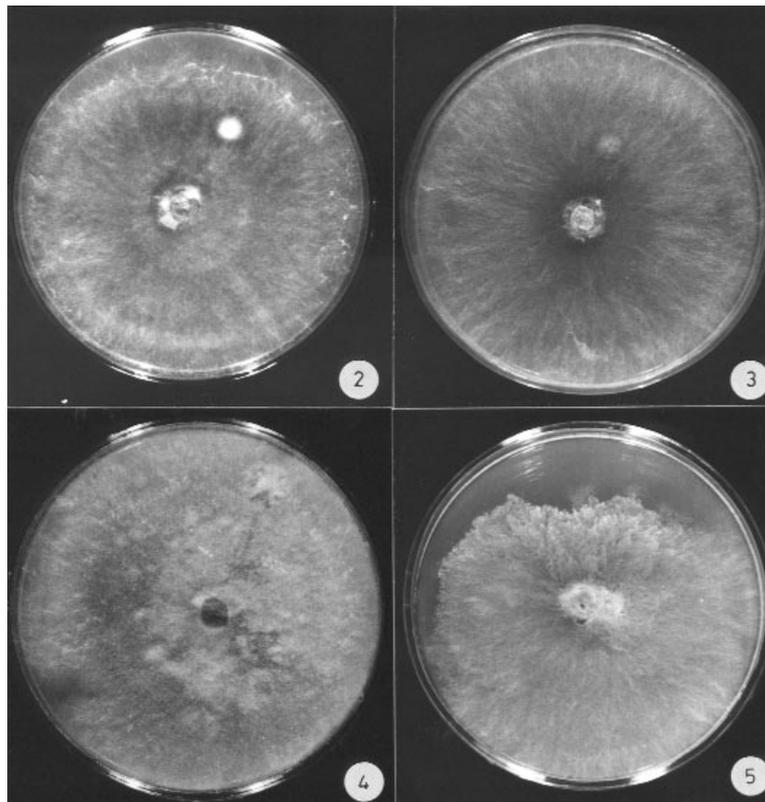


Figure 1. Development curve of *Morchella esculenta* mycelia in agar media of the control groups.



Figures 2-5. Development of *Morchella esculenta* mycelia in agar media of the control groups 2- PDA, 3- 2% MEA, 4- 3% MEA, 5- CYM.

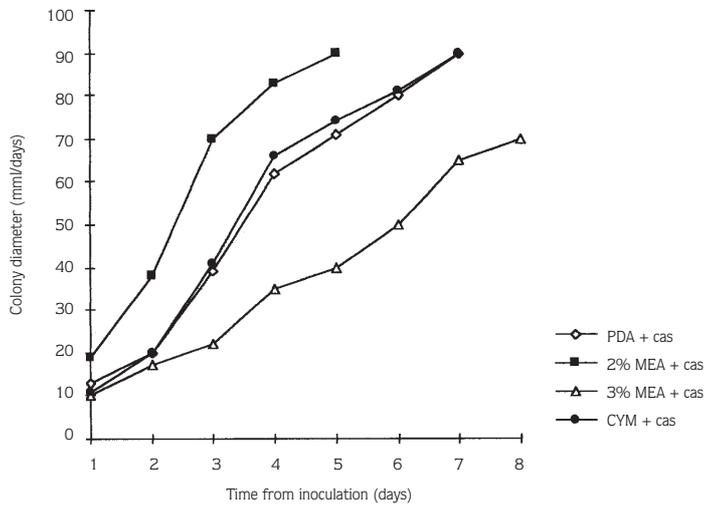
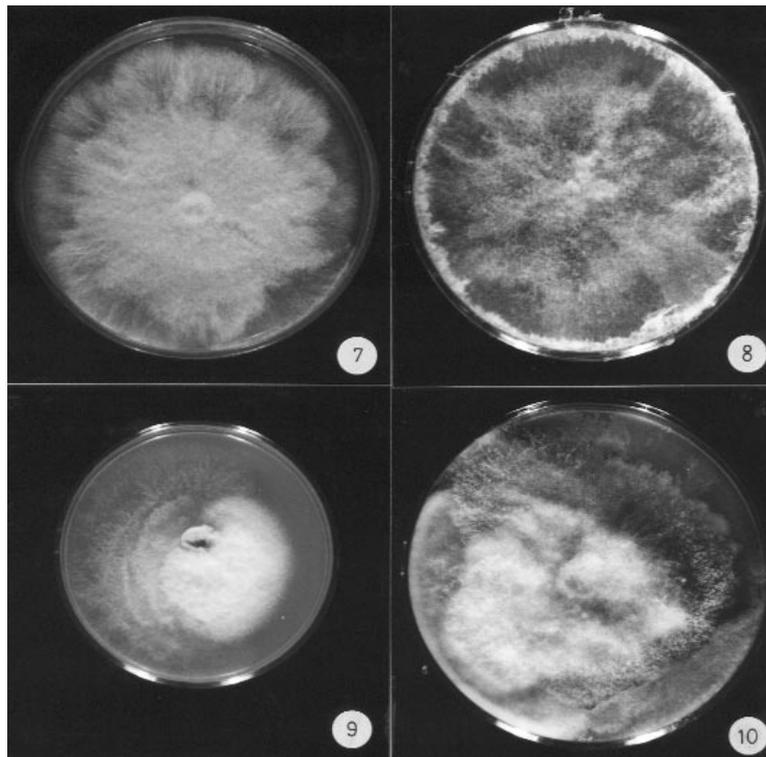


Figure 6. Development curve of *Morchella esculenta* mycelia in agar media with the addition of casein.



Figures 7-10. Development of *Morchella esculenta* mycelia in agar media with the addition of casein 7- PDA+cas, 8- 2 % MEA+cas, 9- 3 % MEA+cas, 10- CYM+cas.

In colonies where aerial and vegetative hyphae formation was observed, with the exception of PDA+cas agar media, the pigmentation that started with yellow color took on a yellow-brown color two days after the inoculation.

### 2.2. The effect of Casamino Acids (caa) on mycelium development

In our research, the fastest mycelial colonization was traced in agar media that had been subjected to the addition of Casamino Acids. Mycelia covered to whole petri dish in 4 days in the PDA+caa agar media. A dark brown pigmentation was observed in mycelia that formed aerial hyphae. In the MEA+caa agar media to which 2% malt extract was added, development of mycelia that formed colonies within 6 d started on the second day after the inoculation, and this development continued with an increase in density. Brown pigmentation was observed. In the MEA+caa agar media to which 3% malt extract was added, mycelia that finished colonization in 7 days formed aerial hyphae. Brown pigmentation was noted.

In the CYM+caa agar media mycelia that formed aerial hyphae, colonization was complete within 6 days. No pigmentation was noted (Fig.11, Figs. 12-15).

### 2.3. The effect of peptone (p) on mycelium development

While mycelia covered the total surface of the petri dish in the agar media to which PDA and 2% MEA had been added with in a week, in the 3% MEA+p agar media this period was 11 days. In the PDA+p agar media, mycelia formed linear vegetative hyphae, and in the 2% MEA+p agar media they formed linear aerial hyphae. In 2% MEA+p and 3% MEA+p agar media, brown pigmentation was observed. Mycelia completed colonization in 6 days and formed aerial hyphae in the CYM+p agar media (Fig. 16, Figs. 17-20).

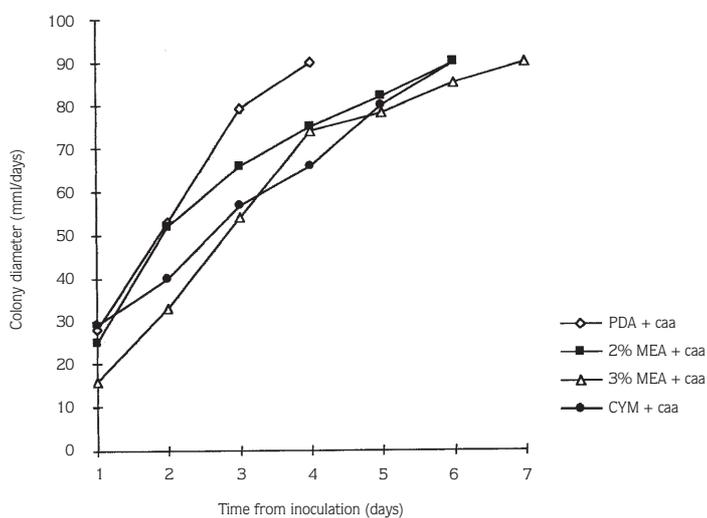
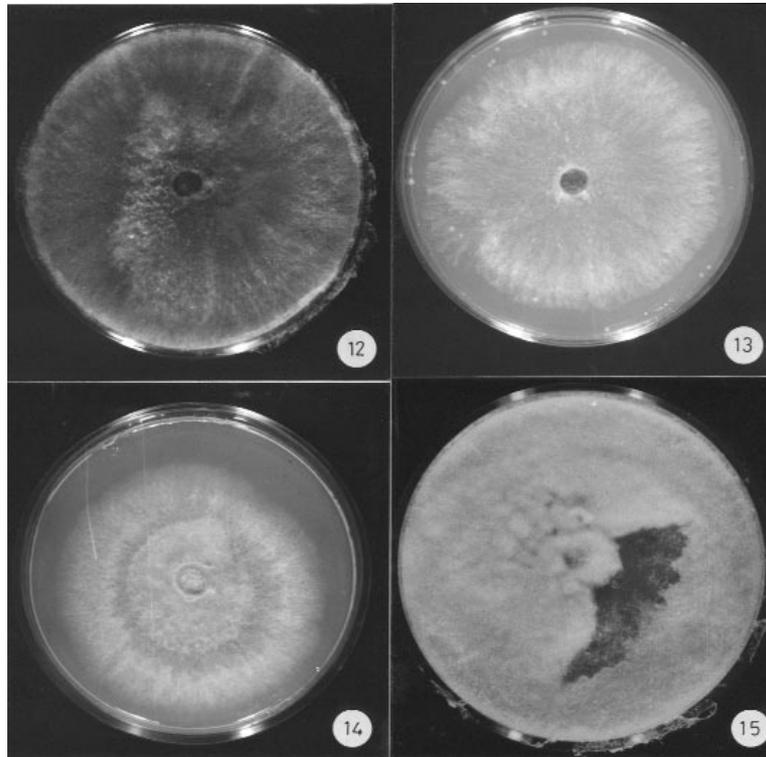


Figure 11. Development curve of *Morchella esculenta* mycelia in agar media with the addition of Casamino Acids.



Figures 12-15. Development of *Morchella esculenta* mycelia in agar media with the addition of Casamino Acids.12- PDA+caa, 13- 2 % MEA+caa, 14- 3 % MEA+caa, 15- CYM+caa.

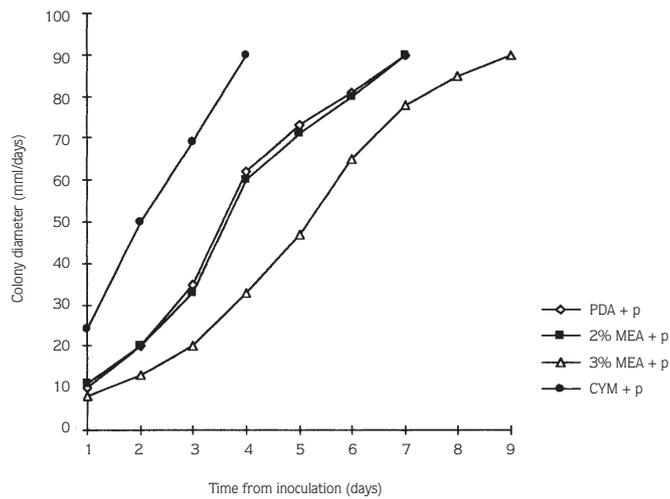
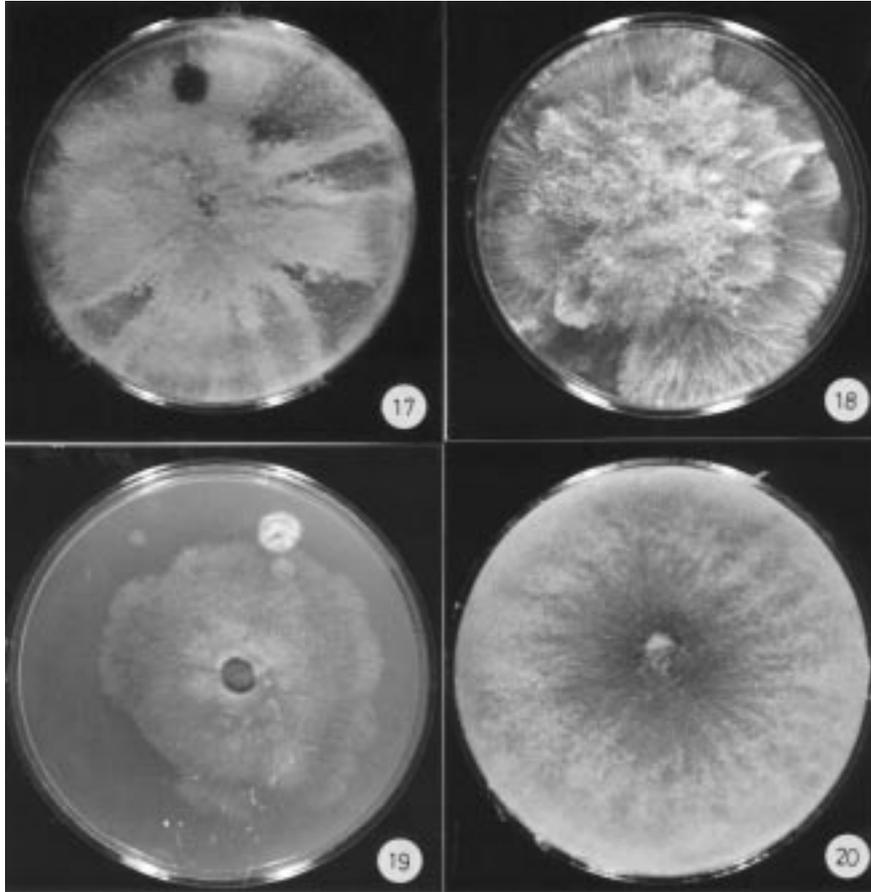


Figure 16. Development curve of *Morchella esculenta* mycelia in agar media with the addition of peptone.



Figures 17-20. Development of *Morchella esculenta* mycelia in agar media with the addition of peptone.17-PDA+p,18-2% MEA+p,19-3 % MEA+p, 20- CYM+p.

#### 2.4. The effect of sodium nitrate ( $\text{NaNO}_3$ ) on mycelium development

Mycelia began to develop on the second day after inoculation and while increasing their density, they continued their development in the form of rings. Meanwhile, they formed ball-like hyphae. The maximum development rate of mycelia in PDA+ $\text{NaNO}_3$ , and CYM+  $\text{NaNO}_3$  was 20 days, in 2% MEA+  $\text{NaNO}_3$  it was 24 days and in 3% MEA+  $\text{NaNO}_3$  the rate was 21 days. The pigmentation observed during the mycelium development was pink in PDA+  $\text{NaNO}_3$ , greenish in CYM+  $\text{NaNO}_3$ , yellow-pink in 2% MEA+  $\text{NaNO}_3$  and black in 3 % MEA+  $\text{NaNO}_3$  (Fig. 21, Figs. 22-25).

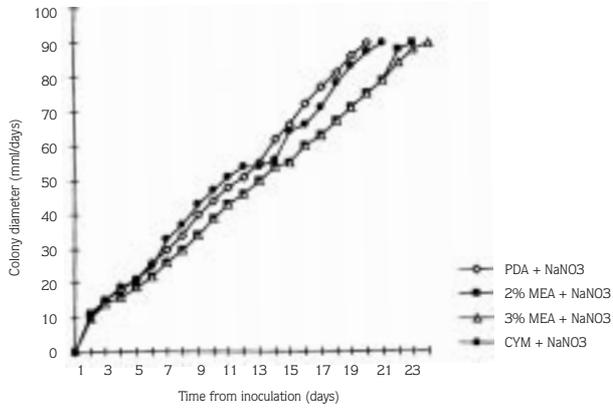
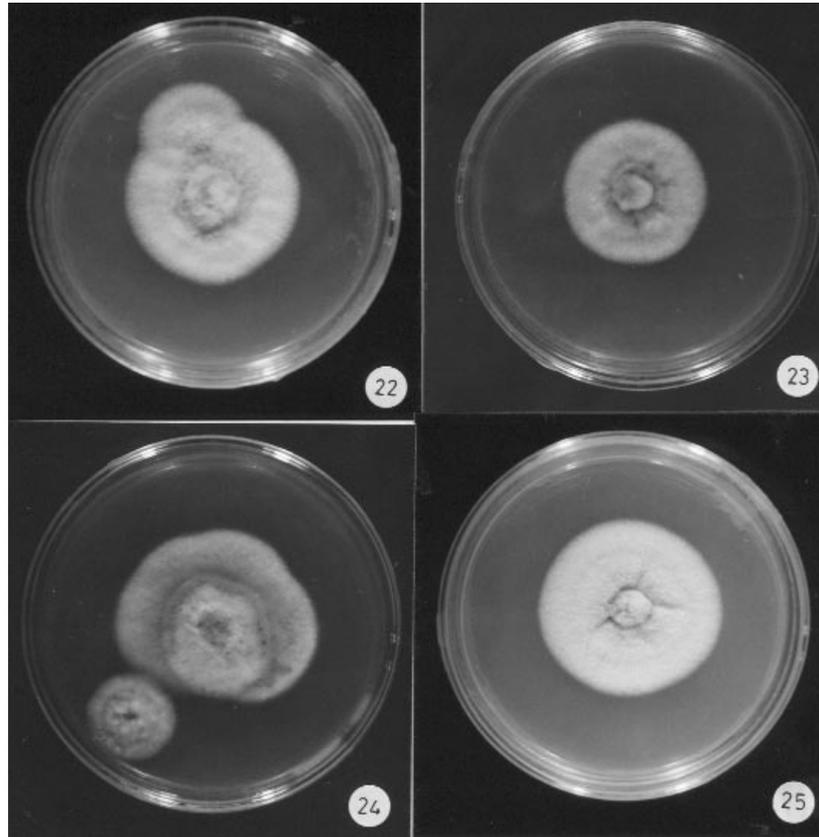


Figure 21. Development curve of *Morchella esculenta* mycelia in agar media with the addition of sodium nitrate.



Figures 22-25. Development of *Morchella esculenta* mycelia in agar media with addition of sodium nitrate. 22- PDA+NaNO<sub>3</sub>, 23- 2 % MEA + NaNO<sub>3</sub>, 24- 3 % MEA+ NaNO<sub>3</sub>, 25- CYM+ NaNO<sub>3</sub>.

## Discussion

The development of *Morchella esculenta* ascospores and mycelia was observed under in vitro conditions and in different agar media.

The effects of sodium nitrate, peptone, casein and Casamino Acids on the development of vegetative mycelium were studied. Sixteen different agar media obtained with the addition of these substances to the control groups had various pH values, which are indicated in Table I. The pH of the control groups was set at 6.5. The necessity to obtain an optimum value of pH of 6.5 for *Morchella* mycel development has been noted in various studies (21,23) when the radial development rate of mycelia in all the agar media is taken into consideration. It has been observed that mycelia have a very slow development rate in agar media to which sodium nitrate has been added and their period of colonization is 20-24 days. It has also been noted by Kaul (1977)(28) that nitrate is used in a limited way by high Basidiomycetes, and their influence on mycelium development has been noted by Volk and Leonard (1989 b) (11).

Kaul (1977) (28) reported that sodium and potassium nitrates give moderate to good growth of different morel species. Brock (1951) (29) also found sodium nitrate to be a moderate source of nitrogen for *Morchella esculenta*. Nitrates are considered excellent sources of nitrogen for many fungi, though inability to utilize it has been reported for higher Basidiomycetes, Saprolegniaceae and Blastocladiales (30). Hurni (1946) (31) found ammonium nitrate to be a moderate source of nitrogen for *M. esculenta*. Casein was used because of its positive effect on the mycelium development of *Agaricus* types in liquid cultures, and its effect on the mycelium development on *Morchella* types was examined. Dijkstra and his colleagues (1972) (32) reported casein's positive effects on *Agaricus*, but no such positive effects were observed on *Morchella* types. Peptone is composed of a mixture of amino acids. A mixture is preferred over single amino acids by organisms in general. Peptone was the only compound besides asparagine which was utilized well by *Morchella esculenta* (28).

It has been observed that the colonization characteristics of vegetative mycelium developed in solid nutritional environments showed different qualities. Three different colony properties of vegetative mycelium development in agar media were noted. Two of these mycelium developments are aerial hyphae and vegetative hyphae, and they were reported by Hervey and his colleagues in 1978 (8). The third colonial characteristic that was observed in our research was in agar media with  $\text{NaNO}_3$  and with which *M. esculenta* mycelial pellet discs have been inoculated. When agar media with  $\text{NaNO}_3$  were studied by transferring mycelial pellets to their centers, a colonization increase in circular forms spreading the center to the edges was observed.

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