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# Morel Culture Study Part I: *Morchela* sp. mycelial growth and nutrient-primed mycelia

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**ABSTRACT**

**Objectives:** Cultivated morels have still not replaced wild morels. Thus, wild-harvested morels are one of the most valuable forest products in the global market. Many researchers have discussed why morels are difficult and expensive to culture and investigated cheaper to grow morels. **Material and methods**: In this study, we researched optimal living conditions for morels using an artificial inexpensive method. We used different substrates, such as peat, potato crust, rice hulls, wheat, sawdust, and chestnut crust, to produce *Morchella* pre-sclerotia. **Results and Conclusions**: We determined mycelial growth using the colony diameter method and *Morchella* nutrient-primed prepared by using only peat and its mixtures with potato crust, rice hulls, wheat, sawdust and chestnut crust in different ratios after removing the spores. The results show that the pre-sclerotial time was accelerated using cheap substrates and that a sclerotia kit could be developed.

## 1. INTRODUCTION

Morels are the edible fruiting bodies of *Morchella* that are often collected for personal consumption and are commercially harvested for sale nationally and internationally. Morels are globally important because their international commerce network is extensive and profitable. Morels grow in all temperate and boreal climate forests in the Northern Hemisphere and also occur in some Mediterranean and subtropical regions, but thay rarely occur in hot climates. Their distribution is associated with live or dead trees, and disrupted soil, and they are tolerance to cold soils [1-3]. Morels have adapted to a wide range of unusual habitats and environmental conditions, including river bottoms, dunes, garbage dumps, abandoned coal mines, cellars and basements, saw mills, wood piles, sand bars in rivers, road cuts, excavations, deer trails, orchards, bomb craters and limed soils [4-6]. Literature on morel classification illustrates the related "look-alike" genera *Verpa* (thimble morels) and *Gyromitra* (false morels) which belong to the *Morchella* (true morels) genus [7].

**Kingdom** Fungi
**Phylum** Ascomycota
**Class** Pezizomycetes
**Order** Pezizales
**Family** Morchellaceae
**Genus** Morchella

The typical morel mycelium differs from many mushroom-producing fungi in Basidiomycetes. Hyphal cells of morels are multikaryotic or multinucleated. They have either multiple copies of the same haploid nuclei, as observed in many Ascomycetes, or many different haploid nuclei co-exist unpaired in a typical morel mycelium. A heterokaryotic mycelium is produced sexually when haploid hyphae of sexually reproducing fungi anastomose with other hyphae containing different haploid nuclei [8,9]. Heterokaryosis allows morels to adapt to a broader range of environmental conditions and may be protective against deleterious mutations because it is more likely that a good copy of the gene exists if the mycelium is heterokaryotic than if was dikaryotic [10,11]. Some *Morchella* sp. produces asexual conidia released by simple hyphal structures clonal propagation [12]. The mycelia of morels, form a variety of other structures, including sclerotia, mycorrhiza, mycelial muffs, and sporocarps. The mycelia have a critical role in the rapid formation of fruiting bodies [13,14]. Changes in soil pH, temperature, moisture and chemistry, microbial diversity, tree death (with or without fire), and other disturbances have also been associated with fruiting morels [15,16] .

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Sclerotial formation is critical for morel fructification. Two kinds of sclerotia exist in culture, such as encrusting sclerotia which tend to aggregate in a circular crust; and isolated sclerotia, which have growing margin[17,18]. The aim of this study was to evaluate some substrate materials for producing nutrient-rich mycelia and pre-scleroids of morels in culture.

## 2. MATERIAL AND METHODS

*Morchella* sp. were collected from forested highlands in Mersin, Turkey. and brought to the laboratory. The fresh fruiting bodies were dried in an incubator and cross-cut a sterilized blade. The pieces were washed within alcohol and the spores were transferred to a sterile container. The spores were transferred to Malt extract agar medium (MEA) and incubated at 23$^{o}$C to grow hyphae. After five inocula were produced, the stock cultures were prepared and maintained at 4$^{o}$C for further studies.

Peat (P), peat + potato crust (PP; domestic waste), peat +rice hulls (PR), peat + rice hulls + sawdust (PRS), peat +wheat (PW), peat + sawdust (PS), and peat + sawdust + chestnut crust (PSC; domestic waste) were used as additional material.

### 2.1 Growth of *Morchella* sp. mycelia

Morel mycelia were grown using a method modified from that used by Stamet [19]. Stock spores were inoculated into the sterile 10 cm diameter petri dishes containing (MYA) malt yeast agar; 20 g.L$^{-1}$ agar, and 10 g.L$^{-1}$ glucose, pH 5.5). Additional substrates were rused to determine the optimal mycelial growth rate on MYA medium. Ground P, PP, PR, PRS, PW, PS, and PSC were added to MYA at concentrations of 0, 1, 2, 3, and 4 g. L$^{-1}$. The mixture of additives was autoclaved at 121$^{o}$C for 15 min and then poured onto plates.

Colonized agar plugs (3 mm) from the stock culture were placed in the center of the petri dishes and incubated at 20$^{o}$C in dark for 10 days. Growth rates of the mycelia were determined by measuring the diameter of the main colony, with four replications per treatment.

### 2.2 Nutrient-primed mycelia time of the *Morchella* sp.

In the second experiment, we determined nutrient-primed mycelia time of the *Morchella* (mean of 10 replicates) prepared using peat alone and PP, PR, PRS, PW, PS, and PSC in different ratios.

In the third experiment, we evaluated the effect of substrate supplementation on pre-sclerotia production, using a modification of the jar method introduced by Ower et al. [20]. Different substrats such as P, PP, PR, PRS, PW, PS, PSC were enriched in 80% wheat bran to observe the formation of sclerotial during mycelial growth without a physical barrier (perforated aluminum sheet). We photographed the earliest pre-sclerotial structure that formed (Fig 1).

The mycelial growth results and mean values of nutrient-mycelia time were compared using analysis of variance and Tukey's test (P<0.001).

## 3. RESULTS AND DISCUSSION

Our chosen study area, with C4–5–6 grid squares was located in the hills of the Taurus Mountains, Mersin, southern Turkey. Morels collected from this area were transported to the laboratory, and their spores were isolated. The effect of different substrate materials on the growth of morel mycelia and initial scleroidal formation has been reported previously. In this study, mycelial growth and nutrient -primed mycelia time of *Morchella* sp. in P, PP, PR, PRS, PW, PS, PSC were studied. The largest mean colony diameters occurred with P, PP and PSC, whereas PR, PRS, PW, and PS produced the shortest diameters (Table 1). In addition, all substrates had a positive effect on mycelial growth. Mycelia formed 2-3 times more rapidly in morels with PP than in those grown with the other substrate groups and was attributed to the suitability of PP compared with the other substrates for preparing sclerotia. Adding wheat bran to the PP medium may have initiated the formation of the pre-sclerotial structure more quickly (Table 2).

**Table 1:** Mycelial growth of *Morchella* sp. in malt extract agar (MYA) medium supplemented with (1, 2, 3, and 4 g. L$^{-1}$) peat (P), peat + potato crust (PP; domestic waste), peat +rice hulls (PR), Peat + rice hull + sawdust (PRS), peat +wheat (PW), peat + sawdust (PS), and peat + sawdust + chestnut crust (PSC; domestic waste). Growth was measured as the mean of two colony diameters taken at right angles, after a 15 day incubation in dark at 20$^{o}$C. Means followed by the same letter are not significantly different (p <0.001).

| Materials for substrate | Mean colony diameter | | | | |
|---|---|---|---|---|---|
| | control | 1 g.L$^{-1}$ | 2 g.L$^{-1}$ | 3 g.L$^{-1}$ | 4 g.L$^{-1}$ |
| Malt extract agar (control) (MYA) | 3.25a | - | - | - | - |
| (MYA)+ P | - | 3.26a | 3.32a | 3.25a | 3.25a |
| (MYA)+ (PP) | - | 6.95b | 6.92b | 6.98b | 7.80b |
| (MYA)+ (PR) | - | 3.25a | 3.28a | 3.50a | 3.25a |
| (MYA)+ (PRS) | - | 3.55a | 3.56a | 3.60a | 3.44a |
| (MYA)+ (PW) | - | 4.25a | 4.27a | 4.00a | 4.26a |
| (MYA)+ (PS) | - | 4.44a | 4.36a | 4.46a | 4.51a |
| (MYA)+ (PSC) | - | 5.26b | 5.32b | 5.33b | 5.35b |

**Table 2:** Nutrient-primed mycelia time (N-PMT) of the *Morchella* (means of 10 replicates) prepared with only peat alone and a mixtures of peat + potato crust (PP; domestic waste), peat +rice hulls (PR), Peat + rice hulls + sawdust (PRS), peat +wheat (PW), peat + sawdust (PS), peat + sawdust + chestnut crust (PSC; domestic waste).

| Materials for substrate | %V/V | N-PMT |
|---|---|---|
| Peat | 100 | 73 ±1.5 |
| peat + potato crust | 25/75 | 20 ±1.0 |
| peat +rice hull | 25/75 | 43 ±1.9 |
| Peat + rice hull + sawdust | 25/37,5/37,5 | 65 ±1.2 |
| peat +wheat | 25/75 | 60 ±1.1 |
| peat + sawdust | 25/75 | 62 ±1.3 |
| peat + sawdust + Chestnut crust | 25/37,5/37,5 | 55 ±1.3 |

A bran rich substratum (wheat bran) was directly cultured to grow ascocarps as an alternative to nutrient-rich mycelia for further investigation. In the present study, autoclaved-pasterized wheat bran was added to the surface of the mycelial mass to initiate the formation of the pre-scleroidal structure. Nutrient-primed mycelia in P, PP, PR, PRS, PW, PS, and PSC were enriched with 80% wheat bran on day 20 to observe early pre-sclerotial formation without a physical barrier (perforated aluminum sheet). The earliest formation of a pre-sclerotial

structure occured in PP on day 30 (Figure 1). Guler and Ozkaya [21] reported that various carbon sources spherical and thick morel sclerotial structures *in vitro*. In detail, Many studies have reported that suitable concentrations of some minerals, heavy metals and toxic metallic elements play an important role in metabolic processes, during the growth of morel mushrooms [22,23]. In particular, iron, calcium, and magnesium are abundant in several *Morchella* sp. [24]. We modified the best best-known method reported by Ower et al. [20] to grow sclerotia This method involves filling a jar half full with a carbon source without a soil layer and dividing the material into layers containing different nitrogen substrates. This method produced sclerotia in approximately 4 weeks. Similarly, Volk and Leonard [8] and Amir et al. [25] obtained sclerotia in aging culture media.

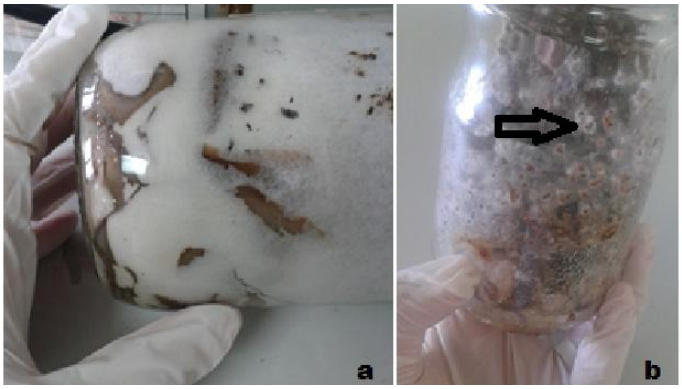


**Fig. 1: a** Nutrient-primed mycelia in peat + potato crust (domestic waste). b) Formation of pre-sclerotia during mycelial growth in peat + potato crust (domestic waste) enriched with 80% wheat bran without a physical barrier (perforated aluminum sheet)

The morel scleroidal structure characterized as spherical thick-walled, and darkly pigmented and its structure is an important factor during production [26,27]. Sclerotia formation time is critical factor related to morel culture production cost, and using inexpensive substrates (industrial waste) to form sclerotia quickly is the most economical approach.

## 4. CONCLUSION

In this study, a pre-sclerotial structure was produced quickly and inexpensively using wheat bran and PP Sclerotia kits could be commercially developed to shorten culture time.

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**Conflict of Interests:** There are no conflicts of interest.

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