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Morphological development of *Morchella conica* mycelium on different agar media

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Abstract: *The present study presents the development of mycelium of Morchella conica where different concentration of sucrose added at different agar media. For this sucrose have been added as 0.25, 0.50, 0.75, 1.00 and 1.25% concentration to wheat agar, potato dextrose agar, malt extract agar and complete medium yeast agar. The radial growth speed, morphologic specifications, radial growth radius and pigmentation of mycelium were taken as criteria, the development period of mycelium in wheat agar was completed in 4 days and mycelium were very thin. The colonization period of the mycelium was determined; 7 days in potato dextrose agar, 5 days in malt extract agar and 5 days at complete medium yeast agar. The development of the mycelium; at potato dextrose agar was dense and circular; at malt extract agar and at completed medium yeast agar was rhizomorphic. Mycelium has developed very well at sucrose medium and formed creamy and light yellow pigmentation.*

Key words: *Morchella conica, Mycelium development, Sucrose*
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Introduction

Morchella species were known as edible mushrooms but can not be produced for trade purposes. The morel life cycle not present in the other cultivated mushrooms: the sclerotium (Volk and Leonard, 1989). The sclerotium of the morel is a relatively large structure (1mm -5 cm diameter) composed of large cells with very thick walls that allow the fungus to survive in adverse natural conditions, such as winter (Volk and Leonard, 1990). In the spring, the sclerotium has two options for germination; to form a new mycelium or to form a fruiting body. Unfortunately for the potential grower, it is very easy to get the sclerotia to form a new mycelium but very difficult to force it to form a fruiting body. Very specific conditions of nutrition, humidity, carbon dioxide levels and temperature must be met for primordia to form (Volk, 2008). The determination of the food value of *Morchella* can be based on the food value of vegetative hyphae (Hawker, 1956). A lot of different factors can effect the development of *Morchella* hyphae. Fron (1905) reported the carbon nutrition and the effects of pH on mycelial growth. The sucrose is a natural sweetener, traditionally used in human nourishment due to its pleasant taste, nutritious value and low cost production (Glazer and Nikaido, 1995). Maheshwari and Balasubramanyam (1988) examined utilization of glucose and sucrose by thermophilic fungi. Goldenbaum and Siddiqi (1995) found that liquid fungal medium comprises from 0.5 to 1.0 percent (weight / volume) of one or more sugars selected from the group consisting of glucose, sucrose, and maltose, in an amount that is effective to stimulate fungal growth. Glucose, the most abundant monosaccharide in nature, is the principal and preferred carbon and energy source for nearly all cells. In addition to being a major nutrient, glucose can act as a "growth hormone" to regulate several aspects of cell growth, metabolism, and development (Ozcan and Johnston, 1999). Chung and Tzeng (2004) examined yeast extract by equivalent carbon content to that of sucrose itself supported significant growth and served as sources. Overall, among the 14 carbon sources tested in the presence of thiamine, the most favorable in order were sucrose, raffinose, fructose, glucose, maltose and galactose. These carbon sources were utilized only in the presence of thiamine, indicative of the major role of thiamine for sugar metabolism.

This paper presents, morphological specification of *Morchella conica* mycelium on different agar media containing sucrose that have different concentrations as 0.25, 0.50, 0.75, 1.00 and 1.25%. Our purpose and aim is to sight if there is any change in the development of *Morchella conica* mycelium in different media where different concentrations of sucrose added to and compare them with the control group. Also define the varieties during the incubation period and pigmentation formation during the *Morchella conica* mycelium development.

Materials and Methods

Organism: In this study; the *Morchella conica* Pers. that is included in Ascomycetes class is used.

Agar media: In this study; as agar medium, potato dextrose agar (PDA), malt extract agar (MEA) (Gunay, 1995) complete medium yeast agar (CYM) (Volk and Leonard, 1989) and wheat agar (WA) (Guler, 1998) were used. These agar medium were used as control groups in the study. In these control groups, sucrose were separately added as 0.25, 0.50, 0.75, 1.0 and 1.25 percentages. All prepared agar medium were sterilized in the autoclave at 121°C for 15 min.

Mycelium transfers: The spores taken from the *Morchella conica*s were inoculated to PDA by multiple spore method and vegetative

Table - 1: The mycelium specifications of control groups

Agar media	CP (days)	RGR	Mycelium specification
WA	4	Poor	Linear, Light yellow pigmentation, Aerial hyphae and no Sclerotia
PDA	7	Good	Circular, in age ring shape, Young mycelium are white, than dark brown color, Aerial hyphae is only seen at the center. Yes sclerotia
MEA	5	Good	As beam, than turned surface hypae, Pigmentation yellow. Yes sclerotia
CYM	5	Very good	As rhizomorfic and pretty dense, No aerial hypae. Yes sclerotia

CP = Colonization period, RGR = Radial growth ratios

Table - 2: The mycelium specifications of wheat agar (WA) media

Concentration	CP (days)	RGR	Mycelium specifications
0.25	4	Poor	Linear and white light yellow Pigmentation at the center. No aerial hypae. Yes sclerotia
0.50	4	Poor	Linear, light yellow pigmentation. No aerial hypae and sclerotia
0.75	4	Poor	Linear and dense light yellow pigmentation. No aerial hypae and sclerotia
1.00	4	Poor	Linear and dense. Light yellow pigmentation at the center. No aerial hypae and sclerotia
1.25	4	Poor	Linear and white. No aerial hypae and sclerotia

CP = Colonization period, RGR = Radial growth ratios

Table - 3: The mycelium specifications of potato dextrose agar (PDA) media

Concentration	CP (days)	RGR	Mycelium specifications
0.25	6	Medium	Circular and dense. There is yellow pigmentation at the center of the mycelium that was white at the start. Than mycelium color changed to cream. No aerial hypae and sclerotia
0.50	6	Medium	Dense and circular. Mycelium has grown in age rings. At the start there are light yellow pigmentation at the center. Than dark brown color occurred. Aerial hypae is seen. No sclerotia
0.75	6	Medium	At the beginning at the side of the colony uneven but as development continues age circles were formed. There were systematic aerial hypae at the center
1.00	7	Medium	As the development continues age circles were formed. Light yellow colored pigmentation. At the center dense cottony mycelium aerial hypae was seen. No sclerotia. Medium Smooth circular, colony sides uneven. In the center yellow-cream color
1.25	7	Medium	Pigmentation, as development continues turned to light yellow pigmentation. No sclerotia

CP = Colonization period, RGR = Radial growth ratios

Table - 4: The mycelium specifications of malt extract agar (MEA) media

Concentration	CP (days)	RGR	Mycelium specifications
0.25	7	Good	Rhizomorfic, at the beginning light yellow pigmentation formed. Turned to dark brown color as the growth continues. No aerial hypae and sclerotia
0.50	6	Good	Rhizomorfic, yellow light pigmentation, locational dark brown pigmentation
0.75	6	Good	Rhizomorfic, light yellow pigmentation. No aerial hypae. Yes sclerotia
1.00	5	Good	Rhizomorfic, Pigmentation at the center dark yellow, seen light. Yellow at the sides. Yes sclerotia
1.25	5	Good	Rhizomorfic, light yellow pigmentation. No aerial hypae. Yes sclerotia

CP = Colonization period, RGR = Radial growth ratios

Table - 5: The mycelium specifications of complete medium yeast agar (CYM) media

Concentration	CP (days)	RGR	Mycelium specifications
0.25	5	Very good	Rhizomorfic, cream color pigmentation. No aerial hypae, Yes sclerotia
0.50	5	Very good	Rhizomorfic, no aerial hypae, cream color pigmentation, Yes sclerotia
0.75	5	Very good	Rhizomorfic, cream color pigmentation. No aerial hypae, Yes sclerotia
1.00	5	Very good	Rhizomorfic, light yellow pigmentation. No aerial hypae, Yes sclerotia
1.25	5	Very good	Rhizomorfic, light yellow pigmentation, No aerial hypae, Yes sclerotia

CP = Colonization period, RGR = Radial growth ratios

primer spores were gained. From these primer spores mycelium agar discus in 8 mm in radius were taken. They were separately inoculated to potato dextrose agar (PDA), malt extract agar (MEA) complete medium yeast agar (CYM) and wheat agar (WA) at the centre which are located in the 9mm Petri dishes. (The control groups of the study). In the same way also the 8 mm mycelium agars discs were separately inoculated to the agars where different concentrations of sucrose added. The vegetative growth of the primary mycelium, which was developed by using the multiple spore method, was researched. During the development the radial growth speed were taken as criteria. In the study for all groups the terminologies weak, medium, good and very good were used for the radial growth percentages.

Weak: The mycelium, which is not clear on the agar surface and develops early.

Medium: The mycelium, which is clear on the surface of the agar and develops circularly.

Good: The mycelium which develops densely and beamy on the surface of the agar.

Very good: The mycelium, which develops very densely rhizomorfic on the surface of the agar.

Results and Discussion

In this study; the morphologic specifications, radial growth ratios (RGR) and colonization periods (CP) of the control groups and the agar mediums which sucrose was added at different concentrations, were researched. At the mycelium specifications, mycelium growth ratio, variety of mycelium growths, sclerotia formation and pigmentation were also researched. At the end of this study; all the results were given in Tables.

Control groups: When the control groups are compared with each other; the fastest development period was seen at wheat agar. But, the mycelium development in wheat agar is considerably delicate. The colonization period at the CYM medium agar where the best mycelium development seen was 5 days. Except WA agar medium, sclerotium was composed in all other groups. The results are given asTables. The mycelium specifications of control groups were given at Table 1.

Sucrose added agar media: The mycelium specifications at sucrose added wheat agar (WA) medium, potato dextrose agar (PDA) medium, malt extract agar (MEA) medium and complete medium yeast agar (CYM) medium are given in Tables 2,3,4 and 5 respectively.

Wheat agar's being the right agar medium for spore germination and mycelium growth was stated by Gunay (1995). Guler (1998) has stated that wheat agar is the right agar for *Agaricus bitorquis* too.

At the researches done on *M. conica* , the researchers have studied the mycelium development at PDA colonization formation. Guler *et al.* (1995,1996) have defined this development period as 5 days, Karaboz and Oner (1988) have stated the same period as 7 days. Kaul (1981) have investigated growth characters and rate of growth of *Morchella* spp on PDA.

Arkan *et al.* (1997) have seen *M. conica* mycelium colonization period as 4 days in MEA. Guler *et al.* (1996) have stated that the *M. conica* mycelium complete their colonization in 7 days without forming pigmentation as aerial hyphae. Guler and Sorkun (2001) found that the *M. conica* mycelium had completed their colonization period in 4-5 days as cotton mycelium without creating pigmentation. The mycelium colonization period was defined as 6 days by Sonmez (1998) in her research. Hayes (1972) found that MEA as the only natural medium suitable for primordium formation. Iqbal *et al.* (1988) found that best growth of *Agaricus bitorquis* was on MEA and PDA. Takaaki and Hiroko (2004) found a method for culturing an edible fungus providing a liquid culture medium containing sucrose as a carbon source inoculating the medium with an *Agaricus* mycelium.

Guler *et al.* (1996) have stated that in CYM the mycelium covered the petri dishes in 4 days by forming pigmentation. Sonmez (1998) in her study says that the *M. conica* mycelium complete their colonization in CYM in 5-6 days. Arkan and Guler (2000) in their other study have mentioned that the mycelium development was completed in 7 days forming aerial hyphae without pigmentation. Volk and Leonard (1989) have investigated mycelial characters of *Morchella* spp on CYM.

Pessoni *et al.* (2005) reported *Penicillium jancewskii,* a filamentous fungus grows rapidly on media, containing either sucrose or inulin as carbon sources. Sati and Bisht (2006) in their study; have investigated four isolates of *Tetracheatum elegans*, *Tetracladium marchalianum*, *Pestalotiopsis submersus* and *Flagellospora penicillioides* for their carbon requirement, using glucose, fructose, sucrose, xylose, starch, cellulose, dextrin and lactose and they reported that glucose and sucrose were found to be the suitable sources of carbon for all four fungal isolates. Maltose and sucrose appeared as sources of carbon same as other carbon sources like glucose and lactose (Khan *et al.*, 1991). Ramasawamy and Kandaswamy (1978) have studied the effect of carbon compounds on the mycelial growth of *Podaxis pistillaris* and found maltose and sucrose as good sources of carbon.

In this study the *Morchella conica* mycelium development in agars where different concentrations of sucrose were added to was investigated. The poorest development was seen in wheat agar and the best mycelium development was seen both in the control groups and in sucrose added agars in CYM agar.

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