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Research article

MYCELIUM DEVELOPMENT OF *HELVELLA CRISPA* (Scop.) Fr. ON DIFFERENT AGAR MEDIA

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ABSTRACT: In this study, the development of mycelium of *Helvella crispa* (Scop.) Fr. which added the different concentration of glucose and lactose to potato dextrose agar and malt extract agar. For this; glucose and lactose have been added as 0.5, 1.5 and 2.5% concentration to potato dextrose agar and malt extract agar separately. The radial growth speed, morphologic characterizations and pigmentation of mycelium were taken as criteria. The colonization period of mycelium in potato dextrose agar and malt extract agar was completed in 11 and 15 days approximately. Mycelium developed as dense at the potato dextrose agar medium with glucose (at the 0.5 and 1.5% concentration) and developed very dense with glucose (at the 2.5% concentration). But mycelium developed as dense at the malt extract agar medium with lactose (at the 0.5 and 1.5% concentration) and developed very dense with lactose (at the 2.5% concentration). The development of the mycelium was obtained as linear at the both two agar medium. Mycelium has formed yellowish-brown pigmentation.

Key words: *Helvella crispa*, Mycelium development, Glucose, Lactose

INTRODUCTION

Helvella is a genus of ascomycete fungus of the Helvellaceae family. The mushrooms, commonly known as elfin saddles, are identified by their irregularly shaped caps, fluted stems. Well known species include the whitish *H. crispa* and the grey *H. lacunosa*. (Ammirati et. al., 1985). A lot of different factors can affect the development of macro fungus. Fron (1905) explained that the carbon nutrition and the effects of pH on mycelial growth. Glucose, the most abundant monosaccharide in nature, is the principal and preferred carbon and energy source for cells. In addition, glucose can act as a "growth hormone" to regulate aspects of cell growth, metabolism, and development (Ozcan and Johnston- 1999). Maheshwari and Balasubramanyam (1988) reported utilization of glucose and sucrose by thermophilic fungi. Liquid fungal medium comprises from 0.5 to 1.0 percent sugars selected (glucose, sucrose, and maltose) that is effective to stimulate fungal growth. (Goldenbaum and Siddiqi, 1995). The aim of this paper, the effects of different concentrations of glucose and lactose were examined on *Helvella crispa* (Scop.) Fr. mycelium. Our purpose is to sight if there is any change in the development of *Helvella crispa* mycelium in different media where different concentrations sugars and compare them with the control group.

MATERIALS AND METHODS

Organism

The *Helvella crispa* (Scop.) Fr is used that is included in Ascomycetes class-Helvellaceae family.

Agar media

The potato dextrose agar (PDA), malt extract agar (MEA) (Gunay, 1995) were used as agar medium, these agar medium were used as control groups in the study. In these control groups, glucose and lactose were separately added as 0.50, 1.5 and 2.5 percentages. All prepared agar mediums were sterilized in the autoclave at 121°C for 15 min.

Mycelium transfers

The piece of tissue taken from the *Helvella crispa* were inoculated to PDA and MEA and vegetative prime mycelium was gained. From these primer mycelium agar discs in 8 mm (Ø) were taken. They were separately inoculated to potato dextrose agar (PDA) and malt extract agar (MEA) at the centre which is located in the 9mm Petri dishes.

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These are control groups of the study. In the same way also the 8 mm radius mycelium agars discs were separately inoculated to the agars where different concentration glucose and lactose added too. All prepared mediums with inoculated *H.crispa* mycelium were incubated at 28°C and dark. Prepared all different medium were given at Table 1. During the development the radial growth speed were taken as criteria.

Table 1. All agar medium in this study

Agar Medium	Carbohydrates	Concentrations	Abbreviations
Potato dextrose agar (PDA) (Control)	-	-	PDA-C
PDA	Glucose	0.5	PDA+0.5-Glu
PDA	Glucose	1.5	PDA+1.5-Glu
PDA	Glucose	2.5	PDA+2.5-Glu
PDA	Lactose	0.5	PDA+0.5-Lac
PDA	Lactose	1.5	PDA+1.5-Lac
PDA	Lactose	2.5	PDA+2.5-Lac
Malt extract agar (MEA) (Control)	-	-	MEA-C
MEA	Glucose	0.5	MEA+0.5-Glu
MEA	Glucose	1.5	MEA+1.5-Glu
MEA	Glucose	2.5	MEA+2.5-Glu
MEA	Lactose	0.5	MEA+0.5-Lac
MEA	Lactose	1.5	MEA+1.5-Lac
MEA	Lactose	2.5	MEA+2.5-Lac

RESULTS AND DISCUSSION

In this study; the morphologic characteristics, radial growth ratios (RGR) and colonization periods (CP) of the control groups and the agar mediums which glucose and lactose were added at different concentrations to the potato dextrose agar and malt extract agar were researched. At the mycelium characterizations, mycelium growth ratio, variety of mycelium growths and pigmentation were also researched. All the results were given in Tables.

Control groups: When the control groups are examined that, during the incubation, 24 h later, the development of the mycelium started from the center, towards the edges proceeded in parallel. The colonization period was 15 d at the PDA-C and MEA-C agar. The mycelium characterizations of control groups were given at Table 2. During the incubation of mycelium development of *Helvella crispa* was given Figure 1.

Table 2. The mycelium characterizations of control groups

Agar medium	CP (days)	RGR	Mycelial characterizations
PDA	15	Good	Linear, Yellowish-brown pigmentation. Aerial hyphae
MEA	15	Good	Linear, Light yellowish-brown pigmentation. Aerial hyphae

CP= Colonization period RGR= Radial growth radios

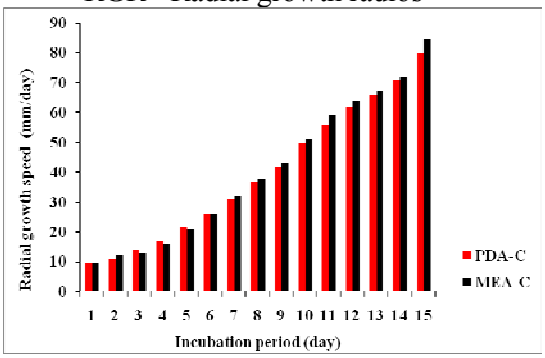

Figure 1. The mycelium development curve of control groups

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As seen from Figure 1; first 5 d; mycelium developed more speed at the PDA-C agar according to MEA-C agar. At 6th d, mycelium development was equalized both the mediums, but at the last 9d mycelium developed more speed at the MEA-C agar according to PDA-C agar.

Glucose added agar media: The mycelium characterizations at glucose added potato dextrose agar (PDA) medium and malt extract agar (MEA) were given in Table 3 respectively. During the incubation of mycelium development of *Helvella crispa* at different agar media with glucose were given Figure 2.

Table 3. The mycelium characterizations at different agar medium with glucose

Agar medium	CP (days)	RGR	Mycelial characterizations
PDA+0.5-Glu	15	Good	Dense, Linear, No aerial hyphae Yellowish-brown pigmentation.
PDA+1.5-Glu	13	Good	Dense, Linear, No aerial hyphae Light yellowish-brown pigmentation.
PDA+2.5-Glu	11	Very good	Very dense. Mycelium has grown in age rings. Firstly, yellow pigmentation at centre. Than brown pigmentation formed. No aerial hyphae
MEA+0.5-Glu	15	Medium	Linear. Yellowish and white pigmentation. No aerial hypae
MEA+1.5-Glu	15	Medium	Linear. Yellowish and white pigmentation. No aerial hypae
MEA+2.5-Glu	15	Medium	Linear. Yellowish and white pigmentation. No aerial hypae

CP= Colonization period RGR= Radial growth radios

As seen from Table 3; at the all mediums with added glucose; mycelium developed more speed especially at the PDA+2.5-Glu according to all MEA mediums.

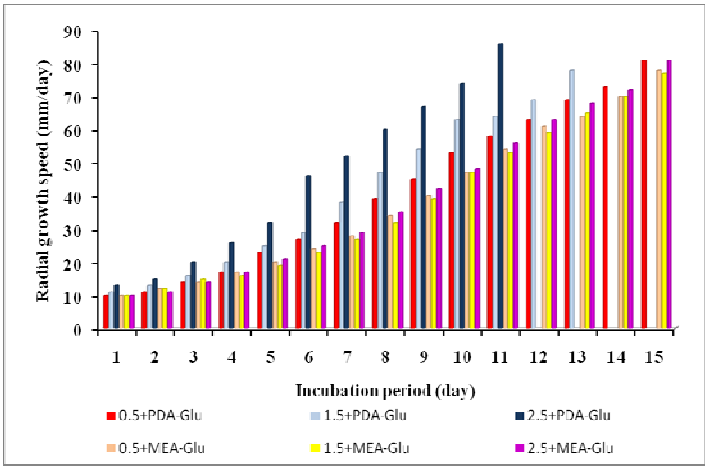

Figure 2. The mycelium development curve of *Helvella crispa* at PDA and MEA with glucose

Lactose added agar media: The mycelium characterizations at lactose added potato dextrose agar (PDA) medium and malt extract agar (MEA) were given in Table 4 respectively. During the incubation of mycelium development of *Helvella crispa* at different agar media with glucose were given Figure 3.

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Table 4. The mycelium characterizations at different agar medium with lactose

Agar medium	CP (days)	RGR	Mycelial characterizations
MEA+0.5-Lac	15	Good	Dense, Aerial hyphae, Yellowish-brown pigmentation.
MEA+1.5-Lac	13	Good	Dense, Aerial hyphae. Yellowish-brown pigmentation.
MEA+2.5-Lac	11	Very good	Very dense, Aerial hyphae. Yellowish-brown pigmentation.
PDA+0.5-Lac	15	Medium	Linear, Yellowish-brown pigmentation. Aerial hyphae
PDA+1.5-Lac	15	Medium	Linear, Light yellowish-brown pigmentation. Aerial hyphae
PDA+2.5-Lac	15	Medium	Linear, Light yellowish-brown pigmentation, aerial hyphae

CP= Colonization period RGR= Radial growth radios

As seen from Table 4; at the all mediums with added lactose; mycelium developed more speed especially at the MEA+2.5-Lac according to all PDA mediums.

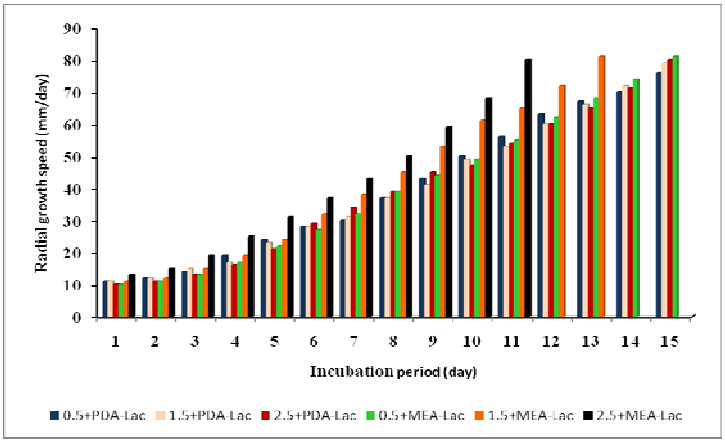

Figure 3. The mycelium development curve of *Helvella crispa* at PDA and MEA with lactose

Some researchers done on Ascomycetes species, especially, have studied the mycelium development of *Morchella* conica 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.

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.

In this study glucose and lactose were used as carbon sources. These carbohydrates were added different concentration at PDA and MEA medium. As a result; when 2.5% glucose and lactose have been added, at the both PDA and MEA agar medium the mycelial growth was more rapid compared to control groups. There were no differences between the other groups to forming colonization 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. They reported that glucose and sucrose were found to be the suitable sources of carbon for all four fungal isolates. In this study the *Helvella crispa* mycelium developments that in different agar and different concentrations of monosaccharide (glucose) and disaccharide (lactose) were added to be investigated. The development mycelium was seen well almost all agar medium.

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