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DETERMINATION OF OPTIMUM CULTURE CONDITIONS FOR MYCELIAL GROWTH OF *Macrolepiota procera* MUSHROOM

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ABSTRACT

Macrolepiota procera, commonly called the Parasol Mushroom, is a delicious mushroom collected from the nature and commonly consumed by the public in many regions of Turkey. This study was conducted to determine the optimum culture conditions (pH, temperature, carbon and nitrogen sources) for mycelial growth of *M. procera*. Three pH values (pH 5.0, 5.5 and 6.0), four incubation temperatures (15, 20, 25 and 30°C), seven carbon (C) sources (dextrose, glucose, lactose, maltose, mannitol, sucrose and xylose) and six nitrogen (N) sources ($(NH_4)_2HPO_4$, NH_4NO_3 and $Ca(NO_3)_2$, malt extract, peptone and yeast extract) were investigated. In the second step of the study, the effect of seven pH values (4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0) on the mycelial colony diameter was examined at 20 and 25°C since these temperatures gave the best mycelial growth in the previously conducted temperature experiment. The best mycelial growth was determined at pH 6.0. The optimum temperature for mycelial growth of *M. procera* was found as 25°C. The use of glucose as carbon source and yeast extract and peptone as nitrogen source in the culture medium gave the best results for mycelial growth. Determining of optimum culture conditions for mycelial growth of *M. procera* will provide important contributions to the fortcoming studies on it's commercially cultivation in Turkey.

Key words: carbon and nitrogen sources, *Macrolepiota procera,* mycelial growth, pH, temperature

INTRODUCTION

Macrolepiota procera (Scop. ex Fr.) Singer is an edible saprophytic mushroom and commonly known as the Parasol Mushroom. *M. procera* belongs to the phylum Basidiomycota, the class Agaricomycetes, the order Agaricales and the family Agaricaceae [Phillips 1994]. It is a highly delicious mushroom with good taste, pleasant smell, delicate texture and faint nutty aroma of the cap. This mushroom is an important source of protein, vitamins, minerals and dietary fiber, and its medicinal value is quite high [Falandysz et al. 2008, Kułdo et al. 2014, Kumari and Atri 2014]. *M. procera* has a very big and magnificent sporocarp. It resembles a parasol at maturity. The cap diameter ranges between 11 and 40 cm. It is initially spherical and opens at maturity. The colour of cap is grayish brown and the middle of cap is convex. The cap flesh is white and soft. The gills are white or pale cream, crowded, remote from the stipe. *M. procera* has a quite long stipe (10–40 cm). The stipe is smooth, hollow, cylindrical, grayish brown and decorated with small brown scales snakeskin appearance. The base of the stipe is swollen. The only cap of *M. procera* can be eaten as its stem is very fibrous and tough. A large and movable ring is present on the stipe. The spores are ellipsoidal, smooth, thick-walled and 15–20 × 10–12 µm. The spore print is white or very pale cream

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[Pekşen et al. 2008]. *M. procera* grows naturally as single or in groups and forms fruiting bodies on soil surface in the forests, the edge of forests, meadows, parks and gardens during late summer or autumn.

M. procera commonly grows in Turkey [Sesli and Denchev 2014] and gladly consumed by the peoples and sold in the local markets [Solak and Gücin 1992, Pekşen and Karaca 2000, Pekşen et al. 2016, Yılmaz and Zencirci 2016, Pekşen and Kaplan 2017]. It is called as "Dede mantarı", "Dedebör", "Şemsiye mantarı", "Şalvarlı" and "Turna bacağı" in different regions of Turkey. *M. procera* is a substantial source of income and a valuable food for especially population in rural areas of Turkey [Pekşen and Kibar 2008, 2016, 2017]. However, the commercial cultivation of *M. procera* could not be achieved yet in Turkey and it is just collected from the nature [Pekşen and Kibar 2017]. Recently, natural flora has been destroyed due to eccessively and unconsciously collection of wild edible mushrooms including *M. procera*. As a result of human impact, drastic decreases have been occurred in the quantities of mushrooms collected from the nature. Thus, preservation of wild edible mushrooms such as *M. procera* for future generation may be provided with their cultivation. The cultivation of *M. procera* can provide considerable contributions to the general economy by opening new business opportunities for especially small family businesses and forest villagers. In addition to, an important source in the nutrition of public will be created.

The first stage for cultivation of wild edible mushrooms is the production of mycelial starter culture [Jonathan and Adeoyo 2011]. Afterwards, optimum mycelial growth conditions should be determined. The factors such as culture medium, temperature, pH, carbon and nitrogen sources, availability of nutrients and genetic potential greatly affect the mycelial growth of mushrooms [Calam 1971, Hoa and Wang 2015]. For this reason, it is very important to detect the necessary factors for the optimum mycelial growth of *M. procera.* There is a limited number of studies on the mycelial growth and cultivation of *M. procera* in Turkey [Pekşen and Kibar 2008, 2016, 2017]. The objective of this study was to determine the most proper culture conditions such as pH, temperature, carbon and nitrogen sources for mycelial growth of *M. procera*, an important wild edible mushroom in Turkey.

MATERIALS AND METHODS

This study was carried out in the mushroom mycelia production laboratory, Department of Horticulture, Faculty of Agriculture, Ondokuz Mayis University, Samsun, Turkey.

The collection, identification and isolation of mushroom samples. The fruiting bodies of *M. procera* were collected from the campus of Ondokuz Mayis University in autumn. Identification of mushroom was done by conventional methods [Phillips 1994]. The pure mycelial cultures were obtained using the method described by Jonathan and Fasidi [2003]. Fungal cultures were maintained on Malt Extract Agar (MEA) medium at 25°C in the dark. The cultures were maintained at 4°C and subcultured regularly to use in the later investigations [Brundrett et al. 1996].

The determination of the most suitable pH and temperature values for mycelial growth. The effect of different pH values (pH 5.0, 5.5 and 6.0) and temperatures (15, 20, 25 and 30°C) on mycelial growth of *M. procera* was investigated on MEA media. The pH values of the media were adjusted to 5.0, 5.5 and 6.0 with the addition of 1 N NaOH or HCl prior to autoclaving. Then, the prepared media were sterilized by autoclaving at 121°C for 30 min. After cooling, media were poured into the 9 cm diameter Petri dishes and each dish was aseptically inoculated with a 5 mm in diameter mycelial agar disc of *M. procera*. The inoculated petri dishes were sealed with parafilm and incubated at 25°C in complete darkness. The MEA medium adjusted to pH 6.0 was similarly prepared for the temperature experiment. The inoculated petri dishes incubated at 15, 20, 25 and 30°C in complete darkness. The experiments related to pH and temperature were set in a completely randomized design with five replications. In the next step, the effect of seven pH values (pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0) on mycelial growth were examined by expanding the range of the pH values on MEA medium at 20 and 25°C, since the best mycelial growth was obtained at 20 and 25°C.

The determination of the most suitable carbon and nitrogen sources for mycelial growth. In order to determine the most suitable carbon and nitrogen sources for the mycelial growth of *M. procera*, seven carbon (dextrose, glucose, lactose, maltose, mannitol, sucrose and xylose) and six nitrogen sources

($(NH_4)_2HPO_4$, NH_4NO_3 $Ca(NO_3)_2$, malt extract, peptone and yeast extract) were tested. For this purpose, Sabouraud Agar (SB) medium was used because it contains both carbon and nitrogen. SB medium was composed of glucose 40 g, peptone 10 g, agar 20 g and 1000 ml of distilled water. Each of the carbon sources was separately added into the SB medium at the rate of 40 g/l. The SB medium without carbon was used as C-free medium in the experiment. Each of the nitrogen sources was individually supplemented to the SB medium at the concentration of 10 g/l. The SB medium without nitrogen served as N-free medium in the experiment. The sterilisation and inoculation of the media prepared with different carbon and nitrogen sources were performed as mentioned above for the pH and temperature experiment. Afterwards, the inoculated petri dishes were incubated at 25°C under dark conditions. The experiments related to carbon and nitrogen sources were arranged in a completely randomised design with seven replications. With respect to mycelial growth parameters in the experiments, mycelial growth rate, mycelial growth area and mycelial colony diameter were measured as described by Pekşen et al. [2013].

Statistical analysis. Data were subjected to analysis of variance using the SPSS statistical program. The means showing statistical significance were compared by Duncan's multiple range test. Square root transformation was applied to data about mycelial growth of *M. procera* in various nitrogen sources prior to statistical analysis because no mycelial growth was observed in the medium containing $(NH_4)_2HPO_4$.

RESULTS AND DISCUSSION

The mycelial growth rate and colony diameter ranged from 0.15 to 0.16 cm day^{-1} and 4.66 to 4.98 cm, respectively. No statistically significant difference was found among the investigated pH values with regard to mycelial growth rate and mycelial colony diameter (Tab. 1).

The results of variance analysis revealed that there were significant differences ($P < 0.01$) among temperatures for mycelial growth rate and mycelial

Table 1. Effect of different pH values on mycelial growth of *M. procera*

pH	Mycelial growth rate (cm day^{-1})	Mycelial colony diameter (cm)[a]
5.0	0.16[ns]	4.80[ns]
5.5	0.15	4.66
6.0	0.16	4.98

[a] Mycelial colony diameter was measured on the 28[th] day after mycelial inoculation for the present study. [ns] Non significant.
There are no statistically significant differences among means within the same columns according to Duncan's multiple range test

Table 2. Effect of different temperatures on mycelial growth of *M. procera*

Temperature (°C)	Mycelial growth rate (cm day^{-1})	Mycelial colony diameter (cm)[a]
15	0.11b*	3.53b*
20	0.20a	6.01a
25	0.21a	6.28a
30	0.11b	3.43b

[a] Mycelial colony diameter was measured on the 28[th] day after mycelial inoculation for the present study. ** Significant at $P < 0.01$.
Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test

colony diameter. The highest mycelial growth rate and mycelial colony diameter (0.21 cm day^{-1} and 6.28 cm, respectively) was obtained at 25°C, followed closely by 20°C without statistically significant differences between them. On the other hand, mycelial growth rate and mycelial colony diameter evidently decreased at 15 and 30°C (Tab. 2).

In the present study, reduction of mycelial growth at 15 and 30°C could be attributed to decrease in the absorption of important nutrients needed for fungal growth at low temperatures [Garraway and Evans 1984] and denaturation of important enzymes for fungal metabolic processes at high temperatures [Jonathan et al. 2004].

As pH values of 5.0, 5.5 and 6.0 did not show any significant effect on the mycelial growth, range of the pH values was expanded in the consequent study conducted at 20 and 25°C which the best mycelial growth was obtained. The effect of different pH values and temperatures on mycelial colony diameter was significant ($P < 0.01$). At the end of incubation period, mycelial colony diameter determined in different pH values were between 6.38 and 7.51 cm. The highest mycelial colony diameter was measured at pH 6.0. This was followed by pH 6.5, 7.0 and 5.5 which were not statistically different with pH 6. The mycelial growth markedly decreased at pH below 5.5. Accordingly, the most suitable pH range for the best mycelial growth was between 5.5 and 7.0. The mycelial colony diameter at 25°C (7.23 cm) was significantly higher than that of 20°C (6.41 cm). There was a significant ($P < 0.01$) interaction between pH and temperature in terms of mycelial colony diameter. The highest mycelial colony diameter was recorded at pH 6.0 and 20°C (7.72 cm), and pH 7.0 and 25°C (7.63 cm) combination (Tab. 3).

Johansson [2002] stated that temperature and pH are highly important and critical environmental factors that affect the mycelial growth of mushrooms. Mushrooms can grow over a wide range of pH range, with an optimum between pH 5.5 to 8.0 [Deacon 1984]. Acidic pH was more favorable for both mycelial growth and production of metabolites in most of Ascomycetes and Basidiomycetes [Park et al. 2001, Shih et al. 2007].

The optimum pH and temperature values for mycelial growth can vary depending on muhroom species and even the fungal strain. Petcharat and Khuntong [1999] found that optimal temperature and pH for mycelial growth of *M. gracilenta* were 25°C and pH 5.0, respectively. The best mycelial growth of *M. procera* was observed at pH 6.5 [Jonathan 2002]. The best mycelial growth in *M. bonaerensis* was determined at 25°C and mycelial growth was not affected by pH [Maki and Meirelles 2002]. The optimal mycelial growth of *M. procera* was obtained at 30°C and pH 7.0 by Shim et al. [2005]. The most suitable temperature and pH for mycelial growth of *M. detersa* were at 30°C and pH 7.0 or 8.0, respectively [Rizal et al. 2014]. The results of this study were generally in agreement with the findings of the mentioned above researchers.

Significant differences ($P < 0.01$) were found among the carbon sources with regards to mycelial growth parameters. The mycelial growth rate, mycelial growth area and mycelial colony diameter in media prepared with various carbon sources varied from 0.42 to 1.23 mm day^{-1}, 42.71 to 247.33 mm^2 and 3.95 to 8.81 cm, respectively. The maximum mycelial growth rate, mycelial growth area and mycelial colony diameter was obtained when glucose was used as the carbon source (Fig. 1). This was sourced from *M. procera* produces enzymes utilizing glucose better than other carbon sources in the study. Glucose was followed by maltose and dextrose which also considerably promoted mycelial growth. Conversely, minimum mycelial growth rate, mycelial growth area and mycelial colony diameter was recorded in lactose and C-free medium. The low mycelial growth in the C-free medium could be explained by limited nutrition due to carbon deficiency. Since mycelial growth started late (on the 8th day) in the medium containing xylose, mycelial growth area (on the 13th day) was found low (Tab. 4).

Generally, mycelia of mushrooms can grow on many carbon sources [Yang et al. 2003]. The mycelial growth of mushrooms shows differences depending on carbon sources. It is reported that the most widely used carbon sources by mushrooms are glucose, mannitol and fructose [Griffin 1994]. Glucose and starch were the most suitable carbon sources for the mycelial growth of *M. gracilenta* [Petcharat and Khuntong 1999]. The best carbon source for mycelial growth of *M. procera* was maltose [Shim et al. 2005]. Gbolagade [2006] suggested that glucose and mannose were the best carbon compounds for mycelial biomass produc-

Table 3. Effect of different pH values and temperatures on mycelial colony diameter (cm) of *M. procera*[a]

pH	Temperature		Mean
	20°C	25°C	
4.0	5.84cd*	7.33ab	6.58b*
4.5	5.79d	7.35ab	6.57b
5.0	5.60d	7.16ab	6.38b
5.5	7.06abc	6.46a-d	6.76ab
6.0	7.72a	7.30ab	7.51a
6.5	6.67a-d	7.37ab	7.02ab
7.0	6.16bcd	7.63a	6.89ab
Mean	6.41b*	7.23a	–

[a] Mycelial colony diameter was measured on the 28th day after mycelial inoculation for the present study. ** Significant at $P < 0.01$. Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test

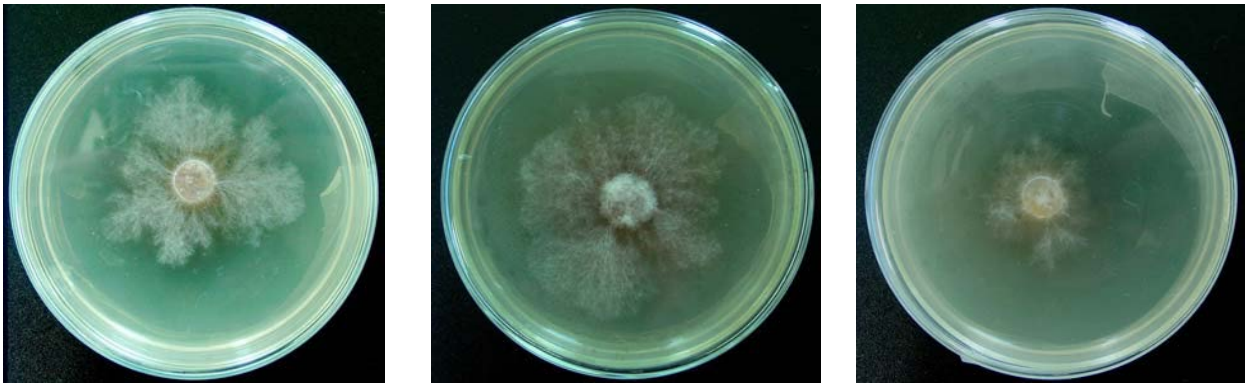
Table 4. Effect of different carbon sources on the mycelial growth of *M. procera*

Carbon sources	Mycelial growth rate (mm day^{-1})	Mycelial growth area (mm^2)[a]	Mycelial colony diameter (cm)[b]
Dextrose	0.93bc*	184.00b*	7.86ab*
Glucose	1.23a	247.33a	8.81a
Lactose	0.42d	78.71c	4.28d
Maltose	0.91bc	206.14ab	7.64ab
Mannitol	0.73c	93.14c	6.01c
Sucrose	0.81c	157.57b	7.39bc
Xylose	1.11ab	42.71c	6.55bc
C-free	0.43d	61.33c	3.95d

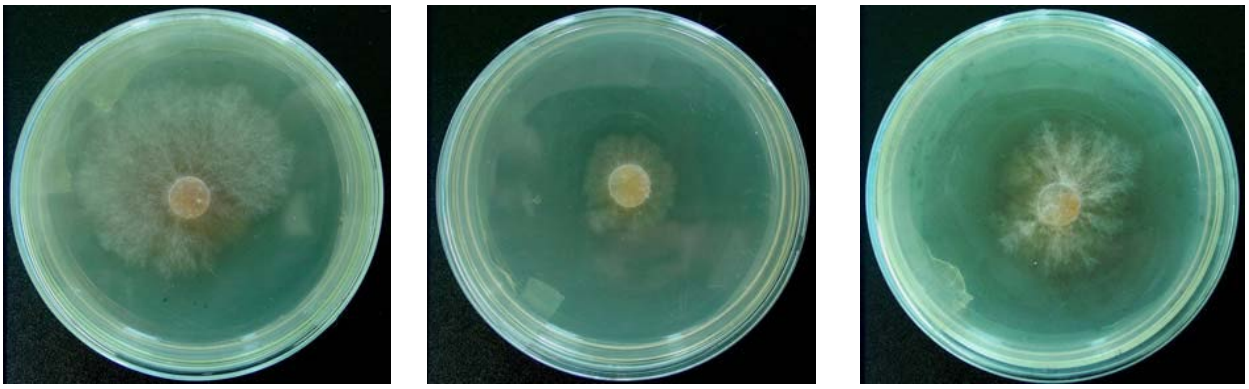
[a] Mycelial growth area was measured on the 13th day after mycelial inoculation for the present study. [b] Mycelial colony diameter was measured when mycelial growth was completed in any of Petri dishes containing different carbon sources, on the 28th day after mycelial inoculation for the present study. ** Significant at $P < 0.01$. Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test

tion of *Lepiota procera.* Shih et al. [2008] determined that glucose promoted good biomass production in various mushroom species. Pekşen and Kibar [2016] also found that the best carbon source for mycelial biomass production of *M. procera* in submerged culture was dextrose and it was followed by glucose, mannitol, sucrose and lactose. In the present study, similar results were found with those findings regarding carbon sources.

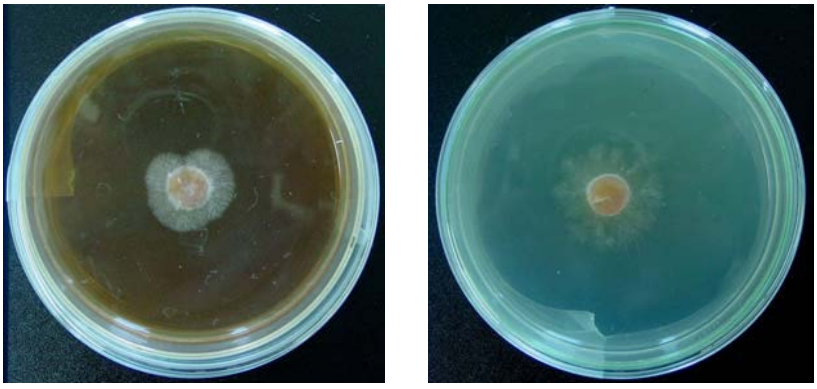
Nitrogen sources had significant effect ($P < 0.01$) on the mycelial growth. Among the nitrogen sources, the highest mycelial growth rate, mycelial growth area and mycelial colony diameter was found in the media containing yeast extract (1.20 mm day^{-1}, 170.57 mm^2 and 8.26 cm, respectively) and peptone (0.98 mm day^{-1}, 171.50 mm^2 and 7.21 cm, respectively) without statistical difference between them. Malt extract supported moderate mycelial growth. However, no mycelial growth was observed in the medium with $(NH_4)_2HPO_4$. The mycelial growth drastically decreased in NH_4NO_3 medium. It was weak and quite slow in this medium when compared with other nitrogen sources (Fig. 2). The media containing $(NH_4)_2HPO_4$, NH_4NO_3 and $Ca(NO_3)_2$ gave lower values than the N-free



Dextrose Glucose Lactose



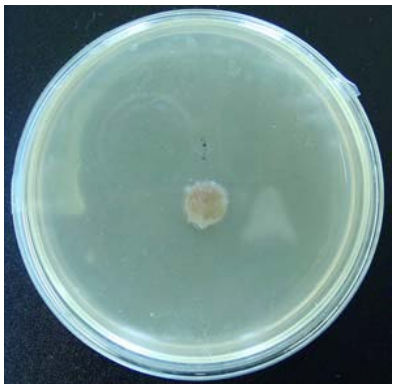
Maltose Mannitol Sucrose



Xylose C-free

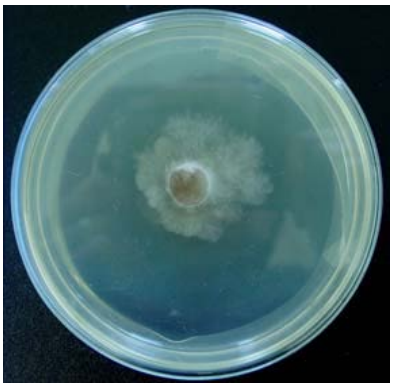
Fig. 1. The mycelial growth of *M. procera* on the media prepared with different carbon sources

$(NH_4)_2HPO_4$

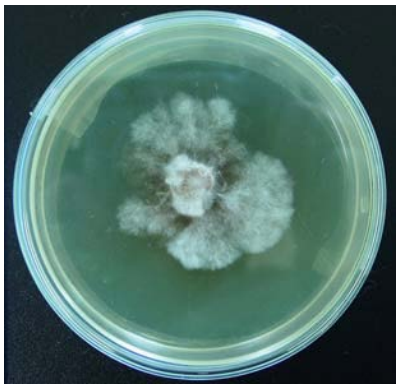


NH_4NO_3

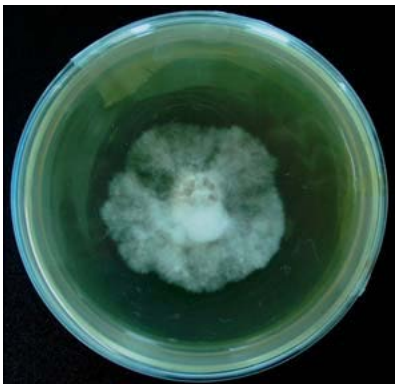
$Ca(NO_3)_2$



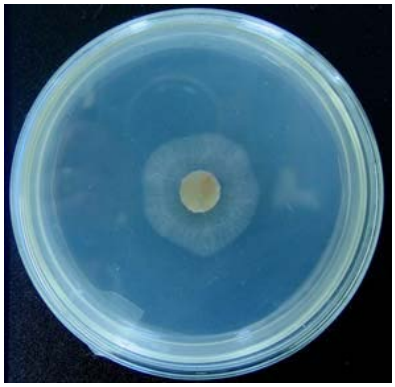
Malt extract



Peptone



Yeast extract



N-free

Fig. 2. The mycelial growth of *M. procera* on the media prepared with different nitrogen sources

Table 5. Effect of different nitrogen sources on the mycelial growth of *M. procera*[a]

Nitrogen sources	Mycelial growth rate (mm day^{-1})	Mycelial growth area (mm^2)[b]	Mycelial colony diameter (cm)[c]
$(NH_4)_2HPO_4$	0.00d*	0.00d*	0.00d*
NH_4NO_3	0.27c	11.83d	1.43c
$Ca(NO_3)_2$	0.70b	86.86c	5.73b
Malt extract	0.78b	129.29abc	6.30ab
Peptone	0.98ab	171.50a	7.21ab
Yeast extract	1.20a	170.57a	8.26a
N-free	0.73b	109.86bc	6.41ab

[a] Square root transformation was applied to data about mycelial growth prior to statistical analysis. [b] Mycelial growth area was measured on the 13th day after mycelial inoculation for the present study. [c] Mycelial colony diameter was measured when mycelial growth was completed in any of Petri dishes containing different nitrogen sources, on the 28th day after mycelial inoculation for the present study. ** Significant at $P < 0.01$. Means followed by different letters within the same columns are statistically different according to Duncan's multiple range test

medium for the mycelial growth rate, mycelial growth area and mycelial colony diameter (Tab. 5).

Kim et al. [2005] informed that nitrogen is very important nutrient for fungal growth, synthesis of enzymes and metabolite production. Various nitrogen sources have different effects on mycelial growth of different fungal species [Lin and Yang 2006]. The results of present study were in agreement with Gbolagade [2006] who reported that good biomass production was obtained by D-alanine, yeast extract and peptone in submerged cultures of *Lepiota procera* and were also similar with Pekşen and Kibar [2016] who stated that peptone and malt extract were the most favorable nitrogen sources for mycelial biomass production of *M. procera* in submerged culture. In the study, the promotive effect of yeast extract and peptone on mycelial growth may be due to their high carbohydrates, vitamins and amino acids composition [Garraway and Evans 1984, Gbolagade et al. 2006]. The optimal nitrogen source for mycelial growth of *M. procera* was glycine [Shim et al. 2005]. Ammonium was one of the most commonly used nitrogen sources by many mushrooms [Rangel-Castro et al. 2002]. In *M. gracilenta*, the maximum mycelial growth was determined in the medium with ammonium sulphate among the nitrogen sources [Petcharat and Khuntong 1999]. However, a sufficient and rapid mycelial growth could not be achieved in the media with ammonium ($(NH_4)_2HPO_4$ and NH_4NO_3) in this study. Our results were also in accordance with the results of Kibar and Pekşen [2011] and Pekşen et al. [2013] who reported that the lowest mycelial growth was determined in NH_4NO_3 and $(NH_4)_2HPO_4$ among the nitrogen sources for different mushroom species. Our findings were similar with the reports of Finlay et al. [1992] and Griffin [1994] who stated that mycelial growth was generally slower in the medium with nitrate and nitrate could be prevented mycelial growth of some Basidiomycetes. It was suggested that Basidiomycetes generally prefer organic nitrogen sources for mycelial growth rather than inorganic ones [Shih et al. 2006]. In the current study, mycelial growth determined in the organic nitrogen sources such as yeast extract, peptone and malt extract was better than that of inorganic nitrogen sources ($(NH_4)_2HPO_4$, NH_4NO_3 and $Ca(NO_3)_2$). Similarly, organic nitrogen sources were more suitable for the mycelial growth of different mushrooms compared to inorganic nitrogen sources [Manjunathan and Kaviyarasan 2011, Joshi et al. 2013, Pekşen and Kibar 2016].

CONCLUSIONS

In the present study, optimum culture conditions including pH, temperature, carbon and nitrogen sources for mycelial growth of *M. procera* were investigated. Consequently, the optimum pH and temperature for mycelial growth were found pH 6.0 and 25°C, respectively. Whereas, the lowest mycelial growth was

determined at 15 and 30°C. The best carbon source for mycelial growth was glucose. Study results also indicated that yeast extract and peptone were the most suitable nitrogen sources for mycelial growth. On the other hand, the poorest mycelial growth was noted in lactose and C-free medium among carbon sources and in $(NH_4)_2HPO_4$ and NH_4NO_3 among nitrogen sources. Thus, this study provided basic and valuable information on the most suitable culture conditions for mycelial growth of *M. procera*. However, more comprehensive studies on the effect of carbon and nitrogen ratio, macro and micro elements, amino acids, vitamins, phytohormones on mycelial growth in this mushroom species may be recommended. In conclusion, gaining of basic information on optimum environmental and nutritional conditions for mycelial growth of *M. procera* is very important to be able to cultivate it successfully.

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