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Research article

Investigations on the Cultivation of Wild Edible Mushroom *Macrolepiota procera*

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Abstract. *Macrolepiota procera* is a mushroom collected from the nature during usually in spring and autumn in Turkey and also a delicious mushroom widely consumed. In this study, artificial cultivation possibility of *M. procera* was investigated. As a first step, 4 different cereal grains such as barley, wheat, oat and millet were tested to determine the most suitable materials for spawn production. In the next step, different substrates (commercial compost used in the cultivation of *Agaricus bisporus*, wheat straw, oak leaves, peat and the mixtures of these materials at different ratios) and different treatments (shocking, casing material and different temperatures) were evaluated for the artificial cultivation of *M. procera*. In the result of the study, wheat was determined as the most suitable material for spawn production of *M. procera*. The mycelial growth of this mushroom has been succeeded in the substrates prepared from wheat straw, peat, oak leaf, wheat straw and peat mixtures, oak leaf and peat mixture and oak leaf and wheat bran mixtures. However, fruiting bodies has not been obtained from all tested substrates and treatments. The results of this study revealed basic information for the further researches on cultivation of *M. procera* in Turkey.

Yabani Yenebilir Mantar *Macrolepiota procera*'nın Yetiştiriciliği Üzerine Araştırmalar

Anahtar kelimeler:
Macrolepiota procera, misel gelişimi, tohumluk misel, kültüre alma, yetiştirme ortamı

Özet. *Macrolepiota procera* Türkiye'de genellikle ilkbahar ve sonbahar aylarında doğadan toplanan ve aynı zamanda yaygın olarak tüketilen lezzetli bir mantardır. Bu çalışmada yenebilir doğa mantarı *M. procera*'nın kültüre alınabilme olanakları araştırılmıştır. İlk adım olarak, tohumluk misel üretimine en uygun sardırma materyalini belirlemek için buğday, arpa, yulaf ve darı gibi 4 farklı tahıl taneleri test edilmiştir. Bir sonraki adımda, *M. procera*'nın yetiştiriciliği için farklı yetiştirme ortamları (*Agaricus bisporus* yetiştiriciliğinde kullanılan ticari hazır kompost, buğday samanı, meşe yaprakları, torf ve farklı oranlarda bunların karışımları) ve değişik uygulamalar (şoklama, örtü toprağı ve farklı sıcaklıklar) değerlendirilmiştir. Çalışma sonucunda, buğday *M. procera*'nın tohumluk misel üretimi için en uygun sardırma materyali olarak belirlenmiştir. Mantarın misel gelişimi buğday samanı, torf, meşe yaprağı, buğday samanı ve torf karışımı, meşe yaprağı ve torf karışımı ile meşe yaprağı ve kepek karışımından hazırlanan yetiştirme ortamlarında sağlanmıştır. Bununla birlikte, bu çalışmada ele alınan tüm yetiştirme ortamları ve uygulamalarda mantar oluşumu sağlanamamıştır. Bu çalışmanın sonuçları, Türkiye'de *M. procera*'nın yetiştiriciliği konusunda yapılacak daha ileri araştırmalar için bazı temel bilgileri ortaya koymuştur.

INTRODUCTION

Macrolepiota procera (Scop. ex Fr.) Singer, commonly called the Parasol Mushroom, is an edible saprophytic mushroom. It belongs to phylum Basidiomycota, order Agaricales and family *Agaricaceae*. *M. procera,* which grow as alone or small scattered groups, forms fruiting bodies during late summer and autumn on soil surface in forests, pastures, meadows, lawns, roadsides, parks and gardens in temperate regions. *M. procera* has a very large and stately sporocarp. The cap is about 10 to 30 cm in diameter and has a beautiful snakeskin pattern. The cap is egg-shaped or spherical in the early stage of its development and gradually opens at maturity. When it is fully developed it resembles a parasol. The basic cap colour is grayish brown and the surface of cap is covered with shaggy brown scales with a white background. The middle of cap is convex and dark brown. The gills are crowded, remote from the stipe and white, but pinkish in matured fruiting bodies. The stipe is slender, hollow, cylindrical, long (10-20 cm) and grayish brown. The stipe reaches full height before the cap has expanded and its bottom part is swollen. The annulus is thick, tough, persistent and movable. The spores are oval or ellipsoidal, smooth and 15-20 × 10-13 µm in size. The spore print is creamy white. The flesh is thin, soft and white.

M. procera was formerly known as *Lepiota procera*. It commonly grows and consumed in Europe, North America, Asia and North Africa (Vellinga 2003; Vellinga e*t al*., 2003). This mushroom is highly appreciated due to its delicious and delicate texture, good taste, pleasant smell and faint nutty aroma of the cap. *M. procera* is relatively rich in proteins, minerals, vitamins and carbohydrates, contains high amounts of dietary fiber and has also low fat content and good medicinal value (Falandysz *et al*., 2001; Barros *et al*., 2007; Ouzouni and Riganakos 2007; Falandysz *et al*., 2008; Kuldo *et al*., 2014; Kumari and Atri 2014). This mushroom is edible and of excellent quality but only its cap can be used because its stem is very fibrous, tough and inedible. The cap has to be cooked before eaten. Nevertheless, it should be carefully consumed because *M. procera* resemble in appearance poisonous species such as *Chlorophyllum molybdites*, some *Amanita* and *Macrolepiota* species.

M. procera is much sought after and a fairly common species in Turkey. It has been reported that this mushroom has been distributed in different regions of Turkey (Sesli and Denchev 2014). *M. procera* is widely consumed by the public, of economic importance and sold at the local markets in the Black Sea Region of Turkey which it has highly mild and rainy climate (Pekşen and Karaca 2000; Pekşen *et al*., 2008; Pekşen and Kibar 2016). However, the commercial cultivation of this mushroom is not yet available in Turkey and it is only collected from nature during the fructification seasons.

Cultivation of edible mushrooms generally involves three principal steps. The first step is the production of mycelial starter culture. The second stage is the preparation of spawn. The last step is the determination of compost to produce fruiting bodies (Jonathan and Adeoyo 2011). *M. procera* can decompose agricultural wastes such as straw, sawdust and bran, since it is saprophyte (Jones *et al*., 2004). *M. procera* is cultivated in some countries (Shim *et al*., 2005; Kwon and Thatithatgoon 2004; Thawthong *et al*., 2014). There are few studies on mycelial growth of *M. procera* in Turkey (Pekşen and Kibar 2008; 2016). To our knowledge, there is no research carried out on the cultivation of *M. procera* in our country. Therefore, the objective of the present study was to determine the most suitable media for spawn production and to evaluate different substrates (commercial compost used in the cultivation of *A. bisporus*, wheat straw, oak leaf, peat and their mixtures in different ratios) and various treatments (shocking, casing soil, different temperatures) for cultivation of wild edible mushroom *M. procera*.

MATERIALS AND METHODS

The present study was conducted in the mycelial production laboratory and mushroom growing room of Department of Horticulture, Faculty of Agriculture, Ondokuz Mayıs University, Samsun, Turkey, between 2006 and 2009.

Collection, Identification and Isolation of M. procera

The sporocarps of *M. procera* (Figure 1a) were collected from a mixed deciduous forest at the campus of Ondokuz Mayıs University, Samsun, Turkey in autumn 2006. Identification of *M. procera* was done using conventional methods (Phillips 1994). The pure mycelial cultures of *M. procera* (Figure 1b) was obtained by tissue culture method (Jonathan and Fasidi 2003). For this purpose, the tissue pieces isolated from the internal part of the cap were transferred to Malt Extract Agar (MEA) medium and fungal cultures were incubated at 25 °C in the dark. Stock cultures were stored at 4 °C and subcultured every three months for further studies.

Figure 1. Sporocarps (a) and pure culture (b) of *M. procera* and views from mushroom production experiments (c).
Şekil 1. M. procera'nın sporokarpları (a) ve saf kültürü (b) ve mantar üretim denemelerinden görüntüler (c).

Spawn Production

To determine the best cereal grain for spawn production of *M. procera*, 4 different cereal grains such as barley, wheat, oat and millet were tested. In order to prepare the spawn, each cereal grain was washed, boiled for 15 min, filtered through a screen to drain the excess water. The moisture content of the spawning media was around 60%. After cooling, to prevent sticking to each other of grains and to adjust the pH value the mixture of gypsum:lime (4:1, on the basis of dry weight) added to the spawning media. The media were filled into 250 mL bottles, the mouth of each bottle was sealed with a cotton plug and covered with aluminium foil. The bottles were autoclaved at 121 °C for 30 min, allowed to cool and aseptically inoculated with two mycelial plugs (5 mm in diameter) of *M. procera*. The inoculated bottles were then incubated at 23±2 °C in the dark until full colonisation. The experiment was performed in a completely randomized design with 6 replications. In the experiment, linear mycelial growth (cm) on the 5, 11, 20 and 31th days after inoculation was determined. In addition to, the number of days from inoculation to time that bottle completely covered by mycelium was recorded as spawn run period (day).

Preparation of Substrates and Mushroom Production Experiments

Mycelial growth in the substrates prepared from commercial compost, straw and their mixtures with peat

Substrate formulations used in the experiment are given in Table 1. Commercial compost used in the cultivation of *A. bisporus* was obtained from the MÜPA company. In the substrates prepared using straw, all materials were weighed. Straw was wetted with water until its moisture content reached up to 70%, the other materials were added to the substrate and the mixture

was homogenized. The prepared substrates were filled into the heat-resistant polypropylene bags (28×40 cm) with 1 kg wet substrate per bag. Afterwards, the mouth of bags was sealed with a cotton plug and covered with aluminium foil. Then, the bags were sterilized at 121 °C for 1.5 h, cooled down to room temperature and inoculated with spawn (0.7% of the wet weight of the substrate) in sterile conditions. In substrates used commercial compost, peat was sterilized at 121 °C for 1.5 h before being added to the mixture. The heat-resistant polypropylene bags were filled with 1 kg of substrates. The inoculation were made by spreading spawn (0.7% wet weight) on the surface of the substrate in bags. The top of substrates was covered with paper and moistened from time to time to prevent drying. The inoculated bags were placed in mushroom growing room and incubated at 24±2 °C under dark conditions until fully mycelial colonization of the substrate. The experiment was carried out in a completely randomized design with 16 replications. Ash, carbon (C), nitrogen (N) contents and C:N ratios of substrates were determined. In the experiment, the mycelial growth (cm) was determined by measurements made every five days after mycelium inoculation. The spawn run period (day) was expressed as days from the inoculation to completed mycelial colonization in the bags (Figure 1c).

Mycelial growth in different substrates

In another experiment carried out in jars, mycelial growth in the substrates prepared by using commercial compost (unused or spent) and peat was investigated. Spent commercial compost used in this experiment was stored in the open for a year after *A. bisporus* production. The substrates used spent commercial compost and peat were wetted with water until its moisture content was about 70%. The prepared substrates were transferred into the small jars of 250 g. Except for unused commercial compost, all the substrates were sterilized at 121 °C for 1.5 h. Mycelial inoculation with spawn 0.7% of the wet weight of the substrate were made to upper part of the jar under steril conditions. After inoculation, the jars were maintained at 24±2 °C under dark conditions. The experiment was replicated 6 times for each substrate. In the experiment, the spawn run period was determined as mentioned above (Figure 1c).

Mycelial growth in the substrates prepared from straw, straw and peat mixtures

In the previous experiments, the mycelial growth in the commercial compost used for the production of *A. bisporus* could not be obtained. Therefore, the commercial compost was not used as the substrate in the following experiments and the substrates shown in Table 2 were evaluated in this experiment. The preparation of substrates, sterilization, inoculation (spawn at the rate of 0.7%) and incubation were performed as described above. But, the heat-resistant polypropylene bags (20×30 cm) were filled to be 0.5 kg with the substrates. The experiment was carried out in a completely randomized design with 16 replications. In the experiment, moisture, pH, ash, C, N contents of substrates were determined and their C:N ratios were calculated. The linear mycelial growth in the bags was identified by measuring the observable progression of mycelia into the substrate every three days. In addition, the spawn run period was detected (Figure 1c).

Table 1. Substrates prepared from commercial compost, straw and their mixtures with peat and their contents.
Çizelge 1. Ticari hazır kompost, saman ve onların torf ile karışımlarından hazırlanan yetiştirme ortamları ve içerikleri.

Substrates	Contents
Straw	Wheat straw, 0.5% urea, 1% lime, 2% gypsum, 0.2% $MgSO_4$, 4% wheat bran
Straw:Peat (1:1)	Wheat straw, 0.5% urea, 1% lime, 2% gypsum, 0.2% $MgSO_4$, 4% wheat bran: peat (1:1, w/w)
Straw:Peat (2:1)	Wheat straw, 0.5% urea, 1% lime, 2% gypsum, 0.2% $MgSO_4$, 4% wheat bran: peat (2:1, w/w)
Commercial compost	Compost used for cultivation of *A. bisporus*
Commercial compost:Peat (1:1)	CC:P (1:1, w/w)
Commercial compost:Peat (2:1)	CC:P (2:1, w/w)

CC: Commercial compost, P: Peat.

Table 2. Substrates prepared from straw, straw and peat mixtures and their contents.
Çizelge 2. Saman ile saman ve torf karışımlarından hazırlanan yetiştirme ortamları ve içerikleri.

Substrates	Contents
Straw	Wheat straw, 0.5% urea, 1% lime, 2% gypsum, 0.2% $MgSO_4$, 4% wheat bran
Straw:Peat (1:1)	Wheat straw, 0.5% urea, 1% lime, 2% gypsum, 0.2% $MgSO_4$, 4% wheat bran: peat (1:1, w/w)
Straw:Peat (1:2)	Wheat straw, 0.5% urea, 1% lime, 2% gypsum, 0.2% $MgSO_4$, 4% wheat bran: peat (1:2, w/w)

Mycelial growth in different substrates prepared from oak leaf and wheat bran mixtures

Oak leaves used in this experiment were collected from the area where *M. procera* mushroom grows naturally at the campus. Firstly, three different substrates prepared from oak leaf, peat and oak leaf:peat (1:1) mixture were examined. Prepared mixtures were wetted with water until its moisture content reached up to 70%. Thereafter, the substrates were filled into the heat-resistant polypropylene bags (20×30 cm), with 300 g wet substrate per bag. The bags were sterilized at 121 °C for 1.5 h, cooled and inoculated using 2% spawn. The inoculated bags were moved to mushroom growing room and incubated at 24±2 °C in absence of light until the completion of mycelial growth on substrate. The experiment was set in a completely randomized design with 10 replications. Measurements for mycelial growth were made every two days. Also, the spawn run period was determined.

Secondly, wheat bran at the rate of 10, 20 and 30% was supplemented to oak leaf (Table 3). Oak leaf and wheat bran mixtures were prepared and wetted with water. The same substrate formulations were subjected to fermentation for 5 days. For fermentation, substrates were moistened, stacked and covered. The substrates were mixed daily for 5 days and composted. Filling of the bags, inoculation and incubation were performed as previously mentioned. The experiment was replicated 10 times for each substrate. Mycelial growth in the bags was measured every three days and the spawn run period was also determined in the experiment.

Mushroom production (fructification) experiments

After the bags were fully colonized by the mycelium, various treatments were made to promote mushroom formation. In this period, cold was applied to half of the bags at +4 °C for 48 hours. Casing soil was laid on half of the bags that shocking was made and not made. The bags belonging to these treatments were exposed to different room temperatures (15, 18 and 24 °C) in order to induce fruiting body formation. During this process, relative humidity in the mushroom production room was 80-90% and lighting was made for 8 hours a day. Irrigation and ventilation in the production room were monitored daily. During the experiments, hygienic measures against diseases and harms were taken, when necessary chemical fight was performed.

Statistical Analysis

The data obtained from these experiments were subjected to analysis of variance using SPSS 10.0 software and results were expressed as mean values. The means showing statistical significance were compared using Duncan's multiple range test.

Table 3. The ratios and abbreviations of substrates prepared from oak leaf and wheat bran mixtures (fermented or not fermented).
Çizelge 3. Meşe yaprağı ve buğday kepeği karışımlarından (fermantasyon uygulanan veya uygulanmayan) hazırlanan yetiştirme ortamlarına ait oranlar ve kısaltmalar.

Substrates	Ratios (w/w)	Abbreviations
Oak leaf:Wheat Bran	90:10	90OL:10B
Oak leaf:Wheat Bran	80:20	80OL:20B
Oak leaf:Wheat Bran	70:30	70OL:30B
Oak leaf:Wheat Bran (Fermented)	90:10	90OL:10B F
Oak leaf:Wheat Bran (Fermented)	80:20	80OL:20B F
Oak leaf:Wheat Bran (Fermented)	70:30	70OL:30B F

RESULTS AND DISCUSSION

The effect of different cereal grains used for spawn production on mycelial growth are showed in Table 4 and Figure 2a. The mycelial growth in wheat and millet was completed on the 31 and 35th days, respectively. However, it was determined that mycelial growth continued in barley and oat on the 35th day. Therefore, spawn run periods in barley and oat were not given. Based on the 5, 11, 20 and 31th days, significant differences were found among different cereal grains in terms of mycelial growth rates ($P<0.01$). On the fifth day, the best mycelial growth (1.76 cm) was obtained from barley, followed by wheat and oat. On the other hand, the best mycelial growth on the 11, 20 and 31th days was recorded in wheat (3.59, 8.25 and 12.28 cm, respectively). When considering mycelial growth rate and spawn run period, wheat was determined to be the most suitable cereal grain for spawn production of *M. procera* (Table 4 and Figure 2a).

In general, cereal grains such as wheat, barley, millet, oat, corn, rye and sorghum grains are used for spawn production in the most cultivated mushroom species (Barreto *et al*., 2008; Elhami and Ansari 2008). These materials used for large-scale spawn production have many important advantages, as they are easily available, cheap and their use is easy. Nwanze *et al.* (2005) reported that spawn grains such as wheat, corn and millet affect carpophore production. In previous studies, different spawning media for various *Macrolepiota* species came to the forefront. Among the seven spawning substrates tested (rice straw, rice bran, rice hull, groundnut hull, sawdust, soybean and red sorghum grains), red sorghum was determined to be the best substrate for spawn production of *M. dolichaula* (Rizal *et al*., 2016). In another study, barley and red sorghum were the most suitable media for spawn production of *M. detersa* (Rizal *et al*., 2014).

Some properties of different substrate formulations investigated for mycelial growth are presented in Table 5. N and ash contents of substrates containing straw and prepared by autoclaving were lower than that of the substrates containing fermented mushroom compost used in the cultivation of *A. bisporus*. Conversely, C contents and C:N ratios in the substrates prepared using straw was found higher than that of the substrates containing fermented mushroom compost.

Table 4. The effect of different cereal grains used for spawn production on mycelial growth.
Çizelge 4. Tohumluk misel üretimi için kullanılan farklı hububat danelerinin misel gelişimine etkisi.

Cereal grains	Mycelial growth on the 5th day (cm)	Mycelial growth on the 11th day (cm)	Mycelial growth on the 20th day (cm)	Mycelial growth on the 31th day (cm)
Barley	1.76a**	3.32ab**	6.08b**	9.64c**
Wheat	1.58a	3.59a	8.25a	12.28a
Millet	0.38b	3.25ab	6.58b	10.83b
Oat	1.05ab	2.72b	5.07c	7.76d

**: Significant at $P<0.01$, Means followed by different letters in the columns are statistically different by Duncan's multiple range test.

Table 5. Some properties of substrates prepared from commercial compost, straw and their mixtures with peat, and the effect on mycelial growth.
Çizelge 5. Ticari hazır kompost, saman ve onların torf ile karışımlarından hazırlanan yetiştirme ortamlarının bazı özellikleri ve misel gelişimine etkisi.

Substrates	Ash (%)	C (%)	N (%)	C:N	The number of bags completed mycelial growth	Spawn run period (day)
Straw	12.59	43.71	1.60	27.27	4	48
Straw:Peat (1:1)	12.10	43.95	1.57	27.92	2	43
Straw:Peat (2:1)	12.40	43.80	1.53	28.63	2	45
Commercial compost	24.99	37.50	3.30	11.35	-	-
Commercial compost:Peat (1:1)	17.72	41.14	2.37	17.38	-	-
Commercial compost:Peat (2:1)	20.20	39.90	3.14	12.69	-	-

-: There is no mycelial growth.

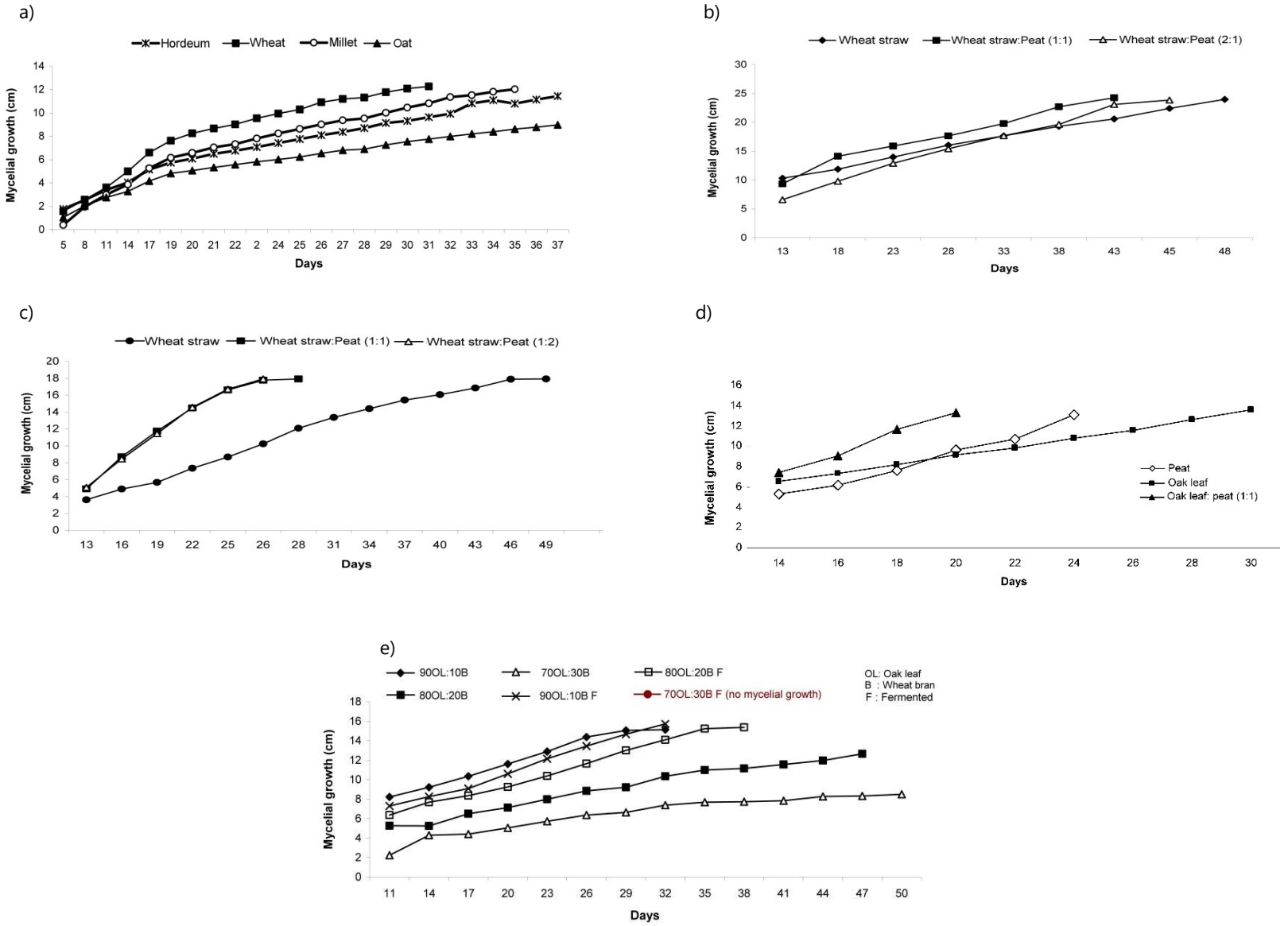

Figure 2. The effect of different cereal grains used for spawn production on mycelial growth (a), mycelial growth of different substrates (b, c, d and e).
Şekil 2. Tohumluk misel üretimi için farklı tahıl tanelerinin misel gelişimi üzerine etkisi (a), farklı yetiştirme ortamlarının misel gelişimleri (b, c, d ve e).

In this experiment, spawn run periods in the substrates prepared using straw ranged from 43 to 48 days. However, no mycelial growth was observed in the substrates containing commercial compost used in the cultivation of *A. bisporus*. Except for 4 bags in straw, 2 bags in straw:peat (1:1) mixture and 2 bags in straw:peat (2:1) mixture, all the bags were infected with *Fusarium poae* pathogen. The number of bags without disease was not found to be sufficient for statistical evaluations. The spawn run period in straw:peat (1:1) was shorter than the others. The spawn run periods were prolonged with the increase amount of straw in the substrate (Table 5 and Figure 2b). But, mycelial growth in straw was more dense according to straw:peat (1:1 and 2:1) mixtures.

In the experiment carried out in jars, spawn run periods in different substrates prepared using commercial compost (unused or spent) and peat were determined between 24 and 39 days. Mycelial growth in the unused commercial compost was observed in only one jar. The mycelial growth and spawn run period in the peat substrate was better and shorter than the other substrates, respectively (Table 6).

Table 6. The effect of substrates prepared using commercial compost (unused or spent) and peat on mycelial growth.
Çizelge 6. Ticari hazır kompost (kullanılmış veya kullanılmamış) ve torf kullanılarak hazırlanan yetiştirme ortamlarının misel gelişimine etkisi.

Substrates	The number of jars completed mycelial growth	Spawn run period (day)
Commercial compost	1	37
Peat	4	24
Spent commercial compost	3	39
Spent commercial compost:Peat	3	28

Moisture contents and pH values of substrates prepared with straw and peat ranged from 74.14 to 78.73% to 6.00 to 7.24, respectively (Table 7). The C:N ratio in the substrate used alone straw was found to be 44.92. The C:N ratios in the substrates prepared by adding peat to straw were about half of that in the straw. The ash and C contents of the substrates were found to rather close to each other. The nitrogen content of straw used in this experiment (0.99%) were found to be lower than that of the previous experiment (1.60%). This is due to the different N contents of the straws supplied from different locations. It is reported that the suitable mycelial growth of *M. procera* was obtained in a wide range of pH 5-8. In addition, its mycelial growth was the best at pH 7 (Shim *et al*., 2005). Jonathan (2002) stated that *Lepiota procera* grew best at pH 6.5. The pH values of substrates in the experiment are within these limits. The effect of substrates prepared with straw and peat on mycelial growth of *M. procera* was significant ($P<0.01$). The mycelial growth was observed at all the substrates examined in this experiment. When compared with substrates prepared from straw and peat (1:1 and 1:2) mixtures, the substrate used alone straw had slower the mycelial growth and longer the spawn run period. The mycelial growth in the straw and peat mixtures (1:1 and 1:2) was quite similar (Table 7 and Figure 2c).

As seen in Table 8, the moisture content of peat was higher than that of substrates prepared from oak leaf and oak leaf:peat (1:1) mixture. This is owing to the high water holding capacity of the peat. The pH contents of substrates varied between 5.37 and 5.48. Shim *et al*. (2005) suggested that *M. procera* can grow at a wide range of pH values (5-8), although the optimum pH for the mycelial growth was pH 7.0. Chang and Miles (1989) stated that mushroom production was influenced by pH of substrate. The mycelia of *M. procera* colonized in all the substrates within 20-30 days following inoculation. The shortest spawn run period was recorded in oak leaf:peat (1:1) substrate, while the longest spawn run period was obtained from the substrate containing only oak leaf. In parallel with this, mycelial growth on the 20th day was the highest in oak leaf:peat (1:1) substrate (13.32 cm) (Table 8 and Figure 2d).

Some chemical properties of substrates prepared with oak leaf, peat and wheat bran are given in Table 9. It was determined that C values of all the substrates examined (49.71-49.89%) were very close to each other. The N contents of substrates increased with the increase amount of wheat bran in the substrates due to the high N content of wheat bran. Depending on increasing in the N amount, the C:N ratios of substrates decreased. Likewise, as the amount of wheat bran in the substrates increases, the P contents of substrates increased. The chemical properties of the different substrates used for the production of mushrooms may influence the mycelial growth.

There were significant differences ($P<0.01$) among substrates shown in Table 10 with regards to mycelial growth on the 20 and 35th day and spawn run period. The best mycelial growth on the 20th day was obtained from not fermented 90OL:10B substrate (11.63 cm). This was closely followed by fermented 90OL:10B substrate (10.61 cm). Compared to mycelial growth on

Table 7. Some properties of substrates prepared from straw, straw and peat mixtures, and the effect on mycelial growth.
Çizelge 7. Saman ile saman ve torf karışımlarından hazırlanan yetiştirme ortamlarının bazı özellikleri ve misel gelişimine etkisi.

Substrates	Moisture (%)	pH	Ash (%)	C (%)	N (%)	C:N	Mycelial growth on the 25th day (cm)	Spawn run period (day)
Straw	78.73	7.24	11.33	44.33	0.99	44.92	8.72b**	50.00a**
Straw:Peat (1:1)	76.64	6.55	11.22	44.39	1.56	28.37	16.31a	27.67b
Straw:Peat (1:2)	74.14	6.00	11.26	44.37	1.67	26.56	16.47a	26.00b

**: Significant at P<0.01, Means followed by different letters in the columns are statistically different by Duncan's multiple range test.

Table 8. Moisture and pH values of substrates prepared from oak leaf, peat and their mixture, and the effect on mycelial growth.
Çizelge 8. Meşe yaprağı, torf ve onların karışımından hazırlanan yetiştirme ortamlarının nem ve pH değerleri ve misel gelişimine etkisi.

Substrates	Moisture (%)	pH	Mycelial growth on the 20th day (cm)	Spawn run period (day)
Peat	68.76	5.37	9.60b**	24b**
Oak leaf	63.54	5.47	9.12b	30a
Oak leaf:Peat (1:1)	65.91	5.48	13.32a	20b

**: Significant at P<0.01, Means followed by different letters in the columns are statistically different by Duncan's multiple range test.

Table 9. Some chemical properties of different substrates prepared with oak leaf, peat and wheat bran.
Çizelge 9. Meşe yaprağı, torf ve buğday kepeği ile hazırlanan farklı yetiştirme ortamlarının bazı kimyasal özellikleri.

Substrates	C (%)	N (%)	C:N	P (%)	K (%)	Ca (%)	Mg (%)	Na (%)	Fe (ppm)	Zn (ppm)
P	49.89	1.34	37.23	0.23	0.16	2.18	0.39	0.06	77.28	8.30
OL	49.71	1.43	36.36	0.26	0.20	1.17	0.34	0.08	659.40	36.35
OL:P (1:1)	49.83	1.55	32.16	0.22	0.16	0.77	0.34	0.05	343.48	26.50
90OL:10B	49.86	1.93	25.83	0.44	0.31	0.68	0.32	0.06	308.83	37.40
80OL:20B	49.86	1.93	25.81	0.94	0.55	0.69	0.35	0.06	225.50	30.50
70OL:30B	49.88	2.29	21.86	1.20	0.49	0.50	0.37	0.05	264.55	41.20
90OL:10B F	49.79	1.83	27.21	0.67	0.36	1.02	0.49	0.07	530.25	40.90
80OL:20B F	49.87	2.07	24.12	0.75	0.06	0.38	0.30	0.05	307.18	39.75
70OL:30B F	49.81	2.38	20.94	1.15	0.57	0.51	0.35	0.06	466.20	79.40

P: Peat, OL: Oak Leaf, B: Wheat Bran, F: Fermented.

Table 10. The effect of substrates prepared from oak leaf and wheat bran mixtures (fermented or not fermented) on mycelial growth.
Çizelge 10. Meşe yaprağı ve buğday kepeği karışımlarından (fermantasyon uygulanan veya uygulanmayan) hazırlanan yetiştirme ortamlarının misel gelişimine etkisi.

Substrates	Mycelial growth on the 20th day (cm)	Mycelial growth on the 35th day (cm)	Spawn run period (day)
90OL:10B	11.63a**	15.32a**	31.10c**
80OL:20B	7.13c	11.00b	45.80a
70OL:30B	5.06d	7.70c	50.00a
90OL:10B F	10.61ab	16.12a	33.50bc
80OL:20B F	9.26b	15.25a	35.90b
70OL:30B F	0.00e	0.00d	0.00d

**: Significant at P<0.01, Means followed by different letters in the columns are statistically different by Duncan's multiple range test, OL: Oak Leaf, B: Wheat Bran, F: Fermented.

the 35th day, the highest mycelial growth was determined in fermented 90OL:10B (16.12 cm), 80OL:20B (15.25 cm) and not fermented 90OL:10B (15.32 cm) substrates. No mycelial growth was observed in fermented 70OL:30B substrate. In addition to, not fermented 70OL:30B substrate showed the lowest mycelial growth on the 20 and 35th days and the longest the spawn run period (Table 10 and Figure 2e). In parallel with decreasing of the C:N ratio in the substrates, the mycelial growth also decreased. The slow or no mycelial growth in 70OL:30B substrate (fermented or not fermented) may be due to high nitrogen content in the substrate (Table 9 and 10). The spawn run periods in the substrates varied from 31.1 to 50.0 days. Philippoussis *et al.* (2001) reported that high nitrogen content in substrate has a negative effect on mycelial growth. The findings related to the spawn run period in this experiment were in agreement with Sharma *et al.* (2008) who reported that the spawn run period was completed in 30-35 days in *M. procera*.

To provide mushroom formation, various treatments (shocking, casing soil, different temperatures) were made in the bags completed mycelial growth. However, fructification in all the substrates and treatments tested in this study could not be achieved (Table 11).

The cultivation of different *Macrolepiota* species was investigated by various researchers. Felgel (2002) investigated cultivation of *M. gracilenta* on different substrates. The rice straw compost was the most suitable substrate for this mushroom. Acoording to Sharma *et al.* (2008), *M. procera* was grown successfully on compost prepared by the short method of composting. Rizal *et al.* (2016) used a composted mixture of rice straw, rice bran, gypsum, calcium carbonate, urea and diammonium phosphate for production of *M. dolichaula*. *M. procera* was cultivated on the substrate containing a mixture of *Agaricus* compost and sawdust (Anonymous 2017). It is reported that *Macrolepiota* species such as, *M. procera*, *M. dolichaula* and *M. gracilenta* are cultivated in Thailand, nowadays (Kwon and Thatithatgoon 2004; Thawthong *et al.*, 2014).

Most cultivable mushrooms have specific requirements for the successful fructification. In general, it is known that mushroom formation is effected by various environmental factors (temperature, humidity, light and aeration), nutritional factors (carbohydrate, nitrogen and vitamins), cultural practices and biotic factors (Sohi and Upadhyay 1989; Zervakis *et al.*, 2001; Boddy et al., 2013). In the present study, the failure of *M. procera* to produce fruiting body despite mycelial colonization may be attributed to the environmental, nutritional and cultural conditions which did not favour for mushroom formation.

Table 11. The substrates and treatments examined for mushroom formation.
Çizelge 10. Mantar oluşumu için ele alınan yetiştirme ortamları ve uygulamalar.

Substrates	**Shocking Casing soil**			**Without shocking Without casing soil**		
	15 °C	18 °C	24 °C	15 °C	18 °C	24 °C
Straw (S)	-	-	-	-	-	-
Straw:Peat (1:1)	-	-	-	-	-	-
Straw:Peat (1:2)	-	-	-	-	-	-
Peat (P)	-	-	-	-	-	-
Oak Leaf (OL)	-	-	-	-	-	-
OL:P (1:1)	-	-	-	-	-	-
90OL:10B	-	*	-	-	*	-
80OL:20B	-	*	-	-	*	-
90OL:10B F	-	*	-	-	*	-
80OL:20B F	-	*	-	-	*	-

*:Treatment has not been made, -: There is no fruiting body, S: Straw, P: Peat, OL: Oak Leaf, B: Wheat Bran, F: Fermented.

CONCLUSION

Wild edible mushrooms are not only delicious foods but also an important source of income for people who collect mushrooms from nature. Cultivation of edible mushrooms supply nutritious food for humans and help in preservation of valuable wild edible species such as *M. procera* for future generation. In addition, it can also provide significant contributions to the country's economy by creating new business opportunities. This study is the first attempt for production of wild edible mushroom *M. procera* in Turkey.

In the current study, the different cereal grains (barley, wheat, oat and millet) were tested for spawn production of *M. procera* and different materials such as commercial compost used in the cultivation of *A. bisporus*, wheat straw, oak leaf, peat and their mixtures at different ratios were investigated for cultivation of *M. procera*. Furthermore, various treatments (shocking, casing soil, different temperatures) were tried to promote mushroom formation. Consequently, wheat was the best cereal grain for spawn production of *M. procera*. The mycelial growth was provided in the substrates prepared from wheat straw, peat, oak leaf, wheat straw and peat mixtures, oak leaf and peat mixture and oak leaf and wheat bran mixtures. However, mushroom formation was not achieved. It is expected that these results may be useful for further investigations on cultivation of this mushroom in our country. Additionally, more detailed studies are needed to be performed on different nutrient sources, substrate materials, growing conditions and cultural practices in order to provide mushroom formation.

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