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PROPOLIS EFFECT ON SCLEROTIAL FORMATIONS OF *MORCHELLA CONICA* PERS.

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Abstract

In this study, the effects of propolis at different concentrations on sclerotial formations of *Morchella conica* Pers., were examined. The propolis used was obtained from three different regions of Turkey. The propolis extracts were prepared at 0.5, 1.0, 1.5 and 2.0 EEP concentrations and they were added to malt extract agar. During the incubation period of 10 days, the mycelium of control group developed rhizomorphic and parallel on the surface of agar medium and there was no pigmentation. On the other hand, the mycelium did not develop normally on the agar medium in the presence of propolis but the sclerotial formations were observed. The sclerotial cells were spherical in form, with thick walls and they were characterised with different pigmentations.

Introductıon

Morchella spp., grows in natural condition in Turkey (Güler,1993). This species can not be grown under the controlled laboratory conditions (Volk & Leonard, 1989, 1990). The developmental stages of the *Morchella* lifecycle was first described by Volk & Leonard (1990) as the vegetative hyphae, sclerotia, primordia and fruiting bodies. These researchers reported that the sclerotia formations form at the negative nature and nutritive conditions and it may germinate miseliogenically at the positive conditions. Sclerotium was formed by mushroom hyphae that was resistant against the negative conditions and it was dormant for a long time (Willets, 1972; Arkan & Güler, 1992).The growing period of the primary mycelium is induced to round up and to form the thick, darkly pigmented walls that are characteristics of sclerotium (Volk & Leonard, 1990; Buscot & Bernillion, 1991; Buscot, 1993).The biology of sclerotia has not yet been explained completely. On the other hand, propolis is a kind of bee product collected mainly from *Populus, Betula, Castanea* plants etc. In many investigations (Burdock,1998; Stangacıu, 1998; Bankova *et al.,* 1995; Yuqiang *et al.,* 1999), the effects of propolis were determined as antiallergic, antimicrobial, antiparasitic, antiseptic, antimicotic, antiviral, local anestesic etc. But there was not any study of propolis in relation to the edible mushrooms.

In the present study, some Turkish propolis collected from various parts of Turkey was added at different concentrations to malt extract agar and its effect on sclerotia formation of *Morchella conica* Pers., was examined.

Materials and Method

Morchella conica Pers., fructifications used were collected from East Anatolia in Turkey. The malt extract agar (MEA) was used as agar medium. The propolis was obtained from Trabzon Bursa and Erzurum cities of Turkey in 1997. Propolis extracts

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were prepared as 0.5 EEP, 1.0 EEP, 1.5 EEP and 2.0 EEP concentrations. For the preparation of propolis extracts about 8.96 ml and 6.7 ml propolis was taken from stock 1 propolis respectively and added to 1.04 ml and 3.3 ml ethyl alcohol (99%) respectively. In this way, stock 2 propolis extracts were obtained as 10 ml. For each group, 0.5 ml and 2.0 ml propolis solution were taken from stock 2 and completed to 100 ml with distilled water prepared (Sorkun *et al*., 1996).

Chemical characterization of the propolis samples were determined by Sorkun *et al*., (2001). The ascospores of *Morchella conica* were inoculated on the MEA by multispore method (Fritsche, 1972) and they were germinated, thus primer mycelium was obtained. The agar discs 8 mm in diameter were taken from the primer mycelium of 5 days. The discs were inoculated at the centre of MEA for control group and the other agar media containing propolis extracts at different concentrations. All groups were incubated in the dark at 22-24°C.

Results

The development of mycelium began after 15 h from inoculation in the control group. Mycelium developed rhizomorphic and parallel to surface of agar media. Mycelium showed anastomosis with one another. The colonisation period was completed at 8 days. No pigmentation was observed. The macroscopic and microscopic developments of mycelium at the control group are shown in Fig. 1.

In the development of mycelium at 0.5 EEP containing agar media, the sclerotial initials were observed at all agar media containing 0.5 EEP. The development of mycelium began after 1 day from inoculation at the propolis of Erzurum and Trabzon; and after 2 days from inoculation at the propolis of Bursa. The mycelium developed as parallel to the surface and regularly at all agars in 2 days, but then the mycelium development stopped, and it did not develop for further 3 days. Then the second development began on 6^{th} day of inoculation again. At the second development, mycelium has not grown regularly. During this period, the mycelium has developed like the age-rings of a tree. The ring started to grow from the centre of mycelium and the density increased gradually. On the other hand, the mycelium developed as miscellaneous aerial hyphae at the propolis of Bursa. The maximum development was observed at the propolis of Erzurum. Yellow-green pigmentation was observed at each of the three groups. The macroscopic and microscopic developments of mycelium at the 0.5 EEP are shown in Fig. 2 and Fig. 3.

In the development of mycelium at 1.0 EEP containing agar media, the sclerotial initials were observed at all agar media containing 1.0 EEP. The development of mycelium began after 1 day from inoculation at the propolis of Erzurum and Trabzon; and 6 days from inoculation at the propolis of Bursa. The mycelium developed regularly in 2 days, but it stopped on 3^{rd} day of inoculation at the propolis of Erzurum and Trabzon and 8^{th} day of inoculation at the propolis of Bursa. The mycelium did not develop for 3 days. Then the development began again. At the second development, the mycelium increased their density and developed as circular at the propolis of Trabzon. The maximum development was observed at the propolis of Erzurum. In this group, the mycelium developed as miscellaneous and light circular. At the propolis of Bursa; the mycelium was thinner and more regularly than the other groups. The pigmentation was observed in each group as the centre was yellow and the vicinity was green. The macroscopic and microscopic developments of mycelium at the 1.0 EEP are shown in Fig. 4 and Fig. 5.

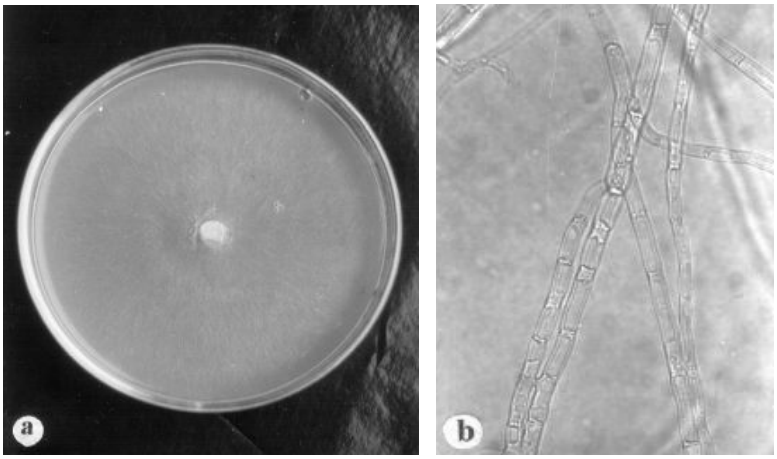
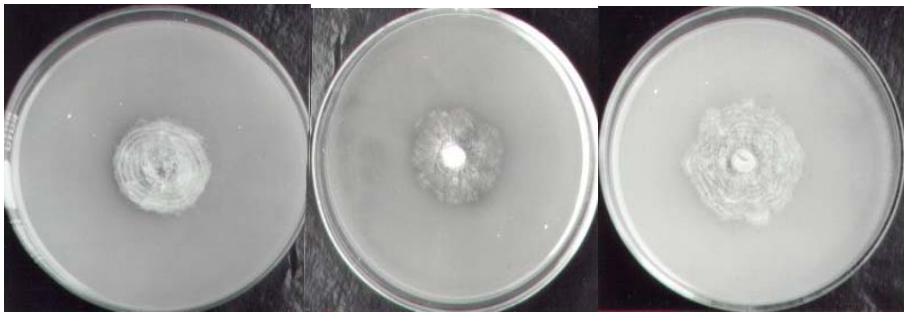


Fig. 1. The development of mycelium at the control group:
a- macroscopic, b- microscopic (40X)



TRABZON **BURSA** **ERZURUM**

Fig. 2. The macroscopic development of mycelium at the 0.5 EEP of three different propolis.

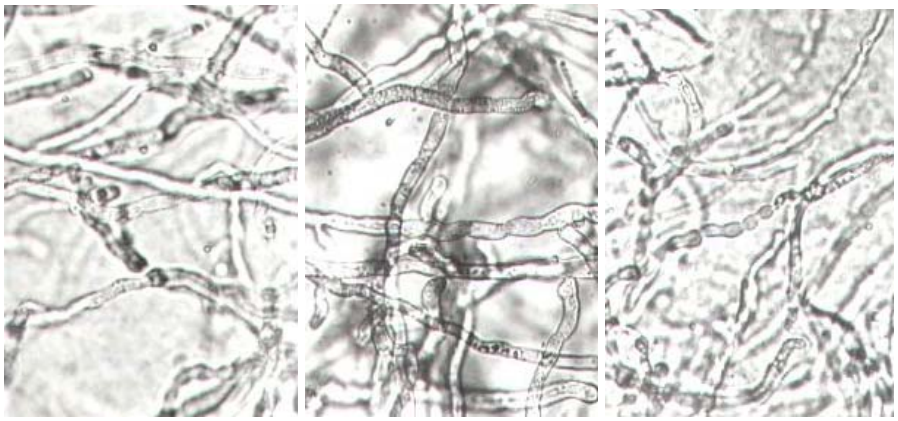


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Fig. 3. The microscopic development of mycelium at the 0.5 EEP of three different propolis (40X).

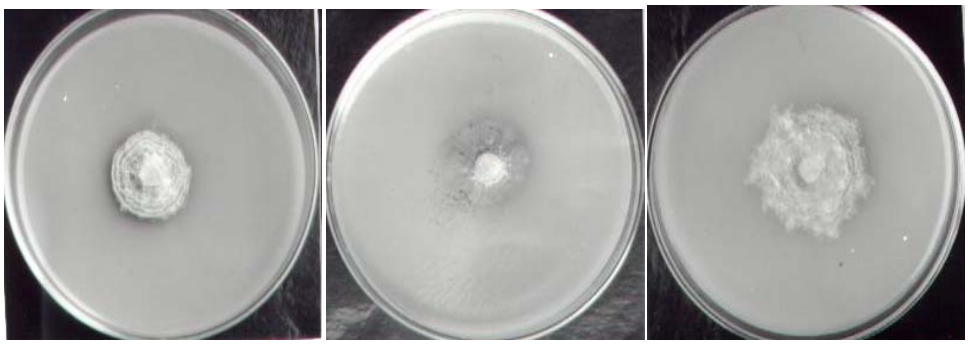

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Fig. 4. The macroscopic development of mycelium at the 1.0 EEP of three different propolis.

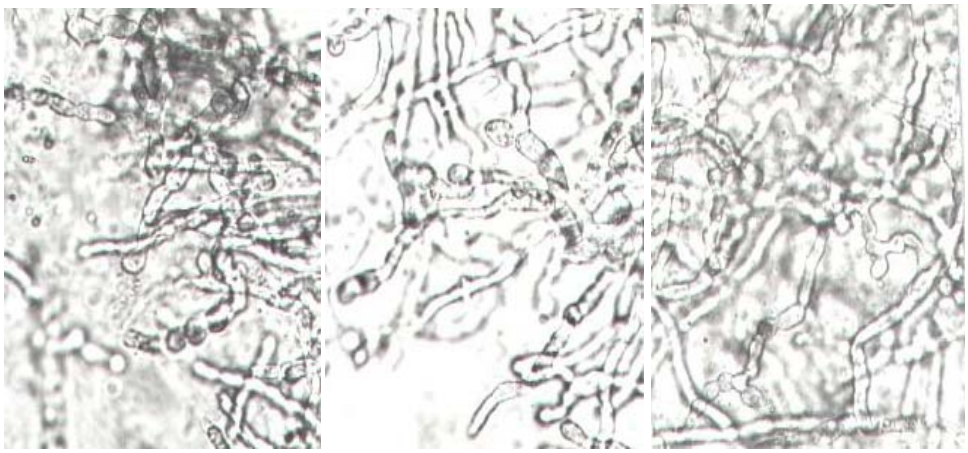

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Fig. 5. The microscopic development of mycelium at the 1.0 EEP of three different propolis (40X).

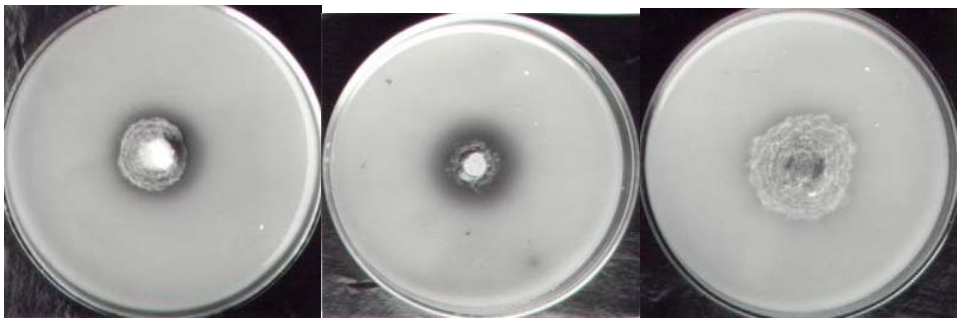

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Fig. 6. The macroscopic development of mycelium at the 1.5 EEP of three different propolis.

In the development of mycelium at 1.5 EEP containing agar media, the sclerotial initials were observed at all agar media containing 1.5 EEP. The development of mycelium began after 1 day, 7 days and 6 days from inoculation at the propolis of Erzurum, Trabzon and Bursa, respectively. At the propolis of Erzurum; the mycelium has developed regularly for 2 days along, but stopped on 3rd day of inoculation. After 3 days, the second development started. At this second developmental period the mycelium did not develop regularly. During this period, the mycelium developed as circular from centre and the density of mycelium increased. The pigmentation was dark yellow on the centre and the vicinity was light green. At the propolis of Trabzon, the density of mycelium increased at the beginning. The mycelium has developed as light circular and the purple pigmentation was observed. Furthermore at the propolis of Bursa, the mycelium developed very slowly in comparison to the other groups. The pigmentation was more dense and dark purple in colour. The macroscopic and microscopic observations were shown in Fig. 6 and Fig. 7.

In the development of mycelium at 2.0 EEP containing agar media, the development of mycelium began after 2 days from inoculation at the propolis of Erzurum. The mycelium developed regularly for 2 days along, but stopped on 4th day of inoculation and the mycelium did not develop for 3 days along. Then the second development started. At this second developmental period the mycelium was not regularly. The mycelium developed like the age-rings of a tree. The density of mycelium increased at the centre. The pigmentation was yellow at the centre and the vicinity was light brown. No development was observed at the propolis of Bursa. At the propolis of Trabzon the mycelium developed very slowly in comparison to the propolis of Erzurum. In this group, light purple pigmentation was observed. But no scleriotal initial was observed neither at propolis of Bursa nor that of Trabzon. The macroscopic and microscopic studies are shown in Fig. 8 and Fig. 9.

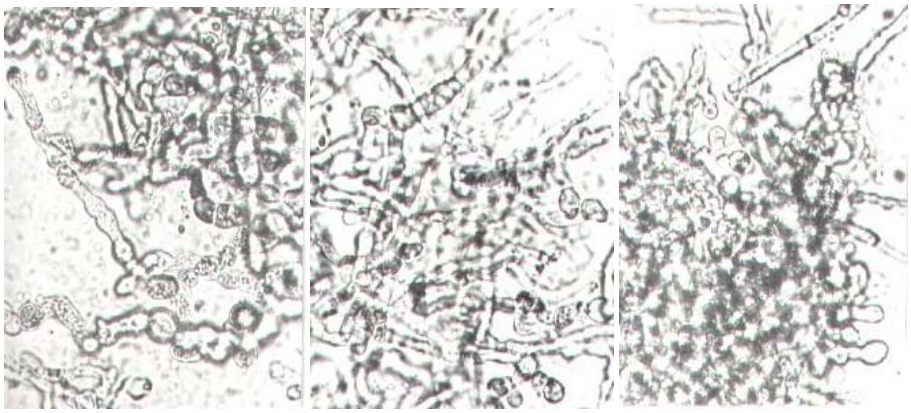
Discussion

In this study, the effects of propolis at different concentrations on sclerotial formations of *Morchella conica* Pers., were determined. Propolis samples were obtained from three regions (Bursa, Erzurum and Trabzon) of Turkey. The propolis solutions were prepared as 0.5, 1.0, 1.5 and 2.0 EEP and they were added to malt extract agar. During 10 days incubation period; the mycelium developed as regularly, parallel to the surface of agar and rhizomorphic at the control group. On the other hand, the mycelium formed the sclerotial initials in all EEP applied groups.

At the propolis of Erzurum; the mycelium has developed after only 1 day from inoculation 0.5, 1.0 and 1.5 EEP while it has developed after 2 days from inoculation at the group of 2.0 EEP. In their microscopic observations; both development of hyphae and sclerotial initials were observed especially at the lower concentrations of propolis.

At the propolis of Bursa the development of mycelium was not observed only first 2 days of inoculation at 0.5 EEP while the development of mycelium was not observed at the first 6 days of inoculation at 1.0 and 1.5 EEP. But no development of mycelium was observed at 2.0 EEP throughout the experimental period.

At the same period with the propolis of Trabzon, the development was observed normally at 0.5 and 1.0 EEP while the development was not observed at the first 7 days of inoculation at 1.5 EEP. No development was determined at 2.0 EEP. The pigmentation increased prominently at the beginning of sclerotial formation during 10 days of incubation period in all groups treated with propolis. Our results showed that the development was slower at the higher concentrations of propolis. This fact is due to



TRABZON **BURSA** **ERZURUM**

Fig. 7. The microscopic development of mycelium at the 1.5 EEP of three different propolis (40X).

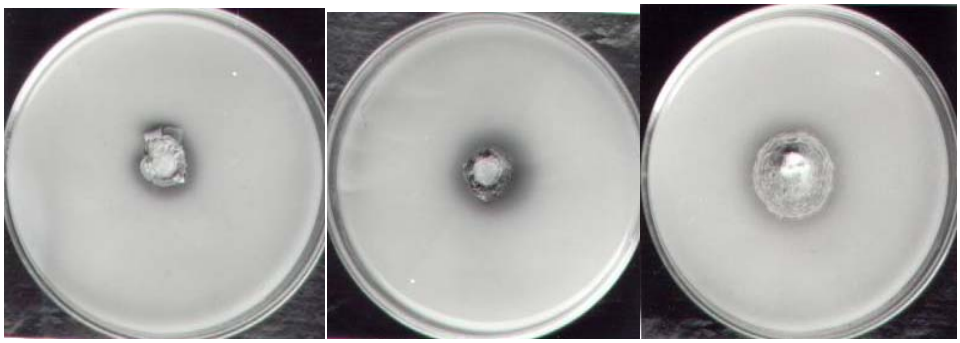


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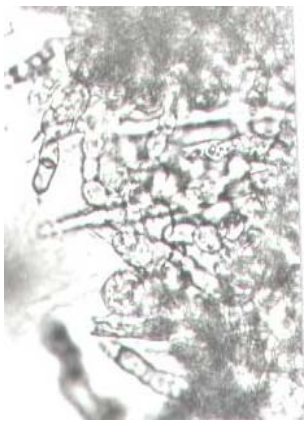
Fig. 8. The macroscopic development of mycelium at the 2.0 EEP of three different propolis.



ERZURUM

Fig. 9. The microscopic development of mycelium at the 2.0 EEP of Erzurum (40X).
Note: Sclerotial initials were not observed at propolis of Bursa and Trabzon.

differences in the chemical composition of the propolis samples. According to chemical characterization Erzurum propolis has very high aromatic acid esters (31.86 %) and very low flavanoids content (4.72%) than other samples (Sorkun *et al*., 2001).

Arkan *et al*., (1997) reported that; the development of mycelium for *Morchella conica* was not obtained at 2.5, 5.0 and 7.5 EEP.

Buscot (1993) showed that two kinds of sclerotia exist in morel and he reported that the first kind of sclerotia of morel: 'early, encrusting sclerotia', which tended to aggregate into circular crust, rapidly became pigmented and formed 2 day after inoculation. The second kind of sclerotia of morel: 'late, isolated sclerotia', began to form 12^{th} day after inoculation and their central part became pigmented while their margin remained growing.

Amir *et al*., (1993) used noble agar and potato dextrose agar for sclerotial formations in their study and they reported that sclerotia of *M.esculenta* can be defined as pseudosclerotia of the lateral type.

Ower (1982); Ower *et al*., (1988) reported that the sclerotial formations was the critical point at the fructification of morel. Willett (1972), Güler (1993), Volk & Leonard (1989, 1990), Arkan and Güler (1992); showed that the sclerotial formations of *Morchella* started from the repeated branching of a terminal hyphae and characterised with pigmentation and thick walls. Volk & Leonard (1989) reported that, if conditions become unfavourable for further growth the heterokaryotic hyphae may form a sclerotium.

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