This article is cited as: Allı, H.(2023). Effects of Olive Mill Wastewater on Mycelial Growth of Some Macrofungi. *Mantar Dergisi* 14(2), 55-59.

Geliş(Recevied) :02.06.2023 ***Research Article***
Kabul(Accepted) :26.07.2023 **Doi: 10.30708.mantar.1308983**

Effects of Olive Mill Wastewater on Mycelial Growth of Some Macrofungi

Hakan ALLI[1*]

** Corresponding Author: hakanalli@gmail.com*

[1] Muğla Sıtkı Koçman University, Science Faculty, Biology Department, Muğla - TURKİYE / hakanalli@gmail.com

Abstract: Olive oil mills produce a liquid waste called olive black water in the olive oil production process. Olive mill waste water from factories specifically in Turkey and surrounding Mediterranean countries where olive oil is produced the most in the world is being disposed of into the environment such as seas and rivers without any precautions being taken. It was focused on determination of effect of olive mill waste water which containing high organic matter on mycelial growth of several macrofungi in this study. It was found that the mycelia of *Rhodonia placenta*, *Trametes versicolor* and *Pleurotus ostreatus* showed the best development in 5%, concentration of olive mill wastewater. On the other hand, *Schizophyllum commune* mycelia grew best in 10% concentration. The lowest mycelial growth rate for all of the fungal species were observed in 70% concentration. Our aim is to cultivate various mushroom species using blackwater with high organic matter content.

Keywords: Environment, Fungi, Mycelial, Olive, Olive Blackwater

Zeytin Karasuyunun Bazı Makromantarların Misel Gelişimi Üzerine Etkileri

Öz: Zeytin üretimininin dünyada en fazla olduğu ülkemiz ve diğer Akdeniz ülkelerinde bulunan zeytinyağı fabrikaları, üretim sürecinde zeytin kara suyu adı verilen bir sıvı atık üretmektedir. Zeytin karasuyunun deniz ve nehir gibi alıcı su kaynaklarına hiçbir önlem alınmadan atılması ciddi çevre sorunlarına neden olmaktadır. Bu çalışmanın amacı yüksek organik madde içeriğine sahip zeytin karasuyunun bazı makrofungusların misel gelişimine etkisinin belirlenmesidir. *Rhodonia placenta*, *Trametes versicolor* ve *Pleurotus ostreatus* misellerinin gelişimi %5 zeytin karasuyu içeren ortamlarda, *Schizophyllum commune* misellerinin gelişiminin ise %10 zeytin karasuyu içeren ortamlarda yüksek olduğu belirlenmiştir. Tüm mantarlarda en düşük misel gelişimin ise %70 zeytin karasuyu içeren ortamlarda olduğu gözlemlenmiştir.

Anahtar kelimeler: Çevre, Mantarlar, Misel, Zeytin, Zeytin Karasuyu

Introduction

Olive and olive oil are two important agricultural products in Mediterranean countries, and almost 95% of global olive oil is produced and consumed within these countries. Türkiye is also among the most important olive producing countries after Spain, Italy and Greece. With about one million hectares of olive land (approximately 123 million fruit bearing and 43 million non-fruiting olive trees), Türkiye is the second producer of table olives and the fourth in olive oil production in the world (Anonynus, 2018, 2019; Özaltaş et al., 2016). Olives and olive oil are produced primarily in the Aegean and Marmara regions, especially within the boundaries of Aydın, Balıkesir, Bursa, Çanakkale, İzmir, Manisa and Muğla provinces (Şengül et al., 2000).

During olive oil production process, olive mill wastewater (OMW) is also produced as a by-product.

Dispose of OMW to the environment without any precautions, serious environmental problems are encountered. Due to its high organic matter content, discharged black water decreases the dissolved oxygen concentration of natural water resources such as rivers, lakes and seas, very quickly. Therefore OMW becomes very difficult for all the macro and microorganisms to survive in their own habitats (Oruç, 1995). The dark color of the OMW also spoils the bright appearance of the water, and reproduction of photosynthetic aquatic plants and alg populations were decreased, or completely stopped by preventing the penetration of sunlight into the water.

The film layer formed the oil contained in OMW also prevents the penetration of atmospheric oxygen into the water (Şengül et al., 2000). Over time, only anaerobic microorganisms develop in the water and putrefaction begins. At the same time, it also causes soil pollution due to its acidic nature and high salt and phenolic content (Oruç, 2012).

The black water produced during the olive processing process is a highly complex mixture and contains organic and inorganic components as well as polyphenols, tannins, amino acids and other biochemical compounds. It is also known that the resulting water exhibits acidic properties and contains high amounts of organic acids and phenols. The release of these compounds into the environment can lead to serious problems with water resources and soil fertility (Diamantis et al., 2022). OMW is also rich in suspended solids (AKM), pectins, sugar, phenol compounds and vegetable oils. However, its environmental impacts have become more pronounced in recent years due to the significant increase in production in the last 35 years, the small sized and very scattered production facilities, and the direct discharge of OMW into the soil or groundwater. For this reason, the attention paid to the treatment of OMW has increased gradually (Rozzi & Malpei, 1997; Chowdhury et al., 2013; Galanakis, 2017). On the other hand, OMW has a high energy source potential due to the simple and complex sugars and aromatic compounds it contains (Oruç, 2012).

Many studies have been carried out in Türkiye to both reduce the phenol content of OMW and to use it as a waste (Zervakis et al., 1996; Yesilada et al., 1995; Yürekli et al.,1999; Yeşilada et al., 1999; Kahraman &Yeşilada 2001; Aypar et al., 2011; Özcan & Topçuoğlu 2001; Apohan & Yesilada 2017; Cibelli et al., 2017). On the other hand there is not any published report to investigate the effect of OMW on mycelial growth of macrofungi in Türkiye.

This study was conducted to investigate the effect of OMW mycelial growth mycelium oroduction by some fungal species, such as *Trametes versicolor* (L.) Lloyd (Turkish name: Hindikuyruğu), *Pleurotus ostreatus* (Jacq.) P. Kumm. (Turkish name: İstiridye mantarı), *Schizophyllum commune* Fr. (Turkish name: Kımuk), *Rhodonia placenta* (Fr.) Niemelä, K.H. Larss. & Schigel. (Turkish name: Ağaçpeteği) (Sesli ve ark., 2020).

Material and Metod

Mycelia of some macrofungi (Table 1), obtained from Muğla Sıtkı Koçman University Mushroom Research and Application Center (MUMMER), were inoculated to the media containing the OMW at different proportions (Table 1).

Table 1. The species and codes of macrofungi

Fungus code	Type name	Collection
1	*Rhodonia placenta*	MAD-698-R
2	*Trametes versicolor*	MAD-697
3	*Pleurotus ostreatus*	MUMMER-3
4	*Schizophyllum commune*	MUMMER-8

Five liters of OMW were obtained from an olive oil factory from Muğla. The OMW is composed of olive washing waters, olive pulp water, water added to olive paste in the centrifugation step, and water coming from washing extraction plants. The OMW stored at 4°C until used. Test solutions of different concentrations were prepared by adding distilled water to the OMW with the ratios given (Table 2).

Table 2. Experiment codes for the tested concentrations

Solution code	Waste fluid (%)	Distilled water (%)
Control	0	100
A	5	95
B	10	90
C	20	80
D	30	70
E	50	50
F	70	30

Malt extract agar (Merck, Germany) was prepared with each solution (X, A, B, C, D, E, F) as experiment medium. Media pH was adjusted to 6.0. After sterilization of the solutions for 15 minutes in an autoclave, set to 121°C and 1.5psi, they were poured to 10 cm petri plates and left to cool at room temperature. The petri dishes were put in an incubator at 35°C for 24 hours to control the presence of any contamination.

Fungal mycelial discs (6.0 mm) were placed in the centre of petri dishes contain prepared media. Then, the inoculated media were incubated at 28°C. The horizontal growth diameter of each fungus colony were measured with a caliper every 24 hours over a period of 7 days.

Results

The growth rate of fungal isolates were presented in figures 1-4.

The mycelium of *Rhodonia placenta* showed the best growth in A1 (5%) which was followed by B1 (10%), C1 (20%), D1 (30%), E1 (50%), X1 (0%) and F1 (70%) respectively (Fig. 1).

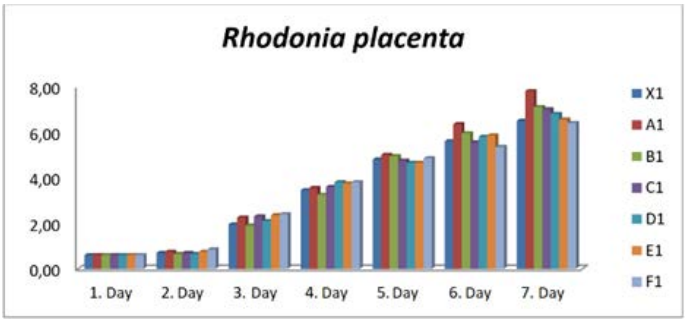

Figure 1. Growth of *Rhodonia placenta* during 7 days

The colony diameter of *Trametes versicolor* was the largest in A2 (5%) which was followed by the others within the order X2 (0%), B2 (10%), C2 (20%), D2 (30%), E2 (50%) and F2 (70%) (Fig. 2).

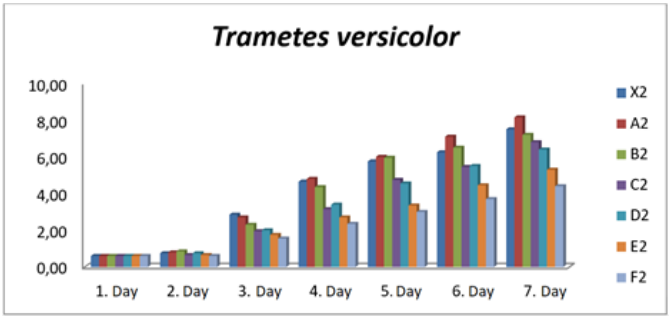

Figure 2. Growth of the fungus of the Turkey Tail (*Trametes versicolor*) over 7 days

Pleurotus ostreatus mycelium showed the most growth in A3 (5%) which was followed by B3 (10%), C3 (20%), X3 (0%), D3 (30%), E3 (50%), and F3 (70%) respectively (Fig. 3).

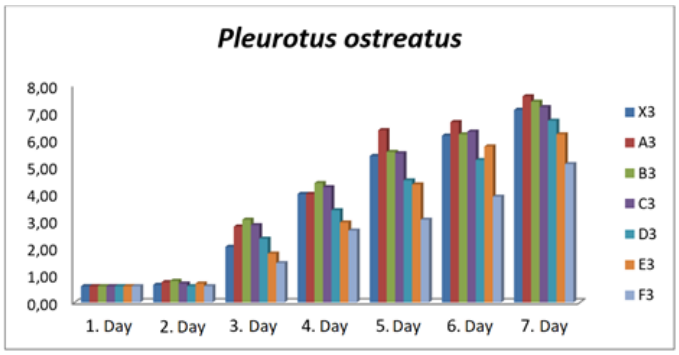

Figure 3. Growth of the fungus of the Oyster Mushroom (*Pleurotus ostreatus*) over 7 days.

The mycelium of *Schizophyllum commune* reached the largest growth diameter in B4 (10%) which were followed by C4 (20%), D4 (30%), E4 (50%), A4 (5%), X4 (0%) and F4 (70%) (Fig. 4).

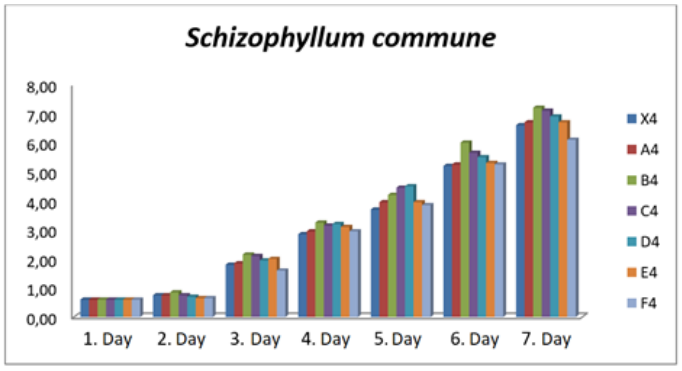

Figure 4. Growth of the fungus of Splitgill (*Schizophyllum commune*) over 7 days.

Discussions

Olive mill wastewater, mostly discharged from the olive oil factories in Türkiye and other Mediterranean countries, pose an important threat to aquatic environments by damaging living things and their ecosystems. In this study, the effect of olive mill wastewater on fungal mycelium growth was investigated.

It was found that, the mycelia of *Rhodonia placenta*, *Trametes versicolor* and *Pleurotus ostreatus* showed the best development in 5% (A) concentration of olive mill wastewater. On the other hand, *Schizophyllum commune* grew best in 10% (B) concentration. The lowest growth rate for all of the fungal mycelia were observed in 70% (F) concentration.

The data obtained for *Trametes versicolor* and *Pleurotus ostreatus* are in agreement with those reported by Galli et al., (1988) and Saiz-Jimmenez and Gomez-Alarcon (1986).

As a result of our study, the positive effect of OMW on fungal mycelial growth was seen as determined as a result of the research.

Since it has a possitive effect on mycelial growth of *Pleurotus ostreatus*, which is popularly known as oyster, beech or poplar mushroom, addition of olive mill wastewater to the compost of *P. ostreatus* as a nitrogen source will allow more efficiency to be obtained. In some countries, *T. versicolor*, *P. ostreatus* and *S. commune* have also been grown for medical purposes, and have been used in capsule form, especially in far east countries (Rogers, R. 2011; Hobbs, C.1986). An increase in the yield of the mushroom produced will also contribute to the economies of the countries in mediterranean basin.

Author Contributions

All authors have equal contribution.

Conflict of Interest

There is no conflict of interest with any institution or person

Ethical Statement: It is declared that scientific and ethical principles have been followed while carrying out and writing this study and that all the sources used have been properly cited (Hakan ALLI).

Acknowledgement

The authors would like to thank to Ferah Yılmaz and Hasan Berk Allı for their help.

References

Anonynus (2018). 2017 Yılı Zeytin ve Zeytinyağı Raporu, Gümrük ve Ticaret Bakanlığı Kooperatifçilik Genel Müdürlüğü, Ankara, Nisan 2018, 29s.

Anonynus (2019). https://webdosya.csb.gov.tr/db/zeytinay/webmenu/webmenu15701. (Erişim:14.05.2023).

Apohan, E., Yesilada, O. (2017). Production of laccase in olive oil mill wastewater and vinasse by immobilized *Trametes versicolor* and *Trametes trogii* with different supports. *Fresenius Environ Bull*, 26, 4261-4267.

Aytar, P., Gedikli, S., Çelikdemir, M., Uzuner, S., Farizoğlu, B., Mesut, Ş. A. M. & Sağlam, N. (2011). Dephenolization of olive mill wastewater by pellets of some white rot fungi. *Hacettepe Journal of Biology and Chemistry*, 39(4), 379-390.

Chowdhury, A. K. M. M. B., Akratos, C. S., Vayenas, D. V. & Pavlou, S. (2013). Olive mill waste composting: A review. International *Biodeterioration & Biodegradation*, 85, 108-119.

Cibelli, F., Bevilacqua, A., Raimondo, M. L., Campaniello, D., Carlucci, Ciccarone, A. & Corbo, M. R. (2017). Evaluation of fungal growth on olive-mill wastewaters treated at high temperature and by high-pressure homogenization. *Frontiers in Microbiology*, 8, 2515.

Diamantis, I., Melanouri, E. M., Dedousi, M., Panagopoulou, I., Papanikolaou, S., Stoforos, N. G. & Diamantopoulou, P. (2022). Sustainable and eco-friendly conversions of olive mill wastewater-based media by *Pleurotus pulmonarius* cultures. *Fermentation*, 8(3), 129.

Galanakis, C. M. (2017). Sustainable Management of Olive Mill Wastewater: Treatment or Valorisation. http://scitechconnect.elsevier.com/sustainablemanagementolive-mill-wastewater/.(Erişim Tarihi: 10/09/2017).

Galli, E., Tomati, U., Grappelli, A. & Buffone, R. (1988). Recycle of olive oil waste for *Pleurotus* mycelium production of submerged culture. *Agrochimica* 32: 451-455

Hobbs, C. (1986). Medicinal Mushrooms: An exploration of Tradition, Healing & Culture, Botanica Press, 415 Farm Road P.O. Box 99 Summertown, Tennessee 38483888-260-8458.

Kahraman, S., Yeşilada, O. (2001). Industrial and agricultural wastes as substrates for laccase production by white-rot fungi. *Folia microbiologica*, 46, 133-136.

Oruç, N. (1995). Aydın İlinde Zeytinyağı İşletmeleri atık suyunun (Karasu) Çevreye Etkisi ve Giderilmesi, *Çevre Sempozyumu*, Bildiri Kitabı, S: 275-282. Atatürk Üniversitesi, Müh. Mim. Fak. Çevre Müh. Bölümü, Erzurum.

Oruç, N. (2012). Zeytinyağı Fabrikası Atığı Karasu Ekolojik Kirlilik Yerine Toprak Düzenleyici olabilir. *SAÜ Fen Fakültesi Dergisi*, (2012-1).

Özaltaş, M., Savran, K.H., Ulaş, M, Kaptan, S. ve Köktürk, H. (2016). *Türkiye Zeytincilik Sektör Raporu*, İzmir, Ekim, 301s.

Özcan, B., Topçuoğlu, S.F. (2001). GA3, ABA and cytokinin production by Lentinus tigrinus and *Laetiporus sulphureus* fungi cultured in the medium of olive oil mill waste. *Turkish Journal of Biology*, 25(4), 453-462.

Rogers, R. (2011). *The Fungal Pharmacy: The Complete Guide to Medicinal Mushrooms & Lichens of North America*, North Atlantic Books, P.O. Box 12327 Berkeley, California 94712 USA.

Rozzi, A., Malpei, F. (1997). Treatment and Disposal of Olive Mill Effuluents, *International Biodeterioration & Biodegradation*, pp, 135-144.

Saiz-Jimmenez, C., Gomez-Alarcon, G. (1986). Effect of vegetarian water on fungal microflora *International Symposion on Olive By-Products Valorition* (ed. FAO), pp. 61-76. Madrid.

Sesli, E., Asan, A., ve Selçuk, F. Abacı Günyar, Ö., Akata, I., Akgül, H., Aktaş, S., Alkan, S., Aydoğdu, H., Berikten, D., Demirel, K., Demirel, R., Doğan, H.H., Erdoğdu, M., Ergül, C.C., Eroğlu, G., Giray, G., Haliki Ustan, A., Keleş, A., Kırbağ, S., Kıvanç, M., Ocak, İ., Ökten, S., Özkale, E., Öztürk, C., Sevindik, M., Şen, B., Şen, İ., Türkekul, İ., Ulukapı, M., Uzun, Ya., Uzun, Yu. ve Yoltaş, A. (2020). Türkiye Mantarları Listesi (The Checklist of Fungi of Turkey). Ali Nihat Gökyiğit Vakfı Yayını. İstanbul. 1177 sayfa.

Şengül, F., Oktay, E. ve Cokay, E. (2000). Zeytinyağı Üretimi Atık sularının Arıtımı ve Yan Ürünlerinin Değerlendirilmesi. *2000 Gap Çevre Kongresi*, Bildiriler Kitabı, Cilt II, S: 713-722, 16-18 Ekim 2000, Şanlıurfa.

Yesilada, Ö., Fiskin, K. & Yesilada, E. (1995). The use of white rot fungus *Funalia trogii* (Malatya) for the decolourization and phenol removal from olive mill wastewater. *Environmental technology*, 16(1), 95-100.

Yeşilada, Ö., Şık, S. & Şam, M. (1999). Treatment of olive oil mill wastewater with fungi. *Turkish Journal of Biology*, 23(2), 231-240.

Yürekli, F., Yesilada, O., Yürekli, M. & Topcuoglu, S. F. (1999). Plant growth hormone production from olive oil mill and alcohol factory wastewaters by white rot fungi. *World Journal of Microbiology and Biotechnology*, 15, 503-505.

Zervakis, G., Yiatras, P. & Balis, C. (1996). Edible mushrooms from olive oil mill wastes. *International Biodeterioration & Biodegradation*, 38(3-4), 237-243.