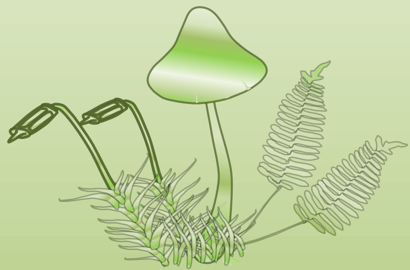
Anatolian Journal of Botany



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The protective role of resveratrol against zinc oxide induced nanotoxicity

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Resveratrolün çinko oksit indüklü nanotoksisiteye karşı koruyucu rolü

Abstract: Zinc oxide (ZnO) is a compound that has harmful effects as well as being used in many different areas. Numerous studies have been carried out to minimize the toxic effects of ZnO nanoparticles (NPs). In the present study, the protective role of resveratrol (RSV), a potent antioxidant polyphenol substance, was examined against ZnO-induced nanotoxicity on human pulmonary alveolar epithelial cells (HPAEpiC). In this context, the cytotoxic and genotoxic effects of different concentrations of RSV (5, 10, 20 mg/L) and ZnO NPs on the cells were measured alone and in combination. At the same time, the effects of aforementioned applications on the total antioxidant capacity (TAC) level in HPAEpiC were assessed. The results obtained showed that ZnO NPs alone significantly increased cytotoxicity and genotoxicity on cells compared to negative control (control (-)). In the experiments performed with RSV + ZnO NP combination, cytotoxic and genotoxic activity decreased at the level of $p < 0.05$ especially at 20 mg/L application of RSV. When the level of TAC in cells was examined, a concentration-dependent increase was detected between TAC and RSV. It was determined that ZnO NPs reduced the TAC level statistically ($p < 0.05$) in comparison with control (-). In conclusion, the present study revealed that RSV, a natural antioxidant, showed protective property against genotoxic and cytotoxic damage induced by ZnO NPs on HPAEpiC.

Key words: Antioxidant, Nanotoxicity, Resveratrol, Zinc oxide

Özet: Çinko oksit (ZnO) birçok farklı alanda kullanılmakla beraber zararlı etkileri de olan bir bileşiktir. ZnO nanopartiküllerinin (NPler) gösterdiği toksik etkileri en az seviyeye indirmek için çok sayıda çalışma gerçekleştirilmektedir. Mevcut çalışmada, güçlü bir antioksidan polifenol madde olan resveratrolün (RSV) insan akciğer alveol epitel hücreleri (İAAEpiH) üzerinde ZnO indüklü nanotoksisiteye karşı koruyucu rolü incelenmiştir. Bu kapsamda, RSV'nin farklı konsantrasyonlarının (5, 10, 20 mg/L) ve ZnO NPler'in hücreler üzerindeki sitotoksik ve genotoksik etkileri tek başlarına ve kombine olarak ölçülmüştür. Aynı zamanda, bahsi geçen uygulamaların İAAEpiH'deki toplam antioksidan kapasite (TAK) düzeyine etkileri değerlendirilmiştir. Elde edilen sonuçlar, ZnO NPler'in tek başına hücreler üzerinde sitotoksisite ve genotoksisiteyi negatif kontrole (kontrol (-)) kıyasla önemli derecede yükselttiğini göstermiştir. RSV + ZnO NP kombineli gerçekleştirilen denemelerde, özellikle RSV'nin 20 mg/L'lik uygulamasında sitotoksik ve genotoksik aktivite $p < 0.05$ seviyesinde düşmüştür. Hücrelerdeki TAK seviyesi incelendiğinde, TAK ile RSV arasında konsantrasyona bağlı bir artış tespit edilmiştir. ZnO NPlerin ise TAK düzeyini kontrol (-)'ye kıyasla istatistiki ($p < 0.05$) açıdan yüksek derecede düşürdüğü belirlenmiştir. Sonuç olarak, mevcut çalışma, doğal bir antioksidan olan RSV'nin İAAEpiH'leri üzerinde ZnO NPleri tarafından indüklenen genotoksik ve sitotoksik hasara karşı koruyucu özellik gösterdiğini ortaya çıkarmıştır.

Anahtar Kelimeler: Antioksidan, Nanotoksisite, Resveratrol, Çinko oksit

1. Introduction

Zinc oxide (ZnO) has very key properties. This compound has many industrial applications due to its unique properties such as high refractive index, high thermal conductivity, antibacterial property and low expansion coefficient (Moezzi et al., 2012). ZnO has proven itself in many sectors such as porcelain, ceramic, rubber, paint, cosmetics, paint, fertilizer, ink and flame retardant. ZnO-containing creams are used to aid to the treatment of sunburn, insect bites and itchiness, rashes and skin irritations (Özgür et al., 2005). On the other hand, side effects of ZnO-containing products can not be overlooked. Especially cosmetic products containing ZnO nanomaterials have a significant risk of absorption. Toxicology studies have shown that nanomaterials can have various side effects on the central nervous system, the immune system and the lungs (Dwivedi et al., 2009; Zhao and Castranova, 2011).

The most important entry and target organ of the nanoparticles (NPs) in the human body is the lungs. It is well known that the lungs are easily exposed to NP materials (Kendall and Holgate, 2012). One of the main mechanisms of lung damage caused by NPs that are formed as a result of burning is the oxidative stress. Brown et al. (2001) reported that there is a significant correlation between the surface area of NPs and the inflammation caused by oxidative stress. It has also been determined that high-size NPs cause genotoxicity (Donaldson et al., 2010). Antioxidants, especially those of herbal origin, play an important role in reducing of oxidative stress and genetic damage in human body (Birben et al., 2012). Resveratrol (RSV) is one of these important natural components. RSV in the phytoalexin group is obtained as a secondary metabolite from different plants. The rate of this compound is especially high in *Vitis vinifera* L. bark (Savouret and Quesne, 2002). RSV with antioxidant activity inhibits the progression of vascular stiffness by inhibiting low density lipoprotein oxidation and reduces lipid peroxidation and reduces lipid peroxidation and production of reactive oxygen species (King et al., 2006).

Based on high antioxidant properties of RSV, in the present study, the toxicity induced by ZnO and the possible beneficial effect of RSV against ZnO-induced cytotoxicity and genotoxicity were investigated.

2. Materials and Method

2.1. NP Synthesis and Characterization

ZnO NPs were synthesized by direct precipitation method using zinc nitrate and KOH as precursors as previously described (Ghorbani et al., 2015). For the synthesis, zinc nitrate hexahydrate ($Zn(NO_3)_2.6H_2O$) and KOH solutions were prepared using deionized water. The KOH was slowly added into $Zn(NO_3)_2.6H_2O$ solution at room temperature under stirring, which resulted in the formation of a white suspension. The white product was centrifuged at 5000 rpm for 25 min and washed three times with distilled water, and one time with absolute alcohol. The obtained product was calcined at 500°C for 4 h. Characterization of NPs UV-Vis spectroscopy was used to prove the existence of nanoparticles. The NPs were characterized by X-ray diffraction (XRD). The average crystallite sizes of the ZnO NPs were calculated from the full width at half maximum of XRD peaks by using Debye-Scherrer's formula and were found to be between 50-100 nm.

2.2. Antioxidant Compound

RSV (Cas: 501-36-0, $C_{14}H_{12}O_3$) that used in this investigation as an antioxidant agent was obtained from Sigma-Aldrich, Germany.

2.3. Cell Cultures and Treatments

Human pulmonary alveolar epithelial cells (HPAEpiC) was provided from American Type Culture Collection (ATCC, USA). The cells were grown and maintained in Alveolar Epithelial Cell Medium (AEpiCM) consists of 500 ml of basal medium, 10 ml of fetal bovine serum, 5 ml of epithelial cell growth supplement and 5 ml of penicillin-streptomycin at 37°C in a 90% humidified incubator with 5% CO_2.

2.4. Testing Cytotoxicity, Genotoxicity and Oxidative Alterations

For 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, the cells were cultured in in 48-well plates. Cells were incubated at 37°C in a humidified 5% CO_2/95% air mixture and treated with ZnO NPs (100 mg/L) and RSV (5 (RSV 1), 10 (RSV 2) and 20 (RSV 3) mg/L) for 72 h (Turkez et al., 2016a; Oztetik et al., 2017). In all assays, dimethyl sulfoxide (DMSO) + relevant cell culture medium (2% DMSO) was used as negative control (control (-)). MTT substrate solution was used according to the manufacturer's instructions (EMD Millipore Corporation, USA). In MTT assays, 1% Triton-X was used as positive control (control (+)).

8-hydroxy-2′-deoxyguanosine (8-OH-dG) assay kits were purchased from Cayman Chemical (Ann Arbor, MI, USA) for determining 8-OH-dG levels in cultures. All procedures were carried out in accordance with the provider’s manual (Turkez et al., 2016b). In genotoxicity assays, mitomycin-C (10^{-7} M) was used as control (+).

The automated Trolox equivalent total antioxidant capacity (TAC) assay was carried out by commercially available kits (Rel Assay Diagnostics, Turkey) (Emsen et al., 2017). In TAC assays, ascorbic acid (20 mg/L) was used as control (+).

2.5. Statistical Analyses

All the assays were carried out at least in triplicate measurements. Protective abilities of antioxidant agent were analyzed using variance (ANOVA) test followed by appropriate post-hoc test (Duncan test) and values with $p < 0.05$ were considered as significantly different. Hierarchical cluster analysis (HCA) with Ward’s minimum variance method was utilized to investigate the similarities and dissimilarities among the cytotoxic effects. All analyses were performed using Statistical Package for Social Sciences (SPSS, version 21.0, IBM Corporation, Armonk, NY, USA).

3. Results

3.1. Cytotoxicity activities

The results of the present study showed that control (+) caused maximum cytotoxicity (85.43%) on HPAEpiC. Next high cytotoxicity (51.79%) value belonged to ZnO NPs. The effects of RSV treatments on viability of the cells were high. Important statistical significances in the reduction of cell viabilities were found in the cultures concomitantly treated with RSV and ZnO NPs as compared to the group ZnO NPs treated alone. Cytotoxic effects of RSV 3 and RSV 3 + ZnO NPs applications were not statistically ($p > 0.05$) different (Figure 1).

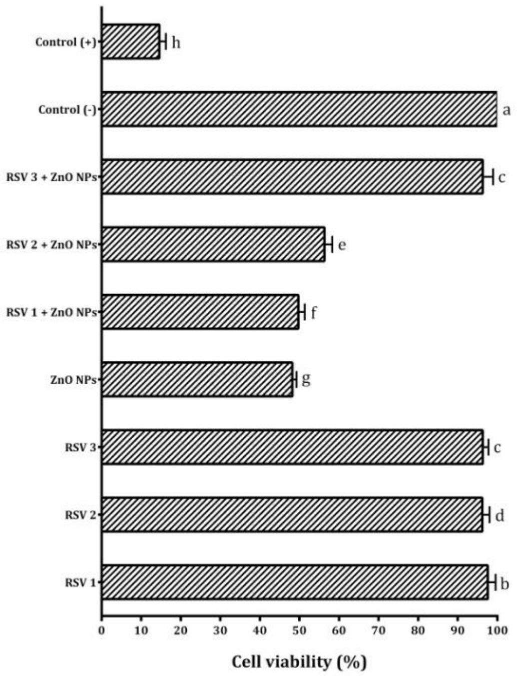

Figure 1. Viability rates in the HPAEpiC exposed to different treatments. Each value is expressed as mean ± standard deviation (n = 3). Values followed by different small letters differ significantly at $p < 0.05$

HCA was performed for cytotoxicity of control groups, RSV and ZnO NPs on HPAEpiC. Cytotoxic activities showed that the nine treatments can be divided into three groups (group A, B and C). Group A was larger than those of groups B and C. Control (+) was single treatment in group C and it had the most distant relationship with the other groups. RSV 3 + ZnO clustered closely together with control (-) in one branch of the dendrogram. These results indicated that cytotoxicity caused by ZnO NPs was highly reduced with RSV 3 treatment (Figure 2).

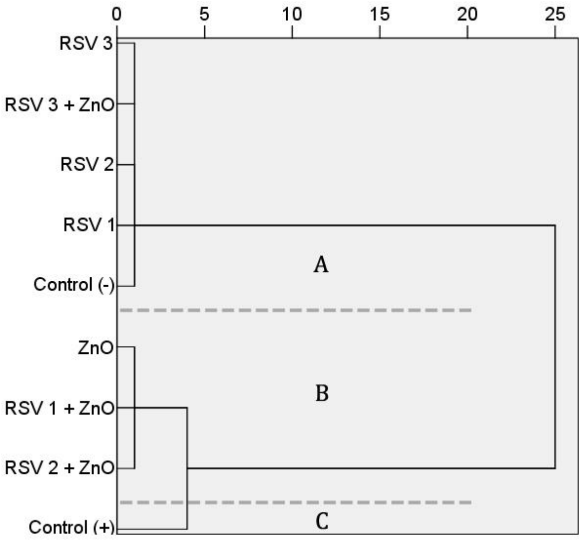

Figure 2. Dendrogram built from cytotoxic effects of different treatments tested on HPAEpiC

3.2. Genotoxicity activities

Oxidative stress induced DNA damage emerged in HPAEpiC by RSV and ZnO NPs was measured with 8-OH-dG level occurring in the cells. It was observed that control (+), RSV 2 and RSV 3 applications significantly ($p < 0.05$) increased 8-OH-dG concentrations in the cells. RSV 1 was statistically ($p > 0.05$) indifferent from control (-). RSVs significantly decreased 8-OH-dG concentrations in ZnO NPs-treated HPAEpiC in a dose dependent manner (Figure 3).

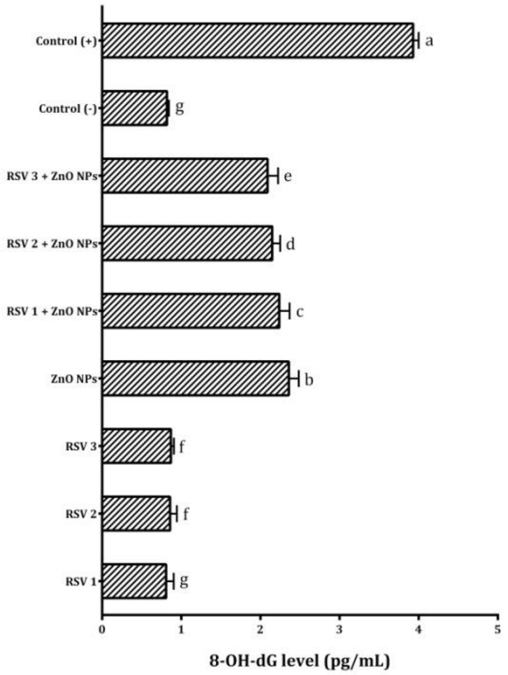

Figure 3. 8-OH-dG adducts in the HPAEpiC exposed to different treatments. Each value is expressed as mean ± standard deviation (n = 3). Values followed by different small letters differ significantly at $p < 0.05$

3.3. Anti-oxidative activities

Antioxidant capacities of different experiments of RSV and ZnO NPs on HPAEpiC were detected with TAC analysis. It was detected that TAC value decreased with the addition of ZnO NPs. Different concentrations of RSV significantly ($p < 0.05$) increased TAC on the cells compared with control (-). TAC levels caused by RSV alone on the cells increased in a concentration-dependent manner. While TAC in HPAEpiC reached the highest level (13.91 mmol Trolox equivalent/L) by ascorbic acid, lowest value (1.13 mmol Trolox equivalent/L) was revealed with ZnO NPs (Figure 4).

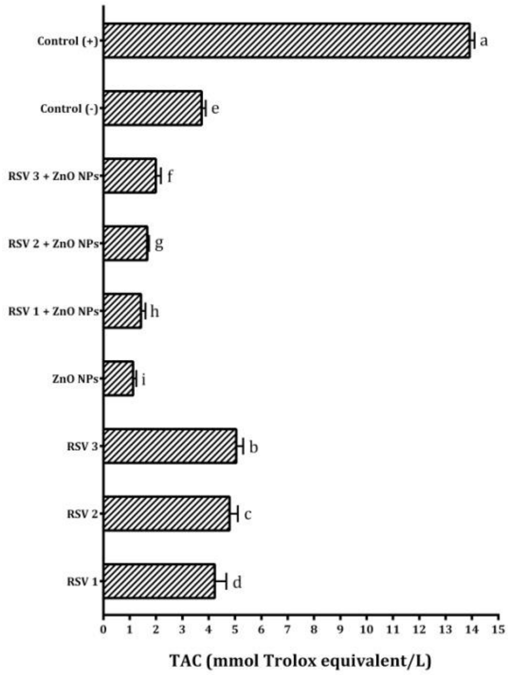

Figure 4. TAC levels in the HPAEpiC exposed to different treatments. Each value is expressed as mean ± standard deviation (n = 3). Values followed by different small letters differ significantly at $p < 0.05$

4. Discussion

There are many studies showing the negative effects of NPs on humans (Tourinho et al., 2012; Fröhlich and Salar-Behzadi, 2014). It has been found that there is a relationship between NPs and respiratory system diseases such as lung cancers, obstructive and interstitial lung diseases (Bonner, 2010). Possible toxic effects of NPs on other organs and systems outside the respiratory system have also been investigated. The toxic effects on the central nervous system are another of these damages. The toxicity that NPs cause as a result of DNA damage disturbs the whole life balance (Glauert et al., 2008). Inhibition of the resulting toxic effects in different areas through natural products is one of the common areas of study (Emsen et al., 2016; Dogan et al., 2017). Especially natural antioxidant components are preferred in this process. Some of these components increase the antioxidant capacity by minimizing oxidative stress-induced damage (Karatas et al., 2015). RSV, one of these important compounds, has been used in many studies. RSV that is a compound in the phenolic structure and has strong antioxidant activity, is used for many treatment process. It was reported that RSV supplementation helped to prevent joint damage by reducing inflation in the body (Hao et al., 2017). It has also been shown that resveratrol may be effective in combating various cancer cells such as liver, breast, pancreas and prostate (Carter et al., 2014).

In the present study, 8-OH-dG level measured in the cells showed that ZnO NPs caused DNA damage in HPAEpiC. At the same time, cytotoxic effects of ZnO NPs were determined. It was detected that cytotoxic and genotoxic activities of these NPs were significantly high. However, RSV supplementation to cultures with ZnO NP increased the viabilities rate of the cells. As shown in dendogram in figure 2, RSV 3 + ZnO treatment was included with control (-) in one branch. So, RSV 3 was the most critical experiment in terms of reducing ZnO NP-induced cytotoxicity. Furthermore, ZnO NP-induced genotoxicity could be reduced significantly by the presence of different concentrations of RSV. In previous studies, it was reported that the cytotoxic and genotoxic activities increased in various cells such as MCF-7 (Elavarasan et al., 2017) and MDA-MB-231 human breast cancer cells (Roshini et al., 2017) treated with ZnO alone. In a research carried out on mouse testicular cells, it was pronounced that ZnO NPs induced apoptosis through DNA damage caused by reactive oxygen species (Han et al., 2016). There are ZnO NPs induced genotoxicity studies in the literature. Kononenko et al. (2017) revealed that ZnO NPs caused genotoxicity *in vitro* in the Madin-Darby canine kidney cells. On the other hand, some investigators reported that high oxidative stress caused by ZnO NPs in the cells generate oxidant injury, reactive oxygen species (ROS) and excitation of inflammation (Xia et al., 2008). The oxidant substances such as free radicals are inhibited through antioxidant compounds. The level of oxidative stress is reduced due to the increase of the antioxidant capacity in the cells (Birben et al., 2012). Our findings were in line with previous reports. The TAC in the tested cells was increased through a natural antioxidant component, RSV.

The findings of this investigation clearly indicated that RSV modulated ZnO-induced genetic damage in HPAEpiC due to its strong antioxidant and detoxifying nature. Therefore, natural antioxidants could be beneficial against heavy metal poisoning including zinc.

Conflicts of interest

There is no conflict of interest in any form between the authors.

References

Birben E, Sahiner UM, Sackesen C, Erzurum S, Kalayci O (2012). Oxidative Stress and Antioxidant Defense. World Allergy Organ J 5: 9–19.

Bonner JC (2010). Nanoparticles as a Potential Cause of Pleural and Interstitial Lung Disease. Proc Am Thorac Soc 7: 138–141.

Brown DM, Wilson MR, MacNee W, Stone V, Donaldson K (2001). Size-Dependent Proinflammatory Effects of Ultrafine Polystyrene Particles: A Role for Surface Area and Oxidative Stress in the Enhanced Activity of Ultrafines. Toxicol Appl Pharmacol 175: 191–199.

Carter LG, D'Orazio JA, Pearson KJ (2014). Resveratrol and Cancer: Focus on *In Vivo* Evidence. Endocr Relat Cancer 21: R209–R225.

Dogan M, Emsen B, Aasim M, Yildirim E (2017). *Ceratophyllum demersum* L. Extract as a Botanical Insecticide for Controlling the Maize Weevil, *Sitophilus zeamais* Motschulsky (Coleoptera: Curculionidae). Egypt J Biol Pest Control 27: 11–15.

Donaldson K, Murphy FA, Duffin R, Poland CA (2010). Asbestos, Carbon Nanotubes and the Pleural Mesothelium: A Review and the Hypothesis Regarding the Role of Long Fibre Retention in the Parietal Pleura, Inflammation and Mesothelioma. Part Fibre Toxicol 7: 5.

Dwivedi PD, Misra A, Shanker R, Das M (2009). Are Nanomaterials a Threat to the Immune System? Nanotoxicology 3: 19–26.

Elavarasan N, Kokila K, Inbasekar G, Sujatha V (2017). Evaluation of Photocatalytic Activity, Antibacterial and Cytotoxic Effects of Green Synthesized ZnO Nanoparticles by *Sechium edule* Leaf Extract. Res Chem Intermed 43: 3361–3376.

Emsen B, Dogan M, Aasim M, Yildirim E (2016). Insecticidal Activity of *In Vitro* Propagated Aquatic Plant *Ceratophyllum demersum* L. against Granary Weevil *Sitophilus granarius* L. (Coleoptera: Curculionidae). Egypt J Biol Pest Control 26: 619–624.

Emsen B, Turkez H, Togar B, Aslan A (2017). Evaluation of Antioxidant and Cytotoxic Effects of Olivetoric and Physodic Acid in Cultured Human Amnion Fibroblasts. Hum Exp Toxicol 36: 376–385.

Fröhlich E, Salar-Behzadi S (2014). Toxicological Assessment of Inhaled Nanoparticles: Role of *In Vivo*, *Ex Vivo*, *In Vitro*, and in Silico Studies. Int J Mol Sci 15: 4795–4822.

Ghorbani H, Mehr F, Pazoki H, Rahmani B (2015). Synthesis of ZnO Nanoparticles by Precipitation Method. Orient J Chem 31: 1219–1221.

Glauert HP, Tharappel JC, Lu Z, Stemm D, Banerjee S, Chan LS, Lee EY, Lehmler HJ, Robertson LW, Spear BT (2008). Role of Oxidative Stress in the Promoting Activities of PCBs. Environ Toxicol Pharmacol 25: 247–250.

Han Z, Yan Q, Ge W, Liu Z-G, Gurunathan S, De Felici M, Shen W, Zhang X-F (2016). Cytotoxic Effects of ZnO Nanoparticles on Mouse Testicular Cells. Int J Nanomedicine 11: 5187–5203.

Hao L, Wan Y, Xiao J, Tang Q, Deng H, Chen L (2017). A Study of Sirt1 Regulation and the Effect of Resveratrol on Synoviocyte Invasion and Associated Joint Destruction in Rheumatoid Arthritis. Mol Med Rep 16: 5099–5106.

Karatas M, Dogan M, Emsen B, Aasim M (2015). Determination of *In Vitro* Free Radical Scavenging Activities of Various Extracts from *In Vitro* Propagated *Ceratophyllum demersum* L.. Fresenius Environ Bull 24: 2946–2952.

Kendall M, Holgate S (2012). Health Impact and Toxicological Effects of Nanomaterials in the Lung. Respirology 17: 743–758.

King RE, Bomser JA, Min DB (2006). Bioactivity of Resveratrol. Compr Rev Food Sci Food Saf 5: 65–70.

Kononenko V, Repar N, Marušič N, Drašler B, Romih T, Hočevar S, Drobne D (2017). Comparative *In Vitro* Genotoxicity Study of ZnO Nanoparticles, ZnO Macroparticles and ZnCl 2 to MDCK Kidney Cells: Size Matters. Toxicol Vitr 40: 256–263.

Moezzi A, McDonagh AM, Cortie MB (2012). Zinc Oxide Particles: Synthesis, Properties and Applications. Chem Eng J 185–186: 1–22.

Oztetik E, Aydin E, Sonmez E, Aydin N, Turkez H (2017). Molecular Genetic Responses in Different Human Primary Cell Cultures Exposed to Aluminum–Zinc Oxide Based Nanoparticles *In Vitro*. J Biotechnol 256: S34–S35.

Özgür Ü, Alivov YI, Liu C, Teke A, Reshchikov MA, Doğan S, Avrutin V, Cho SJ, Morkọ H (2005). A Comprehensive Review of ZnO Materials and Devices. J Appl Phys 98: 1–103.

Roshini A, Jagadeesan S, Cho Y, Lim J, Choi K (2017). Synthesis and Evaluation of the Cytotoxic and Anti-Proliferative Properties of ZnO Quantum Dots against MCF-7 and MDA-MB-231 Human Breast Cancer Cells. Mater Sci Eng C 81: 551–560.

Savouret JF, Quesne M (2002). Resveratrol and Cancer: A Review. Biomed Pharmacother 56: 84–87.

Tourinho PS, van Gestel CAM, Lofts S, Svendsen C, Soares AMVM, Loureiro S (2012). Metal-Based Nanoparticles in Soil: Fate, Behavior, and Effects on Soil Invertebrates. Environ Toxicol Chem 31: 1679–1692.

Turkez H, Geyikoglu F, Yousef MI (2016a). Ameliorative Effects of Docosahexaenoic Acid on the Toxicity Induced by 2,3,7,8-Tetrachlorodibenzo-P-Dioxin in Cultured Rat Hepatocytes. Toxicol Ind Health 32: 1074–1085.

Turkez H, Sonmez E, Di Stefano A, Mokhtar YI (2016b). Health Risk Assessments of Lithium Titanate Nanoparticles in Rat Liver Cell Model for Its Safe Applications in Nanopharmacology and Nanomedicine. Cytotechnology 68: 291–302.

Xia T, Kovochich M, Liong M, Mädler L, Gilbert B, Shi H, Yeh JI, Zink JI, Nel AE (2008). Comparison of the Mechanism of Toxicity of Zinc Oxide and Cerium Oxide Nanoparticles Based on Dissolution and Oxidative Stress Properties. ACS Nano 2: 2121–2134.

Zhao J, Castranova V (2011). Toxicology of Nanomaterials Used in Nanomedicine. J Toxicol Environ Heal - Part B Crit Rev 14: 593–632.

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Ease of Phytochemical Extraction and Analysis from Plants?

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Bitkilerden Fitokimyasal Özütlemenin ve Analizinin Kolaylığı?

Abstract: Plants are vital and sustainable resources for our world. From the ancient times, they are not only supplier of oxygen but also the important part of the food pyramid. Besides, they are sources of thousands of bioactive phytochemicals. With emerging technologies, human being can made synthetic drugs based on phytochemicals to improve their life quality. However, nowadays, Humans have started the search again for natural sources because of emerging new diseases and side effects of drugs. Although lots of chemicals identified and purified from plant materials, it is obvious that there is still more phytochemicals than we discovered especially for foods. Determining full potential nutraceutical value of foods may lead classical breeding or biotechnological studies to more promising area. In concordance, aim of this review is to briefly indicate the bottleneck of analysis of plant phytochemicals by pointing the critical parts of the whole analysis process.

Key words: Phytochemicals, Phenolics, Extraction, Antioxidant Capacity

Özet: Bitkiler, dünyamız için hayati ve sürdürülebilir kaynaklardır. Eski çağlardan beri sadece oksijen tedarikçisi değil aynı zamanda gıda piramidinin önemli bir parçasıdırlar. Ayrıca, binlerce biyoaktif fitokimyasalın kaynağıdırlar. Gelişmekte olan teknolojilerle, insanoğlu fitokimyasallara dayalı sentetik ilaçlar üretebiliyorlar. Ancak günümüzde insanlar ortaya çıkan yeni hastalıklar ve ilaçların yan etkilerinden dolayı doğal kaynakları araştırmak için tekrar arayışa girdiler. Bitki materyalinden tanımlanan ve arıtılmış birçok kimyasal madde olmasına rağmen, özellikle gıdalar için keşfettiklerimizden çok daha fazla fitokimyasalın bulunduğu açıktır. Gıdaların potansiyel nutrasötik değerinin belirlenmesi, klasik ıslah veya biyoteknolojik çalışmaların daha umut verici bir alana gelmesine neden olabilir. Bu amaçla, bu incelemenin amacı, bütün analiz sürecinin kritik kısımlarına işaret ederek bitki fitokimyasal analizinin darboğazını kısaca göstermektir.

Anahtar Kelimeler: Fitokimyasallar, Fenolikler, Özütleme, Antioksidant kapasite

1. Introduction

In addition to being one of the main food sources for humans and animals, plants have been used as the main source of treatment for many diseases throughout history (Cheynier, 2012; Oroian and Escriche, 2015; Shahidi and Ambigaipalan, 2015). The metabolites that the plants produce to protect themselves against biotic and abiotic stresses have turned into medicines that people can use to treat various diseases (Bhattacharya et al., 2010; Elmastas et al., 2017; Galindo et al., 2017; Liu et al., 2017). In ancient times, the treatment is carried out by direct usage or brewing of this plant, on the other hand, currently, the active ingredients are purified and synthetically produced with new technologies (Cheynier et al., 2013; Pezeshkpour et al., 2018).

From past to present day, plants used as natural medicines in the treatment of many diseases. Among these diseases, we can give an example of a wide range from simple injuries to stomach aches to feverish diseases to aging (Bahiense et al., 2017; Dai and Mumper, 2010; Ferhat et al., 2017; Kolakul and Sripanidkulchai, 2017; Li et al., 2018; Mabeku et al., 2017; Marques et al., 2017). In the past years, plants were being used directly and the information about how their mechanisms and dosage were decided by means of trial and error. Moreover the information flow from one generation to next is by means of hearsay. However, with today's technology, active substances can now be identified and purified and effects and dosage can be determined individually (Azmir et al., 2013; Barb a et al., 2016; Bhat and Riar, 2017; Dominguez-Rodriguez et al., 2017; Nadpala et al., 2018; Wang and Zhu, 2017).

The importance of dosage of any substances is indicated by “*Sola dosis facit venenum* “ in Latin which means “the dose makes the poison “ in English. The phrase is the condensed form of Paracelsus statement “All things are poison and nothing is without poison; only the dose makes a thing not a poison " (Siddiqui et al., 2003; Stall et al., 2008). In addition, in 1907, Paul Ehrlich introduced the chemotherapy concept using Arsphenamine, the first synthetic drug, in medical therapy (Siddiqui et al., 2003). With the concept of chemotherapy, dosing was become widespread around the world. We can illustrate the significance of the dose through vitamin C, which everyone knows very well. Everyone can easily mention the benefits of vitamin C, but the less known part is the reversal of the antioxidant properties, especially when taken at high doses, where it acts like free radicals (Oroian and Escriche, 2015). Similar situations apply to all medicines we actually use. In synthetic drugs, the dose concept is determined by bio-accessibility as measuring the absorption level, the serum value and the excretion of the drug. However, it is not possible to carry out a similar research method to measure the bio-accessibility of direct usage of plants. Firstly, the plant has many bioactive substances. Secondly, the absorption of bioactive substances depends on the material of the plant, the way of use and the interactions of bioactive chemicals. Phytochemicals in the plants can exhibit synergistic effect and increase the effect or show antagonistic interaction and reduce the efficiency (Acosta-Estrada et al., 2014; Burgos-Edwards et al., 2017; Celep et al., 2017). Therefore, the usage of plants and/or their extracts, and the phytochemical combinations should be considered carefully.

With the added concern of antibiotic multiple drug resistance and the side effects of synthetic drugs, research has turned to finding new and natural resources. The high potential of plants as having bioactive phytochemicals, decrease in the cost and time of the research for finding, and assessing antioxidant substances have been resulted in focusing on finding and determining the phytochemicals in plants and their usage as natural sources.

Antioxidants prevent damage to lipids, proteins and DNA by means of preventing radicals initiation, breaking chain propagation or suppressing formation of them. Thus, they can prevent deteriorating disease (i.e. cancer) and slowing down the aging process (Bahiense et al., 2017; Iseri et.al., 2011; Kada et al., 2017; Nunes et al., 2017; Oke-Altuntas et al., 2017; Pisoschi and Pop, 2015; Sadi and Sadi, 2010). The phytochemicals having antioxidant activity can be grouped in 3: vitamins, carotenoids and phenolics (Oroian and Escriche, 2015).

Plants are used for food and medical treatment from ancient years. Although, thousands of plant bioactive compound have been determined, purified and used as drug substance, there is still need for further investigation to reveal the full potential of plants especially for foods. Therefore, the aim of this paper was to briefly cover the bottleneck of the discovering new chemicals from plants by focusing on phenolics and their extraction methods and analysis procedures.

2. Phenolics in Plants

Although the phenolic and polyphenolic terms are used as synonymously, the definition of polyphenolic should be as the phenolics that contains at least 2 phenol rings. The definition of polyphenol was first used in 1962 and was later revised in 1994 and 2011 and accepted as "term polyphenol should be used to define the plant secondary metabolites derived from the shikimate derived phenylpropanoid and/or the polyketide pathway(s), featuring more than one phenolic ring and being devoid of any nitrogen-based functional group in their most basic structural expression" (Cheynier, 2012) (Figure 1).

Although the secondary metabolites are not considered as vital metabolites, they help the organism in its survival. Plants produce many phenolic substances as secondary metabolites. However, the type and amount of phenolic substances varies according to plant material, the biotic and abiotic stresses that the plant is experiencing with regard to the physical and biological interaction of the plant with the soil microorganisms, season, and environment (Demir et al., 2014; Elmastas et al., 2017; Galindo et al., 2017; Majdoub et al., 2017; Martini et al., 2017; Mirto et al., 2018; Ojeda-Amador et al., 2018; Petropoulos et al., 2017; Petropoulos et al., 2018; Silva and Sirasa, 2018; Sutay Kocabas et al., 2015; Tupec et al., 2017). Some phenolic species are common and widespread among plants, while some are species-specific. Because, these substances are the metabolites of plants and their production is related to the metabolic activity, thus, their genetic background (Cheynier, 2012; Cheynier et al., 2013) (Figure 2).

The phenolic substance identified from the plants has exceeded 8000 and increasing in number every day (Cheynier, 2012). Although the extraction and analysis of phenolic substances from plants seems to be very easy, it is not true that they can be obtained by a single process or by a single method. As mentioned, the quantity and type of phenolic substances are influenced by many factors, so it is not possible to obtain them by a single method (Acosta-Estrada et al., 2014; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017; Pezeshkpour et al., 2018). Similarly, antioxidant capacity assays cannot be assessed through a single method since they can be water soluble or bound to cell materials such as cell membrane. It was also indicated in literature that bound phenolics have higher antioxidant capacity than water soluble ones (Acosta-Estrada et al., 2014; Dominguez-Rodriguez et al., 2017; Shahidi and Zhong, 2015). Therefore, both for extraction and analysis at least more than one method or combination of methods should be used to precisely determine phenolics.

3. Extraction Methods

Along with the anxiety caused by the side effects of synthetic drugs, recent research has focused on the nutraceutical properties of plants and foods. The most prominent features of these properties are the antioxidant capacities and the most studied and investigated molecules among the antioxidants are phenolic molecules (Acosta-Estrada et al., 2014; Cheynier, 2012; Cheynier et al., 2013).

Obtaining a molecule from a plant takes place in five steps: i) macroscopic matrix pretreatment, ii) molecule separation, iii) molecule extraction, iv) purification, and v) product formation (Galanakis, 2012; Oroian and Escriche, 2015). It should be noted that the most important step is the extraction. Because being easy and cheap at this stage, the organic solvent extraction method is the most cited method in the literature. In addition, pulsed electric fields, ultrasound, high pressure processing methods are cited as more technological and effective processes to increase the yield (Azmir et al., 2013; Barba et al., 2016; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017; Pezeshkpour et al., 2018). Therefore, it will be the best to select the combination of methods and process depending on the plant material and desired molecules.

3.1 Organic Solvent Extraction

The organic solvent extraction phase uses either a single solvent (water, ethanol, ethyl acetate and hexane) as solid-liquid extraction or an aqueous form of these solvents as a two phase extraction method. In general, ethanol is preferred because at the point of consumption of the molecules obtained, ethanol is considered as "Generally Accepted As Safe (GRAS)" according to American Food and Drug Administration (Azmir et al., 2013; Nunes et al., 2017; Oroian and Escriche, 2015). On the other hand, the studies showed that addition of water to the extraction solvent resulted in increase in the yield of phenolic compound extraction. Moreover, it should be indicated here that for carotenoid extraction, acetone or ethyl acetate had higher yield than ethanol (Oroian and Escriche, 2015). Furthermore, acid addition to organic solvent is recommended for anthocyanin extraction. Another important point for extraction is extraction time and temperature. Especially for longer time, the yield decreases because of the oxidation of the phenolics (Dai and Mumper, 2010). Therefore, although aqueous ethanol extraction is widely used and accepted method, it is wise to consider that the interaction between solute and solvent system determines the solubility. Besides conventional solvent extraction, special extraction methods can be performed.

Super Critical Fluid extraction

Super critical fluid extraction is the usage of the fluid at their super critical stage. Accepted as GRAS, CO_2 was the generally used fluid in this method. Although it requires a special equipment, it is fast process and requires less amount of sample, and solvent than conventional solvent extraction (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

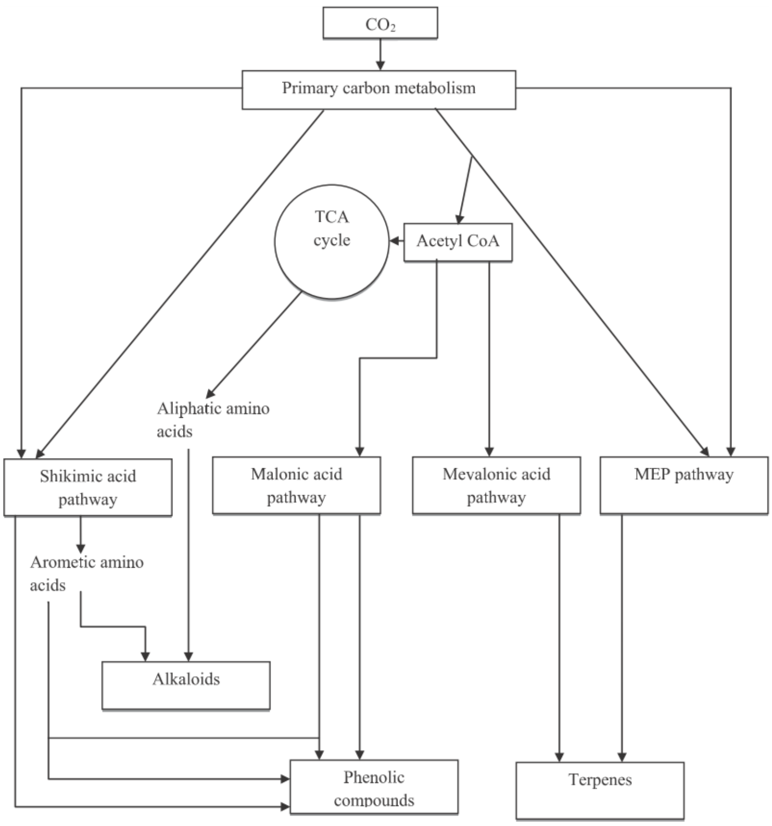

Figure 1. Pathways for production of three major groups of plant bioactive compounds (Azmir et al., 2013).

Subcritical water extraction

It is performed by keeping water in its liquid form at higher degrees of temperature such as 100 – 300 °C by controlling pressure. Therefore, this process reduces water polarity and provides the solubilization of both hydrophobic and hydrophilic molecules. This method also need specialized equipment (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

3.2 Advanced Extraction Techniques

These techniques are generally used to assist the solvent extraction by disrupting the plant materials to increase the yield of phenolics.

Microwave assisted extraction

With controlled pressure and temperature, usage of microwave helps to reduce the time needed for extraction and also decrease the amount of solvent to be used. By this way, it increases the yield of phenolic compounds obtained from plant material (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

Ultrasonic extraction

The process depends on the production of local pressures by making bubbles with ultrasonication. When bubbles exploit, they disrupt the plant cell and cell wall. Therefore, it increases the amount of released phenolic compound (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

Pulsed electric field extraction

It is based on the same principle with electroporation, which give electro-shock to the plant materials. Thus it makes the cell soft and influences cell infrastructure, which resulted in higher yield by increasing the release of phytochemicals (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

Enzyme assisted extraction

This is the most specialized method that uses special enzymes especially for degradation of cell wall. Hence, it causes the release of cell wall bound phytochemicals and reduces the amount of solvent usage (Azmir et al., 2013; Castro-Lopez et al., 2017; Dominguez-Rodriguez et al., 2017).

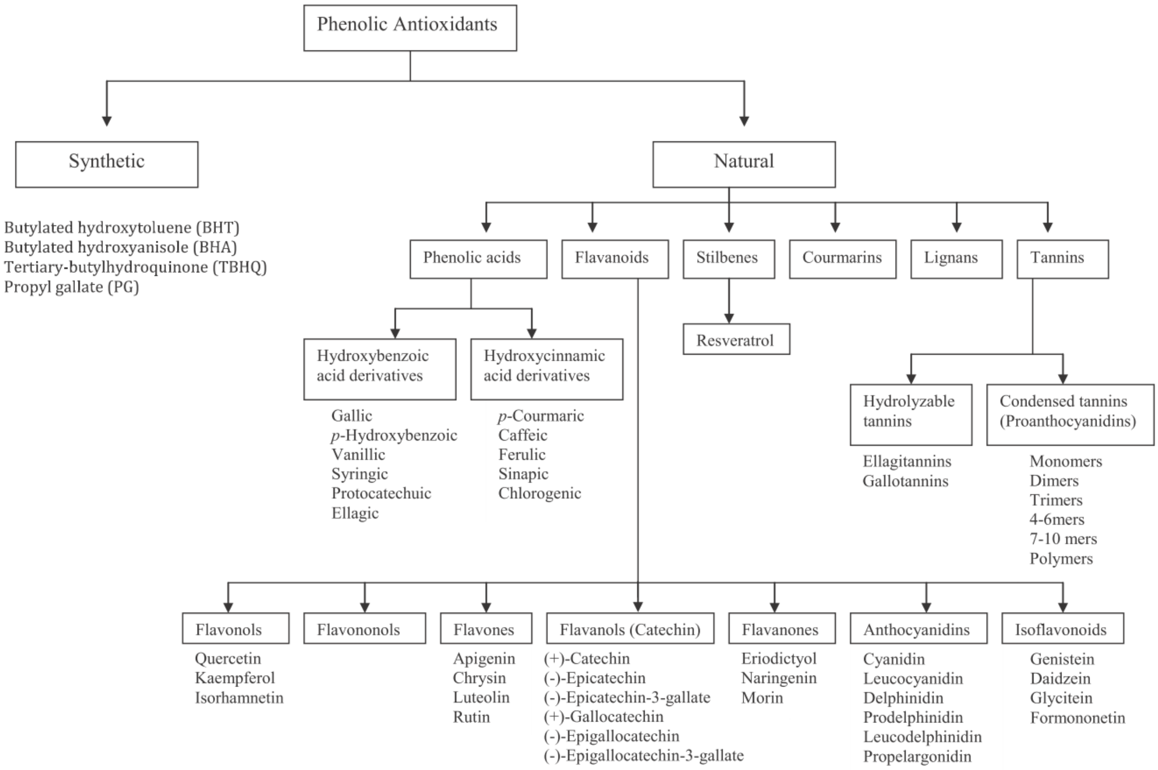

Figure 2. Classification of phenolic antioxidants (Shahidi and Ambigaipalan, 2015)

4. Analysis of phenolic compounds

Analysis on phenolics is performed in three main framework: I) Determination of total phenolic content, II) Determination of antioxidant capacity and III) Optional analysis which can include antimicrobial, anticancer, anti-inflammatory and cytotoxic effects etc.

Although reducing sugars and amino acids (especially ascorbic acid) can react with the Folin-Ciocalteu reagent, the Folin- Ciocalteu assay is widely used and accepted method for total phenolic content determination (Silva and Sirasa, 2018). Besides that, HPLC coupled with the reverse phase C18 is also preferred technique for phenolic content determination and its combination with mass spectroscopy provides chemical analysis of the phytochemicals as both in qualitative and quantitative characterization (Acosta-Estrada et al., 2014; Dominguez-Rodriguez et al., 2017).

On the other hand, for the antioxidant capacity measurement, there are more than one equivalent methods. Conventional methods includes DPPH (1, 1-diphenyl-2-picrylhydrazyl) scavenging activity, ferric reducing/antioxidant power (FRAP), hydroxyl radical-scavenging capacity (HRSCA), Trolox equivalent capacity (TEAC), oxygen radical absorbance capacity (ORAC), cupric ion reducing antioxidant capacity (CUPRAC) assays (Dai and Mumper, 2010; Oroian and Escriche, 2015; Silva and Sirasa, 2018). Although all these five methods are cheap, fast and easy to handle, DPPH and FRAP assays are the most cited and widely used assays for antioxidant capacity measurement. Besides these methods, cyclic voltammetry (especially for beverages), biosensors, chemiluminescent assay, and electron spin resonance spectroscopy are the other methods used for antioxidant capacity determination (Oroian and Escriche, 2015). Although the latter ones are expensive, they are more sensitive than classic methods. It should be noted that all the methods by itself are enough to evaluate the antioxidant effect. Whereas, it should be considered to use more than one method for the precise assessment of to the antioxidant capacity and noted that each analysis has its own standard method.

5. Conclusion

Phytochemical composition of plants can vary depending on the situation that plant experiences at that moment. As a living thing, plants together with their biological conditions react to environmental stimuli. In other words, each factor either autochthonous (biotic) or resulted from environment (abiotic) may cause metabolic changes in the plant. Therefore, development stages of the plant, climatic changes, interaction with microorganisms (either symbiotic or pathogen), injuries, and *etc.* can affect the metabolic composition of a plant. Moreover, since these metabolites have different functions and differ in structure, their location and behavior against a solvent should be distinctive. Thus, pretreatment of plant materials and usage of more than one method based on features of molecules of interest for extraction could help the revealing of plants full potential. Besides, investigation of new methods is required.

References

Acosta-Estrada BA, Gutiérrez-Uribe JA, Serna-Saldívar SO (2014). Bound Phenolics In Foods, A Review. Food Chemistry 152:46–55.

Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM (2013). Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. Journal of Food Engineering 117: 426–436.

Bahiense JB, Marques FM, Figueira MM, Vargas TS, Kondratyuk TP, Endringer DC, Scherer R, Fronza M (2017). Potential Anti-Inflammatory, Antioxidant and Antimicrobial Activities of Sambucus Australis. Pharmaceutical Biology 55:991-997.

Barba FJ, Zhu Z, Koubaa M, Sant'Ana AS, Orlien V (2016). Green Alternative Methods for the Extraction of Antioxidant Bioactive Compounds from Winery Wastes and By-Products: A Review. Trends in Food Science & Technology 49:96e109.

Bhat FM, Riar CS (2017). Extraction, Identification and Assessment of Antioxidative Compounds of Bran Extracts of Traditional Rice Cultivars: An Analytical Approach. Food Chemistry 237:264–274.

Bhattacharya A, Sood P, Citovsky V (2010). The Roles of Plant Phenolics in Defence and Communication during Agrobacterium and Rhizobium Infection. Molecular Plant Pathology 11(5):705–719.

Burgos-Edwards A, Jiménez-Aspee F, Thomas-Valdés S, Schmeda-Hirschmann G , Theoduloz C (2017). Qualitative and Quantitative Changes in Polyphenol Composition and Bioactivity of Ribes Magellanicum and R. Punctatum after In Vitro Gastrointestinal Digestion. Food Chemistry 237:1073–1082.

Castro-López C, Ventura-Sobrevilla JM, González-Hernández MD, Rojas R, Ascacio-Valdés JA, Aguilar CN, Martínez-Ávila GCG (2017). Impact of Extraction Techniques on Antioxidant Capacities and Phytochemical Composition of Polyphenol-Rich Extracts. Food Chemistry 237:1139–1148.

Celep E, İnan Y, Akyüz S, Yesilada E (2017). The Bioaccessible Phenolic Profile and Antioxidant Potential of Hypericum Perfoliatum L. After Simulated Human Digestion Industrial Crops & Products 109:717–723.

Cheynier V (2012). Phenolic Compounds: From Plants to Foods. Phytochem Rev 11:153–177.

Cheynier V, Comte G, Davies KM, Lattanzio V, Martens S (2013). Plant Phenolics: Recent Advances on Their Biosynthesis, Genetics, and Ecophysiology. Plant Physiology and Biochemistry 72:1e20.

Dai J, Mumper RJ (2010). Plant Phenolics: Extraction, Analysis and Their Antioxidant and Anticancer Properties. Molecules 15:7313-7352.

Demir N, Yildiz O, Alpaslan M, Hayaloglu AA (2014). Evaluation of Volatiles, Phenolic Compounds and Antioxidant Activities of Rose Hip (Rosa L.) Fruits in Turkey. LWT - Food Science and Technology 57:126e133.

Domínguez-Rodríguez G, Marina ML, Plaza M (2017). Strategies for the Extraction and Analysis of Non-Extractable Polyphenols from Plants. Journal of Chromatography A, 1514:1–15.

Elmastas M, Demir A, Genç N, Dölek Ü, Günes M (2017). Changes In flavonoid And Phenolic Acid Contents in Some Rosa Species during Ripening. Food Chemistry 235:154–159.

Ferhat M, Erol E, Beladjila KA, Çetintaş Y, Duru ME, Öztürk M, Kabouche A, Kabouche Z (2017). Antioxidant, Anticholinesterase and Antibacterial Activities of Stachys Guyoniana and Mentha Aquatic. Pharmaceutical Biology, 55:324-329.

Galanakis CM (2012). Recovery of high added-value components from food wastes: Conventional, emerging technologies and commercialized applications. Trends in Food Science & Technology 26(2):68-87.

Galindo A, Calín-Sánchez A, Griñán I, Rodríguez P, Cruz ZN, Girón IF, Corell M, Martínez-Font R, Moriana A, Carbonell-Barrachina AA, Torrecillas A, Hernández F (2017). Water Stress At The End Of The Pomegranate Fruit Ripening Stage Produces Earlier Harvest And Improves Fruit Quality. Scientia Horticulturae 226:68–74.

Iseri OD, Körpe DA, Yurtcu E, Sahin FI, Haberal M (2011). Copper-induced oxidative damage, antioxidant response and genotoxicity in Lycopersicum esculentum Mill. and Cucumis sativus L. Plant Cell Reports, 30(9):1713-21.

Kada S, Bouriche H, Senator A, Demirtaş I, Özen T, Toptanci BÇ, Kızıl G, Kızıl M (2017). Protective Activity of Hertia Cheirifolia Extracts against DNA Damage, Lipid Peroxidation and Protein Oxidation. Pharmaceutical Biology, 55:330-337.

Kolakul P, Sripanidkulchai B (2017). Phytochemicals and Anti-Aging Potentials of the Extracts from Lagerstroemia Speciosa and Lagerstroemia floribunda. Industrial Crops & Products 109:707–716.

Li Y, Bao T, Chen W (2018). Comparison of the Protective Effect of Black And White Mulberry against Ethyl Carbamate-Induced Cytotoxicity and Oxidative Damage. Food Chemistry 243:65–73.

Liu B, Zhao S, Tan F, Zhao H, Wang D, Si H, Chen Q (2017). Changes in ROS Production and Antioxidant Capacity during Tuber Sprouting In Potato. Food Chemistry 237:205–213.

Mabeku LBK, Bille BE, Tchouangueu TF, Nguepi E, Leundji H (2017). Treatment of Helicobacter Pylori Infected Mice with Bryophyllum Pinnatum, A Medicinal Plant with Antioxidant and Antimicrobial Properties, Reduces Bacterial Load. Pharmaceutical Biology, 55(1):603-610.

Majdoub N, El-Guendouz S, Rezgui M, Carlier J, Costa C, Kaab LBB, Miguel MG (2017). Growth, Photosynthetic Pigments, Phenolic Content and Biological Activities of Foeniculum Vulgare Mill., Anethum Graveolens L. And Pimpinella Anisum L. (Apiaceae) In Response to Zinc. Industrial Crops & Products 109:627–636.

Marques P, Marto J, Gonçalves LM, Pacheco R, Fitas M, Pinto P, Serralheiro MLM, Ribeiro Cynara H (2017). Scolymus L.: A Promising Mediterranean Extract for Topical Anti-Aging Prevention. Industrial Crops & Products 109:699–706.

Martini S, Conte A, Tagliazucchi D (2017). Phenolic Compounds Profile and Antioxidant Properties of Six Sweet Cherry (Prunus Avium) Cultivars. Food Research International 97:15–26.

Mirto A, Iannuzzi F, Carillo P, Ciarmiello LF, Woodrow P, Fuggi A (2018). Metabolic Characterization and Antioxidant Activity in Sweet Cherry (Prunus Avium L.) Campania Accessions Metabolic Characterization of Sweet Cherry Accessions. Food Chemistry 240:559–566.

Nađpala JD, Lesjak MM, Mrkonjić ZO, Majkić TM, Četojević-Simin DD, Mimica-Dukić NM, Beara IN (2018). Phytochemical Composition and In Vitro Functional Properties of Three Wild Rose Hips and Their Traditional Preserves. Food Chemistry 241:290–300.

Nunes R, Pasko P, Tyszka-Czochara M, Szewczyk A, Szlosarczyk M, Carvalho IS (2017). Antibacterial, Antioxidant and Anti-Proliferative Properties and Zinc Content of Five South Portugal Herbs. Pharmaceutical Biology, 55:114-123.

Ojeda-Amador RM, Fregapane G, Salvador MD (2018). Composition and Properties of Virgin Pistachio Oils and Their By-Products from Different Cultivars. Food Chemistry 240:123–130.

Oke-Altuntas F, Ipekcioglu S, Yaglioglu AS, Behcet L, Demirtas I (2017). Phytochemical Analysis, Antiproliferative and Antioxidant Activities of Chrozophora Tinctoria: A Natural Dye Plant. Pharmaceutical Biology, 55:966-973.

Oroian M, Escriche I (2015). Antioxidants: Characterization, Natural Sources, Extraction and Analysis. Food Research International 74:10–36.

Petropoulos S, Fernandes A, Karkanis A, Ntatsi G, Barros L, Ferreira ICFR (2017). Successive Harvesting Affects Yield, Chemical Composition And Antioxidant Activity Of Cichorium Spinosum L. Food Chemistry 237:83–90.

Petropoulos SA, Fernandes A, Vasileios A, Ntatsi G, Barros L, Ferreira ICFR (2018). Chemical Composition and Antioxidant Activity of Cichorium Spinosum L. Leaves In Relation To Developmental Stage. Food Chemistry 239:946–952.

Pezeshkpour V, Khosravani SA, Ghaedi M, Dashtian K, Zareb F, Sharifi A, Jannesar R, Zoladl M (2018). Ultrasound Assisted Extraction Of Phenolic Acids From Broccoli Vegetable And Using Sonochemistry For Preparation Of MOF-5 Nanocubes: Comparative Study Based On Micro-Dilution Broth And Plate Count Method For Synergism Antibacterial Effect. Ultrasonics - Sonochemistry 40:1031–1038.

Pisoschi AM, Pop A (2015). The Role of Antioxidants in the Chemistry of Oxidative Stress: A Review. European Journal of Medicinal Chemistry 97:55e74.

Sadi G, Sadi Ö (2010). Antioxidants and Regulation of Antioxidant Enzymes by Cellular Redox Status. Turkish Journal and Scientific Reviews 3(2): 95-107.

Shahidi F, Ambigaipalan P (2015). Phenolics and Polyphenolics in Foods, Beverages and Spices: Antioxidant Activity and Health Effects –A Review. Journal of Functional Foods 18:820–897.

Shahidi F, Zhong Y (2015). Measurement of Antioxidant Activity. Journal of Functional Foods 18:757–781.

Siddiqui MA, JMehta N, Khan IA (2003). Paracelsus: the Hippocrates of the Renaissance. Journal of Medical Biography 11: 78-80.

Silva KDRR, Sirasa MSF (2018). Antioxidant Properties of Selected Fruit Cultivars Grown In Sri Lanka Food. Chemistry 238:203–208.

Staal FJT, Pike-Overzet K, Ng YY, van Dongen JJM (2008). Sola Dosis Facit Venenum. Leukemia in Gene Therapy Trials: A Question of Vectors, Inserts and Dosage. Leukemia 22:1849–1852.

Sutay Kocabaş D, Tur E, Kocabas A (2015). Phytochemical Analysis of Some Native Apple Varieties and Valorization of Apple Tree Leaves for Xylanase Production. The Journal of FOOD 40(5):1-8.

Tupec M, Hýsková V, Bělonožníková K, Hraníček J, Červený V, Ryšlavá H (2017). Characterization of Some Potential Medicinal Plants from Central Europe by Their Antioxidant Capacity and the Presence of Metal Elements. Food Bioscience 20:43–50.

Wang S, Zhu F (2017). Chemical Composition and Biological Activity of Staghorn Sumac (Rhus Typhina). Food Chemistry 237:431–443.

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Mineral Contents of Two Wild Morels

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İki Yabani Kuzu Göbeğinin Mineral İçerikleri

Abstract: Mineral (Ni, Cu, Co, Zn, Cr, Mn, Mg, Cd, Fe, Ca and Pb) contents of two wild edible morels, *Morchella deliciosa* Fr. and *Morchella elata* Fr., which are collected and consumed in Gaziantep province, were determined by an atomic absorption spectrophotometer (AAS). Although both of the morelscontained considerable amounts of minerals, all the contents are in the range reported from Turkey.

Key words: Mineral content, mushrooms, Gaziantep, Turkey

Özet: Gaziantep yöresinde toplanıp tüketilmekte olan İki yabani yenilebilir kuzu göbeği türünün, Morchella deliciosa Fr. and Morchella elata Fr., mineral (Ni, Cu, Co, Zn, Cr, Mn, Mg, Cd, Fe, Ca ve Pb) içerikleri atomik absorpsiyon spectrofotometresi kullanılarak belirlenmiştir. Her iki kuzu göbeği de kayda değer mineral içeriğine sahip olmasına rağmen, bütün içeriklerTürkiye'den rapor edilen aralıktadır.

Anahtar Kelimeler: Mineral içerik, mantarlar, Gaziantep, Turkey

1. Introduction

Fungi are an important group of organisms in nature and can be found almost everywhere in terrestrial ecosystems. Some of them with relatively large fruting bodies and varying degrees of edibility are known as mushrooms and have long been used as a source food for human in various cultures. Mushrooms are usually considered as valuable nutrient sources and many of them are also recommended against health problems such as headache, colds, asthma, diabetes etc. (Kalač et al., 1991).

Fruiting bodies of mushrooms are generally known to be rich in mineral contents (Vetter, 1990), because of the environmental factors such as amount of organic matter, pH and metal concentrations of underlying soil (Garcia et al., 1998). Due to such properties, mushrooms are thought to be used to evaluate the level of environmental pollution (Sesli and Tüzen, 1999).

Minerals such as iron, copper, zinc and manganese are essential metals and play important roles in living systems (Tüzen et al., 2007), but they may be hazardous on human if they are taken above threshold concentrations (Olumuyiwa et al., 2007).

Many studies have been carried out ot the metal contents of wild growing mushrooms in many countries. Kalač (2009) published a review about the contents of generally studies elements in fruit bodied of mushrooms. Similar studies were also carried out to determined the mineral contents of naturally growing mushrooms, collected from different regions of Turkey (Işıldak et al., 2004; Türkekul et al., 2004; Sesli et al., 2008; Gençcelep et al., 2009; Uzun et al., 2011; Kaya and Bağ, 2013; Tel et al., 2014; Kaya et al., 2017).

This work aims to determine the mineral contents of the fruiting bodies of two wild morels, *Morchella deliciosa* Fr. and *Morchella elata* Fr.

2. Materials and Method

Dry fruit bodies of *Morchella deliciosa* and *Morchella elat*a (Fig. 1) were obtained from the findings of TOVAG-212T112 which were carried out within the boundaries of Gaziantep province (Turkey). The habitats of the collected samples generally were pine forest, pine-oak mixed forest or pine-fir-cedar mixed forest.

Mushroom samples were prepared for element analysis by following the procedure followed by Khairiah et al. (2004) and Kaçar (1984). First of all the samples were washed with ultrapure water and dried at 80 °C for 8-10 hours. Then the samples were crushed and dried again at same temperature. One g of powdered mushroom samples were put in 50 ml beakers and 15 ml of HNO_3 were added. After waiting 8-10 hours, 4 ml $HClO_4$ were added and heated gently for about 5-6 hours and cooled. Then 5 ml of H_2O_2 was added and heated till the solution is colorless enough. The solution was cooled and distilled water was added on it until the total volume reaches to 10 ml.

Figure 1. Fruit bodies of *M. deliciosa*(a) *and M. elata* (b)

Element analysis of mushroom samples were performed by using flame atomic absorbtion spectrophotometer (FAAS). The absorption measurements of the elements were performed under the conditions recommended by the manufacturer and metal ion concentrations were determined as six replicates.

3. Results

The metal concentrations were determined on dry weight basis. Except Pb, all the minerals were in detectable limits in fruit bodies of both mushrooms. Lead was detected only in *Morchella deliciosa.* The average contents of trace elements in *Morchella deliciosa* samples were 5,05, 14,17, 2,47, 100,1, 0,408, 22,24, 61,09, 1,713, 120, 2036 and 13,69 mg/kg for Ni, Cu, Co, Zn, Cr, Mn, Mg, Cd, Fe, Ca and Pb respectively. The amount of the same minerals within the same order in *Morhella elata* were 9,063, 18,74, 3,353, 95,23, 5,468, 55,54, 61,97, 2,788, 433,6, 1997 and ND. Measured and average concentrations (mg/kg, dry weight basis) of heavy metals in two morels were given in Table 1 and Table 2.

Table 1. Measured and average concentrations of heavy metals in *Morchella elata*

	Ni	Cu	Co	Zn	Cr	Mn	Mg	Cd	Fe	Ca	Pb
Morchella elata	8,835	19,02	3,03	95,49	6,21	51,99	61,61	2,655	440	1976	…
	8,835	19,82	3,18	92,51	5,97	60,06	61,8	2,67	443,7	2120	…
	8,94	19,61	3,81	94,89	6,27	60,11	61,89	2,775	443,6	2133	…
	9,255	18,78	3,405	101	4,695	49,2	62,13	2,865	426,5	1832	…
	9,315	17,6	3,48	94,94	4,905	55,61	62,16	2,82	423,9	1931	…
	9,195	17,61	3,21	92,58	4,755	56,25	62,21	2,94	424,1	1994	…
Mean	9,063	18,74	3,353	95,23	5,468	55,54	61,97	2,788	433,6	1997	…
± SD	±0.218	±0.956	±0.277	±3.080	±0.757	±4.349	±0.239	±0.111	±9.775	±114.7	

Table 2. Measured and average concentrations of heavy metals in *Morchella deliciosa*

	Ni	Cu	Co	Zn	Cr	Mn	Mg	Cd	Fe	Ca	Pb
Morchella deliciosa	4,875	14,64	2,43	101	0,18	22,56	61,19	1,695	120,2	2217	13,16
	4,785	16,37	2,73	104,4	0,54	24,81	61,14	1,65	120,1	2331	13,5
	5,61	16,64	2,595	104,8	0,405	24,98	61,19	1,74	120,3	2267	15,05
	5,085	12,24	2,25	95,34	0,555	19,31	60,96	1,83	116,7	1761	13,73
	5,025	12,41	2,37	97,14	0,405	20,81	61,01	1,785	121,4	1787	13,91
	4,92	12,72	2,445	97,95	0,36	20,96	61,04	1,575	121,6	1851	12,78
Mean	5,05	14,17	2,47	100,1	0,408	22,24	61,09	1,713	120	2036	13,69
± SD	±0.294	±2.003	±0.170	±3.935	±0.137	±2.303	±0.098	±0.093	±1.757	±262.7	±0.779

4. Discussions

Minerals play a vital role in the proper development and health of human body. However, high amounts of certain minerals are also toxic for most organisms (Savas et al., 1995).

In the presented study, although lead was not detected in *Morchella elata*, it was measured as 13.69 mg/kg in *Morchella deliciosa*. The reported Pb values for mushrooms were 0.5–20 mg/kg (Kalač et al., 2001). Common Pb content in many mushroom species from unpolluted sites is below 2 mg/kg, but levels up to 5 mg/kg have been reported for numerous species. Increased levels are common in mushrooms growing around highways. Extremely high Pb levels over 100 mg/kg were observed in the close vicinity of lead smelters (Kalač et al., 2004).

Nickel content ranged from 4.785 (*Morchella deliciosa*) to 9.315 (*Morchella elata*) mg/kg. The reported Ni values for wild-growing mushrooms were 44.6–127, 0.4–15.9, 2.73–19.4, 0.4–2, 8.2–26.7, 1.72–24.1, 44.6–127 mg/kg (Demirbaş, 2001a; Işıldak et al., 2004; Işıloğlu et al., 2001; Kalač et al., 2001; Mendil et al., 2004; Soylak et al., 2005), respectively. Hence, in this study, Ni levels are in agreement with previous studies.

Cadmium levels in morels ranged from 1.575 (*Morchella deliciosa*) to 2.940 (*Morchella elata*) mg/kg. An extremely high content up to 300 mg/kg is reported in the literature (Seeger, 1982). Considerably increased Cd levels were reported in mushrooms growing in the vicinity of metal smelters (Kalač et al., 1996; Svoboda et al., 2000) and within a town (Kalač et al., 2003). It was reported that cadmium is accumulated mainly in kidneys, spleen and liver and its blood serum level increases considerably following mushroom consumption (Kalač et al., 2001). Thus, Cd seems to be the most deleterious among heavy metals in mushrooms (Kalač et al., 2004).

The range of iron concentrations were between 116.7 (*Morchella deliciosa*) and 443.7 (*Morchella elata*) mg/kg in mushroom species. Fe values in mushrooms samples have been reported in the range of 31.3–1190 mg/kg (Kojo et al., 1989), 568–3904 mg/kg (Turkekul et al., 2004), 102–1580 mg/kg (Soylak et al., 2005), 50–842 mg/kg (Gençcelep et al., 2009), and 38.9–499.0 mg/kg (Ouzouni et al., 2009). Observed Fe values are in agreement with those reported in the literature.

The measured manganese content was 19.31 mg/kg in *Morchella deliciosa*, whereas it was 60.11 mg/kg in *Morchella elata*. The reported Mn contents in previous studies for wild-growing mushrooms were 12.9–93.3 mg/kg (Kalač et al., 2000), 5.0–60.0 mg/kg (Tuzen, 2003), 14.5–63.6 mg/kg (Işıldak et al., 2004), 5.5–135 mg/kg (Gençcelep et al., 2009), 7.6–56.2 mg/kg (Demirbaş, 2001b) and 14.5–63.6 mg/kg (Işıloğlu et al., 2001). In this study, Mn levels are in agreement with previous studies. Mn is essential for most physiological functions such as bone and cartilage formation, amino acid, glucose and cholesterol metabolisms and Mn-mediated anti-oxidant enzymes (NAS, 2001).

Zinc has a biological significance for living organisms and mushrooms are known as good zinc accumulators (Işıloğlu et al, 2001). Zn content measured as 92.51mg/kg for *Morchella elata* and 104.8 mg/kg for *Morchella deliciosa*. Content of Zn in mushrooms ranges from 30 to 150 mg/kg (Kalač et al., 2000), 21 to 100 mg/kg (Çayır et al., 2010), and 35 to 136 mg/kg (Radulescu et al., 2010).

Copper were measured (in average) as 12.24 and 19.82 mg/kg in *Morchella deliciosa* and *Morchella elata*, respectively. Kalač and Svoboda (2000) reported that Cu levels in the accumulating species are usually 100–300 mg/kg, which is not considered a health risk. The Cu results of all mushroom species were in agreement with those found in the literature (Chen et al., 2009; Işıldak et al., 2004; Kalač et al., 1996; Svoboda et al., 2000).

Average cobalt contents for two morels were 2.250 and 3.810 mg/kg. The amount of Co was determined as 7.42 mg/kg for *Agrocybe dura* (Kaya et al., 2010), 7.2 mg/kg for Ramaria largentii (Ouzuni et al., 2009) and 5.8 mg/kg for *Agaricus arvensis* (Borovička et al., 2007). The determined Co contents are in agreement with the previous studies.

The determined chromium concentration was 6.27 mg/kg in *Morchella elata*. The Cr concentration was 0.18 mg/kg in *Morchella deliciosa*. Tüzen (2003) reported Cr content as 0.87- 2.66 mg/kg, Sivrikaya et al. (2002) as 7.0-11.0 mg/kg and Kaya et al. (2011) as 0.77-80.03 mg/kg. Because of its ability to increase glucose tolerance in type-II diabetes mellitus patients (Anderson, 2000), Cr is considered essential to man. The recommended dietary intake for chromium is 0.035 mg/day for male and 0.025 mg/day for the female (Anonymous, 2001). Mushrooms could be thought as a potential source of this element.

Minimum and maximum values of magnesium were 60.96 and 62.21 mg/kg. The highest and lowest levels of Mg were found in *Morchella deliciosa* and *Morchella elata*. Demirbaş (2001a) reported the content of this mineral as 330 mg/kg in *Tricholoma anatolicum* and 6560 mg/kg in *Morchella deliciosa*. Turkekul et al. (2004), Sesli et al. (2008) also reported the Mg content within the range of 688 mg/kg and 1150 mg/kg. Compared to earlier published reports the determined levels of Mg is relatively low.

Calcium content ranged from 1761 (*Morchella deliciosa*) to 2331 (*Morchella deliciosa*) mg/kg. Compared to reports of Kalač, (2009) (100-2400 mg/kg) and Sanmeea et al. (2003) (100-2400 mg/kg). The determined Ca contents are in agreement with the previous studies. Gençcelep et al. (2009) reported the Ca content to be 8800 mg/kg for *Lepista nuda*. Compared to earlier published reports the determined levels of Ca is relatively low. Unlike Ca levels in this study are in agreement with the higher value reported in the literature (Lee et al., 2009) (159.8-324.3 mg/kg).

The essential element concentrations (Mn, Fe, Cu, Zn, Ni) in the two morels were determined to be at suitable levels. Kalac et al. (1991) reported that heavy metals such as lead, cadmium and chromium can accumulate in the body and may be harmful for humans after chronic consumption.

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References

Anderson A (2000). Chromum in the preventon and control of diabetes. Diab. Metabol. 26: 22-27.

Anonymous (2001). Food and Nutrition Board (FNB), Dietary Reference Intakes for Vitamn A, Vitamn K, Arsenc, Boron, Chromum, Copper, Iodne, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium and Znc, Washington, DC: Institute of Medicine, National Academy Press, pp. 1-28.

Borovčka - Řanda Z, Jelínek E, Dunn CE (2007). Hyperaccumulaton of slver by Amanta stroblforms and related speces of the secton Lepdella. Mycologcal Research 111: 1339–1344.

Chen XH, Zhou HB, Qiu GZ (2009). Analysis of several heavy metals in wild edible mushrooms from regions of China. Bulletin of Environmental Contamination and Toxicology 83: 280-285.

Çayır A, Coşkun M (2010). The heavy metal content of wild edible mushroom samples collected in Çanakkale Province, Turkey. Biological Trace Element Research 134: 212-219.

Demirbaş A (2001a). Concentrations of 21 metals in 18 species of mushrooms growing in the East Black Sea region. Food Chemistry 75: 453-457.

Demirbaş A (2001b). Heavy metal bioaccumulation by mushrooms from artificially fortified soils. Food Chemistry 74: 293-301.

Genccelep H, Uzun Y, Tuncturk Y, Demirel K (2009). Determination of mineral contents of wild-grown edible mushrooms. Food Chemistry 113: 1033-1036.

Işıldak Ö, Turkekul I, Elmastaş M, Tüzen M (2004). Analysis of heavy metals in some wild-grown edible mushrooms from the middle Black Sea region, Turkey. Food Chemistry 86: 547-552.

Işıloğlu M, Yılmaz F, Merdivan D (2001). Concentrations of trace elements in wild edible mushrooms. Food Chemistry 73: 169-175.

Kacar B (1984). Plant nutrition practice guide. Ankara Univ. Agricultural Fac. Pub: 900, Practice Guides: 214. Ankara, Turkey.

Kalač P (2009). Chemical composition and nutritional value of European species of wild growing mushrooms: A review. Food Chemistry 113: 9-16.

Kalač P, Burda J, Staskova I (1991). Concentrations of lead, cadmium, mercury and copper in mushrooms in the vicinity of a lead smelter. Sci. Total Environ. 105: 109-119.

Kalač P, Nižnanská M, Bevilaqua D, Stašková I (1996). Concentrations of mercury, copper, cadmium and lead in fruiting bodies of edible mushrooms in the vicinity of a mercury smelter and a copper smelter Science of the Total Environment 177: 251-258.

Kalač P, Svoboda L, Havlíčková (2004). Contents of cadmium and mercury in edible mushrooms. Journal of Applied Biomedicine 2: 15-20.

Kaya A, Bağ H (2010). Trace Element Contents of Edible Macrofung Growing in Adıyaman (Turkey). Asian Journal of Chemistry 22: 1515-1521.

Kaya A, Bağ H (2013). Mineral Contents of some Wild Ascomycetous Mushrooms. Asian Journal of Chemistry 25: 1723-1726.

Kaya A, Gençcelep H, Uzun Y, Demirel K (2011). Analysis of trace metal levels in wild mushrooms. Asian Journal of Chemistry 23: 1099-1103.

Khairiah T, Zalifah MK, Yin YH, Aminah A (2004). The uptake of heavy metals by fruit type vegetables grown in selected agricultural areas. Pak. J. Biol. Sci. 7(8):1438-1442.

Kojo MR, Lodenius M (1989). Cadmium and mercury in macrofungi–mechanisms of transport and accumulation. Angewandte Botanik 63: 279-292.

Lee CY, Park JE, Kim BB, Kim SM, Ro HS (2009). Determination of mineral components in the cultivation substrates of edible mushrooms and their uptake into fruiting bodies. Mycobiol 37: 109-113.

Mendil D, Uluözlü ÖD, Hasdemir E, Çağlar A (2004). Determination of trace elements on some wild edible mushroom samples from Kastamonu, Turkey. Food Chemistry 88: 281-285.

National Academy of Sciences (2001). Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc. http://www.nap.edu

Olumuyiwa SF, Oluwatoyin OA, Olanrewaja O, Steve RA (2007). Chemical composition and toxic trace element composition of some Nigerian edible wild mushroom. International Journal of Food Science and Technology 43(1): 24-29.

Ouzouni PK, Petridis D, Koller WD, Kyriakos A, Riganakos KA (2009). Nutritional value and metal content of wild edible mushrooms collected from West Macedonia and Epirus, Greece. Food Chemistry 115: 1575-1580.

Radulescu C, Stihi C, Busuioc G, Gheboianu AI (2010). I.V. Popescu Studies concerning heavy metals bioaccumulation of wild edible mushrooms from industrial area by using spectrometric techniques. Bulletin of Environmental Contamination and Toxicology 84: 641-646.

Sanmeea R, Dellb B, Lumyongc P, Izumord K, Lumyonga S (2003). Nutrtve value of popular wild edible mushrooms from northern Thailand. Food Chemistry 82: 527-532.

Savas H, Kolayli S, Keha E (1995). Copper levels and glutathione reductase activity in workers of Murgul. Turkish Journal of Medical Science 25: 187-188.

Seeger R (1982). Toxische schwermetalle in Pilzen. Deutsche Apotheke Zeitschrift 122: 1835-1844.

Sesli E, Tüzen M (1999). Levels of trace elements in the fruiting bodies of macrofungi growing in the East Black Sea region of Turkey. Food Chemistry 65: 453-460.

Sesli E, Tüzen M, Soylak M (2008). Evaluation of trace metal contents of some wild edible mushrooms from East Black Sea region, Turkey. Journal of Hazardous Materials 160(2-3): 462-467.

Soylak M, Saraçoğlu S, Tüzen M, Mendil D (2005). Determination of trace metals in mushroom samples from Kayseri, Turkey. Food Chemistry 92: 649-652.

Svoboda L, Kalač P (2003). Contamination of two edible Agaricus spp. mushrooms growing in a town with cadmium, lead, and mercury. Bulletin of Environmental Contamination and Toxicology 71: 123-130.

Svoboda L, Zimmermannova K, Kalač P (2000). Concentrations of mercury, cadmium, lead and copper in fruiting bodies of edible mushrooms in an emission area of a copper smelter and a mercury smelter. The Science of the Total Environment 246: 61–67.

Tel G, Çavdar H, Deveci E, Öztürk M, Duru ME, Türkoğlu A (2014). Minerals and metals in mushroom species in Anatolia. Food Additives & Contaminants: Part B 7(3): 226-231.

Turkekul I, Elmastas M, Tüzen M (2004). Determination of iron, copper, manganese, zinc, lead, and cadmium in mushroom samples from Tokat, Turkey. Food Chemistry 84: 389-392.

Tüzen M (2003). Determination of heavy metals in soil, mushroom and plant samples by atomic absorption spectrometry. Microchemical Journal 74: 289-297.

Tüzen M, Sesli E, Soylak M (2007). Trace element levels of mushroom species from East Black Sea region of Turkey. Food Control 18: 806-810.

Uzun Y, Gençcelep H, Kaya A, Akçay ME (2011). The Mineral Contents of Some Wild Edible Mushrooms. Ekoloji 20 (80): 6-12.

Vetter J (1990). Mineral element content of edible and poisonous macrofungi. Acta Alimentaria 19: 27-40.

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Some Mycetozoa (Myxomycetes) Members from Zorkun High Plateau (Osmaniye)

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Zorkun Yaylası (Osmaniye)'ndan Bazı Mycetozoa (Myxomycetes) Üyeleri

Abstract: Myxomycete samples collected from 4 different localities from Zorkun Plateau (Osmaniye-Turkey) between 2014-2016. As a result of field and laboratory studies 28 species belonging to 9 families and 16 genera were determined. Among them, seven species were collected only from nature, 16 were obtained by Moist Chamber Technique in laboratory, and 5 species were obtained both from nature and by Moist Chamber Technique. *Echinostelium minutum*, *Cribraria cancellata*, *Didymium difforme*, *Didymium squamulosum*, *Physarum album*, and *Arcyria cinerea* are the most common species. Ten species were found to be common and 12 are rare or occasional. Species/genus ratio (S/G) is 1.75. The determined species are given together with habitats, substrates, coordinates, collection dates, voucher numbers and prevalencies.

Key words: Myxomycete, Zorkun Hihgh Plateau, Osmaniye, Turkey

Özet: Miksomiset örnekleri 2014-2016 yılları arasında Zorkun Yaylasından (Osmaniye-Türkiye) 4 farklı lokaliteden toplandı. Arazi ve laboratuvar çalışmaları sonucunda 9 familya ve 16 cinse ait 28 tür tespit edilmiştir. Toplanan örneklerden 7 tür sadece doğal ortamdan, 16 tür Nem Odası Tekniği ilelaboratuvar şartlarında ve 5 tür ise hem doğal ortamdan, hem de Nem Odası Tekniği ile elde edilmiştir. *Echinostelium minutum, Cribraria cancellata, Didymium difforme, Didymium squamulosum, Physarum album*, ve *Arcyria cinerea* en yaygın türlerdir. On tür yaygın, 12 tür de arasıra görülen olarak belirlenmiştir. Tür/Cins oranı 1.75 olarak bulunmuştur. Belirlenen türler habitat, substrat, konum koordinatı, toplanma tarihi, toplayıcı numaraları ve yaygınlık durumları ile birlikte listelenmiştir.

Anahtar Kelimeler: Miksomiset, Zorkun Yaylası, Osmaniye, Türkiye

1. Introduction

Mycetozoa; plasmodial slime moulds; Myxomycetes; a small group of organisms with amorphous, multinucleate and protoplasmic mass and about 994 species world-wide (Lado, 2017). Two hundred and sixty two 262 species have been reported from Turkey (Sesli et al., 2016). Molecular and physiological studies support the classification of myxomycetes within eukaryotic organisms in the kingdom Protoctista (Everhart and Keller, 2008). Plasmodial slime molds are generally found on decaying or living plant materials. Mostly cosmopolitan, several microhabitats for these species have also been reported in forest ecosystems.

Osmaniye is situated in the Eastern Mediterranean region of Turkey with a surface area of 3280 km², and surrounded by Gaziantep to the east, Hatay to the south, Adana to the west and Kahramanmaraş to the north. Southeastern parts are also bordered by the Amanos Mountains, extending from the port of Iskenderun to the east, while northwestern parts are bordered by Toros Mountains. Osmaniye has a Mediterranean climate. Summers are very hot and dry while the winters are cool and wet (Anonymous, 2017). The Zorkun high plateau is located in the most eastern side of the Mediterranean region within Osmaniye province (Turkey) (Figure 1). It is one of the most important and old high plateau of Osmaniye and Çukurova region at the northern slopes of the Amanos mountains. Its population reaches almost to 60.000 people during summer. Average altitude is about 1.550 meters. The Zorkun high plateau is of significant research interest because of different ecosystem types in both forests and agricultural lands. The highest point of the plaeau is Keldaz hill (2108 m). The vegetation of Zorkun plateau is very rich; *Pinus brutia* and maquis are mixed with up to 600 meters. *Pinus brutia* forests are dominant between 600-900 meters. Between 900-1100 meters mixed populations of *Pinus nigra* and *Pinus brutia* are visible, together with *Carpinus orientalis*, *Buxus sempervirens* along stream sides. After 1300 meters *Quercus infectoria* takes the place of *P. brutia* together with *Pinus nigra*. Between the altitudes of 1400- 1800 meters, *Pinus nigra* is the most dominated tree in the region. *Fagus orientalis*, is distributed among *P. nigra* above 1800 meters. Some pure *Abies cilicica* populations also exist between 1900-2000 meters (Sevgi, 1984; Gürçınar and Yüceer, 1995).

The work aims to make a contribution to the myxobiota of Turkey.

Figure 1. Map of Osmaniye and Zorkun high plateau

2. Materials and Method

Natural substrates and debris material, like bark of living trees, as well as decaying bark, wood, leaves and litter were collected from four different stations in Zorkun Hihgh Plateau. Natural mature fructifications were directly collected from the substratum and placed in cardboard herbarium boxes. Fructifications of some myxomycetes were obtained from the moist chamber culture in the laboratory. Collected plants materials were moistened with distilled water. The moist chambers were examined every day under a stereo-microscope. When fructification of myxomycetes were detected in the culture, the moist chamber was allowed to dry slowly and the myxomycetes were then dried for one week and prepared as fungarium material.

Microscopic and macroscopic features of the samples were determined in the laboratory. The morphological characters; fruiting bodies shape, size and colour, spore size and ornamentation, capillitium colour and branching, lime crystalsize and morphology, stalk colour and proportion were measured. The specimens were identified according to the relevant references (Martin and Alexopoulos, 1969; Neubert et al., 1993, 1995 and 2000). The samples are stored in fungarium of biology department.

3. Results

In this study 28 species (65 samples) belonging to 6 orders, 9 families and 16 genera were identified. Twenty three samples (7 species) were collected in field, 42 samples (16 species) were developed in moist chamber culture and 5 species were both collected from nature and obtained by moist chamber culture in laboratory.

Abundance indices were applied to all species collected from research area. These indices are categorised as Rare (for species < 0.5% of total number collections), Occasional (for species > 0.5% but < 1.5% of total), Common (for species > 1.5% but < 3.0% of total), and Abundant (for species > 3.0% of total) (Stephenson et al., 1993). In our study, 6 species were found to be abundant (A), 10 species were common (C), 12 species were occasional (O). The mean number of species per genus (S/G) was calculated from the data sets of study area as 1.75.

Mycetozoa
Protostelia
Protosteliida
Ceratiomyxaceae

1. *Ceratiomyxa fruticulosa* (O.F. Müll.) T. Macbr.

Derviş Pınarı, on conifer wood, 1500 m, 36˚58′15″ N; 36˚0′56″ E, 06.11.2014, Baba 18; Çift Mazı, on woody debris, 800 m, 37˚01′10″N; 36˚18′17″E, 26.01.2015, Natural, Baba 21, Common.

Myxogastria
Echinosteliida
Echinosteliaceae

2. *Echinostelium minutum* de Bary

Derviş Pınarı, on wood, 1500 m, 36˚58′15″N; 36˚0′56″E, 06.11.2014, Baba 2, 17, 45; Kent Ormanı, on *Pinus* sp. cone, 800 m, 37˚01′ 19″ N; 36˚16′30″E, 26.01.2015, Baba 11; Çift Mazı, on *Pinus* sp. bark, 800 m, 37˚01′10″N-36˚18′17″E, 26.01.2015, Baba 2, Abundant.

Liceida
Cribrariaceae

3. *Cribraria cancellata* (Batsch) Nann.-Bremek.

Keldaz hill, on woods, 2100 m, 36˚51′12″N-36˚22′58″E, 06.11.2014, Natural, Baba 19, 40, 41, Abundant.

4. *Cribraria microcarpa* (Schrad.) Pers.

Kent Ormanı, on wood, 800 m, 37˚01′19″N-36˚16′30″E, 26.01.2015, Baba 24; Derviş Pınarı, on conifer bark, 1500 m, 36˚58′15″N-36˚0′56″E, 06.11.2014, Natural, Baba 30, Common.

5. *Cribraria violacea* Rex

Çift Mazı, on conifer wood, 800 m, 37˚01′10″N-36˚18′17″E, 26.01.2015, Baba 11, Occasional.

Liceaceae

6. *Licea biforis* Morgan

Kent Ormanı, on *Pinus* sp. bark, 800 m, 37˚01′19″N-36˚16′30″E, 06.11.2014, Baba 26. Occasional.

7. *Licea castanea* G.Lister

Derviş Pınarı, on conifer bark, 1500 m, 36˚58′15″N-36˚0′56″E, 06.11.2014, Baba 30, Occasional.

8. *Licea minima* Fr.

Keldaz hill, on conifer barks, 2100 m, 36˚51′12″N-36˚22′58″E, 06.11.2014, Baba 9, 48, Common.

Reticulariaceae

9. *Lycogala epidendrum* (J.C.Buxb. ex L.) Fr.

Çift Mazı, on decaying bark, 800 m, 37˚01′10″N-36˚18′17″E, 26.01.2015, Natural, Baba 10, Occasional.

Physarida
Didymiaceae

10. *Didymium difforme* (Pers.) S.F.Gray

Kent Ormanı, in coniferous debris leaves, 800 m, 37˚01′19″N-36˚16′30″E, 26.01.2015, Baba 21, 29, 36, 47; Kent ormanı, on pomegranate bark, 800 m, 37˚01′19″N-36˚16′30″E, 26.01.2015, Natural, Baba 28; Çift Mazı, on leaves, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Baba 54, 78; Derviş Pınarı, on conifer leaves, 1500 meter, 36˚58′15″N-36˚0′56″E, 26.01.2015, Natural, Baba 22, 88, Abundant.

11. *Didymium squamulosum* (Alb. & Schwein.) Fr.

Çift Mazı, on leaves, 800 m, 37˚01′10″N-36˚18′17″E, 26.01.2015, Baba 54, 78; Derviş Pınarı, on conifer leaves, 1500 m, 36˚58′15″N-36˚0′56″E, 26.01.2015, Baba 22, 88, Abundant.

Physaraceae

12. *Physarum album* (Bull.) Chevall.

Çift Mazı, on woods, 800 m, 37˚01′10″N-36˚18′17″E, 26.01.2015, Natural, Baba 4, 71; Derviş Pınarı, on conifer barks, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Baba 12, 18, Abundant.

13. *Physarum cinereum* (Batsch) Pers.

Çift Mazı, on debris barks, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Baba 54, 78. Common.

14. *Fuligo septica* (L.) F.H. Wigg.

Kent ormanı, on grass leaves, 800 m, 37˚01′19″N- 36˚16′30″E, 26.01.2015, Natural, Baba 51, 88, Common.

Stemonitida
Stemonitidaceae

15. *Collaria lurida* (Lister) Nann.-Bremek.

Çift Mazı, on conifer barks, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Baba 8, 28, Common.

16. *Comatricha ellae* Härk.

Derviş Pınarı, on conifer barks, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Baba 41, Occasional.

17. *Comatricha pulchella* (C. Bab.) Rostaf.

Derviş Pınarı, on coniferous leaves, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Natural, Baba 26, Occasional.

18. *Comatricha nigra* (Pers. ex J.F. Gmel.) J. Schröt.

Derviş Pınarı, on conifer woods, 1500 m, 36˚58′15″N- 36˚0′ 6″E, 26.01.2015, Baba 2, Occasional.

19. *Enerthenema papillatum* (Pers.) Rostaf.

Derviş Pınarı, on conifer woods, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Natural, Baba 8, Occasional.

20. *Macbrideola cornea* (G. Lister & Cran) Alexop.

Derviş Pınarı, on conifer barks, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Baba 10, Occasional.

21. *Stemonitopsis amoena* (Nann.-Bremek.) Nann.-Bremek.

Derviş Pınarı, on conifer woods, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Baba 22; Çift Mazı, on barks, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Baba 12, Common.

Trichiida
Arcyriaceae

22. *Arcyria cinerea* (Bull.) Pers.

Derviş Pınarı, on conifer woods, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Natural, Baba 22, 47, 58; Çift Mazı, on barks, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Natural, Baba 12, 56; Keldaz hill, on conifer barks, 2100 m, 36˚51′12″N- 36˚22′58″E, 06.11.2014, Baba 9, 48, 59, Abundant.

23. *Arcyria incarnata* (Pers.) Pers.

Derviş Pınarı, on conifer woods, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Baba 22, Occasional.

24. *Arcyria pomiformis* (Leers) Rostaf.

Derviş Pınarı, on conifer woods, 1500 m, 36˚58′15″N- 36˚0′56″E, 26.01.2015, Baba 22; Çift Mazı, on debris barks, 800 m, 37˚01′10″N- 36˚18′17″E, Baba 26, Common.

25. *Arcyria obvelata* (Oeder) Onsberg

Çift Mazı, on woody debris, 800 m, 37˚01′10″N; 36˚18′17″E, 26.01.2015, Natural, Baba 54, 78, Common.

Trichiaceae

26. *Perichaena corticalis* (Batsch) Rostaf.

Kent ormanı, on debris barks, 800 m, 37˚01′19″N- 36˚16′30″E, 26.01.2015, Baba 54, 78, Common.

27. *Perichaena depressa* Lib.

Çift Mazı, on debris barks, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Baba 42, Occasional.

28. *Trichia decipiens* (Pers.) T. Macbr.

Çift Mazı, on debris, 800 m, 37˚01′10″N- 36˚18′17″E, 26.01.2015, Natural, Baba 5, Occasional.

4. Discussions

Our research is the first study on myxomycetes of Osmaniye. Totaly 28 species were determined for Osmaniye myxobiota. *Echinostelium minutum, Cribraria cancellata, Didymium difforme, Didymium squamulosum, Physarum album*, and *Arcyria cinerea* are the most common species (Abundant) in research area, and they comprised the 50.7% of all the samples. Many studies have also reported that the above-mentioned species are cosmopolitan on different substrates (Alexopoulos et al., 1996; Ergül and Akgül, 2011; Stephenson, 2014; Baba et al., 2016). The most common genera to be determined in the region was *Arcyria*. Then comes *Comatricha, Didymium*, *Echinostelium* and *Physarum*. Comprising 5 genera and 7 species, Stemonitidaceae was the largest family (25%) in the region.

The myxomycete samples are generally determined on dead and decaying coniferous wood, bark, leaves and debris materials (Ing and Haynes, 1999; Ergül et al., 2005; Ko et al., 2010; Baba and Özyiğit, 2017). The majority of the samples were also collected from similar substrates and in our study. In research area the percentage of lignicolous myxomycetes is 34%, corticolous myxomycetes is 40%, foliicolous myxomycetes is 23%.

The mean number of species per genus (S/G) is 1.75 in our area. As Simberloff (1970) and others have pointed out, a low value for S/G implies a higher overall diversity. According to Stephenson et al. (1993) this rate in Southern India is 2.24 and 4.13in North America. Alexopoulos (1970) reported that species diversity of myxomycetes is lower in tropical forests than temperate forests (Tee et al., 2014).

With this study, 28 myxomycete species were determined from Zorkun High Plateau, and a considerable contribution was made to the myxobiota of Osmaniye province and Turkey.

References

Alexopoulos CJ, Mims CW, Blackwell M (1996). Introductory Mycology. John Wiley and Sons, Inc, California Üniversity USA.

Anonymous (2017). http://www.dmi.gov.tr/veridegerlendirme/il-ve-ilceler-istatistik.aspx?m=Osmaniye

Baba H, Zumre M, Gelen, M (2016). An Investigation on North Adana (Turkey) Myxomycetes. Chiang Mai Journal of Science 43(1): 54-67.

Baba H, Özyiğit İİ (2017). Three new rare Myxomycetes (Mycetozoa) records from Hatay, Turkey. Fresenius Environmental Bulletin 26(8): 4907-4910.

Ergül CC, Dülger B, Akgül H (2005). Myxomycetes of Mezit stream Valley of Turkey. Mycotaxon, 92: 239-242.

Ergül CC, Akgül H (2011). Myxomycete diversity of Uludağ national park, Turkey. Mycotaxon 116: 479.

Everhart SE, Keller HW (2008). Life history strategies of corticolous myxomycetes: the life cycle, plasmodial types, fruiting bodies, and taxonomic orders. Fungal Diversity 29: 1-16.

Gürçınar Y, Yüceer NS (1995). Çukurova Bölgesindeki yaylaların Genel Çevre Sorunları Zorkun yaylası Orneği. Çukurova Üniv, Müh. ve Mim. Dergisi 10(1,2): 79-90.

Ing B, Haynes C (1999). Corticolous myxomycetes from Belize. Kew Bulletin 54(3): 723-730.

Ko KTW, Tran HTM, Stephenson SL, Mitchell DW, Rojas C, Hyde KD, Lumyong S, Bahkali AH (2010). Myxomycetes of Thailand. Sydowia 62: 243-260.

Lado C (2005-2017). CSIC (An online nomenclatural information system of Eumycetozoa) October 20, 2017 http://www.nomen.eumycetozoa.com.

Martin GW, Alexopoulos CJ (1969). The Myxomycetes. University of Iowa, Iowa City.

Neubert H, Nowotny W, Baumann K (1993). Die Myxomyceten Vol. 1. Ceratiomyxales, Echinosteliales, Liceales und Trichiales. In verlag karlheinz baumann, gomaringen, Germany.

Neubert H, Nowotny W, Baumann K (1995). Die Myxomyceten Vol. 2. Physarales. In Verlag karlheinz baumann, gomaringen, Germany.

Neubert H, Nowotny W, Baumann K (2000). Die Myxomyceten Vol. 3 Stemonitales. In verlag karlheinz baumann, gomaringen Germany.

Sesli E, Akata I, Denchev TT, Denchev CM (2016). Myxomycetes in Turkey, a checklist. Mycobiota 6: 1-20.

Sevgi C (1984). Adana İlinin Kuzeydoğu Kesiminde yaylacılık. Ege Cografya Dergisi 2(1): 177-197.

Stephenson SL, Kalyanasundaram I, Lakhanpal TN (1993). A comparative biogeographical study of Myxomycetes in the Mid-Appalachians of Eastern North America and two regions of India. Journal of Biogeography 20(6): 645-657.

Stephenson SL (2014). A comparative species listing of myxomycetes from tropical (Philippines) and temperate (United States) forests. Mycosphere 5(2): 299-311.

Tee DC, Mad R, Tran HTM, Ko KTW, Stephenson SL (2014). A comparative species listing of myxomycetes from tropical (Philippines) and temperate (United States) forests. Mycosphere 5(2): 299-311.

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Edaphic relations of *Cirsium cassium* Davis & Parris (Asteraceae), a local endemic from Hatay (Turkey)

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Hatay (Türkiye)'dan yerel bir endemik olan *Cirsium cassium* Davis & Parris (Asteraceae)'un edafik ilişkileri

Abstract: This study was carried out during 2012-2015. Our aim was to determine the soil-plant relationships of *Cirsium cassium* distributed in Hatay province of Turkey. This local endemic species was collected from three different localities in Hatay. The analysis of soil samples revealed that this endemic plant prefers clayey-loam, nonsaline, slightly alkaline soils with a high lime content. The nitrogen and potassium contents are of sufficient level but the soils are rich in phosphorus. Analysis of root, stem and leaf shows that nitrogen and phosphorus are below normal values in all plant organs; sodium is above the normal value in all plant parts. The potassium is below normal values in root and stem, but above normal values in leaf.

Key words: Soil-Plant interactions, endemic plant, conservation

Özet: Bu çalışma 2012-2015 yılları arasında gerçekleştirildi. Amaç, Hatay ilinde dağılmış olan *Cirsium cassium*'un toprak-bitki ilişkilerini belirlemeyi amaçladık. Bu lokal endemik tür, araştırma alanında üç farklı lokaliteden toplanmıştır. Toprak örneklerine ait analizlere göre, bu endemik bitki, yüksek kireç içeriğine sahip killi-tınlı, tuzsuz, hafif alkalin toprakları tercih ettiğini ortaya koydu. Azot ve potasyum içeriği yeterli seviyededir, ancak topraklar fosfor bakımından zengindir. Kök, gövde ve yaprak analizleri, tüm bitki organlarında azot ve fosforun normal değerlerin altında olduğunu; sodyum, tüm bitki kısımlarında normal değerin üzerindedir. Potasyum, kök ve gövde normal değerlerin altında ancak yapraklarda normal değerlerin üzerindedir.

Anahtar kelimeler: Bitki-toprak ilişkileri, endemik bitki, koruma

1. Introduction

Phytogeographically Turkiye occupies an important position in the world from the viewpoint of plant genetic resources and genetic diversity (Ozturk et al., 2016). The report published by WWF for Turkiye includes 122 important plant areas but, the nature association has identified 305 key biodiversity areas on the basis of endemism in the country (Eken et al., 2016; Ozturk et al., 2016). The fundamentals of species conservation are determination of distribution and ecology of the species (Çınar and Tuğ, 2015). The effective factors of the distribution areas are the macro and micro climatic features, habitats and soil characteristics of the plant niches together with the interactions with other species (Çınar and Tuğ, 2015; Ozturk et al., 2016).

Many endemic taxa show a restricted distribution, which is one of the main subjects of several ecological and phylogenetical studies (Çınar and Tuğ, 2015; Ozturk et al., 2016). The main reasons for this are listed as low ecological tolerance, specialized habitat requirements, low dispersal ability, and/or low reproductive capacity. Many plant endemics in Turkiye have only one distribution area, their systematics is well-known but information on their ecology, population structure, and reproductive biology is not enough. It is very important that ecology and genetics of target species must be fully evaluated if we want their proper and successful conservation (Çınar and Tuğ, 2015; Oztürk et al., 2016).

Most important stage during the life cycle of a plant is its habitat. In view of this most important step in this connection is an evaluation of its habitat features together with the biological features of species. Latter is also critical for the wildlife management. The habitat of a species is informative about bioclimatic, edaphic, topographic, biotic characteristics of a specific area. All these features need a recognition of biotic and abiotic factors of a living being (Thomas, 1979; Eskin et al., 2013). This type of evaluation forms the basis of autecological studies. Many plant autecological studies have been undertaken in Turkiye notable among these are; Vardar and Ahmet (1967), Ahmet (1968, 1969, 1970), Öztürk (1975, 1979, 1982), Öztürk and Görk (1979a,b), Öztürk and Ataç (1982), Alptekin et al. (1990), Özdemir et al. (1991a,b), Uysal and Öztürk (1991, 1993), Uysal et al. (1991, 1992, 1994a,b,c, 1996), Öztürk and Seçmen (1993, 1999), Özdemir and Öztürk (1996), Çelik et al. (2006a,b, 2008), Eskin et al. (2013), Altay et al. (2013, 2016a,b), Eroğlu et al. (2014) and Oztürk et al. (2016). In this study, an attempt has been made to evaluate the edaphic relations of *Cirsium cassium*.

2. Materials and Method

Plant (root, stem and leaf) samples of *Cirsium cassium* Davis & Parris (Asteraceae) and soil samples from their growth habitats were collected from three different localities in Hatay (especially from the roads extending between Samandağ and Yayladağ Districts). The root, stem, and leaf samples were oven-dried at 80 °C for 48 hours, milled in micro hammer cutter and passed through 1.5-mm sieve. 0.5 g of samples was weighed and transferred into Teflon vessel and 8 ml of 65% HNO_3 was added. The samples were mineralized in microwave oven at 145 ^oC for 5 min., at 165 ^oC for 5 min. and at 175 ^oC for 20 min. After cooling, samples were filtered using Whatman filters. The volume was made up to 50 ml with ultrapure water. Standard solutions were prepared by using multi element stock solutions-1000 ppm (Merck). The potassium and sodium measurements were conducted by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES). Nitrogen and phosphorus were determined by the methods outlined in detail in Öztürk et al. (1997).

500 g of soil samples were taken from a depth of 30 cm from all localities. pH was determined by Hanna 211 pH meter; total soluble salt (TSS) and conductivity values were measured by Hanna E.C. 211 conductivity meter; soil texture was determined with Bouyoucos Hydrometer; $CaCO_3$ was measured by Scheibler calcimeter; modified Kjeldahl method was used for total nitrogen analysis; phosphorus was determined by using Olsen method; all measurements were taken according to the methods outlined in detail in Öztürk et al. (1997), potassium and sodium concentrations were measured by using ICP-AES (Altay et al., 2016a).

3. Results

The soil analysis of *C. cassium* reveals that it generally prefers clayey soils with a soil pH of 7.80-7.92 and EC 242 to 395 (µS/cm). The values for calcium carbonate lie between 11 to 19 (%), nitrogen between 0.010 to 0.282 (%), phosphorus between 15.950 to 16.925 (mg kg^{-1}), sodium 34 to 41 (mg kg^{-1}) and potassium 174.50 to 520.00 (mg kg^{-1}).

Table 1. Physical and chemical analysis of the soil samples from the study sites

	Min.-Max.	Average
pH	7.80-7.92	7.86
N (%)	0.010-0.282	0.101
P (mg/kg)	15.950-16.925	16.342
K (mg/kg)	174.50-520.00	316.83
Na (mg/kg)	34.00-41.00	37.67
$CaCO_3$ (%)	11.00-19.00	14.70
EC (µS/cm)	242.00-395.00	314.70
TSS (%)	0.125-0.140	0.132

The results of analysis of *C. cassium* of roots, stems and leaves collected during flowering season show that on dry weight basis the nitrogen values range between 0.078-0.178 (%), phosphorus between 0-563 (mg kg^{-1}), sodium between 250-370 (mg kg^{-1}) and potassium between 250-370 (mg kg^{-1}) in roots. In the stems the nitrogen values range between 0.044-0.060 (%), phosphorus between 0-100.5 (mg kg^{-1}), sodium between 390-555 (mg kg^{-1}) and potassium between 340-555 (mg kg^{-1}). In the leaves the nitrogen values range between 0.092-0.153 (%), phosphorus between 0-90 (mg kg^{-1}), sodium between 105-615 (mg kg^{-1}), and potassium betwen 635-10,000 (mg kg^{-1}).

Table 2. Chemical analysis of the plant parts (root, stem and leaves) of *C. cassium*

	Root		**Stem**		**Leaves**	
	Min.-Max.	Average	Min.-Max.	Average	Min.-Max.	Average
N (%)	0.078-0.178	0.116	0.044-0.060	0.052	0.092-0.153	0.113
P (mg/kg)	0-563	221.33	0.00-100.50	33.50	0.00-90.00	58.50
K (mg/kg)	250-370	295	340-555	456.67	635-10,000	4873.33
Na (mg/kg)	250-370	295	390-555	473.33	105-615	291.67

The soil analysis data shows that this endemic taxon prefers clayey-loamy, nonsaline, slightly alkaline soils with high lime content, nitrogen and potassium are at sufficient level, and phosphorus is rich in the soil.

Epstein (1999) has reported that N, P, K and Na values follow as: 1.5%, 2,000 (mg/kg), 10,000 (mg/kg) and 10 (mg/kg) respectively in plants. According to our analysis results of root, stem and leaf parts nitrogen and phosphorus are below normal values in all plant organs; sodium is above the normal value in all plant parts. In addition, potassium values are below normal values in root and stem, but above normal values in leaf.

4. Discussions

Endemics and threatened plants are an important part of plant diversity, these are in need of immediate intervention in order to ascertain their long-term survival (Jalli et al., 2015). The strategies for plant conservation include in situ approaches such as, establishing protected areas, national parks, biosphere reserves and gene sanctuaries; whereas ex situ approaches include seed conservation genebanks, field genebanks and in vitro conservation. An application of different conservation approaches will prove helpful in saving plant taxa in danger. It will also widen our knowledge regarding these species. All these will pave way for their sustainable use beneficial for humans (Jalli et al., 2015).

Circium cassium, being a narrow endemic, must be given priority, and monitored carefully in order to preserve the genetic diversity of this species since endemic species are much more vulnerable to extinction at much higher rates than other species. In addition, ecological studies about such endangered endemic taxa should be supported with comprehensive physiological and molecular studies to promote the understanding about the narrow endemics (Altay et al., 2013; 2016a,b; Eroğlu et al., 2014).

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References

Altay V, Ozyigit II, Keskin M, Demir G, Yalçın IE (2013). An ecological study of endemic plant *Polygonum istanbulicum* Keskin and its environs. Pak J Bot 45(S1): 455-459.

Altay V, Karahan F, Oztürk M, Hakeem KR, Ilhan E, Erayman M (2016a) Molecular and ecological investigations on the wild populations of *Glycyrrhiza* L. taxa distributed in the East Mediterranean Area of Turkey. Journal of Plant Research 129(6): 1021-1032.

Altay V, Gülyanar Ş, Ozyigit II (2016b). Autecology of *Cephalaria taurica* Szabó, a narrow endemic from Turkey: Plant-soil interactions. IOSR Journal of Environmental Science, Toxicology and Food Technology 10(9): 90-94.

Ahmet M (1968). Some aspects of the autecology of *Ranunculus arvensis*. Sci Rep Fac Sci Ege Univ 62: 1-19.

Ahmet M (1969) Some autecological studies of *Ranunculus muricatus* L. Sci Rep Fac Sci Ege Univ 62: 1-13.

Ahmet M (1970). Ecology of *Ranunculus laetus*. Phyton 14: 1-8.

Alptekin E, Öztürk M, Zeybek N (1990). *Lupinus angustifolius'*un Ekolojisi. 10. Ulusal Biyoloji Kongresi, 18-20 Temmuz, Erzurum.

Çelik S, Uysal İ, Menemen Y, Özkan K, Öztürk M (2006a). Ecology and conservation of *Centaurea amanicola* Hub.-Mor. (Asteraceae) a vulnerable endemic species from Amanous Mountains, Türkiye, 1st European Congress of Conservation Biology, Hungary, August.

Çelik S, Özkan K, Gokturk RS, Yücel E, Ozturk M (2006b). Determination of indicator species and comparison of soil characteristics of *Centaurea mucronifera* DC. and *Centaurea pyrrohoblephara* Boiss. distributed in Turkey. International Journal of Biology and Tecnology 3(3): 609-617.

Çelik S, Yucel E, Mendes M, Tug GN, Ozturk M (2008). Canonical correlation analysis for studying the relationship between the basic morphological and some soil chemical characteristics of *Centaurea mucronifera* DC. (Asteraceae). Asian Journal of Chemistry 20(3): 2451-2456.

Çinar IB, Tuğ GN (2015). The Morphology, ecology, and conservation status of the local endemic species *Salsola grandis*. Ekoloji 24(96).

Eken G, Isfendiyaroğlu S, Yeniyurt C, Erkol IL, Karataş A, Ataol M (2016) Identifying key biodiversity areas in Turkey: a multi-taxon approach. International Journal of Biodiversity Science, Ecosystem Services & Management 12(3): 181-190.

Epstein E (1999). Silicon. Ann Rev Plant Physiol Plant Mol Biol 50: 641-664.

Eroğlu H, Ozyiğit II, Altay V, Yarcı C (2014). Autecological characteristics of *Centaurea hermannii* F. Herm.: An endemic species from Turkey. Bulgarian Journal of Agricultural Science 20(1): 183-187.

Eskin B, Ozyiğit II, Doğan I, Altay V, Demir G, Serin M (2013). Germination physiology and autecology of *Centaurea kilaea* Boiss. from Turkey. Sains Malaysiana 42(10): 1473-1482.

Jalli R, Aravind J, Pandey A (2015). Conservation and management of endemic and threatened plant species in India: An overview. In: Bir Bahadur et al. (Eds.), Plant Biology and Biotechnology, Vol.: II Plant Genomics and Biotechnology, pp. 461-486, Springer India.

Oztürk M, Altay V, Aksoy A (2016). Ecology of some endangered endemic plant taxa of Turkiye in relation to climate change. International Scientific Conference within "Day of Kazakhistan", September 3, 2016, EXPO-2016, Antalya-Turkey, pp. 12-15.

Özdemir F, Pirdal M, Öztürk M (1991a). *Marrubium rotundifolium* Boiss.'in morfolojisi, anatomisi ve ekolojisi üzerinde araştırmalar. Anadolu Üniversitesi Fen-Ed Fakültesi Dergisi 3/1: 19-26.

Özdemir F, Pirdal M, Öztürk M (1991b). *Astragalus tmoleus* var. *tmoleus* Boiss.'in morfolojisi, anatomisi ve ekolojisi üzerinde araştırmalar. Anadolu Üniversitesi Fen-Ed Fakültesi Dergisi 27-35.

Özdemir F, Öztürk M (1996). Batı Anadolu'da yayılış gösteren *Capparis* L. türlerinin bireysel ekolojisi üzerine bir araştırma. Turkish Journal of Botany 20: 117-125.

Öztürk M (1975). Batı Anadolu'da yayılış gösteren *Inula graveolens*'in autekolojisi hakkında araştırma. Doçentlik Tezi, Ege Üniv Fen Fak Sistematik Botanik Kürsüsü İzmir.

Öztürk MA (1979). Preliminary observation on the edaphic and biotic relations of *Myrtus communis* L. Ege Univ Fen Fak Dergisi Seri B III 1-2-3-4: 137-142.

Öztürk M (1982). Inula graveolens (L.) Desf.'in ekofizyolojisi, II edafik ilişkileri. Ata Univ Fen Fak Der 2(Özel Sayı 1): 480-492.

Öztürk M, Görk G (1979a). Ecology of *Mentha pulegium*. Ege University Science Faculty Journal III: 57-72.

Öztürk M, Görk G (1979b). Edaphic relations of *Mentha* species in West Anatolia. Ege University Science Faculty Journal III: 95-110.

Öztürk MA, Ataç E (1982). Bazı *Pistacia* türlerinin anatomisi ve ekolojisi üzerinde bir çalışma. Atatürk Üniversitesi Fen Fakültesi Dergisi 2(Özel sayı): 493-508.

Öztürk M, Seçmen O (1993). Autecological studies in Turkey. V. OPTIMA Collogium, Istanbul.

Öztürk M, Seçmen Ö (1999). Plant Ecology. 5th ed., Ege Univ. Press, Izmir.

Öztürk M, Pirdal M, Özdemir F (1997). Bitki Ekolojisi Uygulamalari. Ege Universitesi Basimevi Bornova-Izmir.

Thomas JW (1979). Wildlife habitats in managed forests: The Blue Mountains of Oregon a Washington. U.S.D.A. Forest Service. Handbook 553, Washington, D.C.

Uysal İ, Öztürk M (1991). *Digitalis trojana* Ivan. endemik türünün morfolojisi, anatomisi ve ekolojisi. Anadolu Üniversitesi Fen-Edebiyat Fakültesi Dergisi 3(1): 53-61.

Uysal İ, Öztürk M (1993). Morphology, anatomy and ecology of endemic species *Papaver virchowii* Aschers and Sint. S U Fen Edebiyat Fakultesi Fen Dergisi 11: 105-115.

Uysal İ, Ozturk M, Pirdal M (1991). *Sideritis trojana* Bornm, endemik türünün morfolojisi, anatomisi ve ekolojisi. Doğa-Tr J of Botany 15: 371-379.

Uysal İ, Öztürk M, Pirdal M (1992). Morphology, anatomy and ecology of endemic species *Dianthus ingoldbyi* Turril. Ege Univ Science Faculty Journal 14: 30-38.

Uysal İ, Öztürk M, Pirdal M (1994a). Morphology, anatomy and ecology of *Colchicum burttii* Meikle. XII National Biol Congress, 6-8 July, Edirne.

Uysal İ, Öztürk M, Pirdal M (1994b). Morphology, anatomy and ecology of *Campanula lyrata* Lam. subsp. *lyrata*. XII. National Biol. Congress, 6-8 July, Edirne, pp. 247-251.

Uysal İ, Öztürk M, Pirdal M (1994c). Morphology, anatomy and ecology of *Aristolochia hirta* L. XII. National Biol. Congress, 6-8 July, Edirne, pp. 252-256.

Uysal İ, Pirdal M, Öztürk M (1996). *Alyssum pinifolium* (Nyar) Dudley'in morfolojisi, anatomisi ve ekolojisi. Hacettepe Fen ve Mühendislik Bilimleri Dergisi 17: 105-120.

Vardar Y, Ahmet M (1967). Some ecological aspect of *Myrtus communis* L. Bot J Hrb Syst 93: 652-667.

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Effect of organic and syntetic fertilizers on soil productivity in organic tomatoes production

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Organik ve sentetik gübrelerin organik domates yetiştiriciliğinde toprak verimliliğine etkisi

Abstract: Organic fertilizer is a plant nutrient material which is used for obtaining the requirements of nutrients of plants grown in agricultural production. Organic fertilizers increase the organic matter content by providing nutrients to the soil as well as providing a more favourable growth environment for plants by correcting the physical properties of the soil. Organic fertilizers, mainly nitrogen, phosphorus, potassium, such as many important nutrients are brought to the soil. Chemical fertilizers can be obtained from petroleum products, from rocks and even from organic sources. This type of fertilizer helps plants grow, but there is no contribution to the development of the soil. In this study, each of the pots was applied in 500 ml of water at the stage of seedling uptake and 3 times in 3 weeks intervals following the application of the organic, synthetic fertilizer at a dosage of 200cc / da. The experiment was set up in pots with 5 replicates and was grown under greenhouse conditions. At the end of the experiment some physicochemical properties such as pH, salt, lime, phosphorus, potassium, iron and copper contents of the pots were investigated. The effect of these fertilizers on soil productivity has been evaluated. Phosphorus, potassium and iron contents in fertilizer applications were recorded as high compared to the negative control. In addition, the lowest soil pH was obtained in the application of synthetic fertilizer. This study has shown that organic fertilizer is more useful than synthetic fertilizer in terms of soil productivity.

Key words: tomatoes, fertilizer, soil productivity

Özet: Organik gübreler yetiştirilen bitkilerin bitki besin maddesi ihtiyaçlarını sağlamaları amacıyla kullanılan bitki besleme materyalidir. Organik gübreler yüksek organik madde içermesinden dolayı hem topraklara besin maddesi sağlayarak organik madde muhtevasını arttırır hem de toprakların fiziksel özelliklerini düzeltir. Organik gübrelerle, başta azot olmak üzere, fosfor, potasyum, gibi önemli birçok besin elementleri toprağa kazandırılır. Kimyasal gübreler; inorganik, petrol ürünlerinden, kayaçlardan ve hatta organik kaynaklardan elde edilebilirler. Bitkilerin gelişmesine yardımcı olurlar ancak toprağın gelişmesi için hiçbir katkıları yoktur. Bu çalışmada melas kökenli organik ve sentetik gübre 200cc/ da dozajında her bir saksıya 500 ml su içerisinde fide şaşırtma aşamasında ve takibinde 3 er hafta aralıkla 3 kez uygulanmıştır. Deneme saksılarda 5 tekerrürlü olarak tesadüf parselleri deneme desenine göre kurulmuş ve sera koşullarında yetiştirilmiştir. Denemenin sonunda saksılardaki toprakların pH, tuz, kireç, fosfor, potasyum, demir ve bakır içeriği gibi bazı fizikokimyasal özellikleri incelenmiştir. Bu gübrelerin toprak verimliliğine etkisi değerlendirilmiştir. Gübre uygulamalarında ki fosfor, potasyum ve demir içerikleri negatif kontrole göre yüksek olarak kayıt edilmiştir. Buna ek olarak, en düşük toprak pH'ı sentetik gübre uygulamasında elde edilmiştir. Bu çalışma ile toprağın verimliliği açısından organik gübrenin sentetik gübreye göre daha faydalı olduğu gösterilmiştir.

Anahtar Kelimeler: domates, gübre, toprak verimliliği

1. Introduction

Tomatoes are one of the vegetables produced in temperate climate zone in the world. Origin of tomatoes is Peru, it is started to be produced in Anatolia in 1900s. Production is more intense in Trace, Aegean, and Mediterranean Regions (Kandemir et. al., 2017). Deep, well aerated, rich in organic matter soils, pH at 6,5 and EC lower than 2,3 mS are appropriate for tomatoes production (Lewandowski and Zumwinkle, 1999). Addition of organic matter into soil develops the physical properties and provides sustainability (Bender et. al., 1998).

Sustainability of soil fertility is essential for sustainability of plant production. Sustainable plant production is to obtain enough qualified product without exhaust the natural sources and destroy the nature. It needs to be balanced the plant nutrition objects in soil in addition to climatic factors for sustainability of soil fertility. For this purpose; rotation of plant production and controlled soil tilling is suggested. Organic matter content of most of the soils in Turkey is lower (less than 2%) (Karaman et al., 2007; Zengin, 2017).

Therefore; soil fertilizing, using farm fertilizer or synthetic fertilizers, is very important component of plant production in Turkey.

Organic fertilizers are originated from plant and animal wastes, therefore is recycled in the soil. They contain N, P, K and microelements in different rates depending to the source of the fertilizer. Organic fertilizers speed the microbial reactions and provides micro and macro elements for plant production, beside develops the stricture and aerated the soil.

In contrast, inorganic fertilizers are produced by decomposition of petrol derivatives, rocks and organic matters and contain directly micro and macro elements in. Those are useful for plant development but are not for soil fertility (Yetgin, 2010).

Six million tones inorganic fertilizer, 39 million tones synthetic agricultural additional chemicals and hormones are used in Turkey (Yetgin, 2010). Unconscious use of the chemicals in agricultural practices, natural balance is broken down and as a result, it makes treat to livings through the food chain. Due to the negative effects of the inorganic fertilizers, use of the organic fertilizers is encouraged. In this study, the effect of organic and synthetic fertilizers used in tomatoes production on soil fertility was investigated.

2. Materials and Method

Organic tomatoes seeds of "Kokteyl" variety were used from Ekoherb firm. Experiment was set up in 20 cm diameter plastic pots and placed into greenhouse during March-September 2015. The soil used in the pots contained 25,9% calcium and 1,11% organic matter, pH was 7,8 and EC(Electric Conductivity): 0,01%. Organic fertilizer originated from molasses containing 7% N, 7% P and 7% K and synthetic fertilizer(NPK fertilizer solution) containing 10% N, 8% P and 5% K at dosage of 200 cc/ da were applied in 500 ml tap water at seedling transfer and 3 times more with 3 weeks intervals. Negative check without any fertilizer application was included. Experiment was designed according to randomized plot design with five replications. Fruits and plants were harvested after four months later at the end of the experiment and soil in each pot was analyzed for soil productuvitiy. pH (Richards, 1954), EC (Richards, 1954), P (Knudsen, 1975), K (Carson, 1980), Fe, Cu, organic matter (Walkley, 1946) and $CaCO_3$ (Çağlar, 1949) were analyzed for soil fertility. Statistical differences for soil fertility among fertilizer treatments were compared using ANOVA analysis.

3. Results

Statistically important differences among fertilizer applications were obtained for pH, EC, P, K, Fe and Cu content. There was not any statistical difference for $CaCO_3$ and organic matter among the treatments.

The measurement of the soil salt; EC was higher in both fertilizer treatments being maximum in organic fertilizer treatment than negative check treatment. However there was not any statistical difference between fertilizer applications (Figure 1).

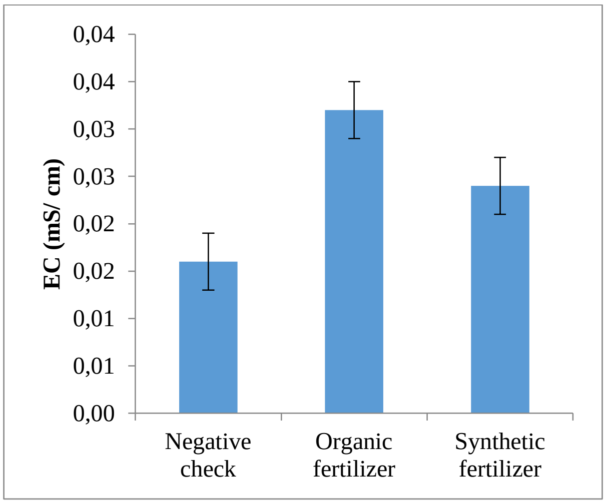

Figure 1. Soil EC (mS/ cm) in fertilizer treatments in the experiment.

Soil pH was statistically importantly lower in synthetic fertilizer treatment being highest in organic fertilizer treatment (Figure 2).

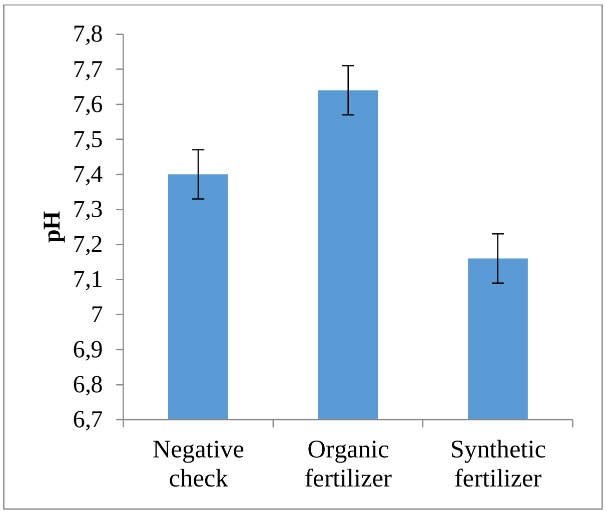

Figure 2. Soil pH in fertilizer treatments in the experiment.

Soil P, K and Fe contents in both fertilizer treatments were higher than negative check (Figure 3, 4 and 5).

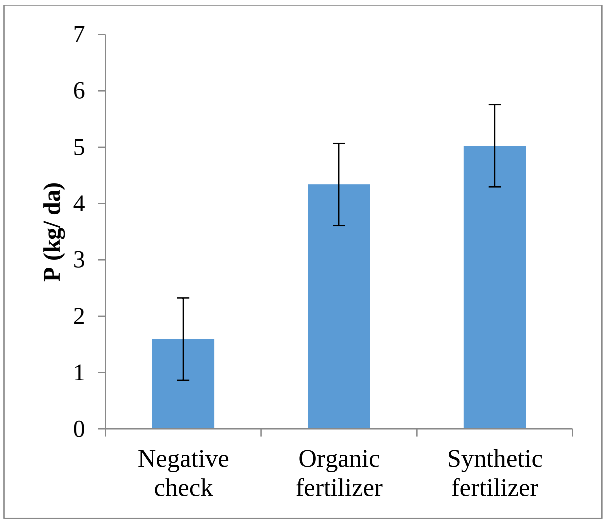

Figure 3. The soil P (kg/ da) in fertilizer treatments in the experiment.

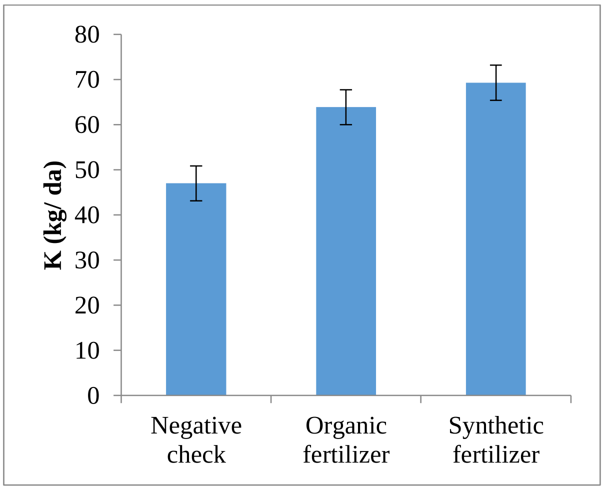

Figure 4. The soil K (kg/ da) in fertilizer treatments in the experiment.

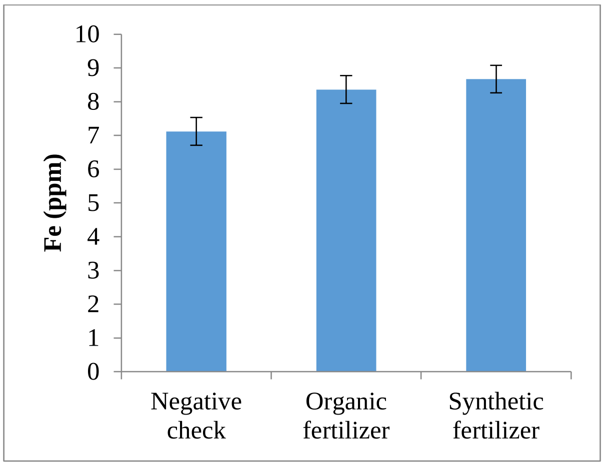

Figure 5. The soil Fe (ppm) in fertilizer treatments in the experiment.

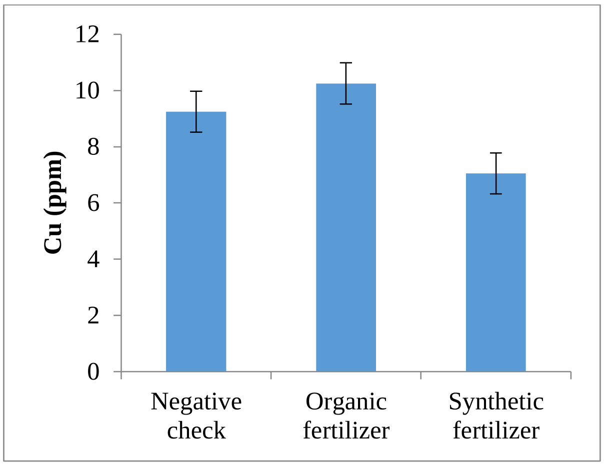

Figure 6. The soil Cu (ppm) in fertilizer treatments in the experiment.

Soil content of Cu was lower in syntetic fertilizer treatment, being the highest in organic matter treatment (Figure 6).

4. Discussions

Tomatoes are produced under greenhouse conditions prevalently in Turkey. Therefore the same land is used continuously for same product causing consumption of macro and micro elements. Soil fertilizing and application of soil regulating materials are frequently applied to avoid from soil exhausting. Synthetic fertilizers are effective only for one growing season, they are moved with underground water in the next year (Yetgin, A. M., 2010). Despite, organic fertilizers are slowly solved in soil water and useful for long time for the plant. Use of organic fertilizers is important for sustainability of soil fertility. Higher salt accumulation with higher EC value in both fertilizer treatments were due to the mineral content of the fertilizers. This is the result of soil fertilizing reported previously in agricultural soils (Ekmekçi et. al., 2005).

The best medium for soil microorganism activity is slight alkali soil reaction (Bender et. al., 1998; Lewandowski and Zumwinkle, 1999). Beside, synthetic fertilizer gives the minerals to the soil ready for the plants and does not have any effect on soil microbial activity. Moreover, causes inappropriate conditions reducing the soil pH level. In the experiment, soil medium was highly acidic in synthetic fertilizer treatments. Therefore, reducing effect on soil microbial activity is shown obviously. Macro and micro elements for plant nutrition were provided succesfully in the soil from organic fertilizer as well as synthetic fertilizer in the experiment.

As a result, organic fertilizer increased soil mineral and organic matter content and developed the soil reaction for microorganism activity. Therefore organic fertilizers originated from molasses are good alternatives to synthetic fertilizers for tomato production under greenhouse conditions.

References

Bender D, Erdal İ, Dengiz O, Gürbüz M, Tarakçıoğlu C (1998). Farklı Organik Materyallerin Killi Bir Toprağın Bazı Fiziksel Özellikleri Üzerine Etkileri. International Symposium On Arid Region Soil. International Agrohydrology Research And Training Center, Menemen, İzmir.

Çağlar KÖ (1949).Toprak Bilgisi. Ankara Üniversitesi Ziraat Fakültesi Yayınları, Ankara.

Carson PL (1980). Recommended potasium test. In: Recommended Chemicalsoil test procedures for the North Central Region. Rev.ed., North Central Regional Publication no.221. North Dakota Agric. Exp. Stn. North Dakota StateUniversity, Fargo. USA.

Ekmekçi E, Apan M, Kara T (2005). Tuzluluğun Bitki Gelişimine Etkisi. Ondokuzmayıs University Journal of Faculty of Agriculture 20(3):118-125.

Kandemir D, Kurtar S E, Demirsoy M (2017). Türkiye Örtüaltı Domates Yetiştiriciliğindeki Gelişmeler. Turktob 17:22-27.

Karaman MR, Brohi AR, Müftüoğlu NM, Öztaş T, Zengin M (2007). Sürdürülebilir Toprak Verimliliği. Detay Yayınlharı, Ankara.

Knudsen A (1975). Recommended phosphorus soil tests. In: Recommend chemical soil test procedure. North Central Regional Publication no: 221, Wisconsin.

Lewandowski A, Zumwinkle M (1999). Assessing the Soil System. A Review of Soil Quality Literature. Minessota Department of Agriculture Energy and Sustainable Agriculture Program, Boulevard.

Richards LA (1954). Diagnosis and Improvement of Saline and Alkaline Soils. U.S. Dep. Agr. Handbook, Washington.

Walkley A (1946). A Critical Examination of a Rapid Method For Determining Organic Carbon in Soils-Effect of variations in digestion conditions and of inorganic constituents. Soil Scence 63: 251-263.

Yetgin MA (2010). Organik Gübreler ve Önemi. İl Tarım Müdürlüğü, Çiftçi Eğitimi ve Yayın Şubesi Yayını, Samsun.

Zengin, M., (2017). Tarımda Toprak Verimliliği Nasıl Artırılır, http://www.kutahyaazot.com/kataloglar/Toprak_Verimliligi_(Mehmet%20ZENG%C4%B0N).pdf (Erişim tarihi: 26.10.2017).

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Leucocoprinus brebissonii (Godey) Locq, A New Record for Turkish Mycobiota

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Leucocoprinus brebissonii (Godey) Locq, Türkiye Mikobiyotası için Yeni Bir Kayıt

Abstract: The genus *Leucocoprinus* Pat. is situated in the order *Agaricales* within the phylum *Basidiomycota.* Though the genus comprises about 40 species worlwide, it is among the genera which are represented with the least taxa in Turkey. About 2400 macrofungi taxa have been determined as a result of the studies carried out in Turkey till now. This number is very low compared to European continent. That's why it is important to determine the macrofungal diversity of Turkey by continuing such studies. In Turkey, *Leucocoprinus brebissonii* (Godey) Locq was determined for the first time from Sürmene (Trabzon). Macroscopic and microscopic properties of the taxon was provided and discussed briefly.

Key words: Biodiversity, *Leucocoprinus,* new record, Trabzon, Turkey

Özet: *Leucocoprinus* Pat. cinsi, *Basidiomycota* bölümünün *Agaricales* ordosu içerisinde yer alır. Cins dünya genelinde yaklaşık 40 tür ile temsil edilmesine rağmen, Türkiye'de en az türle temsil edilen cinsler arasında yer almaktadır. Türkiye'de şu ana kadar gerçekleştirilen çalışmalar sonucunda yaklaşık 2400 makromantar taksonu belirlenmiştir. Bu sayı Avrupa kıtası ile kıyaslandığında oldukça azdır. Bu nedenle bu gibi çalışmalar sürdürülerek Türkiye'nin makromantar çeşitliliğinin ortaya konması önem arz etmektedir. *Leucocoprinus brebissonii* (Godey) Locq türü Türkiye'de ilk kez bu çalışma ile Sürmene (Trabzon)'den tespit edilmiştir.Türün makroskobik ve mikroskobik özellikleri verilerek kısaca tartışılmıştır.

Anahtar Kelimeler: Biyoçeşitlilik, *Leucocoprinus,* yeni kayıt, Trabzon, Turkiye

1. Introduction

Turkey has a diverse plant cover due to its geographical position. Depending on this diversity, it is thought that the country might also have a similar macrofungal diversity. It is also the estimate of Mueller et al. (2007) regarding the plant/macrofungus ratios of temperate regions. Due to its climate and plant cover, Black Sea region is among the richest region of Turkey in terms of macrofungal diversity.

Though there is an important increase in the number of studies carried on the macrofungal diversity, the mycobiota of Turkey has not been completed yet. With such studies, the determined macrofungi species number of our country is being increased.

The last checklists about the determined macrofungi lists of Turkey were presented by Sesli and Denchev (2014), and Solak et al. (2015) in 2014 and 2015 respectively. Since mycodiversity studies are going on in an increasing manner, many contributions (Uzun et al., 2015; Acar and Uzun, 2016; Öztürk et al., 2016; Taşkın et al., 2016; Öztürk et al., 2017) were also made to these checklist.

During a field study in Sürmene district of Trabzon province, some white coprinoid fungi samples were collected. As a result of necessary investigation, they were identifed as *Leucocoprinus brebissonii* (Godey) Locq. The current checklists (Sesli and Denchev, 2014; Solak et al., 2015) and the contributions which were made after the checklist (Acar et al., 2015; Akata et al., 2016; Demirel et al., 2016; Dengiz and Demirel, 2016; Kaya, 2016; Aktaş et al., 2017; Demirel et al., 2017; Işık and Türkekul, 2017; Sesli and Sesli, 2017; Sesli and Vizzini 2017; Uzun and Demirel, 2017; Uzun and Kaya, 2017; Uzun et al., 2017a; 2017b) were checked and it is found that the taxon has not been reported from Turkey.

The aim of this study is to make a contribution to the mycobiota of Turkey.

2. Materials and Method

Macrofungi samples were collected from Sürmene district of Trabzon Province in 2014 during a routine field study. Colour photographs of the samples were taken and some ecologic and morphologic properties of the samples were recorded. After that the macrofungi samples were brought to the fungarium and prepared as fungarium materials according to mycological rules. Dried samples were used to obtain the microscopic data. Microscopic investigations were carried out under a light microscope by mounting in 5% KOH. Basidispore dimensions were determined by at least 10 measurement from each sample.

The macrofungi samples were identified with the help of Moser (1983), Buczacki (1989), Breitenbach and Kränzlin (1995), Rother and Silveira (2009) and Pushpa and Purushothama (2011). They are protected at Yüzüncü Yıl University Fungarium (VANF).

3. Results

The systematic of the taxon is given in accordance with Kirk et al. (2008), and the Index Fungorum (www.indexfungorum.org; accessed 25 October 2017).

Fungi Bartling
Basidiomycota R.T. Moore
Agaricales Underw.
Agaricaceae Chevall.

Leucocoprinus brebissonii (Godey) Locq., Bull. mens. Soc. linn. Soc. Bot. Lyon 12: 95 (1943)
Syn: *Lepiota brebissonii* Godey, *Lepiota cepistipes* var. *cretacea* Grev.

Macroscopic and microscopic features: Pileus 2-4 cm in diameter, ovoid to conic-campanulate when young, convex to plane when mature, slighthly umbonate, surface white, covered by greenish brown to blackish-brown squamulous fibrils at the center, paler to white toward the margin, plicate sulcate to striate at the margin or half-way toward the disk in some members. Flesh thin up to 1 mm and white. Lamellae free, white. Stipe 30-60 x 2-3 mm, cylindric, slighthly bulbous at the base, solid when young, becoming hollow at maturity, surface white, pruinose, annulus membranous, persistent, white, attached half-way zone of the stipe.

Basidia 25-30 x 6-11 µm, clavate with four sterigmata, Cheilocystidia 27-40 x 12-14 µm, cylindric to clavate, hyaline, Spores 9-13 x 6-8 µm, ellipsoid, truncated by an apical germ-pore, hyaline, thick walled.

Ecology: *Leucocoprinus brebissonii* grows on soil among leaf litter or on leaf litter, in hardwood forests, parks and greenhouses (Breitenbach and Kränzlin, 1995; Rother and Silveira, 2009; Pushpa and Purushothama, 2011).

Specimen examined: Trabzon, Sürmene, Çamburnu village, mixed forest clearing, meadow, 40°55'362"N, 40°12'740"E, 70 m, 14.09.2014, O.003.

4. Discussions

Leucocoprinus brebissonii is characterized by having white pileus, covered with dark-brown fibrils at the disk, plicate-striate margin, white spore-print, thick walled and metachromatic basidiospores with a distinct germ pore (Candusso and Lanzoni 1990; Breitenbach and Kränzlin, 1995; Rother and Silveira, 2009; Pushpa and Purushothama, 2011). Our sample fits with all the characters listed above. This taxon is differentiated from the other similar species with above listed characteristics (Candusso and Lanzoni, 1990). The metachromatic spores, absence of clamps, and the striate pileus also distinguishes it from some other similar species such as *Lepiota cristata* and *L. felina*.

Like other *Leucocoprinus* species, *L. brebissonii* have also been recorded from greenhouses, but this one can also fruits in nature freely, especially in warm locations.

Leucocoprinus brebissonii have morphological similarities with *L. venezuelanus* Dennis. But the latter species differs with smaller basidiospores.

In conclusion *Leucocoprinus brebissonii* was added as new record to the mycobiota of Turkey, increasing the number of current members of the genus *Leucocoprinus* from 5 to 6.

Acknowledgements

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Figure 1. Basidiocarps (a) and basidiospores (b) of *Leucocoprinus brebissonii*

References

Acar İ, Uzun Y (2016). *Peziza granularis* Donadini Türkiye Mikobiyotası için Yeni Bir Kayıt. Yüzüncü Yıl University Journal of The Institute of Natural & Applied Sciences, 21(1):39-42.

Acar İ, Uzun Y, Demirel K, Keleş A (2015). Macrofungal diversity of Hani (Diyarbakır/Turkey) district. Biological Diversity and Conservation, 8(1): 28-34.

Akata I, Uzun Y, Kaya A (2016). Macrofungal diversity of Zigana Mountain (Gümüşhane/Turkey). Biological Diversity and Conservation 9(2): 57-69.

Aktaş S, Öztürk C, Pamukçu D (2017). Nallıhan (Ankara) İlçesi Makrofungusları. The Journal of Fungus 8(1): 60-67.

Breitenbach J, Kränzlin F (1995). Fungi of Switzerland. Vol. 4, Verlag Mykologia Lucerne, Switzerland.

Buczacki S (1989). Fungi of Britain and Europe. William Collins Sons & Co Ltd. Glasgow.

Candusso M, Lanzoni G (1990). Lepiota, Fungi Europei 4. Saronno, Giovanna Biella.

Demirel K, Acar İ, Ömeroğlu Boztepe G (2016). Lice (Diyarbakır) Yöresi Makrofungusları. Mantar Dergisi, 7(1): 29-39.

Demirel K, Uzun Y, Keleş A, Akçay ME, Acar İ (2017). Macrofungi of Karagöl–Sahara National Park (Şavşat-Artvin/Turkey). Biological Diversity and Conservation. 10/2: 32-40.

Denğiz Y, Demirel K (2016). Şirvan (Siirt) Yöresinde Yetişen Makrofunguslar Üzerinde Taksonomik Bir Araştırma. Yüzüncü Yıl University Journal of the Institute of Natural & Applied Sciences 21(2): 112-123.

Index Fungorum (2017). http://www.indexfungorum.org/Names/Names.asp. Accessed 25 October 2017.

Işık H, Türkekul İ (2017). A new record for Turkish mycota from Akdağmadeni (Yozgat) province: Russula decolorans (Fr.) Fr. Epicr.. Anatolian Journal of Botany 1(1): 1-3.

Kaya A (2016). Contributions to the macrofungal diversity of Atatürk Dam Lake basin. Turkish Journal of Botany 39(1): 162-172.

Kirk PM, Cannon PF, Minter DW, Stalpers JA (2008). Dictionary of the Fungi. 10th ed. Wallingford, UK: CAB International.

Moser M (1983). Keys to Agarics and Boleti. Gustav Fiscer Verlag, Stuttart.

Mueller GM, Schmit JP, Leacock PR, Buyck B, Cifuentes J, Desjardin DE, Halling RE, Hjortstam K, Iturriaga T, Larsson K-H, Lodge DJ, May TW, Minter D, Rajchenberg M, Redhead SA, Ryvarden L, Trappe JM, Walting R, Wu Q (2007). Global diversity and distribution of macrofungi. Biodivers Conserv 16:37‒48.

Öztürk C, Pamukçu D, Aktaş S (2017). Nallıhan (Ankara) İlçesi Makrofungusları. The Journal of Fungus 8(2): 60-67.

Öztürk Ö, Doğan HH, Şanda MA (2016). Some new additions to Turkish mycobiota from Sakarya region. Biological Diversity and Conservation 9(1): 97-100.

Pushpa H, Purushothama KB (2011). *Leucocoprinus* Pat. (*Agaricaceae, Agaricales, Basidiomycota*) in Bengaluru, Karnataka State, India. World Applied Sciences Jownal14(3): 470-475.

Rother MS, Silveira RMB (2009). *Leucocoprinus* Pat. (*Agaricaceae, Basidiomycota*) no Parque Estadual de Itapuã, Viamão, RS,Brasil. Acta Bot. Bras. 23(3): 720-728.

Sesli E, Denchev CM (2014). Checklists of the myxomycetes, larger ascomycetes, and larger basidiomycetes in Turkey. 6th edn. Mycotaxon Checklists Online. (http://www.mycotaxon.com/resources/checklists/sesli-v106-checklist.pdf): 1-136.

Sesli E, Topcu Sesli A (2017). *Infundibulicybe alkaliviolascens* (*Tricholomataceae*): Türkiye Mikotası için Yeni Bir Kayıt. Mantar Dergisi 8(1): 6-12.

Sesli E, Vizzini A (2017). Two new *Rhodocybe* species (sect. *Rufobrunnea, Entolomataceae*) from the East Black Sea coast of Turkey. Turkish Journal of Botany 41: 200-210.

Solak MH, Işıloğlu M, Kalmış E, Allı H (2015). Macrofungi of Turkey, Checklist, Vol. II. İzmir, Turkey: Üniversiteliler Ofset (in Turkish).

Taşkın H, Doğan HH, Büyükalaca S, Clowez P, Moreau PA, O'Donnell K (2016). Four new morel (*Morchella*) species in the elata subclade (M. sect. Distantes) from Turkey. Mycotaxon 131(2): 467-482.

Uzun Y, Acar İ, Akçay ME, Kaya A (2017a). Contributions to the macrofungi of Bingöl, Turkey. Turkish Journal of Botany 41(5): 516-534.

Uzun Y, Demirel K (2017). A New *Mycena* Record for the Mycobiota of Turkey. Anatolian Journal of Botany 1(1,2): 9-11.

Uzun Y, Kaya A (2017). A Hypogeous *Lactarius* sp., New to Turkish Mycobiota. The Journal of Fungus 8(2): 163-167.

Uzun Y, Kaya A, Karacan İH, Kaya ÖF, Yakar S (2015). Macromycetes determined in Islahiye (Gaziantep/Turkey) district. Biological Diversity and Conservation 8(3): 209-217.

Uzun Y, Kaya A, Karacan İH, Yakar S (2017b). New additions to Turkish Agaricales. Biological Diversity and Conservation 10(2): 8-13.

Cite this article: Keleş A, Oruç Y (2017). *Leucocoprinus brebissonii* (Godey) Locq, A New Record for Turkish Mycobiotab Anatolian Journal of Botany 1(2): 49-51.