



**ISBR-2021**

**3<sup>rd</sup> INTERNATIONAL SYMPOSIUM ON BIODIVERSITY  
RESEARCH**

**THE BOOK OF FULL TEXTS AND ABSTRACTS  
OF THE ISBR-2021**

**20-22 October 2021**



**Erzurum Technical University Erzurum, Turkey**

# 3<sup>rd</sup> International Symposium on Biodiversity Research



## HONORARY PRESIDENT

Prof. Dr. Bülent ÇAKMAK

Erzurum Technical University

Prof. Dr. Sedat MURAT

Canakkale Onsekiz Mart University

## CHAIR OF THE SYMPOSIUM

Prof. Dr. Ümit İNCEKARA

Department of Molecular Biology and Genetic, Faculty of Sciences, Erzurum Technical University

## EDITED BY

Res. Assist. Mesut AKYÜZ

Department of Molecular Biology and Genetic, Faculty of Sciences, Erzurum Technical University

**ISBN: 978-605-82906-2-4**

**Erzurum, 2021**

## **Organization Committee**

### **Honorary Chair**

Prof. Dr. Bülent ÇAKMAK – Rector of Erzurum Tecnical University

Prof. Dr. Sedat MURAT– Rector of Canakkale Onsekiz Mart University

### **Symposium Chair**

Prof. Dr. Ümit İNCEKARA - Erzurum Tecnical University

### **Symposium Secretary**

Assist. Prof. Dr. İsmail BEZİRGANOĞLU - Erzurum Tecnical University

### **Organizing Committee**

Prof Dr. Murat TOSUNOĞLU

Prof. Dr. Arzu GÖRMEZ

Prof. Dr. Levent GÜLTEKİN

Assoc. Prof. Dr. Serkan ÖRTÜCÜ

Assoc. Prof. Dr. Emre İLHAN

Assoc. Prof. Dr. Ömer Faruk KARATAŞ

Assist. Prof. Dr. Üyesi İsmail BEZİRGANOĞLU

Assist. Prof. Dr. Üyesi Elanur AYDIN KARATAŞ

Assist. Prof. Dr. Üyesi Mehmet Enes ARSLAN

Assist. Prof. Dr. Üyesi Ayşenur YAZICI

Assist. Prof. Dr. Üyesi Özlem ÖZDEMİR TOZLU

Assist. Prof. Dr. Üyesi Fatma Necmiye KACI

Assist. Prof. Dr. Üyesi Murat TURAN

Assist. Prof. Dr. Üyesi Gözde Büşra EROĞLU

Assist. Prof. Dr. Üyesi Hasan Onur ÇAĞLAR

Assist. Prof. Dr. Yeşim Bulak KORKMAZ

Ress. Assist. Özge ÇAĞLAR

Ress. Assist. Ayşe ÜSTÜN

Ress. Assist. Damla RÜZGAR

Ress. Assist. Büşra ALBAYRAK

Ress. Assist. Ayşe Gül KASAPOĞLU

Ress. Assist. Emine KARACA

Ress. Assist. Abdulmelik AYTATLI

Ress. Assist. Mesut AKYÜZ

## Scientific Committee

- Dr. Abdullah MART (Osmaniye Korkut Ata University, TURKEY)
- Dr. Ali ERDOĞAN (Akdeniz University, TURKEY)
- Dr. Ali KANDEMİR (Erzincan Binali Yıldırım University, TURKEY)
- Dr. Ahmet POLAT (Atatürk University, TURKEY)
- Dr. Akif IRMAK (Atatürk University, TURKEY)
- Dr. Ayşenur YAZICI (Erzurum Teknik University, TURKEY)
- Dr. Atilla DURMUŞ (Van Yüzüncü Yıl University, TURKEY)
- Dr. Bülent TOPKAYA (Akdeniz University, TURKEY)
- Dr. C. Çiğdem YIĞIN (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. C. Varol TOK (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Cherif ABDENNOUR (University of Annaba, ALGERIA)
- Dr. Çağan ŞEKERCİOĞLU (The University of Utah, USA)
- Dr. Çiğdem GÜL (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Ekrem Lütfi AKSAKAL (Atatürk University, TURKEY)
- Dr. Emre İLHAN (Erzurum Teknik University, TURKEY)
- Dr. Ersin KARABACAK (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Esra KOÇUM (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. F. Güler EKMEKÇİ (Hacettepe University, TURKEY)
- Dr. Ertan YILDIRIM (Atatürk University, TURKEY)
- Dr. Erdoğan ÖZTÜRK (Atatürk University, TURKEY)
- Dr. Erol ATAY (Mustafa Kemal University, TURKEY)
- Dr. Erol YILDIRIM (Atatürk University, TURKEY)
- Dr. Farkhanda MANZOOR (Lahore College for Women, PAKISTAN)
- Dr. Fatih ÖZ (Atatürk University, TURKEY)
- Dr. Gül Nilhan TUĞ (Ankara University, TURKEY)
- Dr. Güray UYAR (Gazi University, TURKEY)
- Dr. Hanife AKYALÇIN (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Hanane KENNOUCHE-BRADAI (University of Tipaza, ALGERIA)
- Dr. Hasan YILMAZ (Atatürk University, TURKEY)

## Scientific Committee

- Dr. Hasan TATLI (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Herdem ASLAN (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Hüseyin Aşkın AKPULAT (Cumhuriyet University, TURKEY)
- Dr. Hossein KAZEMİ (Gorgan University, IRAN)
- Dr. İbrahim UYSAL (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. İlker ANGIN (Atatürk University, TURKEY)
- Dr. İrfan ALBAYRAK (Kırıkkale University, TURKEY)
- Dr. İslam SARUHAN (Ondokuz Mayıs University, TURKEY)
- Dr. İsmail BEZİRGANOĞLU (Erzurum Teknik University, TURKEY)
- Dr. Kenan KAYNAŞ (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Kurtuluş Olgun (Adnan Menderes University, TURKEY)
- Dr. Levent GENÇ (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Lidija POLOVIĆ (Natural History Museum of Montenegro, MONTENEGRO)
- Dr. Mert GÜRKAN (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Meryem Şengül KÖSEOĞLU (Atatürk University, TURKEY)
- Dr. Muhammet TÜRKOĞLU (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Murat BARLAS (Muğla Sıtkı Koçman University, TURKEY)
- Dr. Murat KAYA (Aksaray University, TURKEY)
- Dr. Murat TOSUNOĞLU (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Murat YURTCAN (Trakya University, TURKEY)
- Dr. Musa CABBAROV (Baku State University, AZERBAIJAN)
- Dr. Mustafa KAYA (Trakya University, TURKEY)
- Dr. Mustafa DARILMAZ (Aksaray University, TURKEY)
- Dr. Natalija CADENOVIĆ (Natural History Museum of Montenegro, MONTENEGRO)
- Dr. Neslihan DİKBAŞ (Atatürk University, TURKEY)
- Dr. Nurcihan HACIOĞLU DOĞRU (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Nurşen ÇÖRDÜK (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Nurhayat ÖZDEMİR (Recep Tayyip Erdoğan University, TURKEY)
- Dr. Okan Acar (Çanakkale Onsekiz Mart University, TURKEY)

## Scientific Committee

- Dr. Orhan ERMAN (Fırat University, TURKEY)
- Dr. Özgür EMİNAĞAOĞLU (Artvin Çoruh University, TURKEY)
- Dr. Özer YILMAZ (Uludağ University, TURKEY)
- Dr. Özkan AKSAKAL (Atatürk University, TURKEY)
- Dr. Salih DOĞAN (Erzincan Binali Yıldırım University, TURKEY)
- Dr. Sebastian SALATA (University of Wrocław, POLAND)
- Dr. Sefer DEMİRBAŞ (Namık Kemal University, TURKEY)
- Dr. Sema İşisağ ÜÇÜNCÜ (Ege University, TURKEY)
- Dr. Serdar BİLEN (Atatürk University, TURKEY)
- Dr. Songül KARAKAYA (Atatürk University, TURKEY)
- Dr. Stefano DOGLIO (SRSN Società Romana di Scienze Naturali, ITALY)
- Dr. Sevgi SEVSAY (Erzincan Binali Yıldırım University, TURKEY)
- Dr. Şerife Gülsün KIRANKAYA (Düzce University, TURKEY)
- Dr. Şükran Yalçın Özdilek (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Paulraj Mosae Selvakumar (Asian University for Women, BANGLADESH)
- Dr. Perinçek Seçkinozan ŞEKER (Artvin Çoruh University, TURKEY)
- Dr. Tamer ALBAYRAK (Mehmet Akif Ersoy University, TURKEY)
- Dr. Tanja VUKOV (University of Belgrade, SERBIA)
- Dr. Teoman KANKILIÇ (Niğde Ömer Halis Demir University, TURKEY)
- Dr. Tülay Bican SÜERDEM (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Türker SAVAŞ (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Uğur Cengiz ERİŞMİŞ (Afyon Kocatepe University, TURKEY)
- Dr. Uğur GÖZEL (Çanakkale Onsekiz Mart University, TURKEY)
- Dr. Yalçın Şevki YILDIZ (Erciyes University, TURKEY)
- Dr. Yusuf AYVAZ (Süleyman Demirel University, TURKEY)
- Dr. Yüksel COŞKUN (Dicle University, TURKEY)
- Dr. Yusuf KUMLUTAŞ (Dokuz Eylül University, TURKEY)
- Dr. Zafer TOSUNOĞLU (Ege University, TURKEY)

## PREFACE

Dear Rector and distinguished participants,

As chair of the third international biodiversity research symposium, it is a great pleasure for me to declare open the third international biodiversity research symposium and to welcome the participants from different parts of the World who are here to exchange experience and work together a few days on this exciting symposium hosted by the Department of Molecular Biology, Faculty of Sciences of Erzurum Technical University.

I first wish to extend to you the greetings of the organizing and scientific committee. Without them, this organization will not have been come true.

The first symposium was organized by Department of Biology of Çanakkale Onsekiz Mart University, I would like to take this opportunity to thank the organizing committee of the event, especially Prof. Dr. Murat Tosunoğlu, who also gave us the opportunity to organize the event in our university.

As you know, all our plans were made to hold the symposium face to face. However, due to the pandemic, we decided to have it virtual.

It is a fact that biodiversity is the diversity of life on our planet. It forms the basis of our well-being and economy. Biodiversity is one of the most fundamental factors in the continuation of life on Earth. Unfortunately, biodiversity is declining rapidly day by day. Thus, biodiversity has become one of the most important issues today affecting all living things. Today, biodiversity is disappearing at thousand times the normal rate. Overuse of resources, climate change, excessive increase in air pollution and spread of diseases; accelerates the loss of biodiversity. It is very important to take measures to slow down or stop the decrease in biodiversity and to pass it on to future generations.

Being the first symposium held in our department, with this symposium, we aim to preserve the biological diversity in our country and the world, and to convey our biological richness and natural beauties to future generations.

Before closing, I have to extend further thanks to our Honorary presidents of the symposium, our rector Prof. Dr. Bülent Çakmak and Prof. Dr. Sedat MURAT (rector of Çanakkale Onsekiz Mart university). Secondly, we are indebted to our organizing and scientific committees to have made this event possible. Lastly, I would like to express my gratitude to you who participated in our symposium with presentations from Turkey and abroad.

Prof. Dr. Ümit İNCEKARA

Chair of the Symposium

## **CLOSING DECLARATION**

3<sup>rd</sup> International Symposium on Biodiversity Research hosted by the department of Molecular Biology and Genetics of Erzurum Technical University has been organized virtually between October 20 and 22, 2021.

149 participants from 13 different countries have been recorded the symposium and gathered to supply presentations about variable topics of biodiversity.

I would like to thank each participant for his or her contributions.

Today, we all know that climate change and biodiversity loss are happening before our eyes. The need to give a chance to nature was addressed in the symposium.

As a result of the presentations, we come to this conclusion that we all are responsible for the biodiversity conservation.

I believe that 3<sup>rd</sup> International Biodiversity Research Symposium has achieved its objectives in biodiversity conservation and our responsibilities for passing it on to future generations.

International Biodiversity Research Symposium is planned to be held at different universities in different countries. Thus, the fourth edition of this international event will be hosted by Afyon Kocatepe University, Turkey next year.

Prof. Dr. Ümit İNCEKARA

Chair of the Symposium





ERZURUM TEKNİK ÜNİVERSİTESİ  
ERZURUM TECHNICAL UNIVERSITY  
2010

**3. ULUSLARARASI  
BİYOÇEŞİTLİLİK ARAŞTIRMALARI  
SEMPOZYUMU**

**3<sup>rd</sup> INTERNATIONAL SYMPOSIUM ON  
BIODIVERSITY RESEARCH  
20-22 Ekim / October 2021**



Erzurum  
• 2021 •



**20.10.2021, WEDNESDAY**

**OPENING CEREMONY HALL 1**

**Opening Program**

**11:00 – 11.30**

**Opening Ceremony (Opening Speeches)**

Prof. Dr. Bülent ÇAKMAK, Honorary Chair (Rector of Erzurum Technical University)

Prof. Dr. Sedat MURAT, Honorary Chair (Rector of Canakkale Onsekiz Mart University)

Prof. Dr. Ümit İNCEKARA, Symposium Chair (Dean of Science Faculty, Erzurum Technical University)

20.10.2021, WEDNESDAY

**Keynote: Material Transfer from Nature to Technology**

**Dr. Murat Kaya – TURKEY**

13:00

HALL 1

HALL 2

HALL 3

**Session 1: Diversity of Animal species, Systematics and Phylogeny-1**

**Session 1: Microbial Biodiversity-1**

**Session 1: Diversity of Plant species, Systematics and Phylogeny-1**

**Session Chair: Dr. Nurhayat Özdemir**

**Session Chair: Dr. Arzu Ala Görmez**

**Session Chair: Dr. Sezai Ercişli**

|              |   |              |   |              |   |
|--------------|---|--------------|---|--------------|---|
| <b>13:45</b> | Contribution to the Knowledge of Heteroptera (Hemiptera) Fauna of Eastern Turkey<br>Neslihan Gültekin, Melek Güdek Güçlü, Dilek Doğan*, Mustafa Güllü, Celalettin Gözüaçık          | <b>13:45</b> | Arbuscular Mycorrhizal (AMF) and Disease-Causing Fungus Species Isolated from Dried Tea Seedlings in a Tea Garden<br>Şengül Alpay Karaoğlu*, Fatih Seyis  | <b>13:45</b> | Biodiversity of Sedum L. in Ankara (Turkey)<br>Akın Aras*, Duygu Mermer Doğu, Kamber Erat   |
| <b>14:00</b> | Thrips (Thysanoptera) Species and Distribution Areas in Northern Cyprus Cereal Fields<br>Mustafa Güllü*, Celalettin Gözüaçık  | <b>14:00</b> | Diagnosis of the factors causing the drying of Camellia sinensis seedlings, isolation of the factors and pathogenicity determination by leaf pathogenicity test<br>Şengül Alpay Karaoğlu                          | <b>14:15</b> | Genetic Diversity and Structure of Pea (Pisum sativum L.) Genotypes for Marker-Trait Association of DNA<br>İsmail Bezirganoğlu*, Büşra Yazıcılar, Merve Şimşek Geyik, Doğan İlhan |
| <b>14:15</b> | Variable detection and comparison of supervised machine learning algorithms in classification of two closely related Bufo species<br>Cantekin Dursun*, Serkan Gül, Nurhayat Özdemir | <b>14:15</b> | Identification and characterization of bacteria isolated from apricot trees in the province of Erzurum, Turkey<br>Damla Rüzgar*, Arzu Görmez  | <b>14:30</b> | Investigation of the Important Bee Plants of Uluyayla Plateau (Ulus-Bartın)<br>Bilge Tunçkol  |
| <b>14:30</b> | Aphidofagous Syrphids (Diptera: Syrphidae) from Çardak Lagoon in the Çanakkale Province of the Northwestern Part of Turkey<br>Şahin Kök   | <b>14:30</b> | Isolation and molecular characterization of bacteria from intestinal flora of some Beetles (Coleoptera: Dytiscidae)<br>Ayşenur Yazıcı, Ahmet Polat, Muhammet Çorapçı, Serkan Ortucu*, Mesut Taşkın, Umit Incekara |              |   |

## Keynote: Biodiversity Studies in Turkey

15:00

**Hasan Kanca – TURKEY**

HALL 1

HALL 2

HALL 3

Session 2: Environmental Toxicology-1 & Microbial Biodiversity-2

Session 2: Environmental Stress on Biodiversity

Session 2: Diversity of Plant species, Systematics and Phylogeny-2

Session Chair: Dr. Ömer Faruk Karataş

Session Chair: Dr. Enes Arslan

Session Chair: Dr. İsmail Bezirganoğlu

|       |  |       |  |       |  |
|-------|--|-------|--|-------|--|
| 16:00 | The Effect of Fertilizer Applications on Phenolic Compound Content in <i>Nigella damascena</i> Seeds<br>Funda Ulusu*, Ali Şahin        | 15:45 | Cold-adapted Cellulase Producer <i>Vishniacozyma</i> species from Palandöken Mountain<br>Mehmet Karadayı*, Şeyma Aksu  | 15:45 | Preliminary Data for Plant Biodiversity of the Polog Region of North Macedonia<br>Jusra Reçani*, Ebru Ataşlar  |
| 16:15 | Cytotoxic activity of <i>Nigella damascena</i> seed extracts<br>Funda Ulusu*, Ali Şahin  | 16:15 | Effects of Exogenous Salicylic Acid and Strigolactone applications on Antioxidant Activity in Tomato Seedlings Under Short-Term Drought Stress<br>Gamze Baltacı*, Sevgi Donat, Okan Acar | 16:00 | Evaluation of Genetic Diversity of Eleven <i>Medicago sativa</i> Varieties Cultivated in Turkey by Using Start Codon Targeted Polymorphism<br>Büşra Albayrak*, İsmail Bezirganoğlu     |
| 16:30 | Nano-Encapsulation and Biosynthesis of Metal Nanoparticles by Green Synthesis<br>Ilke Karakaş*, Furkan Öztürk, Nurcihan Hacıoğlu Doğru | 16:30 | The Change of Photosynthetic Pigments of <i>Liquidambar orientalis</i> in Summer Period<br>Fahrettin Atar, Ali Bayraktar*  | 16:15 | A Systematic Study on <i>Crocus gargaricus</i> Herb. Complex<br>Ceyda Yazıcı*, Almıla Çiftçi, Osman Erol   |
| 16:45 | Antimicrobial Activity of Silver Nanoparticles Biosynthesized by Olive Leaves<br>Özge Ceylan*, Nurcihan Hacıoğlu Doğru                 | 16:45 | Changes in Plant Water Potential and Stomatal Conductance Due to Water Stress in <i>Quercus infectoria</i><br>Esra Bayar*, Nevzat Gürlevik, Ayşe Deligöz                                 | 16:30 | Plant Species Diversity, Composition and Vegetation Cover of The Ugtam Nature Reserve, Mongolia<br>Bayanmunkh Tumurkhuu*, Enkhtuvshin Dechinperlii, Uyanga Ariya, Tuguldur Enkhtsetseg |
|       |  |       |  | 16:45 | Tepal Morphology of <i>Persicaria</i> s.str. (Polygonaceae) Taxa in Turkey<br>Suzan Kundakçı*, Serdar Makbul, Mutlu Gültepe, Kamil Coşkunçelebi  |

21.10.2021,

THURSDAY

**Keynote: Palmyraculture: The Role of Palmyra palm in Biodiversity/Sustainable Development**

10:00

*Dr. P. Mosae Selvakumar – BANGLADESH*

HALL 1

HALL 2

HALL 3

**Session 3: Diversity of Animal/Plant Species, Systematics and Phylogeny-2****Session 3: Diversity of Plant species, Systematics and Phylogeny-3****Session 3: Biodiversity, Landscape, Tourism-1, Environmental Toxicology-2****Session Chair: Dr. Salih Doğan****Session Chair: Dr. Ertan Yıldırım****Session Chair: Dr. Ayşenur Yazıcı**

|       |  |       |   |       |   |
|-------|--|-------|---|-------|---|
| 10:45 | An Annotated and Updated Checklist of Turkish Sarcophaga (Liosarcophaga) Enderlein, 1928 with the Comparisons of Male Terminalia<br>Gamze Pekbey                               | 10:45 | Morphological Characteristics of The Genus Lappula Moench. (Boraginaceae Juss.) In Mongolia<br>Munkhzul Tungalag  | 10:45 | Monitoring the Dynamics of the Area of Lake Azegza (Middle Atlas-Morocco) in the Context of Climate Change Using the Techniques of Space Remote Sensing.<br>Amal Raillani*, Lahsen Chillasse, Mhamed Khaffou                          |
| 11:00 | The Cheyletid Mites (Acariformes: Cheyletidae) of Kelkit Valley (Turkey)<br>Burcu Kabasakal*, Salih Doğan  | 11:00 | Horticulture Genetic Resources in Yozgat Province (Turkey)<br>Aysen Koç*, Gülşen Balcı, Emine Sema Çetin, Hakan Keles, Tuğba Kılıç, Selda Daler               | 11:00 | Crop Raiding by Wildlife of the neighbouring conservation area on subsistence homesteads in Northern KwaZulu-Natal Province, South Africa<br>Tlou Raphela*, Pillay Neville  |
| 11:15 | Investigation of Wintering Waterbirds Diversity in Different Wetlands Around the Dardanelles (2021 IWC)<br>İbrahim Uysal*, İbrahim Uysal                                       | 11:15 | Some Morphological Traits of Selected Hawthorn (Crataegus Spp.) Genetic Resources from Coruh Valley<br>Halil İbrahim Sağbaşı*, Sezai Ercişli                  | 11:15 | Determination of the acute effects of olive mill wastewater on Potamopyrgus antipodarum, Melanopsis buccinoidea ve Theodoxus sp. (Gastropoda: Tetridae: Melanopsidae: Neritidae)<br>Deniz Anıl Odabaşı*, Ayтуğ Zilifli, Sevdan Yılmaz |
| 11:30 | Phylogenetic Analysis of Heracleum L. (Apiaceae) Taxa in Turkey Based on nrDNA ITS and cpDNA trnL Intron and trnL-F DNA Sequences<br>Leyla Gürlük, Mustafa Çelik, Özlem Çetin* | 11:30 | Comparison of ATR-FTIR Spectra on Two Endemic Species of Asperula L. (Rubiaceae) Growing at the Same Substrate in Turkey<br>Ayşenur Kayabaşı*, Ertan Yıldırım | 11:30 | In Vivo Biotoxic Effects of Synacryl Black Xfdl Textile Dye on Larval Viability and Lifespan in Drosophila melanogaster Oregon-R<br>Emine Öztürk*, Handan Uysal   |

|  |  |  |   |   |   |  |
|--|--|--|---|---|---|--|
| 13:00  | <b>Keynote: Diversity and Uniformity in Vertebrate Reproduction</b>  |  |   |   |   |  |
|  | <b>Dr. Shai Meiri – ISRAEL</b>   |  |   |   |   |  |
| HALL 1   |  | HALL 2   |   | HALL 3  |   |  |
| <b>Session 4: Diversity of Animal species, Systematics and Phylogeny-3</b> |  | <b>Session 4: Aquatic (Marine and Freshwater) Biodiversity-1</b> |   | <b>Session 4: Conservation Biology, Policy and Strategies &amp; Protected</b> |   |  |
| <b>Session Chair: Dr. Sevgi Sevsay</b>                                     |  | <b>Session Chair: Yunus Esen</b>                                 |   | <b>Session Chair: Dr. F. Necmiye Kacı</b>                                     |   |  |
| 13:45  | New Mite Records (Acari: Erythraeoidea) from Turkey<br>İbrahim Karakurt*, Sevgi Sevsay   | 13:45  | Contribution to the Water Mite Fauna of Bingöl Province, Turkey (Acari, Hydrachnidia)<br>Yunus Esen   | 14:00   | Development of Microplastic Pollution Awareness Scale for Prospective<br>Tuğçe Güleşir*, Ali Gül  |  |
| 14:00  | Determination of The Chromosome Number of The Trombidium holosericeum for The First Time<br>Rümeysa Karağaç*, Halil Erhan Eroğlu, Evren Buğa, Sevgi Sevsay | 14:00  | Comparison of Distribution Altitudes of Some Helophoridae, Hydrochidae and Hydrophilidae Species in Turkey<br>Serhat Özcan1*, Numan Yıldız, Ahmet Polat, Ümit İncekara                                      | 14:15   | Ex-Situ Conservation Sterrgies for Antrodia cinnamomea: An Endemic Medicinal Mushroom in Taiwan<br>K.J. Senthil Kumar*, Büşra Albayrak, Büşra Yazıcılar, Merve Şimşek Geyik |  |
| 14:15  | Parasitism Relationship of Trombidioidea Mites with Spiders<br>Evren Buğa*, Sevgi Sevsay   | 14:15  | Changes in the Blood Cells of the Pelophylax ridibundus (Pallas, 1771) (Amphibia: Ranidae) Living in Different Streams in the Çanakkale<br>Begüm Boran*, Çiğdem Gül   |   |   |  |
| 14:30  | New Locality Records of Trombidoid Mites (Acari: Prostigmata) in Sansa George<br>Evren Buğa*, Sevgi Sevsay   | 14:30  | Isolation and Molecular Characterization of Bacteria from Some Aquatic Beetles (Coleoptera: Hydrophilidae)<br>Ahmet Polat, Ayşenur Yazıcı, A. Muhammet Corapçı, Serkan Ortucu*, Mesut Taşkın, Ümit İncekara | 14:30   |   |  |
| 14:40  | <b>Keynote: The Importance of Biodiversity in Plant Breeding</b>   |  |   |   |   |  |
|  | <b>Dr. Sezai Ercişli – TURKEY</b>  |  |   |   |   |  |
| HALL 1   |  | HALL 2   |   | HALL 3  |   |  |
| <b>Session 5: Effects of Biodiversity to Human Health-1</b>                |  | <b>Session 5: Population Ecology</b>                             |   |   |   |  |

| Session Chair: Dr. Songül Karakaya |  | Session Chair: Dr. Erol Yıldırım |  |  |  |
|------------------------------------|--|----------------------------------|--|--|--|
| 15:15                              | Antioxidant Capacity and Phenolic Composition of <i>Gagea chanae</i> Grossh. and <i>Scilla siberica</i> Haw.<br>Bilge Aydın*, Enes Tekman, Hafize Yuca, Songül Karakaya, Zühal Güvenalp  | 15:15                            | Bioactivity of Essential Oil of <i>Artemisia Herba Alba</i> and Its Effects on <i>Culex Pipiens</i> (Diptera; Culicidae)<br>Salma Kaoutar Abdelali*, Karim Souttou, Linda Aissaoui                   |  |  |
| 15:30                              | In vitro Evaluation of Antidiabetic Activity of <i>Colchicum speciosum</i> Different Parts and Their Anatomical Properties<br>Hafize Yuca  | 15:30                            | Bruchinae Latreille 1802 Species Detected on Edible Grain Legumes and Forage Crops in Southeastern Anatolia Region<br>Melek Güdek Güçlü*, Celalettin Gözüaçık, Neslihan Gültekin, Klaus-Werner Anton |  |  |
| 15:45                              | $\alpha$ -Glucosidase and $\alpha$ -Amylase Inhibitory Potential of <i>Paliurus spinachristi</i> Mill. and Its Main Compounds<br>Hafize Yuca*, Hilal Özbek, L. Ömür Demirezer, Zühal Güvenalp  | 15:45                            | Changes in Carbon Concentration of Tree Components for Calabrian Pine Forests in the Western Black Sea Region of Turkey<br>Şükrü Teoman Güner  |  |  |
| 16:00                              | In Vitro Assessment of Hemostatic Performances of <i>Salvia verticillata</i> , <i>Achillea biebersteinii</i> , <i>Tragopogon aureus</i> , and <i>Cephalaria procera</i><br>Songul Karakaya*, Ozlem Ozdemir Tozlu, Umit Incekara, Hasan Turkez, Ozkan Aksakal | 16:15                            | The Usage of Sage ( <i>Salvia</i> sp.) Taxa as Traditional Folk Medicine<br>Ahmet Efe*, Derya Karakoyun, Çağla Güvenç Biçer, Dudu Özlem Mavi İdman   |  |  |
| 16:15                              | Antimicrobial Activity of Different Parts of <i>Gagea chanae</i> Grossh. and <i>Scilla siberica</i> Haw.<br>Enes Tekman*, Songül Karakaya, Gamze Goger   |                                  |  |  |  |

22.10.2020, FRIDAY

## POSTER PRESENTATIONS

10:00

**Session 6: Diversity of Animal species, Systematics and Phylogeny; Population Ecology; Biodiversity, Landscape, Tourism**

**Session 6: Diversity of Plant species, Systematics and Phylogeny; Environmental Toxicology & Microbial Biodiversity**

**Session Chair: -**

**Session Chair: -**

|             |   |             |   |
|-------------|---|-------------|---|
| 10:00-11:00 | Biodiversity of fresh water Macro Invertebrates from of The Aurès Region, North-Est Algeria<br>Meriem Taferghoust *, Wissem Hezil, Boudjéma Samraoui, Farrah Samraoui                     | 10:00-12:00 | Leaf Geometric morphometrics among a natural population of Norway maple ( <i>Acer platanoides</i> L.) in Northern Algeria<br>Rida Mohammed Mediouni*, Sarra Said, Faiza Ilias, Gaouar Semir Bechir Suheil |
|             | Extensive Road Mortality of <i>Bufo bufo</i> (Linnaeus 1758) in Ikizdere, Rize<br>Cantekin Dursun*, Serkan Gül, Nurhayat Özdemir  |             | Diversite Vegetale De La Cedraie De Belezma -BATNA-<br>Neffar Fahima  |
|             | New Record of biting midge (Diptera: Ceratopogonidae) for Sinop (Turkey): <i>Leptoconops bidentatus</i> Gutsevich, 1960<br>Fethi Turgut   |             | Bio-Ecological & Demo-Ecological Approach of Avifauna at Sector Level'Hamla (Djebel tuggurt) & Fesdis (Kasrou)' of The National Park of Belezma -BATNA<br>Neffar Fahima                                   |
|             | The first record of <i>Atrichopogon infuscus</i> Goetghebuer, 1929 (Diptera: Ceratopogonidae) in Sinop (Turkey)<br>Fethi Turgut   |             | Study of Edaphic Biodiversity Under <i>Allium sativum</i> L Culture Ecosystem in The Semi-Arid Region of Batna in Algeria<br>Nadra Ghanem*, Djihane Zekri, Bouthaina Mokhtari                             |
|             | Morphological Investigation of Some Populations of <i>Podarcis muralis</i> (Laurenti, 1768) (Squamata: Lacertidae) in The Anatolian and Thrace Regions<br>Melis Karakoç*, Murat Tosunoğlu |             | Study of Edaphic Biodiversity Under <i>Olea europea</i> . L Arbori-cultural Ecosystem in The Semi-Arid Region of Batna in Algeria<br>Nadra Ghanem*, Djihane Zekri, Amel Kherbache, Amina Medjoudj         |
|             |   |             | Some New Alien Plant Species and Their Invasive Potential in the Flora of Adjara (Georgia)<br>Mikeladze Irakli*, Bolkvadze Gia, Davitadze Murman  |

|  |   |
|--|---|
| <p>Mapping of <i>Testudo graeca</i> Linnaeus, 1758 (Reptilia: Testudinidae) Living in Bozcaada According to Habitat Preferences<br/>Ceren Nur ÖZGÜL*, Çiğdem GÜL</p>               | <p>Study on Micropropagation of <i>Paeonia mascula</i> subsp. Bodurii<br/>Ebru Cambaz*, Nurşen Çördük, Bahar Kökçü, Ersin Karabacak</p>   |
| <p>Color- Pattern Analysis of <i>Hemidactylus turcicus</i> (Linnaeus 1758) (Sauria: Lacertilia: Gekkonidae) Populations Distributed in Çanakkale<br/>Didem Kurtul*, Çiğdem Gül</p> | <p>Screening for Indole Acetic Acid Production in Halophilic and Halotolerant Gram-Positive Bacteria<br/>Sabrina Behairi*, Nassima Baha, Wafa Achouak, Thierry Heulin, Yahia Kaci</p>   |
| <p>Distribution of Breeding Anatidae Family in Çanakkale Province<br/>İbrahim Uysal*, İbrahim Uysal</p>  | <p>Investigation of the Interaction of Smoke Tree (<i>Cotinus coggygria</i> Scop.) Leaf Extracts with Plasmid DNA by Agarose Gel Electrophoresis Method<br/>Büşra Dalgıç*, Neslihan Demir</p>   |
| <p>Useful plants of mountain xerophytic communities of the Lesser Caucasus (within Azerbaijan)<br/>Cabbarov M.T*, Nebiyeva F.X., Ibrahimov A.S.<sup>2</sup>, Atamov V.V.</p>       | <p>Determination of the acute effects of olive mill wastewater on <i>Gammarus komareki</i> Schäferna, 1923 (Amphipoda: Gammaridae)<br/>Deniz Anıl Odabaşı, Ayтуğ Zilifli, Sevdan Yılmaz</p>   |
| <p>Researches on Bio-Ecology of Small Dove (<i>Spilopelia senegalensis</i> L.) Population in Çanakkale City Center<br/>Sinan Marangoz, Murat Tosunoğlu</p>                         | <p>Greater Inter-Individual than Inter-Population Variability of <i>Calendula suffruticosa</i> subsp. algarbiensis Hexane Extract<br/>Silvana Ohse, Mariza B. Marques, Joaquim J. F. Neto, Paulo C. Silveira*, Diana C.G.A. Pinto</p> |
| <p>Effects of Tourism Activities on Rock Nuthatch (<i>Sitta neumayer</i>) Population in Nevşehir<br/>Bilge Yeni*, Ahmet Karataş</p>  | <p>Use of Rhizobacteria as Salt Stress Protectors in Durum Wheat Plants<br/>Bekkaye M*, Behairi S., Baha N Karaali K., Issad S., Kaci Y.</p>  |
| <p>The Influence of Sluices on Zooplankton Diversity in Canal – Case Study<br/>Nikola Kolarova*, Paweł Napiórkowski</p>  | <p>Palmyraculture: The Role of Palmyra as potential life support for Plant species diversity<br/>Christine Thevamartha*, Sherin Monichan, M. Jefwin Paul, Paulraj Mosae Selvakumar</p>  |
| <p style="text-align: center; color: blue; font-weight: bold; font-size: 1.2em;">SYMPOSIUM EVALUATION</p>  |   |
|  |   |

11:00-  
12:00





**3<sup>rd</sup> International Symposium on Biodiversity Research**

Erzurum, Turkey, 20 - 22 October 2021



**FULL TEXTS AND ABSTRACTS**

**Invited Speaker  
Oral Presentation**

**Palmyraculture: The Role of Palmyra Palm in Biodiversity and Sustainable Development**

Paulraj Mosae Selvakumar<sup>1,2\*</sup>, Sherin Monichan<sup>2</sup>, Christine Thevamirtha<sup>1</sup>,

<sup>\*1</sup>Science and Math program, Asian University for Women, Chittagong, Bangladesh-4000

<sup>2</sup>Panaiyaanmai (Palmyraculture), The Centre for self-reliance and sustainable development, Munnnetram  
Green Industries, Kadayam, Tenkasi, Tamil Nadu, India, 627415

e-mail: p.selvakumar@auw.edu.bd

**Abstract**

Asian Palmyra palm (*Borassus Flabellifer*), a gigantic fan-shaped tree that spreads out to large areas, mainly in South Asian countries such as Tamilnadu and Kerala, northern regions of Srilanka, Bangladesh, Myanmar, Thailand, and Cambodia is an important tree that contributes a lot to biodiversity and sustainability. From ancient times, it has been widely discussed and praised in Tamil classical Sangam literature, which has discussed 801 uses of Palmyra palm (Tala Vilasam). Palmyraculture (in Tamil, Panaiyaanmai) is the self-reliant community living and lifestyle based on Asian palmyra palm towards sustainable development. From 'Palmyraculture' we can promote Sustainable Development through three main pillars: Environment, Economic, and Social. In terms of Environmental Sustainability, Palmyra acts as the main breeding and nesting site for various epiphytes, reptiles, birds and plants and also, it is a natural rainwater harvesting system that stores up water and can turn an arid region into a fertile one. Recent research done on plantations of Tamarind, Pineapple, Cashew, Portia and Neem with young palmyra plants showed that the plants near the palm did not need to be watered in intervals since, the palmyra was the major provider of water, and nutrients. Similarly, its leaves provided shade for plants around its vicinity. Due to its immense ability to nurture plants and animals, it is often mentioned as a "keystone species". In addition, it is also known as "a multi-purpose tree with a great utility because of its wide varieties of commercial uses it has from both its edible parts such as jaggery, sap, toffee, wine, sugar and from non-edible products such as leaves, trunk, tuber coat to make mats, baskets, coir, toys, house construction. In addition to this, Palmyra toddy, a nutritious drink, has gained special attention recently due to its ban in Tamilnadu. When branded alcohol takes months and years to ferment, toddy just takes some days to make a healthy drink unlike the prior. However, overall products from

palmyra contribute significantly to the GDP of a country and also in attaining Economic sustainability that can create a huge impact in the lives of rural communities. Social Sustainability is another key parameter in SDG that can be obtained by depending on the Palmyra tree for nutritious food, shelter and cultural activities. With these parameters, palmyra palm attains a maximum number of SDG goals that directly or indirectly play a major role in attaining equilibrium between present needs and the demand of future generations. Asian Palmyra palm can also be called as “a tree of life” that provides us with all the basic things needed for the survival of humankind on the earth, that includes air, water, food, medicine, shelter, clothing, energy, education, innovation, employment, sports and games, aestheticism, biodiversity and ecosystem development, green economy, and spiritual enlightenment. This paper will cover the wide aspects of how the palmyra tree balances the three pillars of Sustainable Development and its importance towards bringing in sustainability by comparing its services through SDG’s.

**Keywords:** Asian palmyra palm, palmyraculture, self-reliance, biodiversity, key-stone species

**Invited Speaker  
Oral Presentation**

### **The Importance of Biodiversity in Plant Breeding**

Sezai Ercişli<sup>1\*</sup>

<sup>\*1</sup>Ataturk University Agricultural Faculty Department of Horticulture  
25240 Erzurum, Turkey

#### **Abstract**

Agricultural crops in particular horticulture ones characterized by narrow genetic base because a few horticultural commercial cultivars belong to different species have been using in cross breeding studies as parents for centuries and also some horticultural species shows self-fertility, which makes them vulnerable to loss biodiversity. Thus, it is necessary to increase of gene pool for all horticultural species for future climate change scenario that affects food security. One of the solutions to increase biodiversity in horticulture plants is that use of horticulture plants wild relatives that have adaptive characteristics to diverse environmental conditions because they include rich gene or gene combinations that are adapted to climate change. Another solution is to use different breeding methods that positively affects biodiversity. Among them mutation may have an opportunity to increase gene pool. The induced mutation technique is becoming increasingly important to bring about heritable changes in several horticultural plants and offer new genetic variabilities to plant breeders.

**Keywords:** plant, breeding, biodiversity

#### **Importance of Horticultural Plants**

Horticultural plants are gaining more and more importance not only for their attractive crops but also for their important components of the diets for people across the globe (Bowen-Forbes et al., 2010; Gecer et al., 2020). They have been using centuries for food and also for aesthetic purposes by people and accepted one of the rich energy sources. They are rich sources of macro and micronutrients, proteins, fibre, vitamins, bioactive substances (carotenoids, anthocyanins, phenolics), carbohydrates etc. (Liu et al., 2012; Senica et al., 2019). As they exist in the most parts of the world year around, they are also continuously evolving adaptive features. Horticultural crop genetic diversity is considered a source of continuing advances in yield, disease and pest resistance, and crop quality improvement (Dias, 2012; Kobayashi et al., 2027; Casals et al., 2019). Studies strongly showed that

greater diversity within variety and species would enable horticulture to maintain productivity over a wide range of environmental conditions (Luo et al., 2020; Salonia et al., 2020).

### **Importance of Genetic Resources of Horticultural Crops for Plant Breeding**

It is well accepted that to keep and maintaining biodiversity is a global responsibility (Fowler, 2011). The importance of biodiversity in agriculture (agrobiodiversity) accepted and well defined by FAO in 1983. FAO established a commission related to use of plant genetic resources for food security and in this commission comprise with peoples from different countries. The commission members discuss about how people and governments can efficiently use plant genetic resources for mankind in sustainable way. In addition, they can also concentrate how a fair and equitable share of their benefits among different countries. Along with FAO commission, the International Treaty on Plant Genetic Resources for Food and Agriculture, adopted in 2001 as another policymaker. This organization mostly concentrated on present and future food security through the conservation, exchange and sustainable use of the world's plant genetic resources.

More recently the characterization of horticulture plant genetic resources by morphological, biochemical and even molecular methods are accepted an important task that reveal novel variations which can be used for the development of improved cultivars expressing higher yield with better external and inner quality, resistance or tolerance biotic and abiotic stress conditions, storage and transportation capability etc. (Nadeem et al., 2020).

It is clear that horticulture plant genetic resources are vital and playing an ever-growing role on present and future food security. Horticultural plants show great within and between species diversity that indicate strong relationships with biodiversity. As well understood biodiversity is a crucial source for sustainable production. However due to human activities horticultural plants genetic resources are being lost with alarming rate which is dangerous for mankind for future (Fowler and Hodgkin, 2004; Bowen-Forbes et al., 2010; Litaladio et al., 2010).

### **Horticulture Plant Diversity and Plant Breeding Relationships**

Horticultural plants were subjected to several breeding activities in past including conventional breeding techniques including mostly selection breeding, cross breeding, mutation breeding and polyploidy breeding. More recently modern breeding techniques such as gene transfer and genome editing are also widely used for horticulture plant breeding studies. All these breeding techniques rely on crop biodiversity and genetic capacity. Access to genetic variation, biodiversity, is required to achieve crop cultivar improvement (Ghrab et al., 2010; Kaskoniene et al., 2020). The genetic

diversity and genetic variation are differ each other because genetic diversity can be described as the range of genetic characteristics in a crop or species, whereas genetic variation is the genetic differences among individuals for a specific characteristic (Ersoy et al., 2018; Fazenda et al., 2019). In fact, genetic diversity is an important component for plant breeding activities of horticultural crops and it is earth's most important resources for food and agriculture. Without genetic diversity, it is not possible to obtain successful results of any breeding methods to obtain new individuals. Today the genetic diversity can be assessed by more objective methods based on DNA sequence in a population of individuals (Fazenda et al., 2019; Guney et al., 2019).

Most of the horticultural plants have open pollinated nature and this make horticulture plants more diverse. This high variability are more common in wild relatives wild relatives of horticultural plants. They have very rich gene combinations. It is predicted that the future world's food production depends on genetic diversity of wild relatives of horticultural plants. In fact, for centuries people have used, developed and relied on biodiversity for food and agricultural production. The diverse plants in same horticultural crops have a huge potential to provide traits that can help meet future challenges, such as produce plants for changing climatic conditions, obtain plants resistance to abiotic and biotic stress conditions or disease and pests (Sestras et al., 2008; Laurens et al., 2010). In different parts of the world, a high number of plant breeders working on horticultural plant diversity and trying to obtain the most promising genotypes/cultivars with relatively higher yield, biotic and abiotic stress tolerance, and to improve the nutritional quality of foods for a growing world population. The rely on genetic diversity of genetic resources, breeding tools, and methods to incorporate genetic diversity into commercialized cultivars (Galiana-Belaguer et al., 2019).

Plant genomes are frequently be exposed to genetic and epigenetic changes that exhibit large amount of genetic and phenotypic variations is vital for plant breeding (Leitch and Leitch, 2012). It is well documented that plants have greater genetic diversity gives them a remarkable high adaptation ability to environmental changes (Wu et al., 2017; Raza et al., 2019).

Horticultural plant world exhibited a rich source of genetic resources including cultivars, genotypes, landraces and wild relative. In most of the countries field gene banks of above groups are available for scientists and breeders. Another important topic is to determine genetic diversity with proper and objective methods. More recently DNA base technologies are accepted the most proper and objective methods to identify individuals. Various DNA based genomic tools and breeding methods have improved the efficiency and precision of incorporating genetic diversity into commercialized crop cultivars. However, it is true that plant breeding activities belongs to time and resource-intensive

process (Morgante and Salamini, 2003; Vaughan et al., 2007). Traits that breeders have tried to incorporate into horticulture crop plants include: improve yield and quality (nutrition, flavor, appearance), increase environmental tolerance (salinity, low and high temperature, drought, heavy soil etc.), resistance to viruses, fungi, bacteria, increased tolerance to insect pests, increased tolerance of herbicides, prolonged storage period and transport ability. By using different breeding methods, based on genetic diversity, the genetic composition of obtained individual plants are changed and the new individual may have high and sustainable yield capacity, more resistance to biotic and abiotic stress, and enhanced nutrition, taste, or processing attributes (Shah et al., 2018; Galiana-Belaguer et al., 2019; Bulgari et al., 2019).

Horticultural plant genetic resources in particular crop wild relatives (CWR) display a rich genes or gene combinations and provide pests and diseases resistance, efficient use of nutrients and water and minimize external inputs to maintain productivity. CWR is an important elements as natural genetic resources to improve cultivated relatives via plant breeding. CWR strategically important to discover new sources of variation that will enable developing new crop cultivars (Santos et al., 2011; Silva et al., 2017). By using CWR of horticultural crops it is possible to increase yield, disease and pest resistance, and quality. It is widely accepted that greater varietal and species diversity would enable agricultural systems to maintain productivity over a wide range of conditions (Ersoy et al., 2018; Bulgari et al., 2019).

### **Loss of Horticulture Plant Genetic Diversity**

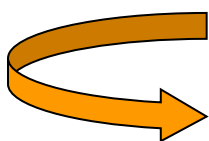
Today's most serious environmental concerns related to horticultural plants is that the loss of horticulture plant biodiversity. The most of the vegetable genetic resources have been lost during the last 50 years of period and it is estimated that on a global scale at the rate of loss is 1-2% per year and FAO reported that around 13% wild relatives of solanaceous plants have been lost (FAO, 2002; Dias, 2010). The loss of genetic resources is described as "genetic erosion", indicating loss of individual genes and of combinations of genes, such as those found in locally adapted landraces. This is the main problem of most Horticulture producer countries and it is clearly defined and determined that the main cause of genetic erosion is the replacement of local cultivars by modern cultivars (Versini et al., 2012; Ozturk and Demirsoy, 2013; Gecer et al., 2020). Moreover, the introduction of high yielded international commercial cultivars into traditional farming systems often leads to a reduction in the number of local or national cultivars (Gecer et al., 2020).

## Domestication of Plants

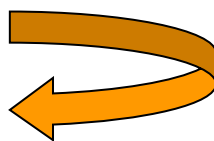
A crucial step for horticultural crop species evolution



Two major consequences on plant diversity



“Domestication syndrome”:  
changes on  
selected traits for  
human use



Reduction of genetic diversity  
in crops relative to their wild  
progenitors due to human  
selection and genetic drift  
through Bottleneck effects

## Domestication of Perennial Fruit Crops

Changing reproductive biology from sexual reproduction (wild forms) to vegetative propagation (cultivated forms)

### How Bottleneck Effects May Reduce the Genetic Diversity of Crops Comparatively to Wild Relatives?

- Genetic erosion of the wild gene pool: 29% of the total diversity were not recovered in wild populations of *Spondias purpurea* within the Mesoamerican center of domestication
- Weak bottleneck effect on diversity between the wild and cultivated forms: Olive and grapevine
- Bottleneck due to plant breeding: 40% at microsatellite loci for Sweet cherry

## Enlarging the Genetic Base Through Biotechnological Methods

The recent advances of plant biotechnology resulted the increase of agro-biodiversity through the use of genomics-led approaches. Some of these techniques transferring desired genes into plant germplasm (Limer et al., 2017; Pompili et al., 2020). More recently the biotechnological approaches mostly concentrate on genome editing and gene transferring. On the other hand, mutation breeding still keep importance and generates massive numbers of putative (commonly accepted) mutants that increase biodiversity (Song et al., 2019; Pompili et al., 2020; Rugini et al., 2020). The genetic variation that is obtained from the biodiversity within horticulture plants' genetic resources helps address many problems in plant breeding. The basic aims of horticulture plant breeding are to



improve crop varieties in terms of yield, quality, adaptability to climate change and biotic and abiotic stress factors in the ecosystem (Botu et al., 2017; Covarrubias-Pazaran et al., 2018; Nsibi et al., 2020).

## REFERENCES

- Casals, J., Rivera, A., Sabaté, J., Romero del Castillo, R., Simó, J., 2019. Cherry and fresh market tomatoes: differences in chemical, morphological, and sensory traits and their implications for consumer acceptance. *Agronomy*, 9(1): 9.
- Bowen-Forbes, C.S., Zhang, Y., Nair, M.G., 2010. Anthocyanin content, antioxidant, anti-inflammatory and anticancer properties of blackberry and raspberry fruits. *Journal of Food Composition and Analysis*, 23: 555-561.
- Covarrubias-Pazaran, G., Schlautman, B., Diaz-Garcia, L., Grygleski, E., Polashocket, J., Johnson-Cicalese, J., Vorsa, N., Iorizzo, M., Zalapa, J., 2018. Multivariate GBLUP improves accuracy of genomic selection for yield and fruit weight in biparental populations of *Vaccinium macrocarpon* Ait. *Frontiers in Plant Science*, 9: 1310.
- Botu, M., Botu, I., Achim, G., Preda, S., Scutelnicu, A., Giura, S., 2017. Conservation of fruit tree genetic resources and their use in the breeding process. *Annals "Valahia" University of Targoviste - Agriculture*, 11(1): 66-69.
- Bulgari, R., Franzoni, G., Ferrante, A., 2019. Biostimulants application in horticultural crops under abiotic stress conditions. *Agronomy*, 9 (6):306.
- Dias, J.S., 2010. Impact of improved vegetable cultivars in overcoming food insecurity. *Euphytica*, 176:125-136.
- Dias, J.S., 2012. Vegetable breeding for nutritional quality and health benefits. In: Carbone, K (Ed.). *Cultivar: chemical properties, antioxidant activities and health benefits*. Nova Science Publishers, Inc., Hauppauge, New York; pp.1-81.
- Ersoy, N., Kupe, M., Sagbas, H.I., Ercisli, S., 2018. Phytochemical diversity among barberry (*Berberis vulgaris* L.), *Natulae Botanicae Horti Agrobotanici Cluj-Napoca*, 46(2):198-204.
- FAO., 2002. World summit on sustainable development. United Nations, August 29. United Nations, New York.
- Fazenda, P., Pereira, R., Fonseca, M., Carlier, J., Leitão, J., 2019. Identification and validation of microsatellite markers in strawberry tree (*Arbutus unedo* L.). *Turkish Journal of Agriculture and Forestry*, 43: 430-436.
- Fowler, C., Hodgkin, T., 2004. Plant genetic resources for food and agriculture: assessing global availability. *Annual Review of Environment and Resources*, 29:143-179.
- Fowler, C., 2011. Conserving diversity: the challenge of cooperation. *Acta Horticulturae*, 916:19-24.
- Galiana-Belaguer, L., Ibanez, G., Cebolla-Cornejo, J., Rosello, S., 2018. Evaluation of germplasm in *Solanum* section *Lycopersicon* for tomato taste improvement. *Turkish Journal of Agriculture and Forestry*, 42: 309-321.
- Gecer, M.K., Kan, T., Gundogdu, M., Ercisli, S., Ilhan, G., Sagbas, H.I., 2020. Physicochemical characteristics of wild and cultivated apricots (*Prunus armeniaca* L.) from Aras valley in Turkey. *Genetic Resources and Crop Evolution*, 67:935-945.
- Ghrab, M., Zribi, F., Ayadi, M., Elloumi, O., Mnaïki, N., Mimoun, M. Ben, 2010. Lipid characterization of local pistachio germoplasm in central and southern Tunisia. *Journal of Food Composition and Analysis*, 23: 606-613.
- Guney, M., Kafkas, S., Koc, A., Aras, S., Keles, H., Karci, H., 2019. Characterization of quince (*Cydonia oblonga* Mill.) accessions by simple sequence repeat markers. *Turkish Journal of Agriculture and Forestry*, 43: 69-79.
- Kaskoniene, V., Bimbiraite-Surviliene, K., Kaskonas, P., Tiso, N., Cesoniene, L., Daubaras, R., Maruska, A.S., 2020. Changes in the biochemical compounds of *Vaccinium myrtillus*, *Vaccinium vitis-idaea*, and forest litter collected from various forest types. *Turkish Journal of Agriculture and Forestry*, 44: 557-566.
- Kobayashi, A.K., Vieira, L.G.E., Besspalhok Filho, J.C., Leite, R.P.Jr., Pereira, L.F.P., Molinari, H.B.C., 2017. Enhanced resistance to citrus canker in transgenic sweet orange expressing the sarcotoxin IA gene. *European Journal of Plant Pathology*, 149: 865-873.
- Laurens, F., Durel, C.E., Patocchi, A., Peil, A., Salvi, S., Tartarini, S., Velasco, R., van de Weg, E., 2010. Review on apple genetics and breeding programmes and presentation of a new European initiative to increase fruit breeding efficiency. *Guoshu Xuebao*, 27: 102-107.
- Leitch, I.J., Leitch, A.R., 2012. Genome size diversity and evolution in land plants. In J. Greilhuber, J. Dolezel, & J. Wendel (Eds.), *Plant genome diversity Vol. 1: Plant genomes, their residents, and their evolutionary dynamics* (pp. 307–322). Vienna: Springer.
- Limera, C., Sabbadini, S., Sweet, J.B., Mezzetti, B., 2017. New biotechnological tools for the genetic improvement of major woody fruit species. *Frontiers in Plant Science*, 8: 1418.

- Liu, Y., Heying, E., Tanumihardjo, S.A., 2012. History, global distribution, and nutritional importance of citrus fruits. *Comprehensive Reviews in Food Science and Food Safety*, 11: 530-545.
- Luo, F., Evans, K., Norelli, J.L., Zhang, Z., Peace, C., 2020. Prospects for achieving durable disease resistance with elite fruit quality in apple breeding. *Tree Genetics & Genomes*, 16:21.
- Lutaladio, N., Burlingame, B., Crews, J., 2010. Horticulture, biodiversity and nutrition *Journal of Food Composition and Analysis*, 23: 481-485.
- Morgante, M., Salamini, F., 2003. From plant genomics to breeding practice. *Current Opinion in Biotechnology*, 14:214-219.
- Nadeem, M.A., Gündoğdu, M., Ercişli, S., Karaköy, T., Saracoğlu, O., Habyarimana, E., Lin, X., Hatipoğlu, R., Nawaz, M.A., Sameeullah, M., Ahmad, F., Jung, B.M., Chung, G., Baloch, F.S., 2020. Uncovering phenotypic diversity and DArTseq marker loci associated with antioxidant activity in common bean. *Genes*, 11: 36.
- Nsibi, M., Gouble, B., Bureau, S., Flutre, T., Sauvage, C., Audergon, J.M., Regnard, J.L., 2020. Adoption and optimization of genomic selection to sustain breeding for apricot fruit quality. *G3 Genes|Genomes|Genetics*, 10 (12): 4513-4529,
- Ozturk, A., Demirsoy, L., 2013. Promising pear genotypes from North Anatolia, Turkey: preliminary observation. *Journal of the American Pomological Society*, 67: 217-227.
- Pompili, V., Dalla Costa, L., Piazza, S., Pindo, M., Malnoy, M., 2020. Reduced fire blight susceptibility in apple cultivars using a high-efficiency CRISPR/Cas9-FLP/FRT-based gene editing system. *Plant Biotechnology Journal*, 18: 845-858.
- Raza, A., Razzaq, A., Mehmood, S. S., Zou, X., Zhang, X., Lv, Y., Xu, J., 2019. Impact of climate change on crops adaptation and strategies to tackle its outcome: A review. *Plants*, 8 (2): 34.
- Rugini, E., Bashir, M.A., Cristofori, V., Ruggiero, B., Silvestri, C., 2020. A review of genetic improvement of main fruit trees through modern biotechnological tools and considerations of the cultivation and research of the engineered plant restrictions. *Pakistan Journal of Agricultural Science*, 57 (1):17-42.
- Salonia, F., Ciacciulli, A., Poles, L., Pappalardo, H. D., La Malfa, S., Licciardello, C., 2020. New plant breeding techniques in citrus for the improvement of important agronomic traits. A Review. *Frontiers in Plant Science*, 11: 1234.
- Santos, A.R.F., Ramos-Cabrera, A.M., Díaz-Hernández, M.B., Pereira-Lorenzo, S., 2011. Genetic variability and diversification process in local pear cultivars from northwestern Spain using microsatellites. *Tree Genetics & Genomes*, 7:1041-1056.
- Senica, M., Stampar, F., Mikulic-Petkovsek, M., 2019. Different extraction processes affect the metabolites in blue honeysuckle (*Lonicera caerulea* L. subsp. *edulis*) food products. *Turkish Journal of Agriculture and Forestry*, 43: 576-585.
- Sestras, A., Sestras, R., Barbos, A., Madalina, M., 2008. The differences among pear genotypes to fire blight (*Erwinia amylovora*) attack, based on observations of natural infection. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 36: 98-103.
- Shah, L.R., Sharma, A., Nabi, J., Prasad, J., 2018. Breeding approaches for abiotic stress management in vegetable crops. *Journal of Pharmacognosy and Phytochemistry*, 7:1023-1028.
- Silva, A.V.C., Amorim, J. A. E., Vitória, M.F., Ledo, A.S., Rabbani, A.R.C., 2017. Characterization of trees, fruits and genetic diversity in natural populations of mangaba. *Ciência e Agrotecnologia*, 41(3): 255-262.
- Song, G., Prieto, H., Orbović, V., 2019. Agrobacterium-mediated transformation of tree fruit crops: methods, progress, and challenges. *Frontiers in Plant Science*, 10: 226.
- Vaughan, D.A., Balazs, E., Heslop-Harrison, J.S., 2007. From crop domestication to super-domestication. *Annals of Botany*, 100: 893-901.
- Versini, G., Franco, M.A., Moser, S., Manca, G., 2012. Characterization of pear distillates from wild and cultivated varieties in Sardinia. *International Journal of Food Science and Technology*, 47(12): 2519-2531.
- Wu, X., Cai, K., Zhang, G., Zeng, F., 2017. Metabolite profiling of barley grains subjected to water stress: To explain the genotypic difference in drought-induced impacts on malting quality. *Frontiers in Plant Science*, 8:1547.

Oral Presentation

Wednesday

Diversity of Animal Species, Systematics, and Phylogeny-1

### Contribution to the Knowledge of Heteroptera (Hemiptera) Fauna of Eastern Turkey

Neslihan Gültekin<sup>1</sup>, Melek Güdek Güçlü<sup>2</sup>, Dilek Doğan<sup>1\*</sup>, Mustafa Güllü<sup>3</sup>, Celalettin Gözüaçık<sup>1</sup>

<sup>1</sup>Iğdır University, Faculty of Agriculture, Department of Plant Protection, Iğdır, Turkey.

<sup>2</sup>Atatürk University, Faculty of Agriculture, Department of Plant Protection, Erzurum, Turkey.

<sup>3</sup>Bingöl University, Faculty of Agriculture, Department of Plant Protection, Bingöl, Turkey.

\*Corresponding author e-mail: dogandilek85@gmail.com

#### Abstract

Hemiptera is a very important order both in terms of species diversity and economically. One of the suborders of this order, Heteroptera Latreille, 1810, includes more than 45,000 species in the world and more than 8,000 of them are distributed in the Palearctic region. According to the latest studies, 1536 species have been recorded from Turkey. In this study, the diversity of Heteroptera species collected from many provinces in Eastern Turkey between 1998 and 2021 is examined. As a result, 128 species belonging to 18 families are identified. These families are Pentatomidae Leach, 1815 (38 species), Miridae Hahn, 1831 (29 species), Alydidae Amyot & Serville, 1843 (3 species), Anthocoridae (1 species), Lygaeidae (9 species), Acanthosomatidae Signoret, 1864 (1 species), Rhopalidae Amyot and Serville, 1843 (6 species), Rhyparochromidae Amyot and Serville 1843 (9 species), Cydnidae Bilberg, 1820 (1 species), Coreidae Leach, 1815 (6 species), Tingidae Laporte, 1832 (1 species), Reduviidae Latreille, 1807 (7 species), Scutelleridae Leach, 1815 (9 species), Plataspidae Dallas, 1851 (1 species), Stenocephalidae Dallas, 1852 (2 species), Heterogastridae Stål, 1872 (1 species), Nabidae Costa, 1853 (2 species), Oxycarenidae Stål, 1862 (1 species) and Pyrrhocoridae Dohrn, 1859 (1 species). In addition, known distributions of these species in Turkey by province and regions are presented.

**Keywords:** Heteroptera fauna, biodiversity, Eastern Turkey.

**Acknowledgement:** We would like to thank Barış Çerçi (Ankara, Turkey) for the identification of examined specimens.

Oral Presentation

Wednesday

Diversity of Animal Species, Systematics, and Phylogeny-1

## Thrips (Thysanoptera) Species and Distribution Areas in Northern Cyprus Cereal Fields

Mustafa Güllü<sup>1\*</sup>, Celalettin Gözüaçık<sup>2</sup>, Ayda Konuksal<sup>3</sup>

<sup>1</sup>Bingöl University Faculty of Agriculture Department of Plant Protection, Bingöl-Turkey

<sup>2</sup>Iğdır University, Faculty of Agriculture Department of Plant Protection, Iğdır-Turkey

<sup>3</sup>Agricultural Research Institute, Lefkoşa-Turkish Republic of Northern Cyprus

\*Corresponding: mgullu83@hotmail.com

### Abstract

Thrips are an important pest group in cereals. This study was carried out to determine the current Thrips (Thysanoptera) species and distribution areas in the cereal fields of the Turkish Republic of Northern Cyprus between 2012 and 2013. For this purpose, a total of 100 sweep nets were swayed 10 times at 10 different places in each field that was randomly entered in the cereal fields in Lefkoşa, Gazimagusa, Girne, Iskele and Güzelyurt districts. Thrips that fell into the sweepnet were taken with a mouth aspirator and kept in small plastic tubes with labels containing 70% ethyl alcohol for to preserve. As a result of the identification made from the collected samples, a total of 11 thrips species were determined, including *Aeolothrips intermedius* Bagnall, 1934, *Melanthrips fuscus* (Sultz, 1776), *Melanthrips pallidus* Priesner, 1919, *Rhipidothrips brunneus* Williams, 1913, and *Rhipidothrips graciosus* Uzel, 1895, species from the Aelothripidae family, *Haplothrips bolacophilus* Priesner, 1938 species from the Phlaeothripidae family, and *Frankliniella tenuicornis* (Uzel, 1895), *Limothrips cerealium* Haliday, 1836, *Sitothrips arabicus* Priesner, 1931, *Stenothrips graminum* Uzel, 1895 and *Thrips angusticeps* Uzel, 1895 species from the Thripidae family. In the literature review, it was understood that all species were the first records for The Northern Cyprus cereal fields and *Aeolothrips intermedius* Bagnall, 1934, *Frankliniella tenuicornis* (Uzel, 1895) and *Rhipidothrips brunneus* Williams, 1913 species were the first records for Cyprus Island.

**Keywords:** Thrips species, cereal, trnc

Oral Presentation

Wednesday

Diversity of Animal Species, Systematics, and Phylogeny-1

## Variable Detection and Comparison of Supervised Machine Learning Algorithms in Classification of Two Closely Related *Bufo* Species

Cantekin Dursun<sup>1\*</sup>, Serkan Gül<sup>1</sup>, Nurhayat Özdemir<sup>1</sup>

<sup>1</sup>Recep Tayyip Erdogan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey.

\*Corresponding author e-mail: cantekin.dursun@erdogan.edu.tr

### Abstract

Machine learning (ML) is the concept that utilizes past experience to learn from and use its knowledge to make future decisions. The main goal of ML is generalizing detectable patterns or revealing unknown rules from given datasets. Supervised learning, a subset of ML, is a method to teach machines to learn the relationship between the target variable and the other variables. In this approach, algorithms refer to techniques in which a model is trained on a range of inputs under a known outcome. In this study, we aimed to determine the accuracy of supervised learning algorithms to classify two closely related species *Bufo bufo* and *B. verrucosissimus*, compare the success rates and reveal informative characters. For this, we used the dataset including 31 different morphological measurements obtained from 220 genetically identified individuals in our previous studies, then we ran downstream analyses following the rules of ML process using the K-Nearest Neighbours (KNN), Support Vector Machine (SVM), Neural Network, Decision Tree and Random Forest algorithms. The results indicated that the algorithms provided reasonable findings with acceptable success rates (all models are over 75%). The most successful method was found as KNN (86%) while the lowest accuracy score was in Support Vector Machine (77%). According to the results of KNN, SVM and Neural Network analyses the most important characters to distinguish the species were more associated with the dimension and shape of inner metatarsal tubercle and dimension and angle of parotoid glands. However, Decision Tree and Random Forest algorithms classified the species following the width of parotoids and eye-related characters. The findings in this study were supported by the results of previous studies based on morphological discrimination and cumulated them in a single framework. Therefore, it is thought that ML algorithms are feasible to classify the species and taxonomically important variable detection.

**Keywords:** caucasian toad, common toad, data science, prediction, Turkey

Oral Presentation

Wednesday

Diversity of Animal Species, Systematics, and Phylogeny-1

**Aphidofagous Syrphids (Diptera: Syrphidae) from Çardak Lagoon in the Çanakkale  
Province of the Northwestern Part of Turkey**

Şahin Kök<sup>1\*</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, Lapseki Vocational School, Department of Plant and Animal  
Production, Çanakkale, Turkey.

\*Corresponding author e-mail: [sahinkok@gmail.com](mailto:sahinkok@gmail.com); [sahinkok@comu.edu.tr](mailto:sahinkok@comu.edu.tr)

**Abstract**

Aphids (Hemiptera: Aphididae) are one of the most important pest insect groups that cause significant economic losses on agricultural crops worldwide. Investigation of the interactions of aphid pests and their natural enemies such as syrphid predators in non-agricultural areas as well as agricultural areas is important in terms of effective and appropriate biological control strategies. The present study aimed to determine the *Aphidophagous syrphids* (Diptera: Syrphidae) in the Çardak Lagoon, which is close to agricultural areas in Çanakkale Province of the northwestern part of Turkey. Sampling was done during the spring and summer in 2020. As a result of the diagnosis of the specimens, five species belonging to five genera from the family Syrphidae (Diptera) associated with seven aphid species from the family Aphididae (Hemiptera) on eight host plants were revealed. Of these, *Episyrphus balteatus* (de Geer) is the most common syrphid with five host aphid species. Also, *Eupeodes corollae* (Fabricius) was determined on only one host aphid species. These results revealed that non-agricultural areas such as lagoon and wetland areas, which are close to the agricultural fields can have rich potential in terms of the presence of aphidophagous syrphids. It is thought that these data will contribute to the use of syrphid species as effective biological control agents against aphid pests in agricultural crops.

**Keywords:** Syrphid, aphid, natural enemy, Çanakkale, Turkey

Oral Presentation  
Wednesday  
Microbial Biodiversity-1

**Arbuscular Mycorrhizal (AMF) and Disease-Causing Fungus Species Isolated from Dried  
Tea Seedlings in a Tea Garden**

Sengül Alpay Karaoğlu<sup>1\*</sup>, Fatih Seyis<sup>2</sup>

<sup>1</sup>Recep Tayyip Erdogan University Faculty of Arts & Sciences, Biology Department, Rize/Turkey

<sup>2</sup>Recep Tayyip Erdogan University Faculty of Agriculture, Field Crops Department, Rize/Turkey

**Abstract**

Tea (*Camellia sinensis*) a perennial plant covering all five continents is grown globally in 58 countries, and its global tea market has reached a volume of approximately 6.40 million tons and an economic value of 20 billion US dollars. Studies on tea plant diseases have started a long time ago but have gained great importance in recent years due to global warming. Our country ranks seventh in the world in terms of the total tea agricultural area (Rize province) and provides employment in the sector to more than 200 thousand farmers. Due to the geographical location of the region, there is no need for important studies on tea diseases. However, factors such as global warming and the application of uniform chemical fertilizers in agriculture have led to the deterioration of the soil ecosystem and the emergence of diseases. In our study, it was aimed to determine the microbial flora and possible disease-causing microorganisms in a sample taken from a tea garden under the threat of desiccation and to create solutions for the prevention of diseases. In the sample taken, 7 different (soil of rhizosphere, the root, root crown, stem and leaves) examination materials were determined, further bacterial and fungal normal flora and disease-causing microorganisms were examined with traditional methods. It has been observed that the bacterial population and diversity of the soil and plant flora have decreased considerably, and the fungal flora can still be considered as wealthy, but plant pathogenic fungi play an important role in the drying of the plant. *Bacillus* sp. were determined as common bacterial species, *Penicillium* sp. as common fungal species, and primarily *Fusarium* sp., *Gliocladium* sp., *Glomerella* sp., *Alternaria* sp., *Pestalotiopsis* sp. were determined as disease-causing agents. It has been observed that the disease causing drying are Daiback and Collar cancer (Canker).

**Keywords:** *Camellia sinensis*, plant disease, microbial flora, microfungi

Oral Presentation  
Wednesday  
Microbial Biodiversity-1

**Diagnosis of The Factors Ausing The Drying of *Camellia Sinensis* Seedlings, Isolation of The Factors and Pathogenicity Determination by Leaf Pathogenicity Test**

Şengül Alpay Karaoğlu<sup>1\*</sup>,

<sup>1</sup>Recep Tayyip Erdogan University Faculty of Arts & Sciences, Biology Department, Rize/Turkey

**Abstract**

Tea cultivation in our country is carried out in some districts of Trabzon, Giresun, and Ordu provinces, especially in Rize and Artvin, and meets a minimum of 85% of the country's tea needs. Disease agents in tea have been investigated for many years in countries such as China, Ceylon, India, which are the leading countries of world tea production. Global warming, climate changes, fertilization - care strategies have been seriously activated of disease factors. For this reason, research on the disease and its factors has gained momentum in recent years. In our study, it was aimed to determine the possible microbial agents of root and shoot drying observed in the garden and the pathogenicity levels of these agents. After the morphological (root, root collar, stem and branches) examinations of the dry tea samples were made, they were brought to the laboratory in sterile bags to be subjected to cultural methods. Potentially causative bacterial and fungal isolates were obtained using culture techniques. Microfungi isolates were identified at genus level by traditional methods. It was observed that the bacterial flora and diversity in the plant rhizosphere decreased, and the fungal flora and diversity increased. The causative bacteria of illness could not be detected. Among the fungal genera containing plant pathogenic species, *Fusarium sp.*, *Paecilomyces sp.*, *Botrytis sp.*, *Gliocladium sp.* and *Colletotrichum sp.* were defined. Of these isolates, which were subjected to leaf pathogenicity test, *Fusarium sp.* (Strain No-7), *Colletotrichum sp.* (11), *Botrytis sp.* (4) and *Gliocladium sp.* The pathogenicity of (9) was confirmed. As a result, it is thought that tea plants, whose resistance is estimated to decrease in environmental conditions, dry out as a result of the disease factor called root neck cancer or collar cancer due to these observed factors.

**Keywords:** *Camellia sinensis*, microbial flora, pathogen microfung



Oral Presentation  
Wednesday  
Microbial Biodiversity-1

## Identification and Characterization of Bacteria Isolated from Apricot Trees in The Province of Erzurum, Turkey

Damla Rüzgar<sup>1\*</sup>, Arzu Görmez<sup>1</sup>

<sup>1</sup>Erzurum Technical University, Faculty of Science, Department of Molecular Biology and Genetics, Erzurum, Turkey

\*Corresponding author e-mail: damla.ruzgar@erzurum.edu.tr

### Abstract

In this study; It was aimed to identify the bacteria isolated from apricot trees grown in Erzurum and to determine the fatty acid compositions of these isolates. According to the results of the isolation, the bacteria were identified as *Ralstonia pickettii* (15), *Pantoea agglomerans* (12), *Chromobacterium violaceum* (6), *Pseudomonas viridiflava* (4), *Hydrogenophaga palleronii* (3), *Pseudomonas coronafaciens* (2), *Kluyvera intermedia* (2), *Pectobacterium atrosepticum* (2), *Acidovorax cattleyae* (1), *Acinetobacter calcoaceticus* (1), *Bacillus subtilis* (1), *Corynebacterium diphtheriae* (1), *Corynebacterium glutamicum* (1), *Tetragenococcus solitarius* (1), *Dickeya chrysanthemi* (1), *Photobacterium leiognathi* (1), *Microbacterium liquefaciens* (1), *Serratia liquefaciens* (1), *Serratia plymuthica* (1), *Stenotrophomonas maltophilia* (1) and *Vibrio cholerae* (1). In summary, it was determined that fatty acid contents might differ according to species, there were quite different bacterial isolates in the flora of apricot trees, contrary to expectations, and some of them were pathogenic strains and biological control agents.

**Keywords:** Apricot, fatty acid analysis, identification, characterization

### INTRODUCTION

Apricot (*Prunus armeniaca L.*) is one of the stone fruits widely grown in Anatolia, belonging to the Rosaceae. Apricot, which are consumed both fresh and dried, is also used in the food

industry for many purposes such as fruit juice, jam. Therefore, it is very important to determine the microbial flora of apricot trees in Turkey, which is one of the world's leading countries in apricot production (Bruno *et al.*, 2021; Ercişli, 2009). As it is known, the microbiome of plants has a very important role in plant germination, promoting plant growth, preventing diseases, protecting the plant and increasing stress resistance. Therefore, the microbial flora of plants is in a strategic position to increase crop production, preserve biodiversity and maintain agroecosystems (Germida *et al.*, 1998; Berg *et al.* 2017).

Microbial Identification System (MIS), which analyzes based on fatty acids and is produced by Microbial ID (MIDI, Newark, DE, USA), is a molecular method used for the identification of microorganisms. This system, the first automated cell fatty acid identification system, is an accurate, relatively rapid and quite efficient, method for the identification of many bacteria. In this method, the fatty acids of the cells are extracted and GC-MS is used to identify and quantitate the fatty acid methyl esters. At the same time, fatty acid profiles are compared to libraries of known microorganisms and thus unknown microorganisms are identified by this system. This method allows to faster identification of bacteria after isolation, and also characterization as it determines the number, diversity and percentage amounts of fatty acids (Buyer, 2002; Buyer, 2006).

In this study, it was aimed to identify the bacteria isolated from apricot trees grown in Erzurum and to determine the fatty acid compositions of these isolates.

## **MATERIALS AND METHODS**

### **Isolation of Bacteria**

The bacteria were isolated from leaves and shoots of apricot trees in Erzurum, Turkey. For this purpose, first plant tissue materials were left in 2 ml sterile saline (0.85% NaCl) test tubes for 30 min and plated onto different medium such as nutrient agar (NA), semi selective King's medium (King *et al.*, 1954) and nutrient agar with 5% sucrose (NSA) (Lelliott *et al.*, 1987). Plates were incubated at 27°C for 2-5 days. Later, different colonies were selected and stored at -80 °C.

### **Identification of Bacteria**

The bacterial isolates were streaked onto trypticase soy broth agar (TSBA) and at 27 °C for 24 h for identification with the MIS. Approximately 40 mg of biomass from the third quadrant was

taken and transferred to teflon capped test tubes. According to standard protocols, the fatty acids of cells were saponified (with sodium hydroxide in methanol), methylated (with hydrochloric acid in methanol), extracted (with hexane in methyltert-butyl ether), and cleaned by base wash (with sodium hydroxide), to cleave the fatty acids from lipids (Sasser, 1990.). Extracted fatty acid methyl esters were analyzed with a Hewlett-Packard model 5890 CG gas chromatograph by using the library TSBA Version 4.0, and were identified by using the MIS software (Miller and Berger 1985; Roy, 1988.).

## RESULTS

A total of 59 bacterial strains was isolated from apricot trees grown in Erzurum. These bacterial strains were identified and characterized according to fatty acid analysis. The results of identification and locations of the isolated bacterial strains are shown in Figure 1. According to the results of FAME, isolated bacteria were identified as *Ralstonia pickettii* (15), *Pantoea agglomerans* (12), *Chromobacterium violaceum* (6), *Pseudomonas viridiflava* (4), *Hydrogenophaga palleronii* (3), *Pseudomonas coronafaciens* (2), *Kluyvera intermedia* (2), *Pectobacterium atrosepticum* (2), *Acidovorax cattleyae* (1), *Acinetobacter calcoaceticus* (1), *Bacillus subtilis* (1), *Corynebacterium diphtheriae* (1), *Corynebacterium glutamicum* (1), *Tetragenococcus solitarius* (1), *Dickeya chrysanthemi* (1), *Photobacterium leiognathi* (1), *Microbacterium liquefaciens* (1), *Serratia liquefaciens* (1), *Serratia plymuthica* (1), *Stenotrophomonas maltophilia* (1) and *Vibrio cholerae* (1).

The percentage of fatty acids compositions are shown in Figure 2. At the same time, it was determined that the identified bacteria generally contained saturated, unsaturated, branched, cyclo, iso, anteiso, hydroxy and methylated ranging in carbon chain length from C9 to C18 fatty acids. Besides, it was also found that the percentage of fatty acids analysis ranged from 0.215 to 0.957 %. Fatty acids of isolated bacteria were detected as 10:0 3OH, 12:0, 12:0 2OH, 12:0 3OH, 16:0, 18:1 w7c in *P. viridiflava*, 14:0, 16:0, 18:1 w7c, 18:1 2OH in *R. pickettii*, 14:0, 16:0, 18:1 w7c in *P. agglomerans* strains (Fig 2). According to the results of analyze, the fatty acids of isolated bacterial strains were consisted predominantly of saturated and unsaturated fatty acids: hexadecenoic acid (16:0) and octadecenoic acid (18:1 w7c), respectively.

**Figure 1. Results of bacterial identification**

| Strain Code | MIS results                                      | SI*   | Locations                      |
|-------------|--|-------|--------------------------------|
| AA-4        | <i>Pseudomonas coronafaciens</i>                 | 0,944 | Erzurum (Hoş-Şenkaya)          |
| AA-28       | <i>Pseudomonas viridiflava</i>                   | 0,926 | Erzurum (Paşalı-Şenkaya)       |
| AA-29       | <i>Pseudomonas viridiflava</i>                   | 0,926 | Erzurum (Ormanağzı-Olur)       |
| AA-31       | <i>Pseudomonas viridiflava</i>                   | 0,877 | Erzurum (Kaledibi-Olur)        |
| AA-33       | <i>Pseudomonas viridiflava</i>                   | 0,917 | Erzurum (Uzundere)             |
| AA-34       | <i>Pseudomonas viridiflava</i>                   | 0,939 | Erzurum (Madenköprübaşı-İspir) |
| AA-47       | <i>Chromobacterium violaceum</i>                 | 0,957 | Erzurum (Başaklı-Oltu)         |
| AA-48       | <i>Chromobacterium violaceum</i>                 | 0,957 | Erzurum (Başaklı-Oltu)         |
| AA-49       | <i>Chromobacterium violaceum</i>                 | 0,957 | Erzurum (Kaledibi-Olur)        |
| AA-51       | <i>Chromobacterium violaceum</i>                 | 0,902 | Erzurum (Oltu)                 |
| AA-52       | <i>Chromobacterium violaceum</i>                 | 0,953 | Erzurum (Uzundere)             |
| AA-53       | <i>Hydrogenophaga palleronii</i>                 | 0,810 | Erzurum (Tortum)               |
| AA-54       | <i>Hydrogenophaga palleronii</i>                 | 0,810 | Erzurum (Kaledibi-Olur)        |
| AA-55       | <i>Hydrogenophaga palleronii</i>                 | 0,810 | Erzurum (Oltu)                 |
| AA-57       | <i>Dickeya chrysanthemi</i>                      | 0,624 | Erzurum (Kirazlı-İspir)        |
| AA-59       | <i>Erwinia caratovora</i> pv. <i>atroseptica</i> | 0,734 | Erzurum (Aksu-Tortum)          |
| AA-60       | <i>Erwinia caratovora</i> pv. <i>atroseptica</i> | 0,684 | Erzurum (Oltu)                 |
| AA-69       | <i>Microbacterium liquefaciens</i>               | 0,615 | Erzurum (Oltu)                 |
| AA-77       | <i>Bacillus subtilis</i>                         | 0,659 | Erzurum (Çamlıbel-Oltu)        |
| AA-83       | <i>Acinetobacter calcoaceticus</i>               | 0,712 | Erzurum (Bardız-Şenkaya)       |
| AA-91       | <i>Serratia plymuthica</i>                       | 0,824 | Erzurum (Tortum)               |
| AA-92       | <i>Serratia liquefaciens</i>                     | 0,636 | Erzurum (Kirazlı-İspir)        |
| AA-95       | <i>Vibrio cholerae</i> non                       | 0,619 | Erzurum (Oltu)                 |
| AA-98       | <i>Corynebacterium diphtheriae</i>               | 0,630 | Erzurum (Ormanağzı-Olur)       |
| AA-100      | <i>Ralstonia pickettii</i>                       | 0,879 | Erzurum (Tortum)               |
| AA-102      | <i>Ralstonia pickettii</i>                       | 0,782 | Erzurum (Uzundere)             |
| AA-103      | <i>Ralstonia pickettii</i>                       | 0,852 | Erzurum (Aksu-Tortum)          |
| AA-104      | <i>Ralstonia pickettii</i>                       | 0,706 | Erzurum (Oltu)                 |
| AA-105      | <i>Ralstonia pickettii</i>                       | 0,631 | Erzurum (Kaledibi-Olur)        |
| AA-106      | <i>Ralstonia pickettii</i>                       | 0,805 | Erzurum (Bardız-Şenkaya)       |
| AA-107      | <i>Ralstonia pickettii</i>                       | 0,790 | Erzurum (Bardız-Şenkaya)       |
| AA-108      | <i>Ralstonia pickettii</i>                       | 0,805 | Erzurum (Hoş-Şenkaya)          |
| AA-109      | <i>Ralstonia pickettii</i>                       | 0,919 | Erzurum (Bardız-Şenkaya)       |
| AA-110      | <i>Ralstonia pickettii</i>                       | 0,819 | Erzurum (Başaklı-Oltu)         |
| AA-111      | <i>Ralstonia pickettii</i>                       | 0,783 | Erzurum (Taht-Şenkaya)         |
| AA-112      | <i>Ralstonia pickettii</i>                       | 0,778 | Erzurum (Taht-Şenkaya)         |
| AA-113      | <i>Pantoeae agglomerans</i>                      | 0,931 | Erzurum (Oltu)                 |
| AA-126      | <i>Pantoeae agglomerans</i>                      | 0,928 | Erzurum (Uzundere)             |
| AA-127      | <i>Pantoeae agglomerans</i>                      | 0,865 | Erzurum (Çamlıbel-Oltu)        |
| AA-128      | <i>Pantoeae agglomerans</i>                      | 0,847 | Erzurum (Ormanağzı-Olur)       |
| AA-129      | <i>Pantoeae agglomerans</i>                      | 0,667 | Erzurum (Taht-Şenkaya)         |
| AA-130      | <i>Pantoeae agglomerans</i>                      | 0,661 | Erzurum (Taşlı-Olur)           |
| AA-131      | <i>Pantoeae agglomerans</i>                      | 0,654 | Erzurum (Taht-Şenkaya)         |
| AA-132      | <i>Pantoeae agglomerans</i>                      | 0,796 | Erzurum (Başaklı-Oltu)         |
| AA-133      | <i>Pantoeae agglomerans</i>                      | 0,921 | Erzurum (Oltu)                 |
| AA-138      | <i>Pantoeae agglomerans</i>                      | 0,887 | Erzurum (Madenköprübaşı-İspir) |
| AA-141      | <i>Pantoeae agglomerans</i>                      | 0,776 | Erzurum (Başaklı-Oltu)         |
| AA-142      | <i>Kluyvera intermedia</i>                       | 0,702 | Erzurum (Uzundere)             |
| AA-143      | <i>Pantoeae agglomerans</i>                      | 0,686 | Erzurum (Oltu)                 |
| AA-144      | <i>Kluyvera intermedia</i>                       | 0,653 | Erzurum (Pazaryolu)            |
| AA-147      | <i>Chromobacterium violaceum</i>                 | 0,662 | Erzurum (Oltu)                 |
| AA-148      | <i>Ralstonia pickettii</i>                       | 0,751 | Erzurum (Madenköprübaşı-İspir) |

|        |                                     |       |                                |
|--------|-------------------------------------|-------|--------------------------------|
| AA-149 | <i>Enterococcus solitarius</i>      | 0,215 | Erzurum (Taşlı-Olur)           |
| AA-151 | <i>Corynebacterium glutamicum</i>   | 0,741 | Erzurum (Madenköprübaşı-İspir) |
| AA-274 | <i>Stenotrophomonas maltophilia</i> | 0,882 | Erzurum (Tortum-Erzurum)       |
| AA-277 | <i>Stenotrophomonas maltophilia</i> | 0,894 | Erzurum (Tortum-Erzurum)       |
| AA-278 | <i>Stenotrophomonas maltophilia</i> | 0,887 | Erzurum (Tortum-Erzurum)       |
| AA-340 | <i>Photobacterium leiognathi</i>    | 0,626 | Erzurum (Ormanağzı-Olur)       |
| AA-342 | <i>Pseudomonas putida</i>           | 0,900 | Erzurum (Taşlı-Olur)           |
| AA-343 | <i>Acinetobacter baumannii</i>      | 0,684 | Erzurum (Ayvalı-Olur)          |

\*Similarity Index

**Figure 2.** Percentage of Fatty Acids Compositions of Bacterial Isolates

| Strains Code   | Fatty Acids |           |              |          |             |          |              |          |            |       |             |          |
|--|-------------|-----------|--------------|----------|-------------|----------|--------------|----------|------------|-------|-------------|----------|
|  | 12:0        | 14:0      | 15:0 ANTEISO | 15:0 ISO | 16:0        | 16:0 ISO | 17:0 ANTEISO | 17:0 ISO | 17:0 CYCLO | 18:0  | 18:1 w7c    | 18:1 w9c |
| AA-4,  |             |           |              |          | 28.09       |          |              |          |            |       | 14.19       |          |
| AA-28, AA-29, AA-31,<br>AA-33, AA-34   |             |           |              |          | 26.49-30.79 |          |              |          |            |       | 20.07-21.00 |          |
| AA-47, AA-48, AA-49,<br>AA-51, AA-52   |             |           |              |          | 30.79-30.97 |          |              |          |            |       | 15.48-17.96 |          |
| AA-53, AA-54, AA-55  |             |           |              |          | 30.28       |          |              |          |            | 28.39 |             |          |
| AA-57  |             |           |              |          | 29.27       |          |              |          |            |       | 13.73       |          |
| AA-59, AA-60   |             |           |              |          | 29.38-29.84 |          |              |          |            |       | 14.22-14.63 |          |
| AA-69  |             |           | 45.14        |          |             | 8.95     | 35.47        |          |            |       |             |          |
| AA-77  |             |           | 38.74        | 32.43    |             |          | 10.52        | 10.71    |            |       |             |          |
| AA-83  | 6.61        |           |              |          | 20.11       |          |              |          |            |       | 20.33       |          |
| AA-91  |             | 6.28      |              |          | 32.32       |          |              |          | 17.51      |       | 10.04       |          |
| AA-92  |             |           |              |          | 30.41       |          |              |          |            |       | 14.33       |          |
| AA-95  |             |           |              |          | 23.07       |          |              |          |            |       | 25.06       |          |
| AA-98  |             |           |              |          | 43.42       |          |              |          |            |       |             |          |
| AA-100, AA-102, AA-103,<br>AA-104, AA-105, AA-106,<br>AA-107, AA-108, AA-109,<br>AA-110, AA-111, AA-112,<br>AA-148 |             |           |              |          | 23.03-27.61 |          |              |          |            |       | 21.20-27.61 |          |
| AA-113, AA-126, AA-127,<br>AA-128, AA-129, AA-130,<br>AA-131, AA-132, AA-133,<br>AA-138, AA-141, AA-143,           |             | 5.01-5.78 |              |          | 27.43-31.11 |          |              |          | 5.16-9.23  |       | 11.42-13.57 |          |
| AA-142, AA-144   |             | 5.59-     |              |          | 28.38       |          |              |          | 5.71       |       | 13.43       |          |
| AA-147   |             |           |              |          | 27.86       |          |              |          |            |       | 19.43       |          |



### 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021



|                        |  |  |  |             |           |  |  |  |  |  |       |       |
|------------------------|--|--|--|-------------|-----------|--|--|--|--|--|-------|-------|
| AA-151                 |  |  |  |             | 41.49     |  |  |  |  |  | 58.51 |       |
| AA-274, AA-277, AA-278 |  |  |  | 35.40-35.60 | 5.74-5.88 |  |  |  |  |  |       |       |
| AA-340                 |  |  |  |             | 26.44     |  |  |  |  |  | 17.61 |       |
| AA-342                 |  |  |  | 74.69       |           |  |  |  |  |  |       |       |
| AA-343                 |  |  |  | 20.03       |           |  |  |  |  |  |       | 38.68 |

\* Less than 5% is neglected.

## DISCUSSION

In this study, most of the bacteria isolated from apricot were identified as *C. violaceum*, *E. intermedius*, *P. agglomerans*, *P. viridiflava* and *S. maltophilia* strains. There have been reported literatures on the causative agents of the disease rather than bacterial diversity in apricots (Lucas, 2009). Therefore, in this study, the bacterial flora of apricot was determined, different from literature. In fact, as such studies on the flora of plants increase, it will be possible to prevent diseases and increase productivity. It was evaluated fatty acids to identify bacteria isolated from apricot tree in this study and was showed relative similarities and differences of fatty acids among bacteria. In the literature, there are some studies on fatty acid analyzes of microorganisms and that some of these fatty acids may be species-specific. For example, it has been reported that branched fatty acids such as a15:0, i15:0, i16:0, a17:0 and i17:0 are markers for Gram-positive bacteria, a15:0 and i15:0 are markers for Gram-negative bacteria isolated from the rhizosphere of *Brassica napus L.* by Ibekwe et al. (1999) and Tunlid *et al.* (1985), respectively. In particular, Ibekwe et al. (1999) showed that some fatty acids (a15:0 and i15:0) were found in all gram-positive bacteria (Ratledge and Wilkinson, 1988) and that cyclopropane (cyc 17:0) was intensely observed in certain groups of gram-negative bacteria in the rhizosphere such as *Campylobacter*, *Cromatium*, *Legionella* and *Rhodospirillum* (Ibekwe and Kennedy 1999). At the same time, it was also announced as component of Gram-negative bacteria particularly *Pseudomonas* species by Wollenweber and Rietschel (1990). Tunlid et al. (1985) reported that it showed high frequency of a15:0 and i15:0 in Gram negative bacteria isolated from the rhizosphere of *Brassica napus L.* In our study; it observed that the presence of i16:0, a17:0, i17:0 in *M. liquefaciens*, a15:0, i15:0, i16:0, i17:0, a17:0 in *B. subtilis* as Gram positive bacteria. But these fatty acids was not determined in Gram positive bacteria such as *C. glutamicum*, *C. diphtheriae*. Vesta and White (1989) reported that cyclopropane fatty acids could be showed as a marker of anaerobic conditions. Our results showed that cyclopropane fatty acids were found a number of Gram-negative bacteria. While these fatty acids were observed in all strains of *P. agglomerans*, they were not founded in all strains of *R. pickettii* and *C. violaceum*. In another study, it has also been reported that 18:1w9c and 16:1w5c may be marker for saprophytic and arbuscular mycorrhizal fungi by Cardinali et al. (2015). However, in our study, 18:1 w9c fatty acid was determined to be present in *A. baumannii*, *A. calcoaceticus*, *C. glutamicum* and *S. maltophilia*. But these fatty acids were not determined in some Gram-positive bacteria such as *C. glutamicum*, *C. diphtheriae*



## CONCLUSIONS

As can be seen from the literature research, the identification and characterization of the bacteria can be performed by determining the fatty acid contents of the cells. In this context, the present study is important in terms of determining the flora of apricot and demonstrating the usability of the MIS system.

## REFERENCES

- Berg G, Köberl M, Rybakova D, Müller H, Grosch R, & Smalla K (2017). Plant microbial diversity is suggested as the key to future biocontrol and health trends. *FEMS microbiology ecology*, 93(5).
- Bruno M R., Russo D, Faraone, I., D'Auria, M., Milella, L & Todaro L (2021). Orchard biomass residues: Chemical composition, biological activity and wood characterization of apricot tree (*Prunus armeniaca* L.). *Biofuels, Bioproducts and Biorefining*, 15(2), 377-391.
- Buyer J S (2002). Identification of bacteria from single colonies by fatty acid analysis. *Journal of microbiological methods*, 48(2-3), 259-265.
- Buyer J S (2006). Rapid and sensitive FAME analysis of bacteria by cold trap injection gas chromatography. *Journal of microbiological methods*, 67(1), 187-190
- Cardinali A, Pizzeghello D & Zanin G (2015). Fatty acid methyl ester (FAME) succession in different substrates as affected by the co-application of three pesticides. *PloS one*, 10(12), e0145501.
- Ercisli S (2009). Apricot culture in Turkey. *Scientific Research and Essay* 4: 715-719
- Germida J J, Siciliano S D, Renato de Freitas J & Seib A M 1998. Diversity of root-associated bacteria associated with field-grown canola (*Brassica napus* L.) and wheat (*Triticum aestivum* L.). *FEMS Microbiology Ecology*, 26(1), 43-50.
- Ibekwe A M & Kennedy A C (1999). Fatty acid methyl ester (FAME) profiles as a tool to investigate community structure of two agricultural soils. *Plant and Soil*, 206(2), 151-161.
- King E O, Ward M K, & Raney D E (1954). Two simple media for the demonstration of pyocyanin and fluorescein. *The Journal of laboratory and clinical medicine*, 44(2), 301-307.
- Lelliott, R. A., & Stead, D. E. (1987). *Methods for the diagnosis of bacterial diseases of plants*. Blackwell Scientific Publications.
- Lucas J A (2009). *Plant pathology and plant pathogens*. John Wiley & Sons.
- Miller L & Berger T (1985). Bacteria identification by gas chromatography of whole cell fatty acids. *Hewlett-Packard application note*, 228(41).
- Roy A 1988. Use of fatty acid for the identification of phyto pathogenic bacteria. *Plant Disease* 72: 4
- Sasser, M (1990). Identification of bacteria through fatty acid analysis. *Methods in phytobacteriology*, 565.
- Ratledge C and Wilkinson S G (1988). *Microbial lipids*, vol. 1. Academic Press. Inc. New York.
- Tunlid A, Baird B H, Trexler M B, Olsson S, Findlay R H, Odham G & White D C (1985). Determination of ester-linked fatty acids and poly  $\beta$ -hydroxybutyrate for the estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* L. *Can.J. Microbiol.* 31, 1113–1119.
- Vestal J R & White D C (1989). Lipid analysis in microbial ecology. *Bioscience*, 39(8), 535-541.
- Wollenweber H. W. and Rietschel E. T. (1990). Analysis of lipopolysaccharides (lipid A) fatty acids. *J. Microbiol. Methods* 11, 195– 211.

Oral Presentation  
Wednesday  
Microbial Biodiversity-1

## Isolation and Molecular Characterization of Bacteria from Intestinal Flora of Some Beetles (Coleoptera: Dytiscidae)

Ayşenur Yazıcı<sup>1</sup>, Ahmet Polat<sup>2</sup>, A.Muhammet Çorapcı<sup>1</sup>, Serkan Örtücü<sup>1\*</sup>, Mesut Taşkın<sup>3</sup>, Ümit İncekara<sup>1,2</sup>

<sup>1</sup>Erzurum Technical University, Department of Molecular Biology and Genetics, Erzurum, Turkey.

<sup>2</sup>Atatürk University, Faculty of Sciences, Department of Biology, Erzurum, Turkey.

<sup>3</sup>Atatürk University, Department of Molecular Biology and Genetics, Erzurum, Turkey.

\*Corresponding author e-mail: serkanortucu@gmail.com

### Abstract

Dytiscidae is one of the largest and most common groups of aquatic beetles. Dytiscidae members are all aquatic and highly adapted for aquatic life. As known, the intestinal flora of insects plays important roles in their environmental adaptation but the microbial flora studies in the gut of Dytiscidae beetles was not completed yet. In the present study, the bacterial flora of some beetles belonging to Dytiscidae was investigated to identify candidate organisms that can be possible new sources for antimicrobial peptides. For this purpose, beetles were collected from Erzurum, Turkey in July-September 2020. To determine the intestinal flora, surface of beetles was sterilized and dissected under aseptic conditions. The gut samples were homogenized in sterile saline water and suspensions were spread on nutrient agar media. The identification of the isolated bacteria was performed using 16S rDNA analysis. After PCR, 16S rDNA genes were sequenced and compared to all known sequences in the GenBank by use of BLASTN 2.2.26+ program. A total eight different species belonging to the family Dytiscidae were obtained; *Dytiscus marginalis*, *Graphoderus cinereus*, *Colymbetes fuscus*, *Hygrotus saginatus*, *Ilybius fuliginosus*, *Laccophilus minutus*, *Agabus labiatus* and *A. bipustulatus*. As a result of the studies conducted, bacterial isolates were identified as *Serratia liquefaciens*, *S. fonticola*, *Bacillus pumilus*, *Carnobacterium divergens*, *Hafnia paralvei*, *Aeromonas rivuli*, *Proteus hauseri* and *Klebsiella pneumoniae*. *S. fonticola* was the most common bacteria isolated from Dytiscidae. All the bacteria isolated in the present study are widely spread in water, soil and air. They can also be found in the intestinal flora of insects because the intestinal flora is influenced by the surrounding environment. These locally isolated bacteria may be the subject of research in future studies in terms of production of some antimicrobial peptides against human pathogens.

**Keywords:** Dytiscidae, intestinal flora, antimicrobial peptides

**Acknowledgement:** This work was supported by the Erzurum Technical University Research Foundation (ETU-BAP: 2020/013).

Oral Presentation

Wednesday

Diversity of Plant species, Systematics and Phylogeny-1

### Biodiversity of *Sedum L.* in Ankara

Akın Aras<sup>1\*</sup>, Duygu Mermer Doğu<sup>1</sup>, Kamber Erat<sup>1</sup>

<sup>1</sup>Türkiye Milli Botanik Bahçesi Müdürlüğü, Ankara, Türkiye.

\*Corresponding author e-mail: akinci36@gmail.com

#### Abstract

Plants are an important part of the biodiversity in the world. Turkey has a rich variety of *Sedum L.* throughout Eurasia due to its geographical location, topographic structure, rocky slopes and climate variability. Succulent plants are seen in almost every region of the world, including *Sedum L.* species. The genus *Sedum L.*, a member of the Crassulaceae family, has 428 species that form plants in different forms, from annuals and creeping plants to shrubs. Floristic studies are important for the conservation of species richness. Detect and protect genetic resources, transfer them to future generations, and for this purpose research filed work is necessary to determine biodiversity studies. *Sedum L.* species will can be used for landscaping and green area arrangement by minimizing water use in the period of water resources are rapidly depleted in the world and in our country. In this study, it was aimed to determine the *Sedum L.* species in Ankara province and its districts. As part of the 2020 fieldland program, surveys were done in Ankara center and its districts according to the available literature studies and screenings were carried out in 8 different locations. *Sedum* species found during survey studies were determined satellite coordinates by taking GPS, and plant samples taken together with their soil were placed in pots. In some locations (according to literature), it has been determined that *Sedum* species no longer live in that location as of 2020. As a result of this study, 5 *Sedum* species were identified and started to be cultivated for use in studies. It is planned that selected plants will be produced in National Botanic Garden of Turkey.

**Keywords:** *Sedum L.*, biodiversity, Ankara

**Acknowledgement:** The research was supported by TAGEM project no: TAGEM/BBAD/Ü/20/A1/P9/



## 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021



Oral Presentation  
Wednesday

Diversity of Plant species, Systematics and Phylogeny-1

### Genetic Diversity and Structure of Pea (*Pisum sativum* L.) Genotypes for Marker-Trait Association of DNA

İsmail Bezirganoğlu<sup>1\*</sup>, Büşra Yazıcılar<sup>1</sup>, Merve Şimşek Geyik<sup>1</sup>, Doğan İlhan<sup>2</sup>

<sup>1</sup>Department of Molecular Biology and Genetics, Erzurum Technical University, 25050 Erzurum, Turkey.

<sup>2</sup>Department of Molecular Biology and Genetics, Kafkas University, Kars, Turkey.

\*Corresponding author: İsmail Bezirganoğlu, Tel: +90-442-666-2150, Fax: +90-442-666-2537

E-mail address: ismail.bezirganoglu@erzurum.edu.tr

#### Abstract

Pea (*Pisum sativum* L.) is a annual plant, which is high in nutritional value and dietary fibres and it is the third most important human food and animal feed grain legume world-wide. Genetic diversity has been determined by DNA, of some *Pisum sativum* L. ecotypes and cultivars. It is aimed to contribute to the improvement programs aimed at developing suitable *Pisum sativum* L. varieties which are economically important in terms of our country. The genetic distances between populations were determined by using simple sequence repeats (SSR) molecular marker technique for eight different ecotypes and eleven *Pisum sativum* L. cultivars. The obtained data were analyzed by NTSYS-pc program and genetic distance dendrogram was created. The 11 SSR markers successfully produced total 66 polymorphic bands by percentage of 89,2% for 18 *Pisum sativum* L. populations. This study initially focuses on the variation of genetic Turkish *Pisum sativum* L. plants by using comprehensive analysis methods.

**Keywords:** *Pisum sativum* l., ssr, genetic diversity

Oral Presentation

Wednesday

Diversity of Plant species, Systematics and Phylogeny-1

### Investigation of the Important Bee Plants of Uluyayla Plateau (Ulus-Bartın)

Bilge Tunçkol<sup>1\*</sup>

<sup>1</sup>Bartın University Ulus Vocational School Department of Forestry, 74600 Bartın, Turkey

e-mail: btunckol@bartin.edu.tr

#### Abstract

In this study, melliferous plants that can be used by the honeybees (*Apis mellifera* L.) in Bartın-Ulus were presented. (*Apis mellifera* L.) This study carried out in years of 2018-2019 in Uluyayla Plateau which is located at Western Black Sea Region of Turkey. The average altitude is 1400 m, annual total precipitation is about 420 mm. In study area, main economic activity is animal husbandry. In addition to agricultural activity, beekeeping and honey production are another important activities. Uluyayla Plateau has wide natural rangelands with abundant honey plant species. Rangelands are important for organic honey production because in this vegetation, chemical fertilization, or other chemicals for weed and pest control are never used. In addition to honey plant species, other flowering plant species were determined. In this study, spread of melliferous plants in Uluyayla location, their families, their Turkish and Latin names, their vegetation period, and their nectar and pollen content were evaluated. Nectar and pollen containing plants among the other plants were defined after investigating the relevant literature. From the investigation of the flora, 170 taxa belonging to 48 families were identified as melliferous plants which have potential to be used by bees for their pollen and nectar. In conclusion, this study aims to create a database showing the data of flowering time and nectar and pollen content of melliferous plants for beekeeper and for the future scientific studies.

**Keywords:** Western Black Sea Region, honeybee, bee plants, nectar, pollen

Oral Presentation

Wednesday

Environmental Toxicology -1 & Microbial Biodiversity -2

## The Effect of Fertilizer Applications on Phenolic Compound Content in *Nigella damascena* Seeds

Funda Ulusu<sup>1\*</sup>, Ali Şahin<sup>2</sup>

<sup>1</sup>Karamanoğlu Mehmetbey University, Vocational School of Technical Sciences Organic Agriculture Program, Karaman, Turkey.

<sup>2</sup>Karamanoğlu Mehmetbey University, Faculty of Health Sciences, Karaman, Turkey.

\* fulusu@kmu.edu.tr

### Abstract

*Nigella damascena* is a valuable medicinal and aromatic plant that contains pharmacologically very important secondary metabolites belonging to the Ranunculaceae family and has many therapeutic properties. In this study, *Nigella damascena* plant was grown under greenhouse conditions, during vegetative and generative development periods. Organic, worm and chemical fertilizers in liquid form were applied in different doses. The total phenolic and flavonoid determination in the structure of the seeds obtained as a result of the application was investigated. In addition, the phenolic compound analysis of the seeds was qualitatively analyzed in HPLC-PDA. The total phenolic compound content of the seeds obtained in the application groups ranged from 0.10 to 0.19 mgGAE/g and the highest chemical fertilizer application was found. In addition, the total flavonoid content of seeds varied between 2.73 – 3.85 mgQE/g and the highest total flavonoid content was determined in organic fertilizer application. Phenolic compounds qualitatively identified by HPLC epicatechin, p-coumaric acid, ferulic acid, rutin hydrate, apigenin and naringenin.

**Keywords:** *Nigella damascena*, phenolic compounds, fertilizer

**Acknowledgement:** This study was supported by Karamanoğlu Mehmetbey University Scientific Research Projects Unit (Project no: 03-D-18).

Oral Presentation

Wednesday

Environmental Toxicology -1 & Microbial Biodiversity -2

### Cytotoxic Activity of *Nigella Damascena* Seed Extracts

Funda Ulusu<sup>1\*</sup>, Ali Şahin<sup>2</sup>

<sup>1</sup>Karamanoğlu Mehmetbey University, Vocational School of Technical Sciences Organic Agriculture Program, Karaman, Turkey.

<sup>2</sup>Karamanoğlu Mehmetbey University, Faculty of Health Sciences, Karaman, Turkey.

\* fulusu@kmu.edu.tr

#### Abstract

*Nigella damascena*, which belongs to the Ranunculaceae family, contains valuable phytochemicals in terms of medicinal and aromatic. In this study, the seeds harvested from the *N. damascena* plant grown with the application of different liquid fertilizers (organic, chemical and vermicompost) (varying ratios) were extracted with solvents of different polarity (n-hexane, methanol). Cell culture studies in MCF-7 and MIA PACA-2 cancer cell lines and HEK 293 cell lines were performed with the alamar blue test to evaluate the cytotoxic activities of the extracts in the concentration range of 0-300 µg/mL. n-hexane seed extracts (0-300 µg/mL) in MIA PACA-2 (IC<sub>50</sub>: 77.65 ± 1.82 µg/mL-24h, IC<sub>50</sub>: 55.16 ± 2.44 µg/mL-48h) in MCF-7 (IC<sub>50</sub>: 141.05 ± 2.76 µg/mL-24h, IC<sub>50</sub>: 89.97 ± 7.98 µg/mL-48h) had cytotoxic activity, whereas it failed to achieve 50% cell inhibition in HEK 293. In the application of methanol seed extract, 50% cell inhibition could not be determined for all 3 cell lines in the 0-300 µg/mL concentration range. In particular, stronger cytotoxic activity was detected in the n-hexane extracts of the seeds of organic and vermicompost fertilizer applications.

**Keywords:** *Nigella damascena*, seed extracts, cytotoxic activity

**Acknowledgement:** This study was supported by Karamanoğlu Mehmetbey University Scientific Research Projects Unit (Project no: 03-D-18).



## 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021



Oral Presentation

Wednesday

Environmental Toxicology -1 & Microbial Biodiversity -2

### Nano-Encapsulation and Biosynthesis of Metal Nanoparticles by Green Synthesis

İlke Karakaş<sup>1\*</sup>, Furkan Öztürk<sup>1</sup>, Nurcihan Hacıoğlu Doğru<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Çanakkale, Turkey

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Art and Sciences, Department of Biology, Çanakkale, Turkey

\*Corresponding author e-mail: ilkekarakass@gmail.com

#### Abstract

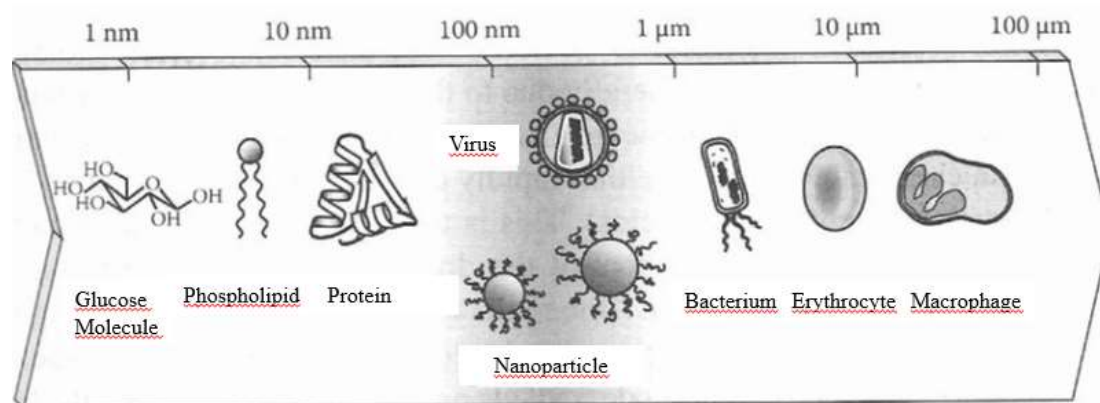
Nanotechnology is one of the most important building blocks of modern science. In recent years, nanotechnology has emerged as a multidisciplinary technology in the fields of biology, chemistry, bioengineering, food engineering, physics and medicine. Green synthesis is an efficient, inexpensive, easily applicable and environmentally friendly method developed as an alternative to existing physical and chemical syntheses. Today, the green synthesis method is frequently used in the production of biomedical and nanomaterials. Encapsulation is defined as the process of covering solid, liquid and gaseous materials with a protective layer or coating material for various purposes. Chitosan, silicium dioxide, iron, gold, zinc and silver nanoparticles/nanomaterials obtained from leaves, flowers, roots, etc. extracts of plants such as peanut, artichoke, liverwort, white mulberry, green tea, ginger, lemon, olive and lavender, agriculture, paint industry It is frequently used with nano-encapsulation method in food industry, pharmacognosy, medicine and health sector. Phenolic acid, flavonoid tannin, terpene, coumarin, lycopene, vitamin, carotenoid and anthocyanin phytochemicals contained in these plant extracts are also used as reducing agents. The aim of this study is to examine the application areas of nanoparticles and nanomaterials obtained from plant extracts in different sectors by nano-encapsulation method.

**Keywords:** Green synthesis, encapsulation,  $SiO_2$ , agnps synthesis, aminopolysaccharide



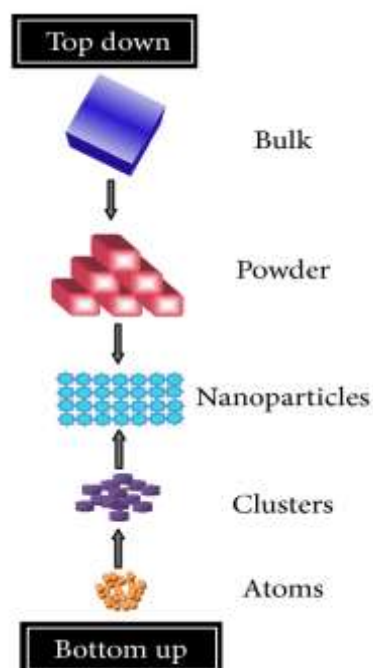
## INTRODUCTION

Nanotechnology, one of the most active and important fields of modern science, is an interdisciplinary technology. Nanotechnology is a scientific field based on the characterization, production and application of nano-scale particles of 1-100 nm size, as well as a new and rapidly developing technology that aims to impart new physicochemical and biological properties to matter at the atomic and molecular level (Ramsden, 2018). Nanoparticle sizes and comparison with other biological molecules are shown in figure 1.



**Figure 1.** Nanoparticle sizes and comparison with biological molecules (Yetişgin and Güney, 2017)

There are two commonly used approaches in nanoparticle synthesis, these approaches are shown in Figure 2. The first is the top-down method and the other is the bottom-up method. In the top-down method, nanoparticle clumps are separated into nanosize at low speed. In the bottom-up method, atoms are combined into molecular structures in the nanometer range. The bottom-up approach is widely used in the physicochemical and biological synthesis of nanoparticles. While changing the physical structures of nanoparticles, differences in the properties of the material can be seen. Applications of nanoparticles in various fields are determined by their size, shape and crystal structure. Therefore, the synthesis of nanoparticles in different sizes and shapes can create various difficulties in nanotechnology (Madhumitha, 2013; Kütük, 2019).



**Figure 2.** Methods used in nanoparticle production (Madhumitha, 2013)

The most practical method used for the easy and environmentally friendly production of nanoparticles is green synthesis, also known as biological synthesis. In green synthesis, there is no need to use high pressure, temperature, energy and toxic chemicals in the production of nanoparticles. Nanoparticles smaller than 100 nanometers are frequently used in many fields such as medicine, pharmacology, food industry, cosmetics industry, pharmaceutical industry and biomedical sectors, since they exhibit different and improved properties compared to bulky materials (Karnani, 2013; Nartop, 2016). The main active reducing agent obtained from plant extracts used in green synthesis is polyphenol (Kharissova, 2013).

Due to the valuable physical and chemical properties of nanoparticles, they are frequently used in various fields. These areas are; coating and paint (Anyago et al., 2008), food industry (Espitia et al., 2012), textile industry (Xue et al., 2009), automotive and agriculture industry (Asmatulu et al., 2013), cosmetics industry (Pardeike et al., 2009), separation and purification of cell fragments and biological molecules (Chiang et al., 2005; Lee et al., 2006), tissue engineering (Peter et al., 2010), tissue engineering (Martinez-Gutierrez et al., 2010), medicine and genetics (Cho et al., 2008), biodetection of pathogens (Zhang et al., 2010), determination of protein and DNA structure (Wang et al., 2006; You, 2007) used in many different fields.

## Biological Synthesis of Nanoparticles

Biological sources used in green synthesis play an active role in reducing the substances that are toxic to them and turning them into non-toxic nanoparticles (Pantidos and Horsfall, 2014). In the synthesis of nanoparticles, enzymes, phenols, flavonoids, sugars, alcohols, etc. phytochemicals take an active role in the reduction process. Some studies on the synthesis of nanoparticles are given in Table 1.

**Table 1.** Synthesis of Nanoparticles with Biological Sources

| Biological Source | Name                              | Nanoparticle |
|-------------------|-----------------------------------|--------------|
| Plant             | <i>Psidium guajava</i>            | Ti           |
| Plant             | <i>Gloriosa superba</i>           | Ru           |
| Plant             | <i>Hypericum triquetrifolium</i>  | Ag           |
| Plant             | <i>Pistacia terebinthus</i>       | Au           |
| Plant             | <i>Capsicum annuum L.</i>         | Au           |
| Fungus            | <i>Aspergillus clavatus</i>       | Ag           |
| Fungus            | <i>Penicillium decumbens</i>      | Ag           |
| Fungus            | <i>Pleurotus ostreatus</i>        | Ag           |
| Fungus            | <i>Pleurotus eryngii</i>          | Ag           |
| Alg               | <i>Dunaliella salina</i>          | Au           |
| Bacteria          | <i>Rhodopseudomonas capsulata</i> | Au           |
| Bacteria          | <i>Pseudomonas stutzeri</i>       | Ag           |
| Waste             | <i>Citrus peels</i>               | Ag           |

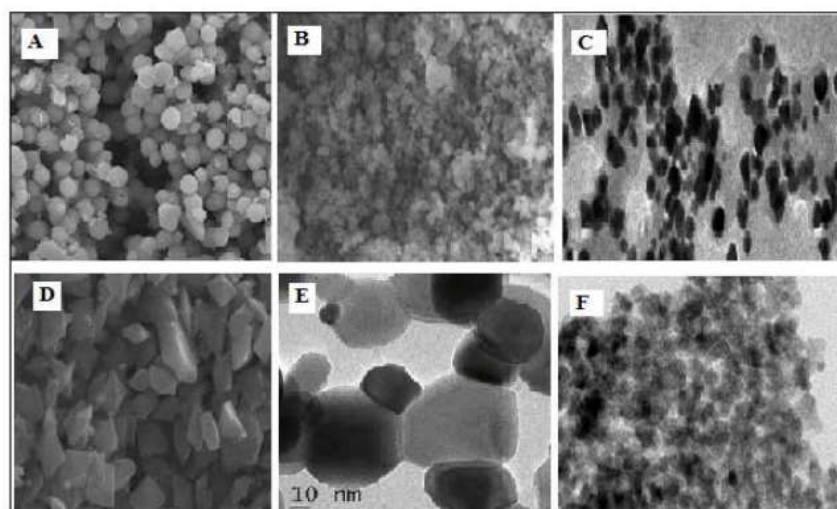
**Table 2.** Advantages and disadvantages of materials used in green synthesis

| Synthesis Using Material | Advantages  | Disadvantages  |
|--------------------------|---|--|
| Plant Extracts           | <ul style="list-style-type: none"> <li>-Environmental friendly.</li> <li>-Easily scaled up for large synthesis of nanoparticles.</li> <li>-No need of high temperature, pressure, energy and toxic chemicals.</li> <li>-More advantageous over use of micro-organisms by less elaborate process of maintaining cultures.</li> <li>-Reduces cost of micro-organism isolation and their culture media.</li> </ul> | <ul style="list-style-type: none"> <li>-Plants can't be manipulated as the choice of nanoparticles through optimized synthesis through genetic engineering.</li> <li>-Plant produce low yield of secreted proteins which decreases the synthesis rate</li> </ul> |
| Waste                    | <ul style="list-style-type: none"> <li>-Easily available and does not require rigorous processing.</li> <li>-Directly used for NP synthesis.</li> <li>-Option for waste management.</li> </ul>  | <ul style="list-style-type: none"> <li>-Parsing problems.</li> <li>-Bad odor during production.</li> <li>-NPs with less features compared to plants extracts and microorganism.</li> </ul>   |

|                           |   |  |
|---------------------------|---|--|
|                           | -Leads to fast and cost-effective approach.<br>-Does not induce toxic NP  |  |
| Enzymes and Microorganism | -Clean, non-toxic, biocompatible and eco-friendly method for synthesis of nanoparticles.<br>-Cost effective, safe and sustainable.<br>-Bacteria are easy to handle and can be easily manipulated. | -Culturing of microorganisms is time consuming.<br>-Difficult to have control over size, shape and crystallinity.<br>-Particles are not mono-dispersed and rate of production is slow. |

### Characterization of Nanoparticles

Nanoparticles can have different physical, chemical and morphological properties (Figure 3). The characterization of nanoparticles is generally performed with various analysis data such as Ultraviolet visible field spectrophotometer, Atomic Power Microscopy, XRD (X-Ray diffractometry), SEM-EDX, Zeta Potential, TGA (Thermo Gravimetric Analysis) and FTIR (Infrared Spectroscopy) (Baran and Keskin, 2020).



**Figure 3.** SEM-TEM images of nanoparticles. A) Au nanoparticle (SEM), B) Ag nanoparticle (SEM), C) Pd nanoparticle (SEM), D) Au nanoparticle (TEM), E) Ag nanoparticle (TEM) F) Pd nanoparticle (TEM) (Baran and Keskin, 2020)

### Metallic Nanoparticle Types and Usage Areas

#### Gold Nanoparticles

It is known that gold nanoparticles are compatible and efficient inorganic structures for drug release and gene therapy applications. Although gold nanoparticles are non-toxic, they are biocompatible and stable. Many studies are carried out with these nanoparticles. Gold nanoparticles are used in medicine and health, especially in photothermal therapies. The physical size of the gold nanoparticle has an important role in drug release, cancer therapy and DNA labeling studies (Chithrani et al.,

2006; Bikram et al., 2007).

### **Calcium Phosphate Nanoparticles**

Calcium phosphate nanoparticles are being investigated for their use in carrier systems such as nucleic acid and drug release due to their high biocompatibility. The interaction between the negatively charged phosphate group in nucleic acids and the positively charged  $\text{Ca}^{2+}$  in calcium phosphate makes these nanoparticles advantageous. However, the large size and low stability of calcium phosphate nanoparticles hinder their release into the cell. These nanoparticles can also be used in imaging and therapy, but more research is needed before they can be used on humans (Sokolova et al., 2006; Morgan et al., 2008).

### **Silica Nanoparticles**

Particle size, shape, structure and porosity have been well demonstrated in extensive studies on silica nanoparticles. In recent studies, the usability of these nanoparticles in drug delivery systems has been examined. Thanks to the large surface area and pore structure of the silica nanoparticles, effective drug encapsulation is ensured and dissolution is prevented until it reaches the target area. In addition, it is possible to interfere with the adjustable particle shape and size and the rate of particle uptake into the cell. In order to use these nanoparticles, which have many advantages, in treatments, more extensive research is required (Jeelani et al., 2020).

### **Zinc Oxide Nanoparticles**

Zinc oxide, a semiconductor nanomaterial, is used in many technological applications thanks to this feature. Thanks to its physicochemical properties, the antimicrobial activity of this nanoparticle, which is used in studies in the field of cancer, is also known. Studies are carried out to monitor simultaneous gene transfer by creating luminescence with surface modification in drug delivery systems with zinc oxide nanoparticles (Zhang and Liu, 2010).

### **Titanium Oxide Nanoparticles**

Titanium oxide nanoparticles are widely used in environmental applications due to their physical and chemical stability, low cost, non-toxicity and corrosion resistance. In addition, titanium oxide nanoparticles, one of the metal nanoparticles widely used in medicine and microbiology, gain photocatalytic properties after UV irradiation. The antibacterial activity of titanium oxide

nanoparticle, which is studied as a part of the drug delivery system in cancer treatment, is also known (Lai et al., 2008; Çakmak and Canbaz, 2020).

### **Silver Nanoparticles**

Silver nanoparticles are frequently used to destroy harmful microorganisms and prevent contamination due to their broad spectrum antimicrobial activity. Antibacterial effect of silver nanoparticle; It is formed by the interaction of Ag<sup>+</sup> ion, which ionizes in air and water, with thiol, carboxyl, amine, phosphate, indole, imidazole, hydroxyl groups in the structure of bacterial cells, and disrupts the structure of the cell and loses its activity (Kumar et al., 2005). Today, silver nanoparticles are frequently preferred in biosensors, wound repair, anticancer and antiviral applications. Silver nanoparticles used in the field of biosensors are used to increase the sensitivity of biosensors (Beykaya and Çağlar, 2016; Umaz et al., 2019; Wu et al., 2020).

### **Advantages of Green Synthesis**

The production of metal nanoparticles by green synthesis has many advantages. Cotton fibers used in green synthesis, industrial milk cans, citrine juices, grape skins, rice bran, watermelon skins and chicken feathers are in the waste class and are used in the production of palladium, gold, silver and iron nanoparticles. In this way, a cheap, environmentally friendly and recyclable system is created and waste management is ensured. There is no need for high temperature, pressure and energy in the production of nanoparticles by green synthesis using plants, waste materials, enzymes and microorganisms. It is possible to produce less toxic nanoparticles with green synthesis, which is easy to apply and is not a very long process.

### **Nano-Encapsulation and Usage Areas**

The process of covering solid, liquid and gaseous materials with a protective layer or coating material for various reasons is known as encapsulation. Chitosan, silicon dioxide, iron, gold, zinc and silver nanoparticles/nanomaterials obtained from the leaves, flowers, roots, etc. extracts of plants are frequently used by nano-encapsulation method in agriculture, paint industry, food industry, pharmacognosy, medicine and health sector. Nano-encapsulation has many benefits such as maintaining the stability of a bioactive substance, trapping aroma-like substances, and providing resistance to chemical agents. The use of nano-encapsulation makes it possible to incorporate bioactive ingredients into most food ingredients. In addition, thanks to a nano-sized encapsulation, the antioxidant is protected against adverse conditions such as low pH and enzymatic deformations.

Encapsulation has started to be used in agriculture with the desire to carry out agricultural activities that will be more efficient and effective and beneficial to human and environmental health in small cultivation areas. With this request, experts have started to develop new techniques in order to prevent the existing and possible future hazards in agricultural activities. The most important application area of nanomaterials created using nano-encapsulation technique is plant protection. The encapsulation technique, which is used to control potential parasitic plants in agricultural areas, is also used to prevent problems related to phytotoxicity. For examples of components encapsulated in the nanoshell are glyphosate, dazolinone, and sulfonylurea. In this way, the transmission of the stimulants required for germination to the plant seed is carried out with the nanocapsule without degradation in the soil. Generally, spherical or cylindrical iron oxide nanoparticles are used by nano-encapsulation method in medicine and health. Nano-encapsulants in medicine and health are used in various applications such as drug release to target tissue, improvement of contrast ratio in magnetic resonance imaging, immunological testing and cellular therapy. In addition, it is used in the development of implants, surgical materials and dental care products from the nano-encapsulation method in dentistry. The nano-encapsulation method is also frequently used in the field of food. It is used to renew the nutrient content and increase the stability of food products without spoiling the taste, aroma and texture of the food. Food materials prepared in this way are known as functional food. Nano-encapsulation technique is used to protect sensitive food ingredients such as aroma chemicals, organic oils and vitamins, to improve their aromas and to mask taste, odor and color in food products. The obtained nano-encapsulants prevent the bioactive compounds in the food from entering into unnecessary interactions without impairing the food quality.

### **Advantages of Nano-Encapsulation**

One of the most important advantages of nano-encapsulation is the ease of sterilization of the applied method. When nano-encapsulants are physically broken down, the products released do not show toxic properties. Due to their high substance-trapping capacity, high amounts of the active substance can be released into the cell. Thanks to this feature, the stability and efficiency of the active substance increase. Due to their small size, they are easier to take into the cell. Nano-encapsulation, which is a simple and easily applicable method, provides advantages in many applications in biological fields by reducing its toxic properties in other regions, as it shows high efficiency in the target region.

### **RESULTS**

Today, different methods, including chemical, physical and biological, have been developed to

obtain metal nanoparticles in various shapes and sizes. The biological method of nanoparticles is an economical and environmentally friendly alternative approach to chemical and physical approaches. Green synthesis provides a new possibility to synthesize nanoparticles using natural reducing and stabilizing agents. While faster synthesis is possible with green synthesis, nanoparticles with controlled toxicity and well-characterized are produced. This method is used in various fields such as medicine, cosmetics, food and medical applications. Nano-encapsulation technique is frequently applied in medicine, agriculture, health, cosmetics, paint and food industry. With the increase in nanotechnological developments, it is planned to increase the usage areas that are limited in the future. It is predicted that new approaches will be formed in the treatment of many diseases, thanks to metallic nanoparticles that play an active role in drug delivery systems, especially in the field of health. In this review, the production of nanoparticles by green synthesis, which is a more applicable, easy and economical technique among nanoparticle production techniques, and the use and advantages of these nanoparticles in various fields by nano-encapsulation are examined. This review is expected to shed light on future studies.

## REFERENCES

- Anyago KC, Fedorov AV & Neckers DC (2008). "Synthesis, characterization, and antifouling potential of functionalized copper nanoparticles". *Langmuir*, 24(8): 4340-4346.
- Asmatulu R, Nguyen P & Asmatulu E (2013). "Nanotechnology safety in the automotive industry". *Nanotechnology Safety*, (1st ed.) 57-72.
- Baran A & Keskin C (2020). Nanopartiküllerin Yeşil Sentezi ve Anti-Mikrobiyal Uygulamaları. *Fen Bilimleri ve Matematik Alanında Akademik Çalışmalar* 2(1): 3-17.
- Baran MF, Eren A & Umaz A (2019). Bazı Mikroorganizmalara Karşı Bitkisel Kaynaklı Olarak Sentezlenmiş Gümüş Nanopartiküllerin Antimikrobiyal Uygulamaları. *Gece Kitaplığı*, (2): 93-100.
- Beykaya M & Çağlar A (2016). Bitkisel Özümler Kullanılarak Gümüş-Nanopartikül (AgNP) Sentezlenmesi ve Antimikrobiyal Etkinlikleri Üzerine Bir Araştırma. *Afyon Kocatepe Üniversitesi Fen ve Mühendislik Bilimleri Dergisi*, 16(3): 631-641.
- Bikram M, Gobin AM, Whitmire RE & West JL (2007). "Temperature-Sensitive Hydrogels with SiO<sub>2</sub>-Au Nanoshells for Controlled Drug Delivery". *Journal of Controlled Release*, 123: 219-227.
- Çakmak NK & Canbaz GT (2020). TiO<sub>2</sub> Nanopartikülü ve TiO<sub>2</sub>/Aktif Çamur Sentezi ile Sulu Çözeltiden Cu (II) İyonlarının Adsorpsiyonu. *Gümüşhane Üniversitesi Fen Bilimleri Enstitüsü Dergisi*, 10(1): 86-98.
- Chiang CL, Sung CS, Wu TF, Chen CY & Hsu CY (2005). "Application of Superparamagnetic Nanoparticles in Purification of Plasmid DNA from Bacterial Cells". *Journal of Chromatography B*, 822(1-2): 54-60.
- Chithrani BD, Ghazani AA & Chan WC (2006). "Determining the size and Shape Dependence of Gold Nanoparticle Uptake into Mammalian Cells". *Nano Letters*, 6: 662-668.
- Cho K, Wang XU, Nie S & Shin DM (2008). "Therapeutic Nanoparticles for Drug Delivery in Cancer". *Clinical Cancer Research*, 14(5): 1310-1316.
- Espitia PJP, Soares NDF, dos Reis Coimbra JS, de Andrade NJ, Cruz RS & Medeiros EAA (2012). "Zinc Oxide Nanoparticles: Synthesis, Antimicrobial Activity and Food Packaging Applications". *Food and Bioprocess Technology*, 5(5): 1447-1464.
- Jeelani PG, Mulay P, Venkat R & Ramalingam C (2020). Silika Nanopartiküllerin Çok Yönlü Uygulaması. *Silikon*, 12(6): 1337-1354.
- Karnani RL & Chowdhary A (2013). Biosynthesis of Silver Nanoparticle by Eco-Friendly Method. *Ind J NanoSci*; 1(2): 25-31.



- Kharissova O, Dias HVR, Kharisov BI, Perez BO, Perez VMJ (2013). The greener Synthesis of Nanoparticles. *Trends in Biotechnology*, 31(4): 240-248.
- Kumar R, Howdle S & Münstedt H (2005). "Polyamide/Silver Antimicrobials: Effect of Filler Types on the Silver Ion Release". *Journal of Biomedical Materials Research Part B: Applied Biomaterials*, 75: 311-319.
- Kütük N & Çetinkaya S (2019). Yeşil Sentez İle Nanomalzeme Üretimini İncelenmesi Ve Kullanım Alanları. 5. *Uluslararası Mühendislik Mimarlık ve Tasarım Kongresi*.
- Lai JC, Lai MB, Jandhyam S, Dukhande VV, Bhushan A, Daniels CK & Leung SW (2008). "Exposure to Titanium Dioxide and Other Metallic Oxide Nanoparticles İnduces Cytotoxicity on Human Neural Cells and Fibroblasts". *International Journal of Nanomedicine*, 2008: 533.
- Lee IS, Lee N, Park J, Kim BH, Yi YW, Kim T & Hyeon T (2006). "Ni/NiO Core/Shell Nanoparticles for Selective Binding and Magnetic Separation of Histidine-Tagged Proteins". *Journal of the American Chemical Society*, 128(33): 10658-10659.
- Madhumitha G & Roopan SM (2013). Devastated Crops: Multifunctional Efficacy for the Production of Nanoparticles. *Journal of Nanomaterials*, 1-12.
- Martinez-Gutierrez F, Olive PL, Banuelos A, Orrantia E, Nino N, Sanchez EM, Ruiz F, Bach H & Av-Gay Y (2010). "Synthesis, Characterization, and Evaluation of Antimicrobial and Cytotoxic Effect of Silver and Titanium Nanoparticles". *Nanomedicine: Nanotechnology, Biology and Medicine*, 6(5): 681-688.
- Nartop P (2016). Biyosentetik Gümüş Nanopartiküllerinin *Pyracantha coccinea* Bitkisinin Gövde Eksplantlarının Yüzey Sterilizasyonunda Kullanımı. *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi* 23(6): 759-761.
- Nartop P Green sterilization of *Rosmarinus officinalis* L. stem surfaces with silver nanoparticles synthesized using *Rubia tinctorum* L. cell culture extracts. *Iranian J Sci Tech*, DOI: 10.1007/s40995-016-0065-0.
- Pal S, Tak YK, Song JM (2007). Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A study of the Gram-Negative Bacterium *Escherichia coli*. *Appl Envir Microbiol* 73(6): 1712–1720.
- Pantidos N & Horsfall LE (2014). Biological Synthesis of Metallic Nanoparticles by Bacteria, Fungi and Plants. *Journal of Nanomedicine & Nanotechnology*, 5(5): 10.
- Pardeike J, Hommoss A & Müller RH (2009). "Lipid Nanoparticles (SLN, NLC) in Cosmetic and Pharmaceutical Dermal Products". *International Journal of Pharmaceutics*, 366(1-2): 170-184.
- Peter M, Binulal NS, Soumya S, Nair SV, Furuike T, Tamura H & Jayakumar R (2010). "Nanocomposite Scaffolds of Bioactive Glass Ceramic Nanoparticles Disseminated Chitosan Matrix for Tissue Engineering Applications". *Carbohydrate Polymers*, 79(2): 284-289.
- Ramsden J (2018). "Applied Nanotechnology: The Conversion of Research Results to Products", William Andrew.
- Umaz A, Koç A, Baran MF, Keskin C & Atalar MN (2019). *Hypericum Triquetrifolium Turra* Bitkisinden Gümüş Nanopartiküllerin Sentezi, Karakterizasyonu ve Antimikrobiyal Etkinliğinin İncelenmesi, 9(3): 1467–1475.
- Wang L, Liu X, Hu X, Song S & Fan C (2006). "Unmodified Gold Nanoparticles as a Colorimetric Probe for Potassium DNA Aptamers". *Chemical Communications*, 36: 3780-3782.
- Wu L, Zhu G, Zhang X & Si Y (2020). Silver Nanoparticles İnhibit Denitrification By Altering the Viability and Metabolic Activity of *Pseudomonas stutzeri*. *Science of The Total Environment*, 706: 135711.
- Xue CH, Jia ST, Zhang J & Tian LQ (2009). "Superhydrophobic Surfaces on Cotton Textiles by Complex Coating of Silica Nanoparticles and Hydrophobization". *Thin Solid Films*, 517(16): 4593-4598.
- Yetişgin AA & Güney CB (2017). Altın Nanopartiküllerinin Tanı ve Tedavide Kullanımı, Lisans Projesi, Yıldız Teknik Üniversitesi, İstanbul, Türkiye.
- You CC, Miranda OR, Gider B, Ghosh PS, Kim IB, Erdogan B, Sai AK, Bunz UHF & Rotello, VM (2007). "Detection and Identification of Proteins Using Nanoparticle–Fluorescent Polymer 'Chemical Nose'sensors". *Nature Nanotechnology*, 2(5): 318.
- Zhang D, Huarng MC & Alocilja EC (2010). "A Multiplex Nanoparticle-Based Bio-Barcoded DNA Sensor for the Simultaneous Detection of Multiple Pathogens". *Biosensors and Bioelectronics*, 26(4): 1736-1742.
- Zhang P & Liu W (2010). "ZnO PMAA-co-PDMAEMA Nonviral Vector for Plasmid DNA Delivery and Bioimaging". *Biomaterials*, 31: 3087-3094.

Oral Presentation

Wednesday

Environmental Toxicology -1 & Microbial Biodiversity -2

### Antimicrobial Activity of Silver Nanoparticles Biosynthesized by Olive Leaves

Özge Ceylan<sup>1\*</sup>, Nurcihan Hacıoğlu Doğru<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Çanakkale, Turkey.

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, Çanakkale, Turkey.

\*Corresponding author e-mail: ozgeee\_10@hotmail.com

#### Abstract

Biosynthesis of nanoparticles as an emerging highlight of the intersection of nanotechnology and biotechnology has received increased attention due to growing need to develop environmentally benign technologies in material synthesis. A great deal of effort has been put into the biosynthesis of inorganic material, especially metal nanoparticle using microorganisms and plants. The silver nanoparticles (AgNPs) synthesized using hot water olive leaf extracts (OLE) as reducing and stabilizing agent were reported and evaluated for antimicrobial activity against test microorganisms in the study. Gram-negative bacteria (*Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NRRLB 3704, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853), Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus haemolyticus* ATCC 43252) and yeast (*Candida albicans* ATCC 10231) were used for determining the antimicrobial activity of AgNPs extract. This extract was showed strong antimicrobial activities with inhibition zones at 8.0-17.0 mm. It was observed that AgNPs may be a good alternative therapeutic approach in future.

**Keywords:** Antimicrobial, silver nanoparticles, olive leaves

#### INTRODUCTION

In the last century, where industrialization and the number of populations increased rapidly, the issue of environmental pollution is of great importance the development of green methods for the synthesis of nanoparticles is evolving into an important branch of nanotechnology, because these methods are

considered safe and ecologically sound the nanomaterials fabrication as an alternative to conventional methods (Bae et al., 2000; Awwad et al., 2012). The green synthesis techniques are generally synthetic routes that utilize relatively nontoxic solvents such as water, biological extracts, biological systems and microwave assisted synthesis. Silver nanoparticles (AgNPs) have become the focus of intensive research owing to their wide range of application in the development of new techniques in the areas of electronics, medicine, materials sciences due to good conductivity and chemical stability, selective coatings of solar energy absorption, intercalation materials for electrical batteries, optical receptors, catalysts in chemical reactions, bio labeling, optoelectronics, medical devices, antibacterial and biomaterials production (Awwad et al., 2012). Many research works are available on the biosynthesis of silver nanoparticles using plant leaves extract, such as *Ficus benghalensis* (Saxena et al., 2012), *Rosa rugose* (Dubey et al., 2010), *Stevia rebaudiana* (Yilmaz et al., 2011), *Chenopodium album* (Dwivedi and Gopal, 2010), *Trianthema de candra* (Geethalakshmi and Sarda, 2010), *Polyalthia longifolia* (Kaviya et al., 2011), *Pinus desiflora*, *Diopyros kaki*, *Ginko biloba*, *Magnolia kobus*, and *Pllatanus orientalis* (Song and Kim, 2009), *Catharanthus roseus* (Mukunthan t al., 2011)], *Pungamia pinnata*, *Hemidesmus indicus*, *Syzygium cumini*, *Allium cepa*, and *Pandaanus odorifer* (Panda et al., 2011), *Olea europaea* (Khalil et al., 2014). *Olea europaea* L. is an olive, a member of the Oleaceae family. Olive leaves contains oleuropein, a sekoiridoide which is responsible from many pharmacological activities including antioxidant, antimicrobial, antiinflammatory, antiatherogenic, anticarcinogenic and antiviral activities (Aslan et al., 2017). The objective of this study was to evaluate antimicrobial activity of AgNP synthesized by *O. europaea* leaves.

## MATERIALS AND METHODS

### Preparation of Agnp by Green Synthesis Method

With the pre-prepared 1mM 500 mL AgNO<sub>3</sub> aqueous solution for AgNP synthesis, 125 mL olive leaf extract will be left to react at room temperature in a 1000 mL bottle under constant conditions. The dark solution formed by the decomposition of silver ions will be centrifuged 5min at 10,000 rpm, the upper liquid phase will be removed and the remaining solid part will be washed with pure water several times. The resulting solid part (AgNP) will be left to dry for 48 hours at 65°C (Bayğu, 2020).

### Preparation of Bacterial Inoculum

Gram-negative bacteria (*Acinetobacter baumannii* ATCC 19606, *Escherichia coli* NRRL B3704, *Proteus vulgaris* ATCC 13315, *Pseudomonas aeruginosa* ATCC 27853), Gram-positive bacteria (*Bacillus subtilis* ATCC 6633, *Staphylococcus aureus* ATCC 6538P, *Staphylococcus haemolyticus* ATCC 43252) and yeast (*Candida albicans* ATCC 10231) were used for determining the antimicrobial of AgNP extracts. A twenty-four-hour Mueller Hinton Broth (MHB) culture of tested microorganism was grown in an incubator, centrifuged, and then standardized to approximately  $10^8$  CFU ml<sup>-1</sup> using broth medium.

### Antimicrobial Activity Test

Standard well agar diffusion method was carried out to detect the activity of AgNP against the clinical bacterial and fungal isolates according to Collins et al., (1989) and Erci, (2018). Studies were performed in triplicate. Treatments with Penicillin (P10) and Nystatin (NYS100) served as positive controls. For antimicrobial activities of the AgNPs, wells were made in plates containing Mueller Hinton Agar (MHA) medium seeded with 100 µl of 24 h of each clinical isolate. Solution, that contains both Ag and olive leaves extracts (OLE), as well as the control, 100 µL was placed in separate wells. The plates were left in room temperature for 2 h then, incubated at 37 °C for 24 h. The diameter of inhibition zones was measured and tabulated.

### RESULTS

The silver nanoparticle synthesized by *O. europaea* showed inhibition zone against all the tested bacteria and yeast. Biosynthesized silver nanoparticle showed excellent antibacterial activity against *P. aeruginosa* ATCC 27853, *P. vulgaris* ATCC 13315 and *S. aureus* ATCC 6538P with inhibition zone values of 12,0 mm, 14,0 mm and 17,0 mm as compared to control antibiotic P10, respectively (Table 1). However, AgNP have a weak antagonistic effect against the other test microorganisms with inhibition zones ranged from 8,0 to 12,0 mm. These values are far below the standard antibiotic P10 and NY100.

**Table 1.** The antimicrobial activity of AgNP synthesized using *O. europaea* leaves extract

| Test Microorganisms             | Zone of inhibition* |      |       |
|---------------------------------|---------------------|------|-------|
|                                 | AgNP                | P10  | NY100 |
| <i>E. coli</i> NRRLB 3704       | 8,0                 | 16,0 | D     |
| <i>P. aeruginosa</i> ATCC 27853 | 12,0                | 8,0  | D     |
| <i>P. vulgaris</i> ATCC 13315   | 14,0                | 13,0 | D     |
| <i>A. baumannii</i> ATCC 19606  | 9,0                 | 12,0 | D     |
| <i>B. subtilis</i> ATCC 6633    | 10,0                | 14,0 | D     |
| <i>S. aureus</i> ATCC 6538P     | 17,0                | 15,0 | D     |

|                                   |      |      |      |
|-----------------------------------|------|------|------|
| <i>S. haemolyticus</i> ATCC 43252 | 12,0 | 14,0 | D    |
| <i>C. albicans</i> ATCC 10231     | 8,0  | D    | 16,0 |

\*Inhibition zone (mm); a includes diameter of disk (6 mm); P10 = Penicillin (10 ug/disc); NY100 Nystatin (10 ug/disc)

## DISCUSSION

Biosynthesis of metal nanoparticles using plant extracts studies have increased considerably in the last 20 years. Plant metabolites are an environmentally friendly thereby stimulating the production of metallic nanoparticles. The potential usage of plant-derived nanoparticles is very high in various fields such as pharmaceuticals, therapeutics, sustainable and renewable energies. Plants will be a very broad perspective for synthesis metallic nanoparticles in health and commercial products in the future. The results of the present investigation show that synthesized AgNP by *O. europaea* have antibacterial potential against test bacteria. Our antibacterial activity findings confirmed the observations of some other investigations about AgNP biosynthesized by various plant species (Dubey et al., 2010; Panda et al., 2011; Yilmaz et al., 2011; Saxena et al., 2012; Khalil et al., 2014; Aritonang et al., 2019). When examining previous studies about AgNP biosynthesized by *O. europaea* Awwad et al. (2012) found that AgNPs showed effective inhibitory activity against pathogens *Listeria monocytogenes*, *Shigella* and *S. aureus*. And in another study, Khalil et al. (2014) showed that The AgNPs at 0.03–0.07 mg/ml concentration significantly inhibited bacterial growth against multi drug resistant *S. aureus*, *P. aeruginosa* and *E. coli*. Our findings are similar to previous studies because of strong antibacterial activity against *S. aureus* ATCC 6538P.

## CONCLUSIONS

AgNP synthesized from olive leaves are highly environmentally friendly and non-toxic materials. Hopefully antimicrobial results in vitro against diseases related to global health problems are expected to contribute to obtaining a new source of medicines. This study developed a rapid, eco-friendly stable silver nanoparticle using the aqueous solution of *O. europaea* leaves extract.

## ACKNOWLEDGEMENTS

This investigation is a part of Master thesis of Özge Ceylan.

## REFERENCES

- Aritonang, HF, Koleangan H & Wuntu AD (2019). Synthesis of silver nanoparticles using aqueous extract of medicinal plants (*Impatiens balsamina* and *Lantana camara*) fresh leaves and analysis of antimicrobial Activity *Hindawi International Journal of Microbiology* 2019: 1-8.
- Arslan AKK, Öztürk E, Yerer MB & Koşar M (2017). Oleuropein in olive leaf and its pharmacological effects. *Journal of Health Sciences*, 25: 89-93
- Awwad AM, Salem NM, & Abdeen AO (2012). Biosynthesis of silver nanoparticles using *Olea europaea* leaves extract

- and its antibacterial activity *Nanoscience and Nanotechnology* 2(6): 164-170.
- Bae CH, Nam SH & Park SM (2000). Formation of silver nanoparticles by laser ablation of a silver target in NaCl solution. *Appl. Surf. Sci.* 197: 628-634
- Bayğu, G. (2020). Cimin Üzüm Yaprağı Kullanılarak Yeşil Sentez Yöntemiyle Elde Edilen Gümüş Nanopartiküllerin Genotoksik Etkisinin Kanat Benek Testi ile Belirlenmesi, Erzincan Binali Yıldırım Üniversitesi Fen Bilimleri Enstitüsü, (Yüksek Lisans Tezi)
- Collins CH, Grange JM & Lyne PM (1989). *Collins and Lyne's Microbiological Methods* (6th ed.) London: Butterworths.
- Dubey SB, Lahtinen M & Sillanpää M (2010). Green synthesis and characterizations of silver and gold nanoparticles using leaf extract of *Rosa rugosa*. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 364: 34-41.
- Dwivedi AD & Gopal K (2010). Biosynthesis of silver and gold nanoparticles using *Chenopodium album* leaf extract. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* 369: 27-33
- Erci, F. (2018). Yeşil sentez ile elde edilen metal nanopartiküllerin antimikrobiyal ve antibiyofilm etkinliklerinin değerlendirilmesi, Doktora Tezi, Yıldız Teknik Üniversitesi Fen Bilimleri Enstitüsü, İstanbul.
- Geethalakshmi R & Sarda DVL (2010). Synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their anti microbial activities. *International Journal of Engineering Science and Technology* 2: 970-975.
- Kaviya S, Santhanalakshmi J & Viswanathan B (2011). Green synthesis of silver nanoparticles using *Polyalthia longifolia* leaf extract along with D-sorbitol: Study of antibacterial activity *Journal of Nanotechnology* doi: 10.1155/2011/1152970.
- Khalil MMH, Ismail EH, El-Baghdady KZ & Mohamed D (2014). Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity. *King Saud University Arabian Journal of Chemistry*, 7: 1131-1139.
- Mukunthan KS, Elumalai EK, Patel TN & Murty VR (2011). *Catharanthus rosea*: a natural source for the synthesis of silver nanoparticles. *Asian Pacific Journal of Tropical Biomedicine* 1: 270-274.
- Panda KK, Achary VM, Krishnaveni R, Padhi BK, Sarangi SN & Sahu BB (2011). In vitro biosynthesis and genotoxicity bioassay of silver nanoparticles using plants. *Toxicology in Vitro* 25: 1097-1105
- Saxena A, Tripathi RM, Zafar F & Singh P (2012). Green synthesis of silver nanoparticles using aqueous solution of *Ficus benghalensis* leaf extract and characterization of their antibacterial activity. *Materials Letters* 67: 91-94
- Song JY & Kim BS (2009). Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst Eng.* 32: 79-84.
- Yilmaz M, Turkdemir H, Bayram HE & Cicek A (2011). Biosynthesis of silver nanoparticles using leaves of *Stevia rebaudiana*. *Materials Chemistry and Physics* 130: 1195-1202.

Oral Presentation  
Wednesday  
Environmental Stress on Biodiversity

### Cold-Adapted Cellulase Producer *Vishniacozyma* Species from Palandöken Mountain

Mehmet Karadayı<sup>1\*</sup>, Şeyma Aksu<sup>2</sup>

<sup>1</sup>Atatürk University, Faculty of Science, Department of Biology, Erzurum, Turkey.

<sup>2</sup>Atatürk University, Institute of Natural and Applied Sciences, Erzurum, Turkey.

\*Corresponding author e-mail: mkaradayi@atauni.edu.tr

#### Abstract

Mountains are unique habitats for microbial biodiversity research as they contain many distinct micro-ecosystems at the same time. This unique biodiversity plays a crucial role in many natural cycles in the ecosystem and also serves as valuable resources for the development of many industrial applications, especially cold-adapted enzymes such as cellulases. In this context, the aim of the present study was determined as isolation and identification of the fungal cold-adapted cellulase producers in Palandöken Mountain (Erzurum). For this aim, soil samples were taken from a cryoconite hole in January 2020. The isolation steps were done according to the literature. The pure cultures were inoculated on carboxymethyl cellulose agar plates and incubated at +4 °C for three weeks. Then, active isolates were determined by the observation of clear halos after Congo red staining and decolorization steps. The molecular identification of the isolates was done by ITS-PCR. The sequence data was evaluated by using the BLAST<sup>®</sup> and deposited at GenBank<sup>®</sup> with unique accession numbers. According to the results, 65 yeast isolates were obtained from a cryoconite hole in Palandöken Mountain. Among them, two isolates were determined as cold-adapted cellulase producers and they were identified as *Vishniacozyma tephrensensis* SAY-1 and *Vishniacozyma victoriae* SAY-2. The accession numbers were MW922829.1 and MW922830.1, respectively. In conclusion, these results of the present study are valuable for the development of cold-adapted cellulase production strategies.

**Keywords:** Cellulase, cryoconite, Palandöken Mountain, *Vishniacozyma*

**Acknowledgement:** This work was supported by Research Fund of the Atatürk University (Project Number: FYL-2021-9054).

Oral Presentation  
Wednesday  
Environmental Stress on Biodiversity

**Effects of Exogenous Salicylic Acid and Strigolactone Applications on Antioxidant Activity  
in Tomato Seedlings Under Short-Term Drought Stress**

Gamze Baltacıer<sup>1\*</sup>, Sevgi Donat<sup>1</sup>, Okan Acar<sup>2</sup>

<sup>1</sup>School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

<sup>2</sup>Department of Biology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

\*Corresponding author e-mail: gamzebaltacier@gmail.com

**Abstract**

Drought, which is one of the abiotic stress factors, is the main stress factor that negatively affects the growth, development, and yield of plants. Salicylic acid (SA) is a plant growth regulator associated with stress tolerance in plants. Exogenous application of SA prevents damage caused by various abiotic stresses (drought, high and low temperatures, salinity) and helps plants develop resistance to stresses. On the other hand, as Strigolactones (SLs) are signal compounds produced by plants, they are known to positively affect plant growth with external applications due to their potential to stimulate the tolerance system of plants under stress conditions. The aim of this study was to determine biochemically [GR (Glutathione Reductase), CAT (Catalase), POX (Peroxidase), APX (Ascorbate Peroxidase), TBARS (lipid peroxidation)] the effects of SA and GR24 on the negative effects of drought stress on Full F1 seedlings, which is the most preferred commercial tomato variety by professional farmers in Çanakkale. 45-day-old Full F1 seedlings were irrigated with Hoagland Nutrient Solution (100%) and grown in pots containing perlite and peat in the laboratory. After 5 days of acclimatization, the seedlings were divided into 2 groups: Control group [Control (C), Salicylic Acid (SA), Strigolactone (GR24) and Salicylic Acid + Strigolactone (SA+GR24)] and Drought group [Drought (D), Salicylic Acid (D-SA), Strigolactone (D-GR24) and Salicylic Acid + Strigolactone (D-SA+GR24)]. Exogenous GR24 (0.015 mM) and SA (0.1 mM) (with Tween 20) were applied to 50-day-old seedlings. All treatments increased protein amounts in all plant samples. On the other hand, it was determined that lipid peroxidation, which increased with drought stress, decreased 38% with SA, 25% with GR24, and 37% with SA+GR24. Our results indicated that the decrease in lipid peroxidation may be associated with increased POX, GR, and APX activities, and SA+GR24 application is more effective than separate treatments.

**Keywords:** drought stress, salicylic acid, gr24, *Lycopersicum esculentum* 'full f1', antioxidant enzymes



Oral Presentation  
Wednesday  
Environmental Stress on Biodiversity

### The Change of Photosynthetic Pigments of *Liquidambar orientalis* in Summer Period

Fahrettin Atar<sup>1</sup>, Ali Bayraktar<sup>1\*</sup>

<sup>1</sup>Karadeniz Technical University, Faculty of Forestry, Department of Forest Engineering, Trabzon, Turkey.

\*Corresponding author e-mail: alibayraktar@ktu.edu.tr

#### Abstract

Oriental sweetgum (*Liquidambar orientalis* Mill.) is an important endemic species for both wood and non-wood use in Turkey. In the present study, it is aimed to reveal the change of photosynthetic pigment content of oriental sweetgum in summer period. The leaves collected from five oriental sweetgum individuals in the Kanuni Campus of Karadeniz Technical University were used as study material within the scope of the study. Spectrometer device was used for photosynthetic pigment determination and chlorophyll a, chlorophyll b, total chlorophyll and total carotenoid amounts were determined. Measurements were carried out in June, July and August. As a result of the analysis of variance (one-way ANOVA), it was revealed that there were statistically significant differences at 99% confidence level between the months in terms of all measured parameters. In addition, it was determined that the amount of chlorophyll a, chlorophyll b and total chlorophyll increased from June to August, while the amount of total carotenoids decreased. While the lowest values for chlorophyll a (1.15 mg/g), chlorophyll b (0.55 mg/g) and total chlorophyll (1.70 mg/g) amounts were determined in June, the highest values were determined as 2.31 mg/g, 1.48 mg/g and 3.79 mg/g in August, respectively. The highest total carotenoid amount was 0.24 mg/g in June and the lowest value was 0.08 mg/g in August. The summer period averages of oriental sweetgum were determined as 1.89 mg/g for chlorophyll a, 0.98 mg/g for chlorophyll b, 2.88 mg/g for total chlorophyll and 0.18 mg/g for total carotenoid. In the study, it was revealed that photosynthetic pigment contents showed significant differences during the summer period.

**Keywords:** carotenoid, chlorophyll, seasonal, spectr

Oral Presentation  
Wednesday  
Environmental Stress on Biodiversity

Changes in Plant Water Potential and Stomatal Conductance Due to Water Stress in  
*Quercus infectoria*

Esra Bayar<sup>1\*</sup>, Nevzat Gürlevik<sup>1</sup>, Ayşe Deligöz<sup>1</sup>

<sup>1</sup>Isparta University of Applied Sciences, Faculty of Forestry, Forest Engineering Department, Isparta,  
Turkey.

\*Corresponding author e-mail: esrabayar@isparta.edu.tr

**Abstract**

In this study, water stress (control and water stress treatment) was applied to 1+0 years old *Quercus infectoria* Olivier subsp. *boissieri* (Reuter) O. Schwarz seedlings, and the changes in water potential, stomatal conductance and specific leaf area were investigated. Gravimetric soil water content, water potential, stomatal conductance and specific leaf area were determined on the 10th, 20th, 30th and 40th days by monitoring the seedlings in the water stress treatment until they dry out in the growth chamber. There were no measurements since the water-stressed seedlings dried on the 50th day. While the control treatment was irrigated regularly, irrigation was not applied in the water-stressed treatment. No statistically significant difference was found in the gravimetric soil water content, water potential, stomatal conductance, and specific leaf area on the 10th day in water stress and control treatments. Water potential and stomatal conductance decreased in water-stressed seedlings due to the decrease in soil water content on the 20th day. On the 30th and 40th days, the water potential continued to decrease in the water-stressed seedlings. As a result, seedlings subjected to water stress on 40th days under controlled conditions significantly reduced water potential, stomatal conductance, and specific leaf area compared to control seedlings.

**Keywords:** Water stress, stomatal conductance, *Quercus infectoria*

Oral Presentation

Wednesday

Diversity of Plant Species, Systematics and Phylogeny-2

**Preliminary Data for Plant Biodiversity of the Polog Region of North Macedonia**Jusra Reçani<sup>1\*</sup>, Ebru Ataşlar<sup>2</sup>

<sup>1</sup>Eskişehir Osmangazi University, Graduate School of Natural and Applied Sciences, Department of Biology, 26040, Eskişehir, Turkey

<sup>2</sup>Eskişehir Osmangazi University, Faculty of Science and Letters, Department of Biology, 26040, Eskişehir, Turkey

\*Corresponding author e-mail: jysra.recani@hotmail.com

**Abstract**

The Republic of North Macedonia, which acts as a bridge between the Balkan states, is a country that is quite diverse in terms of plant diversity. Preliminary data presented in this study covers the Polog region of North Macedonia, located in the central part of the Balkan Peninsula. The plant samples of the study, started in July-2020, were investigated in the cities of Gostivar and Tetovo and their surroundings. In addition to this study, examples of field studies carried out in the Bachelor Thesis studies in 2018 were also added. Until June-2021, there are 300 specimens in total as scientific material, 59 families and 163 genera, 227 species have been identified in line with this plant diversity. The order of the widely distributed families is as follows: Asteraceae, Rosaceae, Brassicaceae, Fabaceae. The representatives with the largest number by genera are: Geranium L., Prunus L., Euphorbia L., Rosa L., Lonicera L.

**Keywords:** Flora, Gostivar, Tetovo, Polog region, North Macedonia

Oral Presentation  
Wednesday

Diversity of Plant Species, Systematics and Phylogeny-2

**Evaluation of Genetic Diversity of Eleven *Medicago sativa* Varieties Cultivated in Turkey  
by Using Start Codon Targeted Polymorphism****Büşra Albayrak<sup>1\*</sup>, İsmail Bezirganoğlu<sup>1</sup>**<sup>1</sup>Erzurum Technical University, Erzurum, Turkey.

\*Corresponding author e-mail: busra.albayrak@erzurum.edu.tr

**Abstract**

*Medicago sativa*, which has been cultivated since ancient times, is the most widely grown forage crop in the world due to its high nutritional value, high protein content and productivity. *M. sativa* is cold-resistant, drought-tolerant and a significant crop because it can maintain a symbiotic life with bacteria, *Rhizobium*, that can fix nitrogen. On the other side, *M. sativa* synthesizes various secondary metabolites such as coumarins, terpenoids, organic acids, and especially flavonoids and saponins. Moreover, it has been used in traditional medicine in different countries. Hence, it has great potential to be used in pharmacology and medicine. For these reasons, there is a need to analyze the genetic diversity and relationship, and to determine the population structure among the varieties for further studies like plant breeding. In the present study, it was attempted for the first time to use of start codon targeted (SCoT) polymorphism for the determination of genetic diversity among eleven *M. sativa* varieties cultivated in Turkey. For SCoT polymorphism, seventeen primers (SCoT-5, 6, 9, 10, 17, 18, 20, 21, 22, 31, 33, 39, 40, 56, 65, 71 and 74) were used. Then, the obtained profiles were analyzed by the use of the Total Lab program. To estimate the genetic diversity within the population, genetic distance analysis was applied using the GenAEx program, and allele frequency, frequency-based distance, and neighbor joining tree analysis were applied using Powermarker program. Finally, the neighbor joining analysis was converted a dendrogram in DENDROSCOPE program. As a result, in terms of genetic distance, we constructed a dendrogram in which similarity coefficients ranged from 0.93 to 0.103 for all tested samples. The dendrogram was composed four main groups. Kayseri cultivar was separated from the other samples. In the first main group, there were Nimet and Ferruh varieties. In the second, cultivars were in the IIB composed of Konya, Bilensoy 8, Kalender and Sazova. In last group, cultivars were in IIIB composed of Savaş, Bilensoy and variety Denizli.

**Keywords:** *Medicago sativa*, scot, polymorphism, genetic diversity

Oral Presentation

Wednesday

Diversity of Plant Species, Systematics and Phylogeny-2

**A Systematic Study on *Crocus gargaricus* Herb. Complex****Ceyda Yazıcı<sup>1\*</sup>, Almıla Çiftçi<sup>1</sup>, Osman Erol<sup>1</sup>**<sup>1</sup>Istanbul University, Faculty of Sciences, Department of Biology, Istanbul, Turkey.

\*Corresponding author e-mail: ceydayazici.96@gmail.com

**Abstract**

*Crocus gargaricus* Herb. is an endemic species belonging the genus *Crocus* in the family Iridaceae, and is distributed in Western Anatolia. The populations in this species complex are distributed in three known locations namely Canakkale, Mugla and Bursa. Although Canakkale (Kazdaglari) and Bursa (Uludag) populations are geographically closer, Kazdag and Mugla populations are the same species (*Crocus gargaricus*) whereas Bursa population belongs to another species (*Crocus thirkeanus* Koch). The main difference of these taxa is having stolons which is a morphological trait seen in plants that depends on physiological conditions. To understand the relationships and systematics of these morphologically similar taxa, we focused on previously mentioned morphological differences among the populations such as occurrence of stolon, fine structure of outer corm tunic and qualitative and quantitative characters of flower parts along with vegetative parts. We collected at least 20 specimens from all known populations during flowering time. The measurements were taken with a digital caliper three times to minimize the errors during field work before pressing the plants to be able to represent the correct nature of plant parts. We performed ANOVA on quantitative morphological data to determine the statistically meaningful traits. We then used PCA on meaningful quantitative characters and all qualitative characters observed. Our results showed that the specimens show high morphological variation including presence of stolon, within and among populations depending on sampling size.

**Keywords:** *Crocus*, morphology, systematics**Acknowledgement:** This work is supported by Istanbul University Research Projects Unit with project number 37948. We would like to thank Prof. Dr. Levent Şık for his valuable help in field work.

Oral Presentation

Wednesday

Diversity of Plant Species, Systematics and Phylogeny-2

## Plant Species Diversity, Composition and Vegetation Cover of The Ugtam Nature Reserve, Mongolia

Bayanmunkh Tumurkhuu<sup>1\*</sup>, Enkhtuvshin Dechinperlii<sup>1</sup>, Uyanga Ariya<sup>2</sup>, Tuguldur  
Enkhtsetseg<sup>2</sup>

<sup>1</sup>Department of Biology, School of Mathematics and Natural Sciences,  
Mongolian National University of Education, Ulaanbaatar 210648, Mongolia,

<sup>2</sup>The Nature Conservancy, Ulaanbaatar 14210, Mongolia,

\*Correspondence: e-mail: mandahbayan@gmail.com

### Abstract

The Ugtam Nature Reserve has a unique natural formation that makes its flora and vegetation community unique. This study aimed to determine vascular plant species diversity, species composition, major plant community and their vegetation cover, and rare species in this area. A total of 350 species belonging to 205 genera, 64 families, and five phyla (Polypodiophyta, Equisetophyta, Gymnospermae, Magnoliophyta, Dicotyledones) were recorded. During this study, an endemic species, 15 subendemic species (4.3%), 13 rare species (3.7%) were recorded in the study area, which comprised 8% of the total species. These species recording indicated the unique flora of the Ugtam Nature Reserve. Forb, Shrub, Grass and forb, and Alpine kitam communities of 4 different communities were commonly recorded in the study area.

**Keywords:** Plant community, forb, grass, shrubs, dry steppe

### INTRODUCTION

Ugtam Nature Reserve is located southeast of the Bayandun soum and Dashbalbar soum in Dornod province, Mongolia (Figure 1). This area is a natural reserve that belongs to the Mongol Daguur forest steppe (National Atlas, 2009) and the dry steppe of Dornod Mongol district (Karamysheva and Khramtsov, 1995). The vegetation of this area is mainly composed of forest steppe and vegetation associated with its geomorphology features. The plant species and their habitats recorded are very unique to this area. Moreover, Unatov (1950) and Ulziikhutag (1989) subdivided

the region into 16 vegetation-geographic districts based on the geography of Mongolian territories and their vegetation cover characteristics. Based on this subdivision, the Ugtam Nature Reserve is located in the Mongola Daguur forest steppe (Figure 1).

Plant species diversity, species composition and vegetation cover of Ugtam Nature Reserve are relatively less studied and there is no plant species inventory for this area. The general features of the vegetation cover of this area are covered by some studies on the vegetation of Mongolia (Unatov 1950; Grubov 1955; 1982; Dariimaa and Oyunchuluun 2016) and by the studies on the vegetation of Khentii, Mongol Daguur, Dundad Khalkh, and Dornod Mongol districts (Dashnyam 1974; Shagdar 2003).

In recent years, there has been an increase in anthropogenic impact such as livestock and mining in this area. Therefore, it is important to create a species inventory of flora, in order to determine species composition and vegetation cover, so as to provide baseline information about vegetation pattern in this area for future research on changes in biological communities caused by land-use impacts and environmental management.

This study aimed to determine vascular plant species diversity, species composition, major plant community and their vegetation cover, and rare species in this area.

## MATERIAL AND METHODS

### Study Area

Ugtam Nature Reserve is located in the Bayandun soum and Dashbalbar soum of the Dornod province and covers a transition zone between forest steppe and dry steppe ecosystems. The landscape of the Ugtam mountain is mainly characterized by small valley, main valley and lakes. Moreover, riparian zone is commonly meadows, which safe habitats for several wild birds, including ruddy shelduck (*Tadorna ferruginea* Pallas, 1764); mandarin duck (*Aix galericulata* Linnaeus, 1758); wild ungulates red deer (*Cervus canadensis* Erxleben, 1777); sibirian roe deer (*Capreolus pygargus* Pallas, 1771); mongolian gazelle (*Procapra gutturosa* Pallas, 1777), alongside various livestock. The reserve provides pasture resources for wild ungulates and livestock (Daehler, 2003; Nyambayar & Tseveenmyadag, 2009; Wingard & Zahler, 2006; Соколов & Орлов, 1980). The climate condition is characterized by very limited precipitation (annually 199-285 mm) with a widely varying temperature range between cold winter (-27.5°C) and hot summer (25.1°C) (Batchuluun et al., 2020). The vegetation of the reserve is dominated by short grasses and forb (*Stipa sibirica* Roshev, *Cleistogenes squarrosa* (Trin.) Keng., *Bupleurum*

*bicaule* Helm., *Potentilla tanacetifolia* Willd. ex Schlecht.), alpine kitam (*Filifolium sibiricum* (L.) Kitam., *Agropyron cristatum* (L.) Beauv.), shrubs (*Armeniaca sibirica* (L.) Lam., *Lespedeza hedysaroides* (Pall.) Kitag.), forbs (*Aconogonon divaricatum* (L.) Nakai ex Mori, *Gypsophilla dahurica* Turcz.) (Dashnyam, 1974; Tuvshintogtokh, 2014).

### **Plant Species Richness, Evenness of The Plant Communities, and Beta Diversities Among Plant Communities**

We surveyed vegetation and collected vegetation samples three replicates in every community from July until August 2020 and 2021. Three quadrats (1m x 1m and 100 grids) were randomly selected within the habitats. All the plant species within the selected quadrats were identified for the herbaceous plant composition and richness of the habitats. The canopy cover of these species was assessed by the Braun-Blanquet cover class scale (Braun-Blanquet, 1932). Moreover, we clipped all plants at ground level inside the quadrats for above-ground plant biomass.

### **Data Analyses**

One-way analysis of variance (ANOVA) were performed to determine whether there is a significant difference among the community type. Non-metric multidimensional scaling (NMDS) was used to visualize the differences in community composition among community based on Bray–Curtis dissimilarity measure.

## **RESULT**

### **Plant Species Composition, Diversity and Dominance**

In this work, a total of 350 species belonging to 205 genera, 64 families, and five phyla (Polypodiophyta, Equisetophyta, Gymnospermae, Magnoliophyta, Dicotyledones) (Table 1). The following eight families were more diverse and accounted for 58.5% Of the total flora recorded including Poaceae (55 species), Asteraceae (48 species), Fabaceae (26 species), Rosaceae (21 species), Chenopodiaceae (17 species), Ranunculaceae (14 species), Lamiaceae (13 species), and Brassicaceae (11 species) (Table 2). These aforementioned families were more diverse families of Mongolian flora and were recorded from the mountain and steppe region. One to thirteen species were within a genus. The most diverse genera were *Artemisia* (13 species), *Potentilla* (12 species) and *Allium* (10 species). An endemic species (*Oxytropis gracillima* Bunge) (0.3%), fifteen subendemic species (4.3%) such as *Potentilla strigosa* Pall. ex Pursh., *Caragana microphylla*



Lam., *Oxytropis oxyphylla* (Pall.) DC. thirteen rare species (3.7%) such as *Ephedra sinica* Stapf., *Phragmites communis* Trin., *Glycyrrhiza uralensis* Fisch.

### Ecological Group

Plant species were divided into 11 ecological groups and the number of species recorded varied between the groups (Figure 2). Xerophytes and meso-xerophytes comprised 48.4% of total plants, mesophytes and meso-phytrophytes 30.3%, xero-phytrophytes and meso-hygrophytes 9.4%, phassimophytes 1.4%, halophytes 1.1%, xero-hygrophytes 0.2%, and phassimo-halophytes 0.2% (Figure 2).

### Vegetation Cover and Its Characteristics

Forest steppe, dry steppe shrub, annual and perennial species were predominant in the vegetation cover of this area. Moreover, shrubs, alpine kitam, forbs and grass-forbs communities of 4 different communities were commonly recorded in the study area.

#### *Shrub community*

This community was relatively diverse, with canopy cover of approximately 10-15% and distributed on the lower slope and lower part of the mountain. The commonly recorded species included *Armeniaca sibirica* (L.) Lam. and *Lespedeza hedysaroides* (Pall.) Kitag.

#### *Alpine kitam community*

This community was relatively diverse, with canopy cover of approximately 40-45% and distributed on the upper slope and upper part of the mountain. The commonly recorded species included *Filifolium sibiricum* (L.) Kitam. and *Bupleurum bicaule* Helm., *Allium senescens* L., *Carex enervis* C.A. Mey.

#### *Forb community*

This community was relatively diverse, with canopy cover of approximately 5-10% and distributed on the meadow and along in river valley. The commonly recorded species included *Equisetum arvense* L. and *Inula britannica* L.

#### *Grass and forb community*

This community was relatively diverse, with canopy cover of approximately 25-30% and distributed on the lower slope and low mountains. The commonly recorded species included *Stipa krylovii* Roshev. and *Cleistogenes squarrosa* (Trin.) Keng., *Leymus chinensis* (Trin.) Tzvel.

To visualize dissimilarities in community composition among four communities, as a measure of beta diversity, the NMDS plot was constructed based on the Bray-Curtis dissimilarity index.

Accordingly, NMDS showed a very strong and consistent difference in the community composition (Bray–Curtis dissimilarity) of four types of community including forbs, shrubs, alpine kitam and grass and forbs community (Figure 3). The beta diversity (variation of the species composition of plant communities) estimates (Bray-Curtis similarities) were non significantly different between vegetation community ( $F=1.252$ ,  $Df=3$ ,  $P<0.291$ ) (Figure 3). The plant species composition differences among communities were generally related to differences in grazing intensity and habitats.

## **DISCUSSION**

The flora of the study area was relatively diverse because of its high elevation and habitat diversity, which included high mountain forests, dry steppe, and meadow. The different flora in a vegetation district reflects the adjacent vegetation district's flora. Ugtam Mountain is located in the forest steppe and dry steppe zone and borders with great steppe in south and east. Thus, a majority of flora consisted of dry steppe, mountain steppe and forest steppe vegetation. For ecological groups, the most commonly recorded group (48.4%) was xerophytes and meso-xerophytes, and the next commonly recorded group was mesophytes and meso-phytrophytes, thus indicating the characteristics of the study area (Figure 2). Community structure is associated with habitat location and its biotic and abiotic factors. Forb, alpine kitam, grass and forb and shrub communities were commonly reported in the study area, indicating dry steppe characteristics (Tuvshintogtokh, 2014). Vegetation cover was mainly shrub, undershrub, annual, and biennial herbaceous plant species of mountain steppe and dry steppe. This study period was short, but we were able to document many plant species and perform visual assessment of vegetation cover in the study area. The study provides baseline information about the vegetation pattern of this area for future studies and management. In the future, additional sampling is required to create a vegetation map of this area.

## **ACKNOWLEDGEMENT**

First of all, we would like to express our sincere gratitude to Galbadrakh Davaa for their guidance during our fieldwork. This study was funded by the The Natural Conservation (TNC). The authors also would like to thank the scientific committee, all the Ugtam Nature Reserve staff, administration of Dornod Mongol, herder family of this area and all the faculties of the Department of Biology, Mongolian National University of Education.

**REFERENCE**

- Dashnyam B (ed) (1974). Flora and vegetation of Dornod Mongol. Ulaanbaatar, Mongolia: Publishing Academy of Sciences. pp. 78-115.
- Dariimaa Sh, Oyunchuluun Ya (2016). Plant species composition and vegetation cover of Kherlen Toono Mountain, Mongolia. *Journal of Asia Pacific Biodiversity*, 16: 1-5.
- Daehler C.C (2003). Performance comparisons of co-occurring native and alien invasive plants: implications for conservation and restoration. *Annual Review of Ecology and Systematics*, 34: 183– 211.
- Grubov VI (ed) (1955). Conspectus of flora Mongolian People's Republic. Proceedings of Mongolian Commission of the USSR Academy of Sciences, Vol. 7. Leningrad, Russia: USSR Academy of Sciences Press.
- Grubov VI (ed) (1982). Key to the vascular plants of Mongolia. In: Leningrad, Russia: Nauka. 63 pp.
- Karamysheva Z.V., Khamtsov V.N. (1995). The steppes of Mongolia. *Braun-Blanquetia* 17, pp. 1–79.
- Nyambayar B, Tseveenmyadag N (eds) (2009). Directory of Important Bird Areas in Mongolia: Key sites for key sites for conservation. Ulaanbaatar, Mongolia: Publishing Academy of Sciences. 103 pp.
- National Atlas of Mongolia, 2009.
- Ulziikhutag N (ed) (1989). Characteristics of flora of Mongolia. In: Synopsis of Mongolian flora. Ulaanbaatar, Mongolia: State Publishing. pp. 107-148.
- Unatov AA (ed) (1950). Botanica-geographical district allocation. In: The main features of the vegetation cover of Mongolia. Leningrad, Russia: Russian Academy of Sciences Press. pp. 124-189.
- Shagdar D (2003). Asteraceae (Asteraceae Dumort.) of Mongolia: Taxonomic composition, ecology, geography, history of development and economic value. Dissertation of Science Doctor. St. Petersburg, Russia: Institute of Botany of Academy of Sciences.
- Sokolov VE, Orlov VN (1980). Key to the mammals Moskva, Russia: Nauka.
- Tuvshintogtokh I (ed) (2014). Dry steppe. In: Steppe vegetation of Mongolia. Ulaanbaatar, Mongolia: Bambi San. pp. 206-240.
- Wingard J.R, Zahler P (2006). Silent Steppe: The Illegal Wildlife Trade Crisis in Mongolia. Mongolia Discussion Papers, East Asia and Pacific Environment and Social Development Department. Washington D.C.: World Bank.

Table and Figure

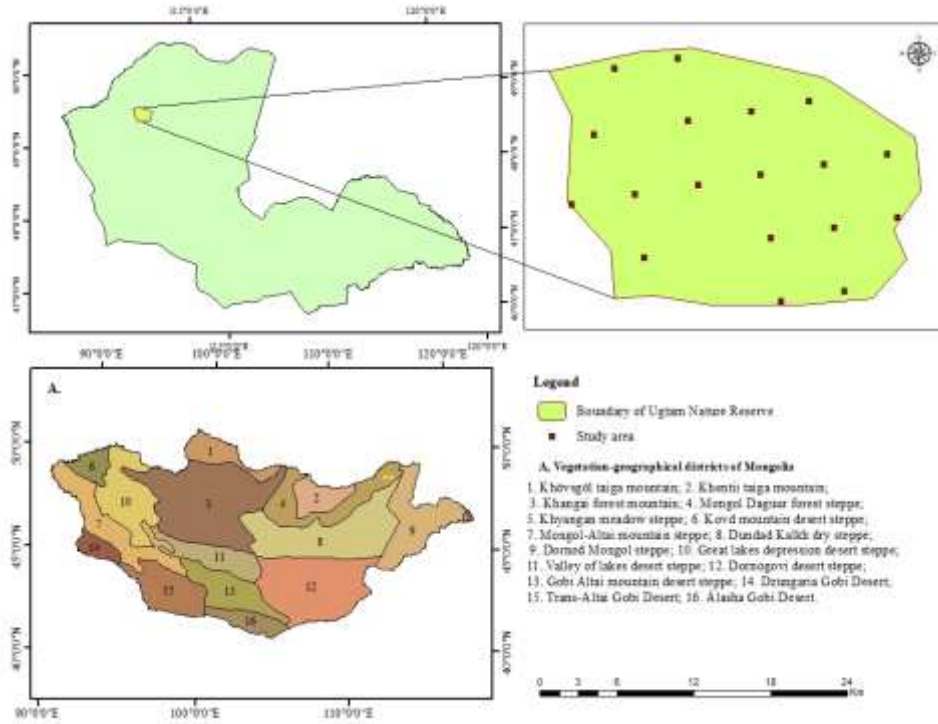


Figure 1. Study area and the sampling sites in the Ugtam Nature Reserve

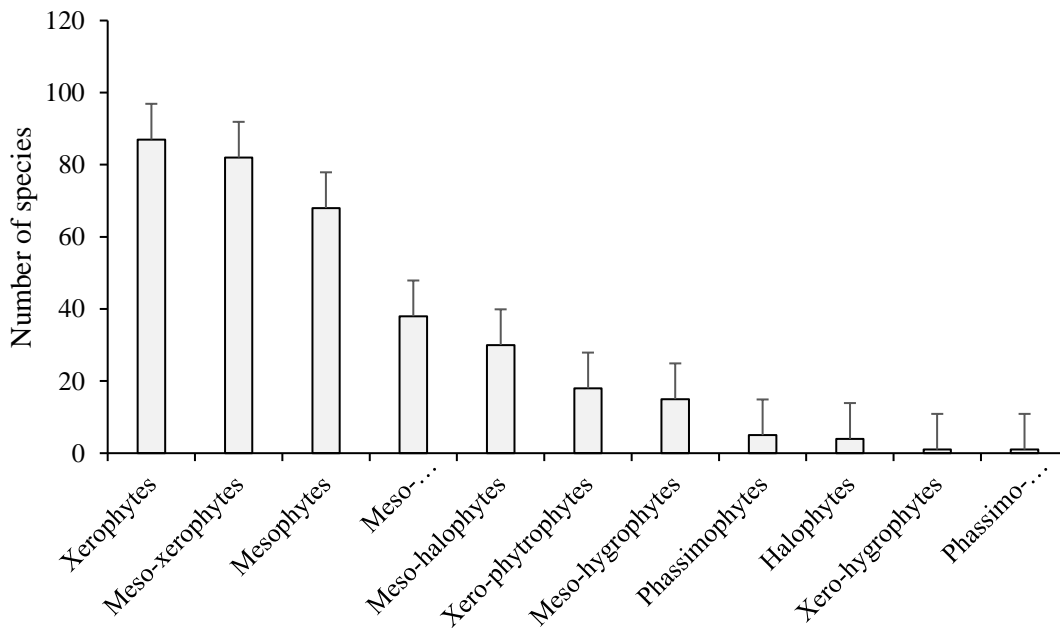
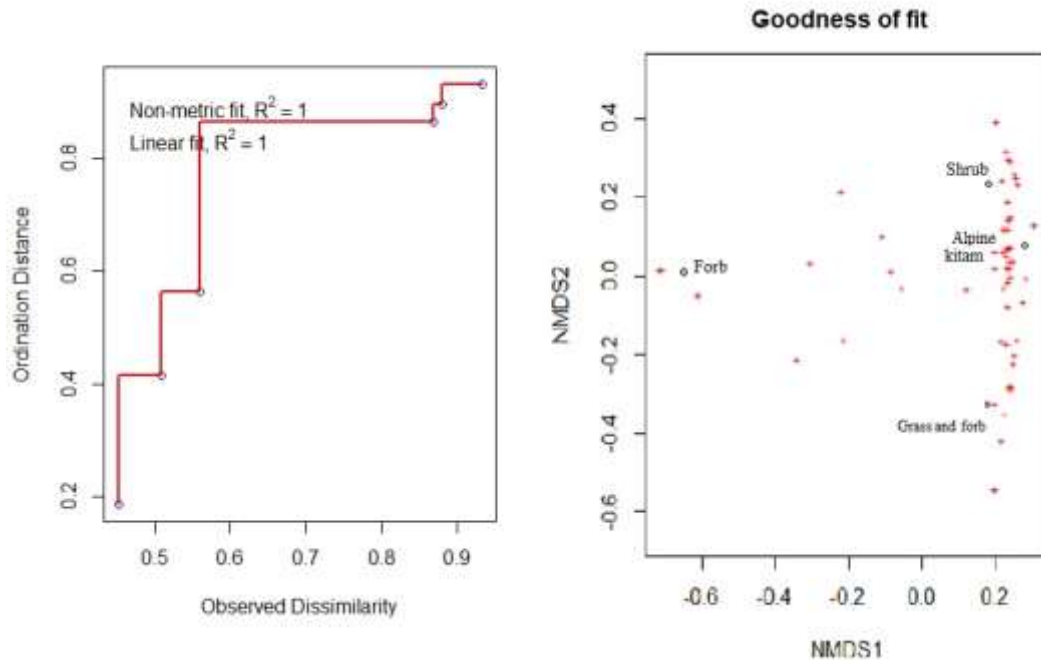


Figure 2. Vascular plant species of Ugtam Nature Reserve by their ecological groups



**Figure 3.** The non-metric multidimensional scaling (NMDS) analysis of plant species composition in different vegetation community. Species names are abbreviated, see Table 2 for full names.

**Table 1.** The group of vascular plant and flora of the Urgam Nature Reserve

| No         | Groups                          |                                    | Number of family | Number of Genera | Number of species | Percent (%) |
|------------|---------------------------------|------------------------------------|------------------|------------------|-------------------|-------------|
| 1          | Polypodiophyta                  |                                    | 3                | 3                | 3                 | 0.85        |
| 2          | Equisetophyta                   |                                    | 1                | 1                | 1                 | 0.28        |
| 3          | Gymnospermae                    |                                    | 2                | 2                | 2                 | 0.57        |
| 4          | Angiospermae<br>Monocotyledones | (Magnoliophyta)<br>(Magnoliopsida) | 13               | 47               | 97                | 27.8        |
| 5          |                                 | Dicotyledones<br>(Liliopsida)      | 45               | 152              | 247               | 70.5        |
| <b>Sum</b> |                                 |                                    | <b>64</b>        | <b>205</b>       | <b>350</b>        | <b>100</b>  |

**Table 2.** Species list of vascular plants at Ugtam Nature Reserve, Mongolia.

| Family                      | Species  |
|-----------------------------|--|
| Sinopteridaceae Koidz.      | 1. <i>Aleuritopteris argentea</i> (S.G.Gmel.) Fee    |
| Woodsiaceae (Deils) Herter. | 2. <i>Woodsia subcordata</i> Turcz.                  |
| Athyriaceae Alston          | 3. <i>Cystopteris fragilis</i> (L.) Bernh.           |
| Equisetaceae Rich. ex DC.   | 4. <i>Equisetum arvense</i> L.                       |
| Pinaceae Lindl.             | 5. <i>Pinus sylvestris</i> L.                        |
| Ephedraceae Dumort.         | 6. <i>Ephedra sinica</i> Stapf.                      |
| Potamogetonaceae Dum.       | 7. <i>Potamogeton gramineus</i> L.                   |
|                             | 8. <i>Potamogeton perfoliatus</i> L.                 |
| Juncaginaceae Rich.         | 9. <i>Triglochin maritimum</i> L.                    |
|                             | 10. <i>Triglochin palustre</i> L.                    |
| Poaceae Barnhart            | 11. <i>Spodiopogon sibiricus</i> Trin.               |
|                             | 12. <i>Echinochloa crusgalli</i> (L.) Beauv.         |
|                             | 13. <i>Panicum miliaceum</i> L.                      |
|                             | 14. <i>Setaria viridis</i> (L.) Beauv.               |
|                             | 15. <i>Hierochloa glabra</i> Trin.                   |
|                             | 16. <i>Achnatherum splendens</i> (Trin.) Nevski      |
|                             | 17. <i>Stipa sibirica</i> (L.) Lam.                  |
|                             | 18. <i>Stipa grandis</i> P.Smirn.                    |
|                             | 19. <i>Stipa krylovii</i> Roshev.                    |
|                             | 20. <i>Alopecurus brachystachyus</i> Bieb.           |
|                             | 21. <i>Alopecurus arundinaceus</i> Poir.             |
|                             | 22. <i>Agrostis clavata</i> Trin.                    |
|                             | 23. <i>Agrostis trinii</i> Turcz.                    |
|                             | 24. <i>Agrostis mongholica</i> Roshev.               |
|                             | 25. <i>Calamagrostis epigeios</i> Tzvel.             |
|                             | 26. <i>Calamagrostis purpurea</i> (Trin.) Trin.      |
|                             | 27. <i>Calamagrostis salina</i> Tzvel.               |
|                             | 28. <i>Helictotrichon schellianum</i> (Hack.) Kitag. |
|                             | 29. <i>Avena sativa</i> L.                           |
|                             | 30. <i>Chloris virgata</i> Sw.                       |
|                             | 31. <i>Tripogon chinensis</i> (Franch.) Hack.        |
|                             | 32. <i>Enneapogon borealis</i> (Griseb.) Honda       |
|                             | 33. <i>Phragmites communis</i> Trin.                 |
|                             | 34. <i>Cleistogenes squarrosa</i> (Trin.) Keng.      |
|                             | 35. <i>Eragrostis pilosa</i> (L.) Beauv.             |
|                             | 36. <i>Eragrostis minor</i> Host                     |

|                  |   |
|------------------|---|
| Cyperaceae Juss. | <p>37. <i>Koeleria cristata</i> (L.) Pers.</p> <p>38. <i>Koeleria macrantha</i> (Ledeb.) Schult.</p> <p>39. <i>Koeleria mukdenensis</i> Domin</p> <p>40. <i>Melica virgata</i> Turcz. ex Trin.</p> <p>41. <i>Poa subfastigiata</i> Trin.</p> <p>42. <i>Poa pratensis</i> L.</p> <p>43. <i>Poa attenuata</i> Trin.</p> <p>44. <i>Poa argunensis</i> Roshev.</p> <p>45. <i>Poa botryoides</i> Trin. ex Griseb</p> <p>46. <i>Poa ochotensis</i> Trin.</p> <p>47. <i>Glyceria triflora</i> (Korsh.) Kom.</p> <p>48. <i>Puccinellia tenuiflora</i> (Griseb.) Scribn. et Merr.</p> <p>49. <i>Puccinellia hauptiana</i> V. Krecz.</p> <p>50. <i>Puccinellia macranthera</i> V. Krecz.</p> <p>51. <i>Festuca sibirica</i> Hack. ex Boiss.</p> <p>52. <i>Festuca dahurica</i> (St.-Yves) V. Krecz.</p> <p>53. <i>Festuca lenensis</i> Drob.</p> <p>54. <i>Bromopsis inermis</i> Leyss.</p> <p>55. <i>Agropyron michnoi</i> Roshev.</p> <p>56. <i>Agropyron cristatum</i> (L.) Beauv.</p> <p>57. <i>Agropyron repens</i> (L.) P.B.</p> <p>58. <i>Hordeum roshevitzii</i> Bowden</p> <p>59. <i>Hordeum brevisubulatum</i> (Trin.) Link</p> <p>60. <i>Hordeum turkestanicum</i> (Nevski) Tzvel.</p> <p>61. <i>Leymus chinensis</i> (Trin.) Tzvel.</p> <p>62. <i>Elymus secalinus</i> (Georgi) Bobr.</p> <p>63. <i>Elymus sibiricus</i> L.</p> <p>64. <i>Elymus dahuricus</i> Turcz. ex Griseb.</p> <p>65. <i>Elymus gmelinii</i> (Ledeb.) Tzvel.</p> <p>66. <i>Cyperus fuscus</i> L.</p> <p>67. <i>Scirpus hippolytii</i> V.Krecz.</p> <p>68. <i>Bolboschoenus planiculmis</i> (Fr. Schmidt) Egor.</p> <p>69. <i>Eleocharis intersita</i> Zinserl.</p> <p>70. <i>Carex duriuscula</i> C.A.Mey.</p> <p>71. <i>Carex stenophylloides</i> V.Krecz.</p> <p>72. <i>Carex enervis</i> C.A.Mey.</p> <p>73. <i>Carex reptabunda</i> (Trautv.) V.Krecz.</p> <p>74. <i>Carex sabulosa</i> Turcz. ex Kunth</p> |
|------------------|---|

|                           |   |
|---------------------------|---|
|                           | 75. <i>Carex korshinskyi</i> Kom.                 |
|                           | 76. <i>Carex pediformis</i> C.A.Mey.              |
|                           | 77. <i>Carex orthostachys</i> C.A.Mey.            |
| Juncaceae Juss.           | 78. <i>Juncus bufonius</i> L.                     |
|                           | 79. <i>Juncus compressus</i> Jacq.                |
| Asphodelaceae Juss.       | 80. <i>Anemarrhena asphodeloides</i> Bunge        |
| Hemerocallidaceae Lindley | 81. <i>Hemerocallis lilio-asphodelus</i> L.       |
|                           | 82. <i>Hemerocallis minor</i> Mill.               |
| Alliaceae Borkh.          | 83. <i>Allium nerinifolium</i> (Herb.) Baker      |
|                           | 84. <i>Allium odorum</i> L.                       |
|                           | 85. <i>Allium leucocephalum</i> Turcz. ex Ledeb.  |
|                           | 86. <i>Allium condensatum</i> Turcz.              |
|                           | 87. <i>Allium senescens</i> L.                    |
|                           | 88. <i>Allium mongolicum</i> Regel.               |
|                           | 89. <i>Allium prostratum</i> Trev.                |
|                           | 90. <i>Allium anisopodium</i> Ledeb.              |
|                           | 91. <i>Allium bidentatum</i> Fisch. ex Prokh.     |
|                           | 92. <i>Allium tenuissimum</i> L.                  |
| Liliaceae Juss.           | 93. <i>Gagea pauciflora</i> Turcz. ex Ledeb.      |
|                           | 94. <i>Lilium pumilum</i> Delile                  |
| Asparagaceae Juss.        | 95. <i>Asparagus dahuricus</i> Fisch. Ex Link.    |
| Convallariaceae Horan.    | 96. <i>Polygonatum sibiricum</i> Delaroché        |
|                           | 97. <i>Polygonatum odoratum</i> (Mill.) Druce.    |
| Iridaceae Juss.           | 98. <i>Iris dichotoma</i> Pall.                   |
|                           | 99. <i>Iris tenuifolia</i> Pall.                  |
|                           | 100. <i>Iris lactea</i> Pall.                     |
|                           | 101. <i>Iris potaninii</i> Maxim.                 |
| Orchidaceae Juss.         | 102. <i>Spiranthes amoena</i> (Bieb.) Spreng.     |
|                           | 103. <i>Orchis salina</i> Turcz. ex Lindl.        |
| Salicaceae Mirb.          | 104. <i>Salix miyabeana</i> Seemen                |
|                           | 105. <i>Populus tremula</i> L.                    |
| Betulaceae S.F. Gray      | 106. <i>Betula mandshurica</i> (Regel) Nakai      |
|                           | 107. <i>Betula platyphylla</i> Sukacz.            |
| Cannabaceae Endl.         | 108. <i>Cannabis sativa</i> L.                    |
| Ulmaceae Mirb.            | 109. <i>Ulmus pumila</i> L.                       |
| Urticaceae Juss.          | 110. <i>Urtica cannabina</i> L.                   |
|                           | 111. <i>Urtica angustifolia</i> Fisch. ex Hornem. |
| Polygonaceae Juss.        | 112. <i>Rheum undulatum</i> L.                    |



|                       |   |
|-----------------------|---|
| Chenopodiaceae Ulbr.  | <p>113. <i>Rumex acetosella</i> L.<br/> 114. <i>Rumex thyrsoifloris</i> Fingerh.<br/> 115. <i>Polygonum aviculare</i> L.<br/> 116. <i>Persicaria amphibia</i> (L.) S.F.Gray<br/> 117. <i>Persicaria lapathifolia</i> (L.) S.F.Gray.<br/> 118. <i>Knorringia sibirica</i> (Laxm.) Tzvel.<br/> 119. <i>Aconogonon angustifolium</i> (Pall.) Hara<br/> 120. <i>Aconogonon divaricatum</i> (L.) Nakai ex Mori<br/> 121. <i>Aconogonon alpinum</i> (All.) Schur<br/> 122. <i>Chenopodium aristatum</i> L.<br/> 123. <i>Chenopodium glaucum</i> L.<br/> 124. <i>Chenopodium acuminatum</i> Willd.<br/> 125. <i>Chenopodium hybridum</i> L.<br/> 126. <i>Chenopodium prostratum</i> Bunge<br/> 127. <i>Chenopodium album</i> L.<br/> 128. <i>Atriplex sibirica</i> L.<br/> 129. <i>Atriplex fera</i> (L.) Bunge<br/> 130. <i>Axyris prostrata</i> L.<br/> 131. <i>Axyris amaranthoides</i> L.<br/> 132. <i>Axyris hybrida</i> L.<br/> 133. <i>Kochia prostrata</i> (L.) Schrad.<br/> 134. <i>Corispermum mongolicum</i> Iljin.<br/> 135. <i>Suaeda corniculata</i> (C.A.Mey.) Bunge<br/> 136. <i>Suaeda salsa</i> (L.) Pall.<br/> 137. <i>Salsola collina</i> Pall.<br/> 138. <i>Salsola pestifera</i> Nels.</p> |
| Amaranthaceae Juss.   | 139. <i>Amaranthus retroflexus</i> L.   |
| Caryophyllaceae Juss. | <p>140. <i>Stellaria dichotoma</i> L.<br/> 141. <i>Stellaria graminea</i> L.<br/> 142. <i>Arenaria capillaris</i> Poir.<br/> 143. <i>Silene juniseensis</i> Willd.<br/> 144. <i>Silene repens</i> Patr.<br/> 145. <i>Lychnis sibirica</i> L.<br/> 146. <i>Melandrium apricum</i> (Turcz. ex Fisch. et Mey.)<br/> 147. <i>Gypsophilla davurica</i> Turcz.<br/> 148. <i>Dianthus versicolor</i> Fisch.</p>  |
| Ranunculaceae DC.     | 149. <i>Caltha palustris</i> L.<br>150. <i>Leptopyrum fumarioides</i> (L.) Reichenb   |

|                           |  |
|---------------------------|--|
|                           | 151. <i>Aquilegia viridiflora</i> Pall.                      |
|                           | 152. <i>Delphinium grandiflorum</i> L.                       |
|                           | 153. <i>Pulsatilla bungeana</i> C.A.Mey                      |
|                           | 154. <i>Pulsatilla turczaninovii</i> Kryl et Serg.           |
|                           | 155. <i>Clematis hexapetala</i> Pall.                        |
|                           | 156. <i>Halerpestes salsuginosa</i> (Pall. ex Georgi) Greene |
|                           | 157. <i>Halerpestes sarmentosa</i> (Adams.) Kom.             |
|                           | 158. <i>Ranunculus sceleratus</i> L.                         |
|                           | 159. <i>Thalictrum petaloideum</i> L.                        |
|                           | 160. <i>Thalictrum foetidum</i> L.                           |
|                           | 161. <i>Thalictrum simplex</i> L.                            |
|                           | 162. <i>Thalictrum minus</i> L.                              |
| Papaveraceae Juss.        | 163. <i>Papaver rubro-aurantiacum</i> (Fisch.) R.Sweet       |
|                           | 164. <i>Papaver nudicaule</i> L.                             |
| Hypocoaceae Wilk et Lange | 165. <i>Hypocoum erectum</i> L.                              |
|                           | 166. <i>Hypocoum lactiflorum</i> (Kar. et Kir.) Pazij        |
| Brassicaceae Burnett      | 167. <i>Lepidium densiflorum</i> Schrad.                     |
|                           | 168. <i>Lepidium ruderale</i> L.                             |
|                           | 169. <i>Lepidium latifolium</i> L.                           |
|                           | 170. <i>Allysum lenense</i> Adams.                           |
|                           | 171. <i>Allysum obovatum</i> (C.A.Mey.) Turcz.               |
|                           | 172. <i>Ptilothrichum canescens</i> (DC.) C.A.Mey.           |
|                           | 173. <i>Ptilothrichum tenuifolium</i> C.A.Mey.               |
|                           | 174. <i>Dontostemon integrifolius</i> (L.) C.A.Mey.          |
|                           | 175. <i>Eryssimum flavum</i> (Georgi) Bobr.                  |
|                           | 176. <i>Eryssimum cheiranthoides</i> L.                      |
|                           | 177. <i>Sisymbrium polymorphum</i> (Murr.) Roth              |
| Crassulaceae J. St.-Hil.  | 178. <i>Sedum aizoon</i> L.                                  |
|                           | 179. <i>Orostachys malacophylla</i> (Pall.) Fisch.           |
|                           | 180. <i>Orostachys fimbriata</i> (Turcz.) Berger.            |
|                           | 181. <i>Orostachys spinosa</i> (L.) C.A.Mey.                 |
| Saxifragaceae Juss.       | 182. <i>Ribes diacantha</i> Pall.                            |
|                           | 183. <i>Parnassia palustris</i> L.                           |
| Rosaceae Juss.            | 184. <i>Spiraea aquilegifolia</i> Pall.                      |
|                           | 185. <i>Cotoneaster melanocarpus</i> Fisch. ex Blytt         |
|                           | 186. <i>Dasiphora fruticosa</i> (L.) Rydb.                   |
|                           | 187. <i>Potentilla anserina</i> L.                           |
|                           | 188. <i>Potentilla bifurca</i> L.                            |

|                 |   |
|-----------------|---|
| Fabaceae Lindl. | <p>189. <i>Potentilla verticillaris</i> Steph.<br/>190. <i>Potentilla multifida</i> L.<br/>191. <i>Potentilla sericea</i> L.<br/>192. <i>Potentilla conferta</i> Bunge<br/>193. <i>Potentilla strigosa</i> Pall. ex Pursh<br/>194. <i>Potentilla supina</i> L.<br/>195. <i>Potentilla viscosa</i> G.Don.<br/>196. <i>Potentilla tanacetifolia</i> Willd. ex Schlecht.<br/>197. <i>Potentilla leucophylla</i> Pall.<br/>198. <i>Potentilla acaulis</i> L.<br/>199. <i>Sibbaldianthe adpressa</i> (Bunge) Juz.<br/>200. <i>Chamaerhodos trifida</i> Ledeb.<br/>201. <i>Chamaerhodos erecta</i> (L.) Bunge<br/>202. <i>Sanguisorba officinalis</i> L.<br/>203. <i>Rosa acicularis</i> Lindl.<br/>204. <i>Armeniaca sibirica</i> (L.) Lam.<br/>205. <i>Thermopsis lanceolata</i> R.Br.<br/>206. <i>Medicago falcata</i> L.<br/>207. <i>Medicago ruthenica</i> (L.) Trautv.<br/>208. <i>Melilotus dentatus</i> (Waldsr. et Kit.) Pers.<br/>209. <i>Melilotus suaveolens</i> Ledeb.<br/>210. <i>Trifolium lupinaster</i> L.<br/>211. <i>Caragana microphylla</i> Lam.<br/>212. <i>Caragana pygmaea</i> (L.) DC.<br/>213. <i>Astragalus davuricus</i> (Pall.) DC.<br/>214. <i>Astragalus chinensis</i> L.<br/>215. <i>Astragalus melilotoides</i> Pall.<br/>216. <i>Astragalus tenuis</i> Turcz.<br/>217. <i>Astragalus adsurgens</i> Pall.<br/>218. <i>Astragalus scaberrimus</i> Bunge<br/>219. <i>Astragalus brevifolius</i> Ledeb.<br/>220. <i>Astragalus galactites</i> Pall.<br/>221. <i>Oxytropis filiformis</i> DC.<br/>222. <i>Oxytropis glabra</i> (Lam.) DC.<br/>223. <i>Oxytropis oxyphylla</i> (Pall.) DC.<br/>224. <i>Oxytropis gracillima</i> Bunge<br/>225. <i>Glycyrrhiza uralensis</i> Fisch.<br/>226. <i>Hedysarum fruticosum</i> Pall.</p> |
|-----------------|---|

|                              |   |
|------------------------------|---|
|                              | 227. <i>Lespedeza hedysaroides</i> (Pall.) Kitag.       |
|                              | 228. <i>Vicia megalotropis</i> Ledeb.                   |
|                              | 229. <i>Vicia cracca</i> L.                             |
|                              | 230. <i>Lathyrus pratensis</i> L.                       |
| Geraniaceae Juss.            | 231. <i>Geranium sibiricum</i> L.                       |
|                              | 232. <i>Geranium wlassowianum</i> Fisch. ex Link.       |
|                              | 233. <i>Erodium stephanianum</i> Willd.                 |
| Linaceae S.F.Gray.           | 234. <i>Linum baicalense</i> Juz.                       |
| Rutaceae Juss.               | 235. <i>Haplophyllum dahuricum</i> (L.) G. Don.         |
| Poygolaceae R.Br.            | 236. <i>Polygala tenuifolia</i> Willd.                  |
| Euphorbiaceae Juss.          | 237. <i>Euphorbia humifusa</i> Willd.                   |
|                              | 238. <i>Euphorbia discolor</i> Ledeb.                   |
| Malvaceae Juss.              | 239. <i>Hibiscus trionum</i> L.                         |
| Violaceae Batsch             | 240. <i>Viola dissecta</i> Ledeb.                       |
| Thymelaceae Juss.            | 241. <i>Stellera chamaejasme</i> L.                     |
| Onograceae Juss.             | 242. <i>Ephelobium palustre</i> L.                      |
|                              | 243. <i>Chamaenerion angustifolium</i> (L.) Scop.       |
| Hippuridaceae Vest           | 244. <i>Hippuris vulgaris</i> L.                        |
| Apiaceae Lindl.              | 245. <i>Anthriscus sylvestris</i> (L.) Hoffm.           |
|                              | 246. <i>Pleurospermum uralense</i> Hoffm.               |
|                              | 247. <i>Bupleurum scorzonerifolium</i> Willd.           |
|                              | 248. <i>Bupleurum bicaule</i> Helm.                     |
|                              | 249. <i>Cicuta virosa</i> L.                            |
|                              | 250. <i>Saposhnikovia divaricata</i> (Turcz.) Schischk. |
| Primulaceae Batsch ex Borkh. | 251. <i>Primula nutans</i> Georgi.                      |
|                              | 252. <i>Androsace septentrionalis</i> L.                |
|                              | 253. <i>Androsace incana</i> Lam.                       |
|                              | 254. <i>Glaux maritima</i> L.                           |
| Plumbaginaceae Juss.         | 255. <i>Goniolimon speciosum</i> (L.) Boiss.            |
|                              | 256. <i>Limonium flexuosum</i> (L.) O. Kuntze.          |
|                              | 257. <i>Limonium bicolor</i> (Bunge) O. Kuntze.         |
| Gentianaceae Juss.           | 258. <i>Gentiana decumbens</i> L.                       |
|                              | 259. <i>Gentiana squarrosa</i> Ledeb.                   |
|                              | 260. <i>Gentianopsis barbata</i> (Froel.) Ma            |
|                              | 261. <i>Lomatogonium carinthiacum</i> (Wulfen) Reichenb |
|                              | 262. <i>Halenia corniculata</i> (L.) Cornaz             |
| Asclepiadaceae Borkh.        |   |
| Convolvulaceae Juss.         |   |

|                         |   |
|-------------------------|---|
| Cuscutaceae Dum.        | 263. <i>Vincetoxicum sibiricum</i> (L.) Decne.        |
| Boraginaceae Juss.      | 264. <i>Convolvulus ammannii</i> Desr.                |
|                         | 265. <i>Convolvulus arvensis</i> L.                   |
|                         | 266. <i>Cuscuta europaea</i> L.                       |
| Verbenaceae Jaume       | 267. <i>Myosotis caespitosa</i> C.F.Schultz           |
| Lamiaceae Martinov      | 268. <i>Lappula intermedia</i> (Ledeb.) M. Pop.       |
|                         | 269. <i>Caryopteris mongholica</i> Bunge              |
|                         | 270. <i>Amythystea coerula</i> L.                     |
|                         | 271. <i>Scutellaria scordifolia</i> Fisch. ex Schrank |
|                         | 272. <i>Scutellaria baicalensis</i> Georgi            |
|                         | 273. <i>Lophanthus chinensis</i> (Raf.) Benth.        |
|                         | 274. <i>Schizonepeta annua</i> (Pall.) Schischk.      |
|                         | 275. <i>Schizonepeta multifida</i> (L.) Briq.         |
|                         | 276. <i>Dracocephalum foetidum</i> Bunge              |
|                         | 277. <i>Phlomis tuberosa</i> L.                       |
|                         | 278. <i>Leonurus sibiricus</i> L.                     |
|                         | 279. <i>Leonurus mongolicus</i> Krecz. et Kupr.       |
|                         | 280. <i>Stachys palustris</i> L.                      |
| Solanaceae Juss.        | 281. <i>Thymus gobicus</i> Tscherneva                 |
|                         | 282. <i>Mentha arvensis</i> L.                        |
| Scrophulariaceae Benth. | 283. <i>Physochlaina physaloides</i> (L.) G. Don.     |
|                         | 284. <i>Hyoscyamus niger</i> L.                       |
|                         | 285. <i>Linaria buriatica</i> Turcz.                  |
|                         | 286. <i>Linaria acutiloba</i> Fisch. ex Rchb.         |
|                         | 287. <i>Scrophularia incisa</i> Weinm.                |
|                         | 288. <i>Veronica anagallis-aquatica</i> L.            |
|                         | 289. <i>Veronica incana</i> L.                        |
|                         | 290. <i>Euphrasia pectinata</i> Ten.                  |
|                         | 291. <i>Odontites rubra</i> (Baumg.) Pers.            |
|                         | 292. <i>Pedicularis resupinata</i> L.                 |
|                         | 293. <i>Pedicularis flava</i> Pall.                   |
| Plantaginaceae Juss.    | 294. <i>Cymbaria dahurica</i> L.                      |
|                         | 295. <i>Plantago salsa</i> Pall.                      |
| Rubiaceae Juss.         | 296. <i>Plantago major</i> L.                         |
|                         | 297. <i>Plantago depressa</i> Schlecht                |
|                         | 298. <i>Rubia cordifolia</i> L.                       |
| Dipsacaceae Juss.       | 299. <i>Galium verum</i> L.                           |
| Campanulaceae Juss.     | 300. <i>Galium boreale</i> L.                         |

|   |  |
|---|--|
| <p>Asteraceae Dumort. Berch. et J.<br/>Presl. Compositae Giseke</p> | <p>301. <i>Scabiosa comosa</i> Fisch. ex Roem. et Schult.<br/>302. <i>Adenophora stenanthina</i> (Ledeb.) Kitag.<br/>303. <i>Heteropappus hispidus</i> (Thunbg.) Less.<br/>304. <i>Aster alpinus</i> L.<br/>305. <i>Aster tataricus</i> L.fil.<br/>306. <i>Arctogeron gramineum</i> (L.) DC.<br/>307. <i>Tripolium vulgare</i> Nees<br/>308. <i>Leontopodium leontopodioides</i> (Willd.) Beauverd<br/>309. <i>Leontopodium ochroleucum</i> Beauverd<br/>310. <i>Inula britannica</i> L.<br/>311. <i>Bidens tripartia</i> L.<br/>312. <i>Achillea asiatica</i> Serg.<br/>313. <i>Chrysanthemum zawadskii</i> Herb.<br/>314. <i>Filifolium sibiricum</i> (L.) Kitam.<br/>315. <i>Artemisia dracunculus</i> L.<br/>316. <i>Artemisia anethifolia</i> Web. ex Stechm.<br/>317. <i>Artemisia macrocephala</i> Jacq. ex Bess.<br/>318. <i>Artemisia sieversiana</i> Willd.<br/>319. <i>Artemisia palustris</i> L.<br/>320. <i>Artemisia scoparia</i> Waldst. et Kit.<br/>321. <i>Artemisia annua</i> L.<br/>322. <i>Artemisia gmelinii</i> Web. ex Stechm.<br/>323. <i>Artemisia lacinata</i> Willd.<br/>324. <i>Artemisia mongolica</i> (Bess.) Fisch. ex Nakai.<br/>325. <i>Artemisia frigida</i> Willd.<br/>326. <i>Artemisia adamsii</i> Bess.<br/>327. <i>Artemisia commutata</i> Bess.<br/>328. <i>Neopallasia pectinata</i> (Pall.) Poljak.<br/>329. <i>Senecio dubius</i> Ledeb.<br/>330. <i>Senecio erucifolius</i> L.<br/>331. <i>Ligularia sibirica</i> (L.) Cass.<br/>332. <i>Echinops latifolius</i> Tausch.<br/>333. <i>Saussurea amara</i> (L.) DC.<br/>334. <i>Saussurea salicifolia</i> (L.) DC.<br/>335. <i>Saussurea salsa</i> (Pall.) Spreng.<br/>336. <i>Cirsium esculentum</i> (Siev.) C.A.Mey<br/>337. <i>Serratula centauroides</i> L.<br/>338. <i>Rhaponticum uniflorum</i> (L.) DC.</p> |
|---|--|

### 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021

|  |   |
|--|---|
|  | <p>339. <i>Scorzonera austriaca</i> Willd.<br/>340. <i>Sonchus arvensis</i> L.<br/>341. <i>Lactuca sibirica</i> (L.) Benth. ex Maxim.<br/>342. <i>Youngia stenoma</i> (Turcz.) Ledeb.<br/>343. <i>Youngia tenuicaulis</i> (Babc. et Stebbins.) Czer.<br/>344. <i>Youngia tenuifolia</i> (Willd.) Babc. et Stebbins.<br/>345. <i>Ixeridium gramineum</i> (Fisch.) Tzvel.<br/>346. <i>Taraxacum dissectum</i> (Ledeb.) Ledeb.<br/>347. <i>Taraxacum collinum</i> DC.<br/>348. <i>Taraxacum leucanthum</i> (Ledeb.) Ledeb.<br/>349. <i>Crepis flexuosa</i> (Ledeb.) Clarke<br/>350. <i>Hieracium virosum</i> Pall.</p> |
|--|---|

Oral Presentation

Wednesday

Diversity of Plant species, Systematics and Phylogeny-2

**Tepal Morphology of *Persicaria* s.str. (Polygonaceae) Taxa in Turkey**Suzan Kundakçı<sup>1\*</sup>, Serdar Makbul<sup>1</sup>, Mutlu Gültepe<sup>2</sup>, Kamil Coşkunçelebi<sup>3</sup><sup>1</sup>Department of Biology, Faculty of Sciences and Art, Recep Tayyip Erdogan University, Rize, Turkey.<sup>2</sup>Department of Forestry, Dereli Vocational School, Giresun University, Giresun, Turkey.<sup>3</sup>Department of Biology, Faculty of Sciences, Karadeniz Technical University, Trabzon, Turkey.

\*(suzan\_kundakci17@erdogan.edu.tr)

**Abstract**

Tepal morphology of the *Persicaria* s.str. (Miller) DC. (Polygonaceae) taxa naturally spreading in Turkey has been studied in detail and the variations among the studied taxa were taxonomically revealed. All Light and Scanning Electron Microscope examinations were performed on the herbarium materials stored in the Herbarium of Biology Department at Recep Tayyip Erdogan University (RUB). It was determined that tepal shape (ovate, lanceolate, obovate or oblong), tepal color (pink, purple, green or cream-white), periclinal walls (concav or convex) and ornamentation (rugose, striate, smooth, ruminant or rough) are important characters to separate the examined *Persicaria* taxa. In this study, variations of the tepal macro-micro morphology among the *Persicaria* taxa were evaluated in detail for the first time by using LM and SEM. The findings showed that the tepal macro-micro morphology varies in the examined taxa and supply taxonomical support to delimiting the examined taxa.

**Keywords:** Tepal morphology, *Persicaria*, sem, Turkey**Acknowledgement:** This study is financially supported by TUBITAK (Project number: 219Z024).



Oral Presentation

Thursday

Diversity of Animal Species, Systematics and Phylogeny-2

**An Annotated and Updated Checklist of Turkish *Sarcophaga (Liosarcophaga) Enderlein, 1928* with the Comparisons of Male Terminalia**

Gamze Pekbey<sup>1\*</sup>

<sup>1</sup>Yozgat Bozok University, Faculty of Agriculture, Department of Plant Protection, Yozgat, Turkey

\*Corresponding author e-mail: gamze.pekbey@bozok.edu.tr

**Abstract**

*Sarcophaga (Liosarcophaga) Enderlein, 1928* is the second most species-richness subgenus of Sarcophagidae, representing with nearly 98 species worldwide. Although a total of ten species of that subgenus have been reported from Turkey so far, the existence of *S. (L.) dux* (Thomson, 1869), *S. (L.) teretirostris* Pandellé, 1896 and *S. (L.) bartaki* (Verves, Radchenko and Khrokalo, 2017) is found doubtful for Turkey. On the other hand, *S. (L.) aegyptica* Salem, 1935 has lastly been added to Turkish fauna. That study aims to provide an updated list of *Sarcophaga (Liosarcophaga) spp.* of Turkey with the description and comparisons of morphological structure of male terminalia of all eight species through the photographs with SEM and stereomicroscope.

**Keywords:** Diptera, flesh flies, *Liosarcophaga*, Sarcophagidae, Turkey

Oral Presentation

Thursday

Diversity of Animal Species, Systematics and Phylogeny-2

**The Cheyletid Mites (Acariformes: Cheyletidae) of Kelkit Valley (Turkey)**Burcu Kabasakal<sup>1\*</sup>, Salih Doğan<sup>1</sup>

<sup>1</sup>Biology Department, Arts and Sciences Faculty, Erzincan Binali Yıldırım University (EBYU),  
Erzincan, Turkey

\*Corresponding author e-mail: burcukabasakal7@gmail.com

**Abstract**

Free-living cheyletid mites (Cheyletidae) live in a broad spectrum of habitats such as plants, soil, vertebrate or arthropod nests. Some members of these mites are permanent ectoparasites of small mammals and birds. Sometimes a few of them cause allergies and dermatitis in humans having close contact with infested pets. The family Cheyletidae have a worldwide distribution. In the present work, it was evaluated cheyletid mites from the materials collected with a field study carried out once a month between May 2020 and April 2021 in Kelkit Valley (Turkey), and from the materials from two previously completed projects: 11BAP18 (EBYU) and 107T183 (TÜBİTAK). As a result, 16 species and 13 genera belonging to Cheyletidae were given. In the oral presentation, the list of all identified species, their brief descriptions and figures were given. This is a faunistic study on cheyletid mites in Kelkit Valley and produced from the first author's PhD thesis.

**Keywords:** Acari, fauna, Cheyletidae, Kelkit Valley, Turkey

Oral Presentation  
Thursday

Diversity of Animal Species, Systematics and Phylogeny-2

**Investigation of Wintering Waterbirds Diversity in Different Wetlands Around the Dardanelles (2021 IWC)**İbrahim Uysal<sup>1\*</sup>, İbrahim Uysal<sup>2</sup><sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Biology, Orcid ID:0000-0001-7180-5488<sup>2</sup>Çanakkale Onsekiz Mart University, Vocational School of Health Services, Orcid ID: 0000-0002-7507-3322

\*Corresponding author e-mail: ibrahimuyysal@hotmail.com

**Abstract**

In wetland ecosystems, waterbirds are one of the most remarkable vertebrate animal groups. With the changes in the wetland ecosystem, the diversity of waterbirds species and populations change rapidly and become an important indicator in the follow-up of sustainability. In the study, mid-winter waterbird counts in 2021 were evaluated in five wetlands on the coast of Çanakkale Strait, which is one of the important migration routes in the Western Palaearctic region, and the number of waterbirds species and densities were compared. Within the scope of the research, a total of 43 species and 8515 waterbird individuals included in 9 order and 12 families were counted. The highest number of species was observed in Çardak Lagoon (31 waterbird species), which is the second study area with the shore arrow feature and the smallest surface area. The highest number of individuals was observed in Gökçeada Salt Lake (3906 waterbirds), which was declared a wetland of national importance and prohibited hunting. Çardak lagoon was also the area where the highest species diversity (Shannon-Wiener Indexes,  $H'$ : 2,473) and the highest species richness (Margalef Index,  $M$ : 4,257) were calculated. The lowest species diversity ( $H'$ : 1,291) and the lowest species richness ( $M$ : 1,58) were detected in Uzunkızılı Pond, the artificial irrigation dam with the smallest surface area and the lowest number of habitats. A significant difference was found between the number of habitats in the wetland and the number of recorded species ( $p < 0.0001$ ). As the number of habitats in the wetland increased, the number of species also increased. A significant difference was found between the wetland area and the total number of individuals ( $p < 0.0001$ ). As the wetland surface area increased, the total number of recorded individuals decreased. It was concluded that the resulting inverse relationship may be related to

parameters such as hunting pressure in the wetland, water quality in the wetland, nutrient abundance, shelter, and the width of shallow water suitable for feeding. As a result, the data obtained revealed the importance of the wetlands for the winter visitor water birds, and pioneering data were presented for the sustainability of the wetlands in future studies.

**Keywords:** Waterbirds, winter, population, diversity index, Çanakkale

**Acknowledgements:** This study was prepared with data collected within the scope of the thesis study to cover part of the master's thesis entitled “Evaluation of Midwinter waterbirds counts and research of breeding waterbirds”, which is being carried out at Çanakkale Onsekiz March University, Graduate Education Institute, Department of Biology.

Oral Presentation

Thursday

Diversity of Animal Species, Systematics and Phylogeny-2

## Phylogenetic Analysis of *Heracleum* L. (Apiaceae) Taxa in Turkey Based on nrDNA ITS and cpDNA trnL Intron and trnL-F DNA Sequences

Leyla Gürlük<sup>1</sup>, Mustafa Çelik<sup>2</sup>, Özlem Çetin<sup>3\*</sup>

<sup>1</sup>Selçuk University, Graduate School of Natural and Applied Sciences, Konya.

<sup>2</sup>Advanced Technology Research and Application Center, Selçuk University, Konya, Turkey.

<sup>3</sup>Department of Biotechnology, Faculty of Science, Selçuk University, Konya, Turkey.

\*Corresponding author e-mail: ozlemcetin8419@gmail.com

### Abstract

The genus *Heracleum* L. comprises about 87 species and is distributed from North America to East Asia. It is represented by 22 taxa in Turkey. The aims of the study were to understand phylogenetic relationships between *Heracleum* taxa distributed in Turkey and to determine which region (ITS, trnL intron and trnL-F) was more powerful to understand evolutionary relationships among *Heracleum* species. Specimens were collected from different localities in Turkey. DNA extractions were performed using the DNeasy Plant Mini Kit (QIAGEN), following manufacturer's instructions. ITS region of nrDNA was amplified using ITS4 (5'TCCTCCGCTTATTGATATGC3') and ITS5 (5'GGAAGGAGAAGTCGTAACAAG3') primers. trnL-F region of cpDNA was amplified using trnL-c (5'CGAAATCGGTAGACGCTACG3') and trnL-f (5'ATTTGAACTGGTGACACGAG3') primers. PCR condition is 95°C for 5 min initial denaturation, 35 cycles of 94°C for 30 s denaturation, 50°C for 30 s annealing, and 72°C for 1 min extension, 72°C for 10 min final extension. PCR products were visualised by agarose gel. The amplified fragments were sequenced using the same primers used for amplification. Alignment of the ITS sequence was done with Bioedit software. The phylogenetic trees were constructed by the Bayesian Interference and Maximum Parsimony methods. In this study, multiple samples of each species collected from various regions in Turkey were examined. ITS regions produced a good resolution of phylogenetics relationship while trnL intron and trnL/F IGS failed to resolve relationship among *Heracleum* species. We have also noticed that some species have identical ITS sequence. For example, the sequences of ITS regions were found to be identical in *H. antasiaticum* Manden. and *H. platytaenium* Boiss. The results of phylogenetic analyses based on ITS sequence confirmed that *Heracleum sensu stricto* is monophyletic group.

**Keywords:** *Heracleum*, phylogeny, umbelliferae

Oral Presentation

Thursday

Diversity of Plant Species, Systematics and Phylogeny-3

## Morphological Characteristics of The Genus *Lappula Moench.* (Boraginaceae Juss.) In Mongolia

Munkhzul Tungalag<sup>1\*</sup>

<sup>\*1</sup>Mongolian National University of Education, Department of Biology, Ulaanbaatar, Mongolia

tmunhzul93@gmail.com

<https://orcid.org/0000-0003-3014-5289>

### Abstract

*Lappula Moench.* (Boraginaceae), a genus comprising 70 species (Ovczinnikova, 2005), has a cosmopolitan distribution. The results show, that keys the identification of some species conspectus of (*Lappula Moench.*) and data, of their habitat and distribution in Mongolia and world's distribution. Morphological features on fruits of some species in Boraginaceae could be useful in solving some taxonomic problems.

**Keywords:** Shape, surface ornemantations, size, nutlet

### INTRODUCTION

*Lappula Moench.* (Boraginaceae), a genus comprising 70 species (Ovczinnikova, 2005), has a cosmopolitan distribution. *Lappula* is the largest genus in the family Boraginaceae with about 19 taxa in Mongolia (Grubov, 1982; Gubanov, 1996; Urgamal et al., 2014), included ornamental, medicinal and high forage plant.

It was concluded that characters of fruit morphology are sufficiently effective for purposes of taxonomy, Nutlet characters are essential for identification and classification of *Lappula* (Ovczinnikova 2006a, 2007a, b; 2008, 2016).

The morphology of the shape and size of the carpobasis, the shape and size of the eremes and the peculiarities of their attachment to the carpobasis, the shape and location of the cicatrix great taxonomic importance in Boraginaceae. When describing the morphological structures

and surfaces of the nutlets used terminology given in the works of Ovczinnikova, 1997, 2007, 2008, 2011. The purpose of this research is to determine the composition of plant species belonging to the genus *Lappula Moench*.

## MATERIALS AND METHODS

This study is based on nutlets taken from herbarium specimens, mostly from UBU, LE, NSK and original collections made during expeditions in different natural regions of Mongolia. By for each species, 10 erems were taken from 1–4 samples. The study used 2000 pages of herbarium, plants were collected during field courses and botanical expeditions of the “Morphological study of pollen and spore in Mongolia” project from 2006-2016, are deposited in the Herbarium of the Laboratory of Palynology at the Mongolian National University of Education and and determined by traditional methods through (Popov, 1953; Grubov, 1982; Ovczinnikova, 2007). Material for comparison research was selected from the herbarium of the Botanical Institute. V. L. Komarov RAS (BIN RAS, NSK), and also collected in natural zones of Mongolia.

Morphological characters of the fruits of 22 species belong to 5 section of *Lappula Moench*. genera of Boraginaceae in Mongolia were examined using under light binocular (MBS-10) and electron microscopy SEM “TM-1000”. Nutlets morphology of the examined specimens exhibits some variation in size, shape and surface ornamentation. Significant morphology of genus *Lappula* classification the features include the shape and surface of the nutlets, carpobasis shape, rows of marginal glochids directly related. The nutlet shape and surface ornamentation were studied following S.V. Ovczinnikova (2007). In order to determine the average nutlet sizes, all mature nutlets from each species were measured.

## RESULTS

We studied nutlet morphology of 22 species belonging to genera *Lappula* found in Mongolia. Morphological variation of the carpobasis and nutlets shape, surface, disk, glochids in *Lappula* is described below. Carpobasis shapes vary from anchor-like, narrowly cone, long narrowly and nutlets shapes from ovoid, narrowly ovoid, acute ovoid, triangular-ovoid, broadly ovoid, narrowly lanceolate. Nutlet surface varies from echinate, tuberculate, wrinkled, granulate, smooth, small anchorlike spines (Table 1.). The *Lappula* (Moench.) species distributed in Mongolia belong to 5 sections in the classification system by S.V. Ovczinnikova (2007).

**Sect 1. *Lappula*:** Carpobasis anchor-like, style completely masked among nutlets. Nutlets easily separated from the carpobase. Nutlets size 3-3.5 mm, ovate. Disk ovate, keel with scabrate. Scar convex at base of groove and center of the abaxial surface. Annual, stem erect, 35-60 sm, grows on steppe, sandy or rocky slopes.

***Lappula anisacantha*** (Turcz. ex Bunge) Gürke.: Ulaanbaatar sity, birch forest behind of the Bogdkhaan mountain, 47°59'23.8" N, 106°56'26.8" E, Khentii aimag, Kherlen river 47°41'08.5" N 108°27'08.5" E, Ovorkhangai aimag, Kharkhorin sum, bridge of the Orkhon river, 47°12'20.04" N, 102°47'02.81" E, Deliin burd 47°02'51.6"N, 103°10'44.2" E, Uyanga sum, Naiman nuur, Khuis nuur, 46°6'15'07''N, 101°07'6'8.71'' E 2231 m, Khujirt sum, mountain slope, 47°01'00.9" N, 102°54'11.9" E, 1698 m, Zavkhan aimag, Tosontsengel sum, the source of the Tegshii river, the mountain slope 48°18'35.2'' N, 97°59'42.6'' E, 2036 m, Palynology laboratory in MSUE.

Typus: described from Tuva Republic Erzinsky District, 11 км S Erzin, Khara-Khaya, A. Yu. Korolyuk, E. A. Korolyuk, Novosibirsk 3.09.2013 (NSK).

Nutlets length 3-3.5 mm, ovoid, surface echinate, tuberculate; disk shape ovate, with keel and 2-3 rows of glochids.

***Lappula consanguinea*** (Fisch. et C.A. Mey) Guerke: Tuv aimag, Khustain nuruu, Hustai National Park (HNP) 07.26. 2018, Uvurkhangai aimag, Kharkhorin sum, Orkhon river, forest upper fringes of the Aguit mountain 47°11'35.44" N, 102°49'52" E, 1733 m, Deliin burd 47°01'19.1"N, 103°14'57.7" E, Zavkhan aimag, Tosontsengel sum, river of Tegsh, mountain steppe, 48°19'29.2'' N, 97°59'45.9'' E, 2009 m, Selenge aimag, Shaamar sum, Zuun Shaamar, fenced plots, 50°05'52.6" N, 106°14'22.6" E 657 m. Mongolian altai, Rashaantyn nuruu, 07.21.1984 № 626, R.V.Kamelin, Sh.Dariimaa, determined S.V. Ovczinnikova.

Nutlets 3-3.5 mm, acute ovoid, adaxially with scattered tubercles; disc narrowly ovate, marginal glochids in 2-3 rows; inner glochids ca. 1.5 mm, thin, hard, ascending to erect, middle glochids shorter.

***Lappula fruticulosa*** Ovczinnikova: Govi-Altai aimag, 20 km northeast of Bugat sum, Mongolian Altai, Uertiin huren uul, 07. 05. 1984. №119. Sh. Dariimaa, P.V. Kamelin, determined S. V. Ovczinnikova. Holotypus: LE

Nutlets 3.5 mm, ovoid, surface wrinkled; disk ovate, with keel and 2 rows of glochids.

***Lappula heteracantha*** (Ledeb.) Guerke:

Zavkhan aimag, Ikh-Uul sum, Bumbatkargana, mountain steppe of Orgikhyn uvur, 48°48'36.9''N, 98°31'36.1'' E, 2122 m. Typus: LE



Cycatrix (Scar shape at base of groove) lanceolate convex, center of the abaxial surface; nutlets 2.5-3.5 mm, narrowly ovoid, surface tuberculate; disk lanceolate, without keel and 2 rows of glochids.

*Lappula intermedia* (Ledeb.) Popov.: Tuv aimag, Batsumber sum, Shatan, stony slopes and tailing of Tsogtkhairkhan mountain, 48°30'52.8"N, 106°50'13.1" E, 1145 m. Khovd aimag, Bulgan sum, north slopes of Baitagbogd, 09.18.1948. №5512, V.I. Grubov, determined S. V. Ovczinnikova.

Nutlets broadly ovoid, 3-3.5 mm, granulose, adaxially wrinkled; disc ovate with a single row of marginal glochids;

*Lappula marginata* (Bieb.) Guerke.: Ovorkhangai aimag, Kharkhorin sum, Deliin burd 47°02'51.6"N, 103°10'44.2" E, meadows 47°00'28.6" N, 103°14'49.4" E, 1586 m, Uyanga sum, Naiman nuur, coasts of Khuis lake, 46°61.5'07''N, 101°76'8.71'' E, 2231 m.

Govi-Altai aimag, 39 km southeast Jargalant, Zavhkan gol, steppe, 1600 m, A. Korolyuk, E. Korolyuk, Novosibirsk 7.15.2017 (NSK). Mongolian Altai, Uertiin huren uul, 20 km northeast Bugat sum, 05.07.1984. №91, P.V. Kamelin, Sh.Dariimaa, determined S. V. Ovczinnikova.

Uvs aimag, Zuungovi sum, sandy of Buurug Del, 07.27.1945, №10402, A.A. Yunatov, determined S. V. Ovczinnikova.

Nutlets ovoid, 3.3-3.5 mm, smooth and nitid. Disc ovate, with a single row of marginal glochids. Glochids on nutlets strongly broadened at base and there fused into flat broad border.

*Lappula patula* (Lehm.) Nalaikh, Enger Shand, roadside 47°47'16.40'' N, 107°16'08.1'' E, Uvurkhangai aimag, Khujirt sum, slopes of hills, 47°01'00.9" N, 102°54'11.9" E, 1698 m, 08.24.2019.

Nutlets ovoid, 2.5 mm, surface tuberculate, base with 4 or 5 small prickles on each side; disc lanceolate, marginal glochids in a single row.

*Lappula redowskii* (Hornem.) Greene: Uvurkhangai aimag, Kharkhorin sum, Orkhon river, forest upper fringes of the Aguit mountain 47°11'35.44" N, 102°43'49.5" E, 1732 m, Deliin burd 47°01'36.4"N, 103°14'52.2" E, Khujirt sum, steppe slopes of hills, 47°00'14.0" N, 102°51'29.0" E, 1915 m, Nalaikh, Terelj, "Khaadyn tamga" camp, mountain slopes 09.13.2019, Bayan-Ulgii aimag, Sagsai sum, coasts of Dayan lake, 48°16'31.8" N, 88°52'08.0" E, 2237 m, Bulgan aimag, stony slopes of Khugnu-Khan mountain, 47°25'29.9" N, 103°41'37.7" E, 1326 m.

Nutlets ovoid, 3-3.5 mm, wrinkled, tuberculate, disc ovate with a single row of marginal glochids.

*Lappula stricta* (Ledeb.) Gurke.: Tuv aimag, Khustain nuruu, 07.26.2018, Uvurkhangai aimag, Kharkhorin sum, Deliin burd, meadow steppe, 47°00'34.1"N, 103°15'0.5" E 1621 m,

Bumbatyn Tsagaan nuur 47<sup>0</sup>04'04.7" N, 103<sup>0</sup>04'41.5" E.

Nutlets oblong-ovoid, 3-3.2 mm, adaxially wrinkled-tuberculate; disc narrowly lanceolate, center line keeled, usually without keel, with a single row of marginal glochids; glochids erect.

***Lappula squarrosa*** (Retz.) Dumort.: Omnogobi province, Dalanzadgad, left bank of the soum, Ya.V. Kalinina. 07.28.1958, determined S. V. Ovczinnikova.

Nutlets ovoid, 3.3-4 mm, tuberculate, disc lanceolate, with 2-3 rows of marginal glochids.

**Sect 2. *Omphalolappula*** (Brand) Ovczinnikova.

Carpobasis anchor-like, style completely masked among nutlets. Nutlets 2.5–3 mm; nutlets narrowly ovoid; adaxially shiny, wrinkled; disc narrow triangular, small, white granulose; Scar position at base of groove, convex-triangular. Herbs annual, much branched, crowded, becoming globose, 4–8 cm tall. Habitat: deserts, semideserts

***Lappula balchaschensis*** Popov ex Pavlov: Khovd, Most sum, river Ulaan erge, 46<sup>0</sup>45'47.8", 92051'39.3", Mankhan sum, tailings of hills Jargalant khairkhan A. Yu. Korolyuk 6.19.2004 (NSK), Bayan-Ulgii, Bulgan sum, Nariin river, Bayankhongor aimag, 35 km south of Shinejinst sum, 6 site, bottom of desert sayrs, 8.06.1979 D. Zumberelmaa, Ch. Sanchir (UBA), Umnugobi aimag, Bulgan sum, in sayrs of hills Baruunsaikhan, 07. 29. 1971, №32 Ch. Sanchir, determined S.V. Ovczinnikova 06.20.2010. Typus: (LE, NSK)

Nutlets narrowly ovoid, 2.7 mm; adaxially shiny, wrinkled; disc narrowly triangular, white granulose; margin thick, with a single row of glochids; spreading outward, slightly widened at base.

**Sect. 3. *Sinaicae*** (Riedl) Ovczinnikova.

Carpobasis wedge, narrowly, style projecting 1–1.5 mm above nutlets. Nutlets ovoid, shiny; disc 2.5-3 mm, oblong-ovate, densely rounded granulose, center line keeled, margin prominent and forming a narrow rib; lateral surfaces granular. Not scar at base of groove. Herbs annual, branched at base. Habitat: rocky slopes, deserts.

***Lappula occultata*** Popov: Khovd aimag, Bulgan sum, river of Bulgan, upper slope of hills Zuun khad, 07.19.1984. №576, P.V. Kamelin, Sh.Dariimaa, determined S. V. Ovczinnikova.

Nutlets ovoid, 1.7-2.2 mm, shiny, lateral surfaces granular; disc oblong-ovate, densely rounded granulose, margin prominent and forming a narrow rib; lateral surfaces granular.

**Sect. 4. *Macranthae*** (Riedl) Ovczinnikova.

Carpobasis anchor-like, style completely masked among nutlets. Nutlets 3.5-4 mm; disk narrowly lanceolate; adaxially tuberculate or glabrous. Scar lanceolate, long, convex at base of groove; Herbs annual, branched at base. Habitat: deserts.

*Lappula semiglabra* (Ledeb.) Gurke.: Umnugovi aimag, Bugan sum, margins of Bayanzag. Mandal-Ovoo, Bayanzag, 07.17.1948. №6788, V.I.Grubov, determined S. V. Ovczinnikova.

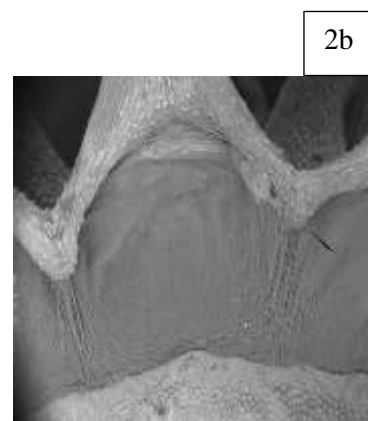
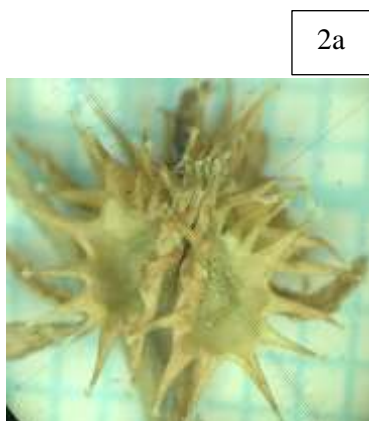
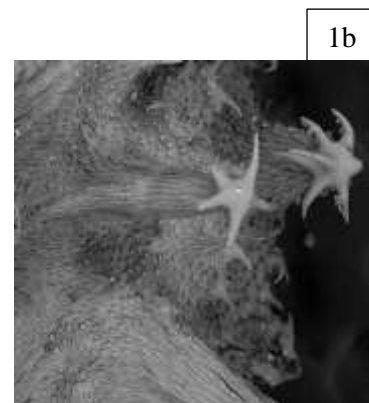
Nutlets homomorphic or heteromorphic, narrowly lanceolate, 4 mm, adaxially tuberculate or glabrous; disc narrowly lanceolate, with scattered tubercles, center line keel usually with short prickles or tubercles;

**Sect. 5. *Microcarpae*** (M. Pop.) Ovczinnikova.

Carpobasis wedge; style projecting 0.5–1 mm above nutlets. Nutlets 2-3.5 mm; disc narrowly ovate, granulose, center line keeled and with short glochids; adaxially tuberculate or glabrous. Scar narrowly triangular, convex at base of groove; Herbs biennial or perennial, erect. Habitat: mountain steppe, meadows, sunny slopes, low mountain canyons, semideserts

*Lappula microcarpa* (Ledeb.) Gurke.: Mongolian Altai, Khovd aimag, Rashaantyn nuruu, 07.21.1984. №672, P.V. Kamelin, Sh.Dariimaa, determined S. V. Ovczinnikova.

Nutlets ovoid, 2.5–2.7 mm; adaxially granulose, sometimes with 2 rows of glochids below; disc narrowly ovate, granulose, center line keeled and with short glochids; marginal glochids in a single row.



3a



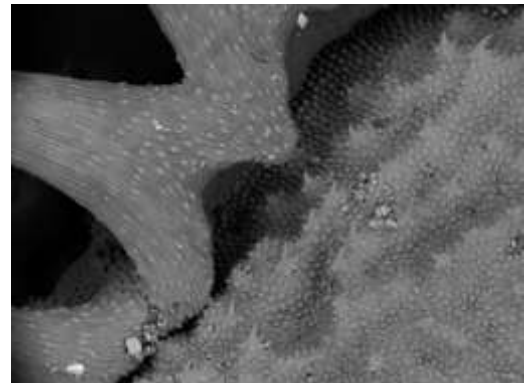
3b



4a



4b



**Figure 1.** *Lappula semiglabra* (Ledeb.) Gurke. 2. *Lappula marginata* (Bieb.) Guerke., 3. *Lappula balchaschensis* Popov ex Pavlov, 4. *Lappula stricta* (Ledeb.) Gurke. a. Nutlets under the light microscope b. SEM micrographs of nutlets *Lappula*.

**Table 1.** A comparison of characteristics studies for *Lappula* taxa nutlets

| Taxa                          | Carpobasis shape | Nutlets shape        | Nutlet surface (ornamentation)          | Nutlet size (mm)                 | Rows of glochids | Disk shape          | Keel in disk                       |                                   |
|-------------------------------|------------------|----------------------|---|----------------------------------|------------------|---------------------|------------------------------------|-----------------------------------|
| <i>Lappula anisacantha</i>    | anchor-like      | ovoid                | echinate, tuberculate                   | 3-3.5                            | 2 or 3           | ovate               | with keel                          |                                   |
| <i>Lappula balchaschensis</i> | narrowly cone    | narrowly ovoid       | adaxially shiny, wrinkled               | 2.7                              | 1                | narrowly triangular | without keel                       |                                   |
| <i>Lappula consanguinea</i>   | anchor-like      | acute ovoid          | echinate, tuberculate, granulate        | 3.3-4                            | 2 or 3           | narrowly ovate      | without keel                       |                                   |
| <i>Lappula diploloma</i>      | narrowly cone    | triangular-ovoid     | smooth                                  | 3                                | 1                | ovate               | without keel                       |                                   |
| <i>Lappula duplicarpa</i>     | anchor-like      | oblong-ovoid         | echinate, tuberculate                   | 3                                | 2                | narrowly ovate      | short glochids                     |                                   |
| <i>Lappula fruticulosa</i>    | anchor-like      | ovoid                | wrinkled                                | 3.5                              | 2                | ovate               | with keel                          |                                   |
| <i>Lappula heterocantha</i>   |                  | narrowly ovoid       | tuberculate                             | 2.5-3.5                          | 2                | lanceolate          | without keel                       |                                   |
| <i>Lappula intermedia</i>     |                  | broadly ovoid        | granulose, adaxially wrinkled           | 3-3.5                            | 1                | ovate               | without keel                       |                                   |
| <i>Lappula Lipskyi</i>        |                  | heteromorphic, ovoid | small tuberculate or smooth             | 3.3                              | 1                | lanceolate          | with keel                          |                                   |
| <i>Lappula macrantha</i>      |                  | narrowly lanceolate  | small anchorlike spines and tuberculate | 3-3.3                            | 1                | narrowly lanceolate | with keel                          |                                   |
| <i>Lappula marginata</i>      |                  | ovoid                | wrinkled                                | 3.3-3.5                          | 1                | ovate               | without keel                       |                                   |
| <i>Lappula microcarpa</i>     |                  | long narrowly        | ovoid                                   | adaxially granulose              | 2.5-2.7          | 2                   | narrowly ovate                     | with keel and with short glochids |
| <i>Lappula occultata</i>      |                  | wedge                | ovoid                                   | shiny, lateral surfaces granular | 1.7-2.2          | 0                   | oblong-ovate                       | without keel                      |
| <i>Lappula patula</i>         | anchor-like      | ovoid                | tuberculate                             | 2.5                              | 1                | lanceolate          | without keel                       |                                   |
| <i>Lappula redowskii</i>      |                  | ovoid                | wrinkled, tuberculate                   | 3.5                              | 1                | ovate               | without keel                       |                                   |
| <i>Lappula semiglabra</i>     |                  | narrowly lanceolate  | tuberculate or glabrous                 | 4                                | 1                | narrowly lanceolate | keel usually with short 5 prickles |                                   |
| <i>Lappula stricta</i>        |                  | oblong-ovoid         | adaxially wrinkled-tuberculate          | 3-3.2                            | 1                | narrowly lanceolate | keel, usually without keel         |                                   |
| <i>Lappula squarrosa</i>      |                  | ovoid                | tuberculate                             | 3.3-4                            | 2 or 3           | lanceolate          | without keel                       |                                   |
| <i>Lappula tenuis</i>         |                  | broadly ovoid        | adaxially granulose or smooth           | 2-2.3                            | 1                | lanceolate          | without keel                       |                                   |
| <i>Lappula tadshikovii</i>    |                  | long narrowly        | ovoid                                   | tuberculate                      | 3                | 2                   | narrowly ovate                     | with keel                         |
| <i>Lappula tianshanica</i>    | ovoid            |                      | echinate, adaxially finely tuberculate  | 3-3.5                            | 2                | narrowly ovate      | with keel                          |                                   |
| <i>Lappula tuvunica</i>       | anchor-like      | oblong-ovoid         | tuberculate                             | 2.5                              | 2                | ovate               | glochids                           |                                   |

## DISCUSSION

Significant morphology of genus *Lappula* classification the features include the shape and surface of the nutlets, carpobasis shape, rows of marginal glochids directly related. The nutlet shape and surface ornamentation were studied following S.V. Ovczinnikova (2007). We studied nutlet morphology of 22 species belonging to genera *Lappula* found in Mongolia. Total of 22 species belong to 5 section of *Lappula* Moench. genera distributed In Mongolia.

## CONCLUSIONS

1. This study indicated that nutlet morphology is useful to reveal relationship at species level.
2. In the future, it is necessary to conduct molecular biological research and ecological, geographical and phylogenetic analysis.

## ACKNOWLEDGEMENTS

The authors thank those botanists that provided plant material, particularly S. Ovchinnikova, Nikiforova O. D. (Central Siberian Botanical Garden), Alisa Grabovskaya-Borodina, L.M. Raenko (BIN RAN), Enkhmaa Ulziikhutag (UBU).

## REFERENCES

- Grubov V. I. 1982. *Opredelitel sosudistykh rasteniy Mongolii* [Key for plants of Mongolia (with an atlas)]. Ed. by E. M. Lavrenko. Nauka, St. Petersburg, 443 pp.
- Овчинникова С.В. (2007) *Труба Eritrichieae (Boraginaceae) во флоре восточной Евразии: систематика, карпология, эволюция*. Диссертация доктора биологических наук. Новосибирск. 645.
- Ovchinnikova S.V. 2005. *The system of the subtribe Echinosperrinae (Boraginaceae)*. Bot. Zhurn. 90: 1153–1172.
- Popov M.G. (1953) *Flora SSSR*. 19. 1.62.
- Urgamal M., et al. (2014) *Conspectus of the vascular plants*. Ulaanbaatar.
- Zhu Ge-ling, Harald Riedl, Rudolf Kamelin. (1995) *Flora China*. 329-427.

Oral Presentation

Thursday

Diversity of Plant Species, Systematics and Phylogeny-3

### Horticulture Genetic Resources in Yozgat

Aysen Koç<sup>1\*</sup>, Gülden Balcı<sup>1</sup>, Emine Sema Çetin<sup>1</sup>, Hakan Keleş<sup>1</sup>, Tuğba Kılıç<sup>1</sup>, Selda Daler<sup>1</sup>

<sup>1</sup>University of Yozgat Bozok, Faculty of Agriculture, Department of Horticulture, Yozgat, Turkey.

\*Corresponding author e-mail: aysen.koc@bozok.edu.tr

#### Abstract

The diversity in genetic resources forms the basis of plant breeding studies. Turkey has a large amount of variations in diversity genetics resources due to the fact that being in a position which enables growing area and being a native land, and has lots of fruits, vegetables, vineyard and ornamentals by reason of geographical and ecological region. Yozgat is located in Kızılırmak Region in Central Anatolia Region on Bozok Plateau. Semi-arid climate dominates in Yozgat however, in Çekerek Valley that incoming in Yeşilirmak basin, has mild climate and effects of Blacksea Region has been seen in it. As a result of our work carried out in the 14 provinces of Yozgat in horticultural products that fruits, vegetables, vineyards and ornamental plants grown naturally as local varieties were identified. The coordinates of these genotypes are determined by GPS device. These genotypes were replicated and started to be preserved by taking samples of fruit, seed, cutting, graft, and corms. Hawthorn, eleagnus and rosehip grown as wild; quinces (Karanlıkdere), pear (Göğsulu, Sarı, Seydiyar, Küp, Yazlık, Kurtdeşen, Kırmızı Aşılama, Orak, Çöpuzunu, Balbardak), apples (Ekşi, Sülümen, Köhne, Danabaşı, Kamyon, Mayhoş, Çandır, Büyük, Cıvıştaklı), plums (Sarı, Bardak, Camız, Sivri, Sarı sivri, Üzüm), vineyards (Siyah, Beyaz, Bulut, Gül), walnuts (Kale, Hisarbey, Akçakışla) species grown as local varieties were selected. Vegetables include red slice tomatoes, pumpkin, Araplı bean, shrub dry bean and donkey dry bean, Bağribütün melon, Topatan melon, summer and winter melon, watermelon seeds were collected. As ornamental plants, wild cyclamen in Aydıncık, wild tulip in Yerköy, peony species known as Cehrilik's tulip in Gelinkayası local, 23 numbers genotypes belong to different orchids species in Akdağmadeni were determined.

**Keywords:** Yozgat, fruit, vegetable, grape, ornamental

## INTRODUCTION

Turkey, which has an important place in the production of horticultural crops in the world, is also the gene center of many fruit species and has rich fruit gene resources (Özbek, 1978). Turkey, due to its geographical structure and different ecological conditions, is in a position where the world's most important gene or origin center overlaps. The fact that 3.708 (34.8%) of the 10.754 taxa in its flora are endemic, further increasing its importance (Şehirli et al., 2005; Karagöz et al., 2010). Breeders are constantly looking for new sources of hereditary material, since modern varieties with high yields but narrow genetic bases lack genes for resistance to environmental pressures (diseases, pests, cold and drought, etc.). In this respect, their quantitative character in long-term programs; their qualitative characters (resistance to diseases, etc.) in short or medium-term programs, in transferring plant genetic resources are used directly or as bridge species (Şehirli and Özgen, 2012).

As a result of natural selection, local varieties or types with some good characteristics have survived to the present day. However, these local varieties are replaced by new varieties developed. Thus, even varieties obtained through natural selection are uprooted and destroyed. However, gene resources are lost due to the opening of new lands to agriculture, the formation of industrial zones and especially the opening of our beaches to the tourism and construction sector. As a result of the inventory studies carried out in 1977-1986 regarding the genetic resources of fruit-vineyards, it was understood that the local fruit-vineyard varieties and types preserved in various institutions lost 19.26% (Tan 1991). However, considering that these resources may be the main material of future breeding studies, the importance of collection and preservation is better understood. For this reason, determining different types of the species in the gene resources available in our country, keeping them under preservation and identification in the gene banks will provide significant convenience for breeding studies.

With this project, the fruit, vegetables, vineyards and ornamentals found in the flora of Yozgat province, which enters the Erciyes basin with Akdağmadeni and Çayıralan districts, the Yeşilirmak basin with Aydıncık, Çekerek and Kadışehir districts, and the Orta Kızılırmak basin with its other 9 districts, has different product groups and endemic plant species. According to Davis (1965-1985), Akdağmadeni, Çulhali Region, Sofular Stream, Büyüknalbant Mountain and Karanlıkdere valley are the regions where genetic diversity is high in Yozgat. Different wild genotypes and local cultivars of plant species were determined, they were reproduced with the propagation method suitable for the species and started to be kept in field gene banks.



## MATERIALS AND METHODS

We have read “The Ethics Statement”.

The plantations in Yozgat province and its districts were visited and wild / local genotypes of fruit, vegetable, vineyard and ornamental plants were evaluated. Genotypes representing the region and having different characteristics from these plants formed the material of the study.

The following methods were used in this study conducted in 2015-2018:

Survey; The program was organized according to the knowledge, publications and the results of the meetings with the Provincial/District Directorate of Agriculture and Forestry.

Selection; The collection of genetic resources from genotypes determined as a result of surveys was made using standard collection forms prepared as indicated in Table 1 (Tan and Tan, 1998, Bilgener et al., 2010).

**Table 1.** Genetic resources collection form

|   |   |  |
|---|---|--|
| Collector number:   | Collection number:  | Date:  |
| Habitat and collection source<br><input type="checkbox"/> Wild <input type="checkbox"/> Farm land<br><input type="checkbox"/> Home garden <input type="checkbox"/> Forest | Botanical name:<br><br>Local name:  | Collection address:<br><br>District:<br><br>Village /location:   |
| Coordinates<br>Latitude:<br>Longitude:<br>Height:<br>Direction:   | Type of material collected<br><input type="checkbox"/> Scion<br><input type="checkbox"/> Bud eye<br><input type="checkbox"/> Sucker shoot<br><input type="checkbox"/> Root cutting<br><input type="checkbox"/> Seed<br><input type="checkbox"/> Onion | Condition of collected material<br><input type="checkbox"/> Wild<br><input type="checkbox"/> Passage or culture form |
| Topography information: (soil, condition of the land, etc.)   | Other common species:   | The size of the population in the region:  |
| Descriptive notes:  |   |  |

In the study carried out to determine the genotypes that stand out with their fruit characteristics, fruit samples were taken from those suitable for the purpose and each plant was considered as a

"Genotype" during the sampling. In the study, the characteristics of being productive, large-fruited and free from disease pests were taken into account in determining the genotypes and were taken as a basis in the preliminary selection. The selected genotypes were named with the initials of the district they were taken from (66Ş01 Şefaati district, 66Y01 Yerköy district).

The selected genotypes were compared by applying the modified weighted grading method. Pomological analyzes were made on the fruits of the selected genotypes. Fruit firmness ( $\text{kg.cm}^{-2}$ ), titratable acidity (in terms of malic acid), Soluble solid content (SSC, %), fruit width and length (cm), fruit weight (g) values in 30 randomly selected fruits from harvested fruits determined. Flesh firmness was measured using a hand penetrometer 8 mm tip (model: GY-1) from two areas of the fruit, along the equatorial circumference, from which the peel was removed. The obtained values are given as  $\text{kg.cm}^{-2}$ . The amount of soluble solid of the fruits was determined by a digital refractometer, and the titratable acidity (%) value was determined by the titration method.

In the study, statistical analyzes were performed using the Duncan multiple comparison test in the SPSS package program.

## RESULTS

### A. Fruit Growing

#### 1. Karanlıkdere Quince (*Cydonia oblonga* Mill.)

In this study, fruit samples were taken from 5 genotypes in Yerköy and 11 genotypes in Şefaati, totally 16 quince genotypes were sampled. In the 1st year of this experiment, 8 promising genotypes were defined based on modified weighted grading evaluation method. In the 2nd year of the selection, the morphological characteristics of the genotypes, selected in the previous year, were examined (Koç and Keles, 2018).



Figure 1. Fruits of quince genotypes (66Ş03 and 66Y04)

## 2. Rosehip Selection (*Rosa* spp.)

Total 54 genotypes selected from Yozgat province were found to be promising as a result of modified weighted grading, they were planted to be grown under the same conditions in Gedikhasanlı Agricultural Application and Research Center (Uçaral and Koç, 2016a, 2016 b, Koc et al., 2018, Koc, 2020).

Studies continue with 6 genotypes that stand out in terms of yield, fruit weight and fruit flesh ratio (Table 2). In addition, studies on thornless rosehip in these genotypes are carried out.

Table 2. Some characteristics of prominent rosehip genotypes

| Item Number | Genotype Name | Yield | Fruit Weight (g) | Fruit Flesh Ratio (%) |
|-------------|---------------|-------|------------------|-----------------------|
| 1           | 66S17         | High  | 3.04 ± 0.29      | 71.51                 |
| 2           | 66Ç03         | High  | 2.90 ± 0.16      | 94.30                 |
| 3           | 66Ş03         | High  | 2.94 ± 0.14      | 64.00                 |
| 4           | 66Y06         | High  | 2.84 ± 0.26      | 69.81                 |
| 5           | 66M30         | High  | 2.70 ± 0.23      | 67.41                 |
| 6           | 66M32         | High  | 2.21 ± 0.28      | 74.53                 |



**Figure 2.** Rosehip genotypes selected and planted in Gedikhasanlı Agricultural Application and Research Center

### 3. Selection of Hawthorn (*Crataegus spp.*)

Genotypes identified in a PhD project were combined with genotypes determined during our project. Some of the morphological and pomological features of the genotypes were examined by considering the UPOV criteria (Keles, 2018). Total 25 genotypes selected from Yozgat province were found to be promising as a result of modified weighted grading, replication studies are in progress (Figure 3).



**Figure 3.** A hawthorn tree selected from Yozgat

#### 4. Walnut Selection (*Juglans regia* L.)

Walnut selection was made in the villages of Musabeyli Strait, Kale, Hisarbey and Akçakışla, where walnut cultivation is intensively carried out in Yozgat. In our selection study, the prominent genotypes were determined by weighted grading according to late leafing, side branch yield, bark fruit weight, yield, ease of internal emergence, internal color, resistance to anthracnose and internal borer (Koc et al., 2019). The selected 16 genotypes will be multiplied and taken to the conservation plot.



**Figure 4.** A walnut tree selected from Yozgat

#### 5. Elaeagnus Selection

Elaeagnus is common in Yozgat provinces and districts. Among the fruit bearing genotypes were selected 3 genotypes in Aydıncık district, 2 genotypes in Şefaati district, 1 genotype in Yerköy district and 5 genotypes in Merkez district.



**Figure 5.** A buckthorn tree selected from Yozgat

**Table 3.** Some fruit characteristics of selected buckthorn genotypes

| Genotype Name | Fruit Weight (g) | Fruit Width (mm) | Fruit Size (mm) | Flesh/Seed Ratio (%) | Flour Yield (%) |
|---------------|------------------|------------------|-----------------|----------------------|-----------------|
| 66A01         | 0.93±0.23        | 13.09±0.92       | 18.58±1.12      | 1.47±0.23            | 59.31±3.38      |
| 66A02         | 1.05±0.13        | 12.88±0.80       | 19.34±1.02      | 2.02±0.25            | 66.68±2.68      |
| 66A03         | 1.06±0.14        | 14.51±0.92       | 21.81±0.71      | 1.59±0.28            | 61.02±4.16      |
| 66M01         | 1.19±0.17        | 11.61±0.64       | 16.50±1.20      | 1.86±0.17            | 64.98±1.98      |
| 66M02         | 0.70±0.13        | 10.25±0.62       | 11.61±0.78      | 2.22±0.46            | 68.46±4.10      |
| 66M03         | 0.37±0.07        | 9.04±0.96        | 11.77±0.56      | 1.04±0.12            | 50.87±2.87      |
| 66M04         | 3.93±0.66        | 18.06±0.67       | 27.02±1.26      | 6.90±0.67            | 87.28±1.03      |
| 66M05         | 3.55±0.80        | 15.57±1.25       | 26.62±2.36      | 6.42±0.97            | 86.33±1.66      |
| 66Ş01         | 1.98±0.24        | 15.23±1.05       | 20.69±1.24      | 3.52±0.51            | 77.66±2.58      |
| 66Ş02         | 2.27±0.41        | 16.39±1.04       | 22.84±1.49      | 3.25±0.38            | 76.34±2.02      |
| 66Y01         | 2.44±0.93        | 15.38±1.47       | 23.68±2.24      | 4.15±1.31            | 79.43±5.52      |

## 6. Terebinth Selection (*Pistacia terebinthus*)

Terebinth population was found in Aydıncık district, and Karanlıkdere, which is located between Yerköy and Şefaattli districts, and a total of 5 genotypes were selected.



**Figure 6.** Terebinth genotypes selected from Yozgat

### 7. Black Mulberry (*Morus nigra*)

Very old black mulberry trees were identified and selected in Büyüknefes, Topaç, Musabeyli and Kurtağılı villages. After applying 6000 ppm IBA in the mist-propagation system, cuttings were planted in peat-filled pots and rooted. Fruit weight, fruit color, Soluble solid content (SSC, %), and acidity values were determined in the fruits taken from the main trees for characterization purposes. Photos of fruits (Figure 4.6) and measurements and analyzes (Table 4) are shown below.



**Figure 7.** Fruit samples of the selected black mulberry genotypes (A: Kurtağılı, B: Musabeyli, C: Topaç, D: Büyüknefes) and tree

In terms of fruit weight, Büyüknefes, Topaç and Musabeyli genotypes were statistically different from the Kurtağılı genotype. In terms of fruit acidity, Kurtağılı was found to be different from other genotypes. SSC was measured as the highest in Musabeyli genotype (Table 4). In the measurements made in fruit juice, the L value varied between 16.08 and 16.85, a value between 1.33 and 3.44, and the b value between 0.43 and 1.34.

**Table 4.** Measurements and analyzes of some characteristics of the selected black mulberry genotypes

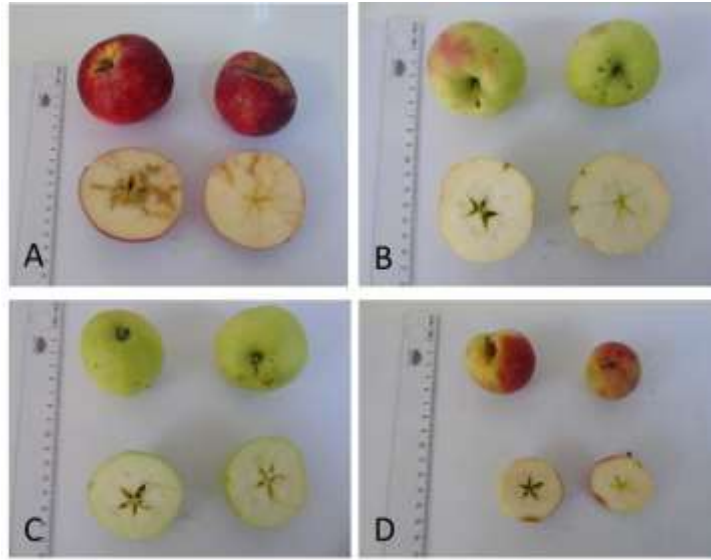
| Özellikler              | Kurtağılı    | Topaç         | Musabeyli    | Büyüknefes    |
|-------------------------|--------------|---------------|--------------|---------------|
| Fruit Weight (g)        | 3.76±0.05 b* | 5.02±0.79 a   | 4.19±0.49 ab | 5.12±0.67 a   |
| Fruits L                | 17.65±0.59 a | 16.61±0.49 ab | 15.54±0.33 b | 16.29±1.84 ab |
| Fruits a                | 9.3±0.11 a   | 4.97±1.18 bc  | 3.42±1.18 c  | 5.91±1.63 b   |
| Fruits b                | 2.21±0.14 a  | 1.3±0.28 bc   | 0.76±0.33 c  | 1.51±0.46 b   |
| Juice L                 | 16.85        | 16.08         | 16.66        | 16.84         |
| Juice a                 | 3.29         | 3.68          | 1.33         | 3.44          |
| Juice b                 | 1.18         | 1.34          | 0.43         | 0.96          |
| SSC (%)                 | 12.4±0.1 c   | 11.1±0.0 d    | 13.9±0.0 a   | 13.7±0.1 b    |
| Titrateable acidity (%) | 1.4±0.13 a   | 0.82±0.08 b   | 0.71±0.07 b  | 0.72±0.22 b   |

\* There is no statistical difference between the means indicated with the same letter (P < 0.05).

## 8. Local Variety Selection

**8.1. Local Apple Selection:** Sour apple, sülümen apple, köhne apple, danabaş apple, kamyon apple, mayhoş apple, big apple, Civistakli apple and Çandır apple grown in Yozgat were taken and grafted (Figure 8, Table 5).





**Figure 8.** Fruit samples of local apple varieties (A: Köhne apple, B: Mahuş apple, C: Cıvıştaklı apple, D: Çandır apple)

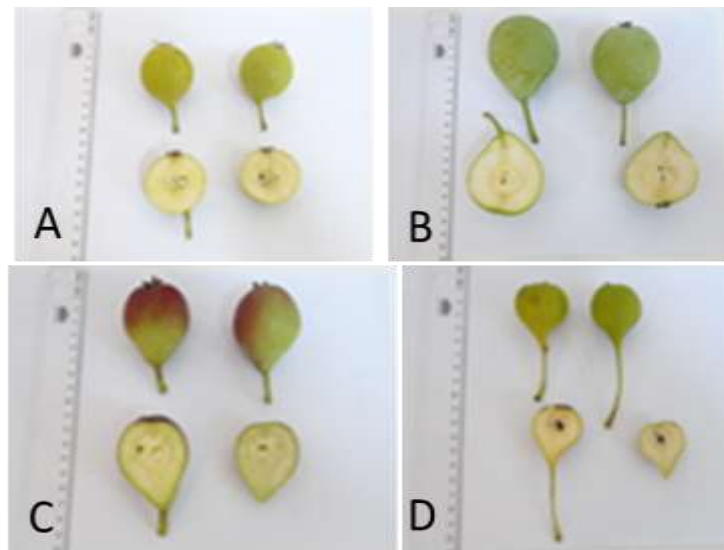
**Table 5.** Some pomological features of local apple cultivars

| Local apple cultivars | L        | a        | b       | Fruit firmness (kg.cm <sup>-2</sup> ) | Fruit Weight (g) | SSC (%) | Titrateable acidity (%) |
|-----------------------|----------|----------|---------|---------------------------------------|------------------|---------|-------------------------|
| Köhne apple           | 37.50 b* | 39.37 a  | 12.96 b | 4.10 c                                | 122.81 a         | 13.63 a | 1.63 a                  |
| Mahuş apple           | 74.25 a  | -7.82 b  | 30.26 a | 7.17 b                                | 85.26 b          | 9.83 c  | 0.29 b                  |
| Cıvıştaklı apple      | 69.86 a  | -16.68 b | 30.70 a | 10.63 a                               | 65.66 b          | 9.33 d  | 0.30 b                  |
| Çandır apple          | 45.84 b  | 31.08 a  | 17.55 b | 3.97 c                                | 27.13 c          | 10.23 b | 0.17 c                  |

\* There is no statistical difference between the means indicated with the same letter ( $P < 0.05$ ).

## 8.2. Local Pear Selection

Gögsulu pear (Taş pear), Yellow pear, Seydiyar pear, Küp pear, Yazlık pear, Seydiyar pear, Kurtdeşen pear, Orak pear, Çöpuzunu pear and Balbardak pear were taken and grafted (Figure 9, Table 6).



**Figure 9.** Fruit samples of local pear varieties (A: Orak pear, B: Suluseydiyar pear, C: Kırmızı aşılama pear, D: Çöp uzunlu pear)

**Table 6.** Some pomological features of local pear cultivars

| Local apple cultivars | L        | a         | b       | Fruit firmness (kg.cm <sup>-2</sup> ) | Fruit Weight (g) | SSC (%) | Titrateable acidity (%) |
|-----------------------|----------|-----------|---------|---------------------------------------|------------------|---------|-------------------------|
| Orak pear             | 57.23 a* | -12.56 bc | 28.49 a | 6.10 c                                | 25.84 c          | 12.47 a | 0.30 b                  |
| Suluseydiyar          | 49.13 b  | -14.38 c  | 22.80 c | 10.70 b                               | 88.48 a          | 8.83 c  | 0.40 a                  |
| Kırmızı Aşılama       | 29.94 c  | 17.35 a   | 7.72 d  | 14.00 a                               | 50.36 b          | 12.50 a | 0.21 c                  |
| Çöpuzunu pear         | 50.64 b  | -12.06 b  | 24.82 b | 2.43 d                                | 21.29 c          | 9.17 b  | 0.24 c                  |

\* There is no statistical difference between the means indicated with the same letter ( $P < 0.05$ ).

### 8.3. Local Plum Selection:

Yellow plum, Bardak plum, Sivri plum, Sarı sivri and Üzüm plum were taken and grafted (Figure 10, Table 7).



**Figure 10.** A tree and fruit samples of local plum varieties (A: Sivri plum, B: Üzüm plum)

**Table 6.** Some pomological features of local plum cultivars

| Local plum cultivars | L         | a        | b        | Fruit firmness (kg.cm <sup>-2</sup> ) | Fruit Weight (g) | SSC (%)   | Titratable acidity (%) |
|----------------------|-----------|----------|----------|---------------------------------------|------------------|-----------|------------------------|
| Sivri plum           | 27.28±3.2 | 9.54±2.6 | 6.61±0.8 | 3.20±0.2                              | 9.88±2.2         | 9.63±0.0  | 1.40±0.0               |
| Üzüm plum            | 27.22±8.7 | 6.40±4.9 | 7.35±6.1 | 5.3±0.9                               | 10.34±2.0        | 14.63±0.5 | 2.02±0.0               |

## B. Viticulture

With the aim of revealing the viticulture potential of Yozgat province has been made the meeting (Çetin and Daler, 2018). Local varieties in Yozgat are grown, such as Parmak, Gök, Karanlıkdere beyazı, Bulut, Tilki, Eldaş, Kabaeldaş, Gelinparmağı, Keçimemesi, Çandır, Zilifder, Çiğitli, Gül, Kaburgalı, Devetüyü, Çıtır, Patpat grapes etc. Genotypes identified in a PhD project was conducted between 2017 and 2020 in order to identification by classical and molecular methods of 50 grape varieties being grown in Yozgat province. Ampelographic definitions were performed using 128 criteria according to "Descriptors for Grapevine (*Vitis* spp.)" norms that have jointly published by BI (Bioversity International), OIV (International Organisation of Vine and Wine) and UPOV (The International Union for the Protection of New Varieties of Plants), and valid worldwide to ensure international method unity (Daler, 2021).



**Figure 11.** Parmak and Gül grapes

### C. Vegetable Cultivation

Vegetable seeds were also collected from producers using their own seeds in survey and selection studies conducted in Yozgat province and its districts (Figure 12). These seeds were replanted in the 2nd year and started to be reproduced.



**Figure 12.** Seeds of some vegetable species collected from Yozgat villages

As vegetables, red sliced tomato, white acur, cucumber, long langa cucumber, pepper, gin pepper, zucchini, pumpkin, okra, Kidney beans from Arapli, Bush dried beans and donkey dried beans, Bağribütün melon, Topatan melon, summer and winter melon, watermelon seeds were collected. With a follow-up project, Bağribütün melon received a geographical indication for Aydıncık/Yozgat.

### D. Ornamental Plants Cultivation

In the survey and selection study, 23 salep orchid genotypes were determined in Akdağmadeni district and these types were found in *Ochis anatolica*, *Orchis morio*, *Orchis mascula ssp. pinetorum*, *Dactylorhiza romana*, *Neotinea maculata*, *Orchis pallens*, *Platanthera chlorontha* and *Orchis purpurea* species. In addition, salep orchids were found on the Erdoğan Akdağ campus of Bozok University, and samples were taken from 4 genotypes of *Limodorum abortivum* (Kılıç et al., 2017).

In the study conducted in Aydıncık district, wild cyclamen (*Cyclamen coum*) was found under trees, on slopes, in damp and shaded places. Wild cyclamen are among our plants that we are obliged to protect in their natural habitats in accordance with the Bern Convention, to which Turkey is also a party. Wild tulips were detected and samples were taken on the Kahya village road in Yerköy. Reproduction studies were carried out by taking the peony type known as Cehrilik tulip together with its rhizomes in Gelinkayası locality in Yozgat Merkez district (Figure 13).



**Figure 13.** Wild cyclamen, wild tulips and the peony type is known as Cehrilik tulip

## DISCUSSION

Understanding the importance of plant biodiversity, European and Asian countries have started to protect these resources with ex-silo conservation method in order to prevent the destruction of these resources. For this purpose, Nikita Botanical Garden, which was established in Soviet Russia in 1812, today belongs to the Ukrainian Academy of Agricultural Sciences. In the Botanical Garden; 1,103 almonds, 790 apples, 783 apricots, 541 cherries, 400 Feijoa, 334 figs, 55 hazelnuts, 10 lemons, 230 olives, 1,284 peaches and nectarines, 351 arnuts, 493 plums, 370 pomegranates, 190 persimmons, 219 quince and 175 walnuts are preserved (Zaurov et al., 2005). Apple, fig, grape, pomegranate fruits were taken from old mixed gardens and valleys in the study conducted on different fruit types to determine fruit characteristics in Italy and Israel and hopeful varieties were selected and improvement studies have been carried out on it (Anikster et al., 1997). Moriguchi et al. (1994) collected 7848 collections of different fruit species in their study in Japan. In the study of the collection of wild apples in Central Asia (Kazakhstan and Kyrgyzstan) by the American Product Recommendation Committee, 7,000 apple types were examined and 54 of them were included in the cultivar development program (Noiton-d, 1994). The first studies on the collection of fruit genetic resources in our country date back to 1930-1940. In these years, first research stations were established and they started to collect fruit genetic resources. Özçağırın (1976) determined the use of cherry and mahaleb rootstocks in our country. Gülcan and Özçağırın (1982) determined different types with growth and development characteristics as a result of the selection studies they carried out in and around the Aegean Region in order to benefit from the mahaleb population in our country. Akbulut (1994) found that 10 types of mahaleb trees in the natural flora of Tokat-Erbaa were hopeful in his preliminary selection study for the determination of those with superior fruit and vegetative characteristics. Küden and Kaşka (1995) conducted studies to determine the cherry varieties and types available in the Central Taurus Mountains, and

they found some types important in terms of fruit size, early fruiting and spur characteristics. Koc et al. (2013) selected 110 cherries, 29 sour cherries, 40 mahaleb and 6 stone cherry (*Cerasus angustifolia* (Spach) Browicz) clones from the Central and Eastern Black Sea Region. They made the morphological and molecular characterization of these types. Koc and Bilgener (2013) Cherry selected from Samsun investigated the morphological characterization and vegetative propagation potential of cherry, sour cherry and mahaleb. In our country, studies have been carried out to determine genotypes with superior characteristics with many Rosehip selections. For this purpose, Erciřli (1996) in Gümüşhane; Güneř (1997) in Tokat; Yazgan (1997) in the Havza district of Samsun; Türkođlu and Muradođlu (2003) in the Van Lake Basin; Kızılcı (2005) in Erzincan; Çelik (2007) in the Van Lake basin; řavir (2008) in Erzincan Munzur Mountain; Dölek (2008) in Amasya; Sađır (2010) in the Akincilar district of Sivas; Özen (2013) in Bolu, Uçaral and Koç (2016a, 2016b) in Yozgat and its districts conducted selection studies. The properties of the selected genotypes such as their thornless, average fruit weights, fruit flesh ratios, soluble solid content (SSC), vitamin C, total dry matter, titratable acidity and pH were determined. Vurgun et al. (2013), in the study they started in 1994, surveyed in the provinces of Van, Erzincan, Erzurum, İđdır, Kars, Ađrı and Gümüşhane determined 32 apples, 36 pears, 16 plums, 6 cherries, 3 quinces, 3 apricots and 14 cherries, and 30 apples, 72 pears, 7 cherries, 6 plums, 1 mulberry type and local varieties from Posof district of Ardahan province. They made grafting in Erzincan Horticultural Research Institute and established a collection garden on the land of the institute. Bostan (1993), Bolat and Güteryüz (1994), Sen et al. (1995) and Guleryüz (1995) Zerdali selection; Kalkışım (1993) and Pırlak (1993) Cranberry selection; Kalyoncu (1990), Balta (2002), řimřek and Osmanođlu (2010a), řimřek et al. (2010), Gülsoy (2012) and Köse (2013) almond selection conducted. Bayazit (2000), Akça and řen (2001), Balci et al. (2001), Aykut (2001), Özkan (2002), Ünver and Çelik (2005), Akça and Körođlu (2005), Beyhan (2005), Arda (2006), Yılmaz (2007), řimřek and Osmanođlu (2010b), Karadađ and Akca (2011) examined the characteristics such as fruit weight, kernel weight, kernel ratio, shell thickness, oil ratio, protein ratio, ash ratio in the genotypes they selected in their selection study. Kellerhals et al. (2004) state that the conservation and protection of apple genetic resources is absolutely essential for apple breeding programs. Researchers have explained with examples the importance of maintaining genetic diversity in terms of breeding programs, resistance breeding, and improving yield and quality. Koc et al. (2009) carried out morphological and molecular characterization studies on types with apple rootstock potential.

## CONCLUSIONS

As a result; studies are continuing on all genotypes determined by this study in Yozgat province. This study, which is the beginning of breeding studies, will contribute to revealing the qualified types in the naturally grown fruit, vegetable, ornamental and vineyard population in our country as a gene source and will contribute to filling the gaps in the practice and literature with the obtained outputs. The results of the research reveal that the species that grow naturally in the region show variation in terms of some physical and chemical properties. Genotypes, which were seen as promising in our research, were kept as gene source material for breeding studies.

## ACKNOWLEDGEMENTS

This study was financially supported by Yozgat Bozok University Scientific Research Projects Division (Project number: 2015ZF/A207). For all my colleagues and our community, I would like to express my condolences to our friend Dr. Cüneyt CİVELEK who is also one of the researchers in the project and passed away in April 2020.

## REFERENCES

- Akbulut M (1994). Selection Breeding of *P. mahaleb* L. in Erbaa. Thesis. Ondokuz Mayıs University, Faculty of Agriculture, Samsun, 87 p.
- Akça Y & Şen SM (2001). Study on the selection of superior walnut trees in Hizan (Bitlis) populations. Acta Hort. (ISHS). 544:115-118.
- Akça Y & Köroğlu E (2005). Çorum ili İskilip ceviz popülasyonu içerisinde üstün özellikli ceviz tiplerinin seleksiyon yolu ile ıslahı. II. Ulusal Ceviz Sempozyumu Özetler, 13-16 Eylül 2005, Bursa. s:10.
- Anikster Y, Feldman M & Horovitz A (1997) The Ammiad experiment. In: Maxted N, Ford-Lloyd, BV, Hawkes, JG (eds), Plant Genetic Conservation: The in-Situ Approach. Chapman and Hall, London, pp. 239-253.
- Arda E (2006). İç Ege Bölgesi'ndeki Ceviz (*Juglans regia* L.) Popülasyonunun Seleksiyon Yolu ile Islahı Üzerinde Araştırmalar. Doktora Tezi, Ege Üniversitesi Fen Bilimleri Enstitüsü, İzmir.
- Aykut N (2001). Van Merkez ve İlçe Cevizlerinin Seleksiyonu. Yüksek Lisans Tezi, Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, 78 s.
- Balci I, Balta F, Kazankaya A & Sen SM 2001. Promising native walnut genotypes (*Juglans regia* L.) of the East Black Sea region of Turkey. Journal American Pomological Society. 55(4):204-208.
- Balta MF (2002). Elazığ Merkez Ve Ağın İlçesi Bademlerinin (*Prunus amygdalus* L.) Seleksiyon Yoluyla Islahı Üzerinde Araştırmalar. Doktora tezi, Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, 262 s. Bayazıt (2000)
- Beyhan Ö (2005). Darendede Cevizlerinin (*Juglans regia* L.) Seleksiyon Yoluyla Islahı Üzerinde Araştırmalar. SAÜ Fen Bilimleri Enstitüsü Dergisi. 9. Cilt, 1. Sayı, 35-42.
- Bilgener Ş, Ercişli S, Gerçekçioğlu R, Eşitken A, Güneş M, Akbulut M, Koç A & Çelik Z, 2010. Orta ve Doğu Karadeniz Bölgesi Kiraz-Vişne Anaç Islahı. TÜBİTAK TOVAG 106 O 031 Sonuç Raporu, 1-113.
- Bolat İ & Güleriyüz M (1994). Erzincan Koşullarında Yetiştirilen Hasanbey Kayısı Çeşidinin Döllenme Biyolojisi Üzerinde Bir Araştırma. Atatürk. Üni. Zir. Fak. Der. 25(4):509- 519.
- Bostan Z (1993). Darendede Zerdalilerinin (*Prunus armeniaca* L.) Seleksiyon Yoluyla Islahı Üzerine Araştırmalar. Doktora Tezi, Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, 182 s.
- Çelik, F (2007). Van Gölü havzası kuşburnu (*Rosa* spp.) genetik kaynaklarının seleksiyonu ve mevcut biyolojik çeşitliliğin tespiti. Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 210 s.
- Çetin ES & Daler S (2018). Yozgat İli Bağcılığının Değerlendirilmesi. Bahçe, 47(Özel Sayı 1), 209 - 218.
- Daler S (2021). Identification by classical and molecular methods of grape varieties grown in Yozgat province. Gaziosmanpaşa University, Institute of Graduate Studies, Department of Horticulture. Supervisor: Prof. Dr.

- Rüstem CANGI, 506 pp.
- Davis PH (ed.) (1965-1985). Flora of Turkey and the East Aegean Islands, Vols. 1-9. Edinburgh, Edinburgh University Press.
- Dölek Ü (2008). Amasya Yöresinde Doğal Olarak Yetişen Kuşburnuların (*Rosa Ssp.*) Seleksiyon Yoluyla Islahı. Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 97 s.
- Ercişli S (1996). Gümüşhane ve İlçelerinde Doğal Olarak Yetişen Kuşburnuların (*Rosa spp.*) Seleksiyon Yoluyla Islahı ve Çelikle Çoğaltma İmkânları Üzerinde Bir Araştırma (Doktora Tezi). Atatürk Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Erzurum.
- Guleryuz M (1995). Selection of the Quality-Fruited Wild Apricot (*Prunus armeniaca L.*) forms Resistant to Late Spring Frosts on Erzincan Plain. Acta Hort. (Ishs) 384:189-194.
- Gülcan R & Özçağırın R (1982). Kiraz İçin İdris Anacı Seleksiyonu. TÜBİTAK TOAG Proje No. 330., 1982: 50 s.
- Gülsoy E (2012). Aydın'ın Yenipazar. Bozdoğan Ve Karacasu İlçelerinde Doğal Olarak Yetişen Bademlerin (*P. amygdalus L.*) Seleksiyonu. Doktora Tezi. Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, 261 s.
- Güneş S (1997). Ümitvar Bir Kuşburnu (*Rosa canina*) Genotipinin Farklı İki Lokasyondaki Fenolojik, Morfolojik ve Pomolojik Özellikleri (Yüksek Lisans Tezi). Gaziosmanpaşa Üniversitesi Fen Bilimleri Enstitüsü Bahçe Bitkileri Anabilim Dalı, Tokat.
- Kalkışım Ö (1993). Samsun'un Vezirköprü İlçesinde Kızılcık'ın (*Cornus Mas L.*) Seleksiyon Yoluyla Islahı Üzerinde Bir Araştırma. Yüksek lisans tezi, Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü, 127 s.
- Kalyoncu İH (1990). Konya Apa Baraj Gölü Çevresinde Yetiştirilen Üstün Özellikli Badem (*Prunus amygdalus L.*) Tiplerinin Belirlenmesi Üzerine Bir Seleksiyon Çalışması. Yüksek lisans tezi, Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü, 70s.
- Karadağ H & Akça Y (2011). Phenological and pomological properties of promising walnut (*Juglans regia L.*) genotypes from selected native population in Amasya province. African Journal of Biotechnology Vol. 10(74). pp. 16763-16768.
- Karagöz A, Zencirci N, Tan A, Taşkın T, Köksel H, Sürek M, Toker C & Özbek K (2010). Bitki Genetik Kaynaklarının Korunması Ve Kullanımı. Türkiye Ziraat Mühendisliği VII. Teknik Kongresi, 11-15 Ocak 2010, Ankara. 155-177.
- Keles H (2018). Selection, Biochemical and Molecular Characterization of Hawthorn (*Crataegus Spp.*) Genetic Resources from Yozgat Province and Districts. Atatürk University, Graduate School of Natural and Applied Sciences, Department of Horticulture. Supervisor: Prof. Dr. Sezai ERCİŞLİ, 174 pp.
- Kellerhals M, Lucas B & Gessler C (2004). Use of Genetic Resources in Apple Breeding and for Sustainable Fruit Production. Journal of Fruit and Ornamental Plant Research vol. 12.
- Kılıç T, Koç A, Balcı G & Koç M (2017). Sustainability of Genetic Resources: Yozgat-Akdağmadeni Salep Orchids. International Final Conference. Interactive Conservation Platform for Orchids Native to Greece and Turkey (ICON). 18-21 April 2017, Antalya/Turkey.
- Kızılcı G (2005). Bazı ümitvar kuşburnu (*Rosa spp.*) tiplerinin Erzincan ekolojik koşullarına adaptasyonu (seleksiyon II). Gaziosmanpaşa Üniversitesi. Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 51 s.
- Koc A, Akbulut M, Orhan E, Çelik Z, Bilgener S & Ercişli S (2009). Identification of Turkish and Standard Apple Rootstocks by Morphological and Molecular Markers. Genet. Mol. Res. 8 (2): 420-425.
- Koc A & Bilgener S (2013). Morphological characterization of cherry rootstock candidates selected from Samsun Province in Turkey. Turk. J. Agric. For., 37 (5): 575-584
- Koc A, Çelik Z, Akbulut M, Bilgener S, Ercişli S, Gunes M, Gerçekcioglu R & Esitken A (2013). Morphological Characterization of Cherry Rootstock Candidates Selected from Central and East Black Sea Regions in Turkey. Hindawi Publishing Corporation the Scientific World Journal, Volume 2013, Article ID 916520, 9 pages, <http://dx.doi.org/10.1155/2013/916520>.
- Koç A & Keles H (2018). Yozgat ili Karanlıkdere Vadisinde Ayva Ön Seleksiyonu. Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi (JAFAG, Journal of Agricultural Faculty of Gaziosmanpaşa University). 35 (Ek Sayı), 23-29.
- Koç A, Balcı G, Keles H & Aras S (2018). Yozgat'tan Selekte Edilen Kuşburnu Genotiplerinin Bazı Fiziksel Özellikleri. III. Uluslararası Bozok Sempozyumu, 3-5 Mayıs 2018, sf. 151-160, Yozgat.
- Koc A, Keles H & Ercişli S (2019). Some Pomological Properties of Promising Seed Propagated Walnut Genotypes from Inner Turkey. Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 47(4), 1094-1099. DOI:10.15835/nbha47411600.
- Koç A (2020). Chemical Changes in Seeds and Fruits of Natural Growing Rosehip (*Rosa Sp.*) from Yozgat (Turkey). Acta Scientiarum Polonorum Hortorum Cultus, 19(2), 123-134. <https://doi.org/10.24326/asphe.2020.2.12>
- Köse M (2013). Erzurum İli İspir İlçesinde Doğal Olarak Yetişen Badem (*Amygdalus communis L.*) Tiplerinin Seleksiyon Yolu İle Islahı Ve Seçilen Tiplerde Rapd Yöntemiyle Genetik Çeşitliliğin Belirlenmesi. Doktora Tezi, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, 201 s.



- Küden A & Kaşka N (1995). Kiraz Çeşit ve Seleksiyon Çalışmaları. Türkiye II. Ulusal Bahçe Bitkileri Kongresi, 3–6 Ekim 1995, Adana, 233–237.
- Moriguchi, T., S. Teramoto ve T. Sanada, 1994. Conservation System of Fruit Tree Genetic Resources and Recently Released Cultivars from Fruit Tree Research Station in Japan. *Fruit Varieties Journal*. 48 (2): 73-80.
- Noiton-D, 1994. Collecting Wild Apples in Central Asia. *Orchardist of New Zealand*. 1994, 67:7, 32-34, 36.
- Özbek S (1978). Özel Meyvecilik. Çukurova Üniversitesi, Ziraat Fakültesi Yayın No: 128, 485 s., Adana.
- Özçağırın R (1976). Kiraz-Vişne Anaçları. *Ege Üniversitesi Ziraat Fakültesi Dergisi* 13(2):163–177.
- Özen MS (2013). Bolu Merkez ilçesinde kuşburnu (*Rosa spp.*) genetik kaynaklarının seleksiyonu ve antioksidan aktivitelerinin tespiti. Selçuk Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 78 s.
- Özkan G (2002). Yenişarbademli (Isparta) yöresindeki ceviz tiplerinin (*Juglans regia L.*) seleksiyonu. Yüksek lisans tezi, Süleyman Demirel Üniversitesi, Fen Bilimleri Enstitüsü, 82 s.
- Pırlak L (1993). Uzundere, Tortum ve Oltu İlçelerinde Doğal Olarak Yetişen Kızılcıkların (*Cornus mas L.*) Seleksiyon Yoluyla Islahı Üzerinde Bir Araştırma. Doktora tezi, Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, 154 s.
- Sağır S (2010). Akıncılar yöresinde doğal olarak yetişen kuşburnu tiplerinin (*Rosa spp.*) seleksiyon yoluyla ıslahı. Ordu Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 56 s.
- Sen SM, Tekintas FE, Askin MA, Cangi R, Bostan SZ, Balta F, Oguz Hİ, Akca Y, Karadeniz T, Kazankaya A, Beyhan O & Nas M (1995). Research on Breeding by Selection of Wild Apricot (*Prunus armeniaca L.*) Forms on Adilcevaz Plain. *Acta Hort. (Ishs)* 384:201-204.
- Şavir Z (2008). Munzur Dağı (Erzincan) Kuşburnu (*Rosa spp.*) Genetik Kaynakları. Yüzüncü Yıl Üniversitesi, Fen Bilimleri Enstitüsü, Yüksek lisans tezi, 66 s.
- Şehirali S, Özgen M, Karagöz A, Sürek M, Adak S, Güvenç İ, Tan A, Burak M, Kaymak HÇ & Kenar D (2005). Bitki genetik kaynaklarının korunma ve kullanımı. TMMOB Ziraat Mühendisleri Odası VI. Teknik Kongresi. Cilt 1. Kozan Ofset, Ankara. 253-273.
- Şehirali S & Özgen M (2012). Bitkisel Gen Kaynakları. Ders Kitabı, A.Ü. Ziraat Fakültesi Yayın No: 557, Ders kitabı No: 1605, A. Ü. Basımevi.
- Şimşek M & Osmanoglu A (2010a). Derik (Mardin) İlçesinde Doğal Olarak Yetişen Bademlerin (*Prunus amygdalus L.*) Seleksiyonu. *Y.Y.Ü., Ziraat Fakültesi Tarım Bilimleri Dergisi*, 20(3): 171-182.
- Şimşek M & Osmanoglu A (2010b). Mazıdağı (Mardin) Yöresindeki Doğal Cevizlerin (*Juglans regia L.*) Seleksiyonu. *YYÜ Tar Bil Derg (YYU J Agr Sci)*. 20(2): 131-137.
- Şimşek M, Çömlekçioğlu S & Osmanoglu A (2010). Çüngüş İlçesinde Doğal Olarak Yetişen Bademlerin Seleksiyonu Üzerinde Bir Araştırma. *Harran Üniversitesi, Ziraat Fakültesi Dergisi*, 14(1): 37-44.
- Tan A (1991). Ülkesel Bitki Genetik Kaynakları Araştırma Projesi. Bitki Gen Kaynakları Araştırma Enstitüsü. Menemen, İzmir.
- Tan A & Tan AŞ (1998). Database management systems for conservation of genetic diversity in Turkey. In: N. Zencirci, Z. Kaya, Y. Anikster, W.T. Adams (Eds.). *The Proceeding of International Symposium on in situ Conservation of Plant Genetic Diversity*. 4-8 November, 1996. Antalya, Turkey.
- Türkoğlu N & Muradoğlu F (2003). Tatvan Yöresinde Doğal Olarak Yetişen Kuşburnu Tiplerinin Üstün Özelliklerinin Belirlenmesi Üzerine Bir Araştırma. *Ulusal Bahçe Bitkileri Kongresi, Antalya*, 256-257, 8–12 Eylül 2003.
- Uçaral H & Koç A (2016a). Yozgat'ta Doğal Olarak Yetişen Kuşburnuların (*Rosa spp.*) Seleksiyon Yoluyla Islahı. *Bahçe, Cilt 45 (2), Özel Sayı (V. Ulusal Üzümsü Meyveler Sempozyumu, 27-30 Eylül, Adana)*, 27-36. Çukurova Üniversitesi, Adana
- Uçaral H & Koç A (2016b). Selections of Natural Growing Rose hips (*Rosa spp.*) from Yozgat Province, Turkey. *Research Journal of Agricultural Sciences* 9 (1): 58-61.
- Ünver H & Çelik M (2005). Ankara yöresi cevizlerinin seleksiyon yoluyla ıslahı. II. Ulusal Ceviz Sempozyumu Özetler. 13-16 Eylül 2005. Bursa. s:28 TÜBİTAK TOGTAG TARP Proje No. 2418. 2002: 1-19.
- Vurgun H, Ünlü HM, Aslantaş R, Keskin S, Kadioğlu Z, Esmek İ, Öz MH, Karadoğan B, Kalkan NN, Dorukoğlu E, Bozbek Ö & Albayrak S (2013). Doğu Anadolu Meyve ve Bağ Genetik Kaynaklarının Belirlenmesi Üzerine Bir Araştırma. *Biyoçeşitlilik ve Genetik Kaynaklar Araştırmaları Program Değerlendirme Toplantısı*. 11-14 Mart 2013. Antalya. sf 94.
- Yazgan İ (1997). Samsun'un Havza İlçesi'nde kuşburnunun (*Rosa spp.*) seleksiyon yoluyla ıslahı üzerine bir araştırma. *Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü, Bahçe Bitkileri Ana Bilim Dalı, Yüksek Lisans Tezi*, 72 sf.
- Yılmaz S (2007). Geç Yapraklanan ve Yan Dallarda Yüksek Oranda Meyve Veren Yeni Ceviz Tiplerinin (*J. regia L.*) Seleksiyon Islahı. Doktora Tezi, Gaziosmanpaşa Üniversitesi, Fen Bilimleri Enstitüsü, Tokat.
- Zaurov, D. E., S. A. Mehlenbacher, T. J. Mainer, J. C. Goffreda, And C. R. Funk, 2005. Genetic Resour–Ces Of Temperate And Subtropical Fruit and Nut Species at The Nikita Botanical Gardens. *Hors- Cience* 40 (1): 5-9.

Oral Presentation

Thursday

Diversity of Plant Species, Systematics and Phylogeny-3

## Some Morphological Traits of Selected Hawthorn (*Crataegus* Spp.) Genetic Resources from Coruh Valley

Halil İbrahim Sağbaş<sup>1\*</sup>, Sezai Ercişli<sup>1</sup>

<sup>1</sup>Atatürk University, Agricultural Faculty, Department of Horticulture 25240 Erzurum, Turkey

\*Corresponding author e-mail: hibrahimsagbas@gmail.com

### Abstract

Currently plant genetic resources are accepted among the natural wealth of the countries and Turkey has special position for plant genetic resources in the world. The country has an important hawthorn (*Crataegus* spp.) genetic resources that distributed throughout the country. In Turkey the number of hawthorn species estimated to be over 25 and hawthorn trees and shrubs are mostly grown spontaneously in Northeast Anatolia, Central Anatolia, the Aegean and the Mediterranean region. Coruh Valley, located in the Northeast Anatolia Region, is the one of the centers of wild grown hawthorn populations yet for different reasons, hawthorn genetic resources are rapidly disappearing in the valley. In this study, 101 hawthorn genotypes with superior fruit characteristics were selected by selection method from the valley and the morphological traits of the genotypes were characterized by using UPOV criteria. The plant height, diameter, shoot length, leaf length, leaf width, leaf length/width, leaf area, leaf lobe depth and petiole length were found between 1.50-9.20 m, 1.20-8.05 m, 1.50-39.50 cm, 24.06-64.61 mm, 24.02-62.75 mm, 0.74-1.71, 2.73-20.75 cm<sup>2</sup>, 8.03-34.05 mm and 4.14-40.87 mm among genotypes, respectively. The results are indicated that hawthorn germplasm in the valley is very diverse and could be ready material for future breeding activities.

**Keywords:** Hawthorn, *Crataegus*, morphology, Çoruh Valley, genetic resources

### INTRODUCTION

Anatolia is one of the foremost world sources of crop plants which have been cultivated for food, and the wild ancestors of many crop plants which now provide staples for mankind still

grow here. The flora of Turkey, same as its fauna, is extremely rich in terms of various species of plants. Currently Turkey is accepted one of the richest countries in terms of plant species with approximately 12140 plant species of vascular plants. The number of endemic plants in Turkey around 3955 (with endemism percentage of 33%). The Northeastern Anatolia Region is one of the leading regions in Turkey in terms of the number of endemic plant species and in the Coruh Valley located in the Northeastern Anatolia, the number of endemic plants is 665 (30% of endemism percentage) (Ministry of Agriculture and Forest, 2021). This high rate of endemism in Turkey made it attraction center of flowering plants because there is no country with such a high rate of endemism in Europe.

The valley is also rich for wild edible fruits including hawthorn. Wild forms develop defense mechanisms against predators, extremes of temperature, flooding, frost and drought. Moreover, they are resistant to the diseases so prevalent among cultivated plants. In addition, they preserve the taste, fragrance, color, hardness and other original characteristics which tend to be lost in the course of cultivation. Today improvement in biotechnology make it possible to transmit useful qualities of this kind to their cultivars. Moreover, wild forms are a fundamental reference source for the development of new cultivars. To put it metaphorically, wild forms of cultivated species are like the national archive of a country, or the core memory of a computer (Ercisli, 2004; Sagbas et al., 2021).

Hawthorn (*Crataegus* spp.) belongs to Rosaceae family and representative by 150-200 species in the world. It is one of the most important wild edible fruit species in Turkey (Donmez, 2004; Ercisli, 2004). In most parts of the country, humans consume hawthorn fruit as fresh after harvest and it is well known that hawthorn fruit is used centuries for treatment of heart disease and blood vessels such as congestive heart failure (CHF), chest pain, and irregular heartbeat. It is also used to treat both low blood pressure and high blood pressure, “hardening of the arteries” (atherosclerosis), and high cholesterol in Turkey (Ercisli, 2004; Caliskan, 2015). Different organs of the hawthorn plant such as leaves, flowers and buds are used in traditional medicine as supportive food for the healing of diseases such as cough, flu, asthma, mild cardiovascular diseases, and also hawthorn flowers and leaves have medical importance in terms of herbal medicine production (Ozyurek et al., 2012; Meriçli and Ergezen, 1994; Ljubuncic et al., 2005; Kùltür, 2007)

Hawthorn is one of the wild-formed fruit species that are widely found in the Coruh valley and not yet cultivated. The diversity we have in hawthorn has not been adequately evaluated until now, and especially in recent years, climate change, human activities, agricultural policies,

globalization, etc., which threaten agricultural biological diversity and thus hawthorn genetic resources are rapidly disappearing in the Coruh Valley.

In the last century in which modern agricultural techniques were used, agricultural production based on a single fruit or vegetable cultivar caused a decrease in genetic diversity and erosion in the gene pool reached serious levels (Miller and Schaal, 2006). Therefore, the determination, protection and use of plant genetic material is of particular importance for future food security in changing environment.

Although it is widely used in landscaping in other countries, the plant also plays an important role in erosion control and wildlife support. In addition, it has been stated that hawthorn can be used as a dwarf rootstock for pome fruits (apple, pear etc.) in arid and calcareous soils (Nas, 2012).

The main purpose of this study is to determine the hawthorn genotypes in the Coruh Valley by selection, taking into account their superior fruit characteristics, and to reveal the diversity of genotypes by examining some morphological features.

## MATERIAL AND METHODS

The material of this study consists of 101 hawthorn (*Crataegus* spp.) genotypes selected from Coruh Valley in Northeastern Turkey.



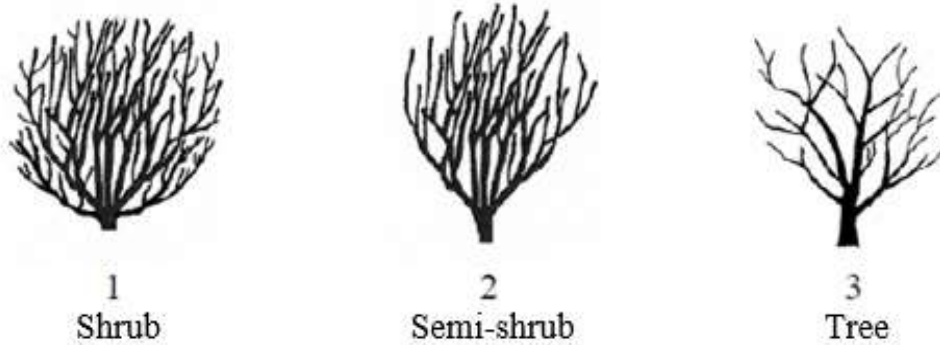
**Figure 1.** Fruit and tree traits of some selected hawthorn genotypes (Original)

For morphological description of genotypes, UPOV (International Union for the Protection of New Varieties of Plants, 2007) for hawthorn was used.

### Plant Features

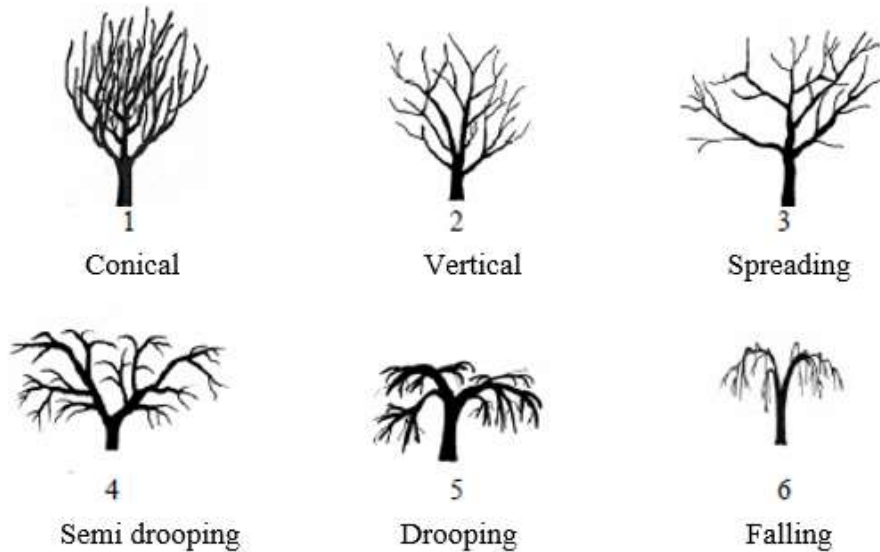
#### -Plant form:

Plant form of hawthorn genotypes were determined as shrub, semi-shrub or tree according to UPOV 1 criterion.



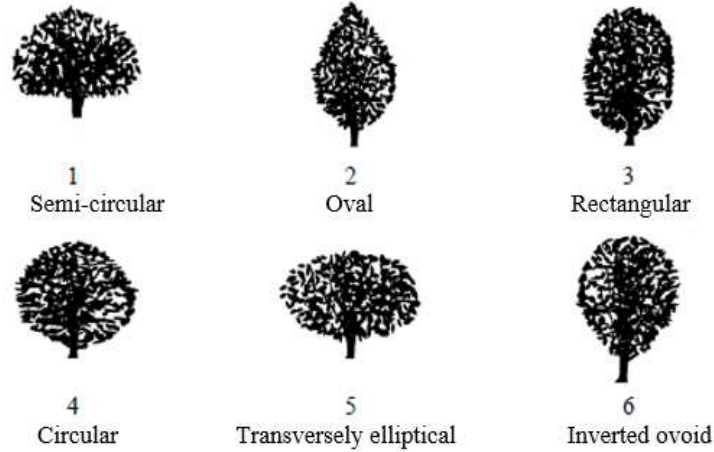
**Figure 2.** Plant form of hawthorn genotypes (UPOV 2007)

**-Growth habit:** Growth habit of hawthorn genotypes determined as conical, vertical, spreading, semi drooping, drooping or falling according to UPOV 2 criteria.



**Figure 3.** Growth habit of hawthorn genotypes (UPOV 2007)

**-Crown shape:** Crown shapes of selected hawthorn genotypes were determined as semi-circular, oval, rectangular, circular, transversely elliptical or inverted ovoid according to UPOV 3 criteria.



**Figure 4.** Crown shape of hawthorn genotypes (UPOV 2007)

**-Plant dimensions:** The height and width of the selected hawthorn plants were measured with the aid of a tape measure.

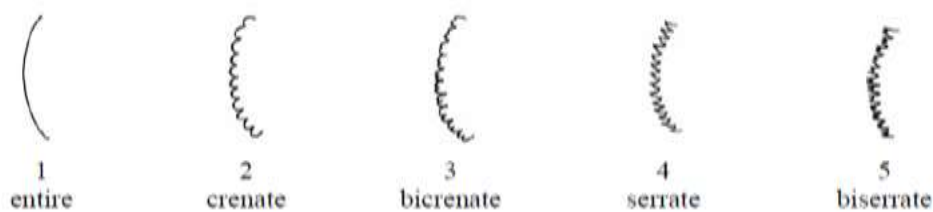
**-Presence of thorn:** The status of thorn in the shoots of the selected plants was evaluated as present or absent according to the criterion 6 of UPOV.

**-Number of thorns:** It is determined by the number of thorns in each plant in the middle age of the branches in 4 different directions, with a length corresponding to 1/3 of the total length of the shoots. 4 different branches in a tree were examined and the average of the number of thorns in the middle of the shoot divided by four. The number of thorns in the shoots of the selected plants was evaluated as thornless, few, medium or many according to the UPOV No 8 criterion.

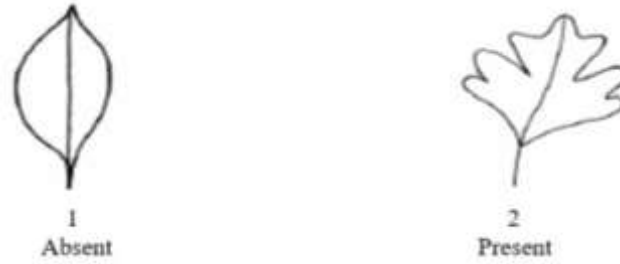
**-Shoot length:** Shoot lengths of selected hawthorn genotypes were measured with a tape measure.

### Leaf Features

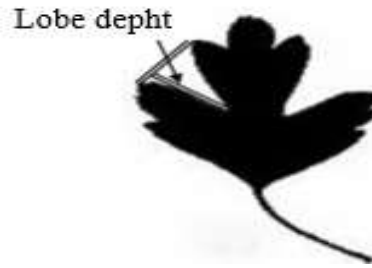
Leaf length, leaf width, lobe depth and petiole length of hawthorn genotypes, which were selected and leaf samples were taken, were measured using AEK-Tech Digital Caliper and leaf areas were measured using the CI-202 leaf area meter device. Leaf margin shapes of selected hawthorn genotypes are entire, crenate, bicrenate, serrate or biserrate according to UPOV 14 criteria; leaf lobe presence was examined as present or absent according to UPOV criterion 15.



**Figure 5.** Leaf margin shapes of hawthorn genotypes (UPOV 2007)



**Figure 6.** Leaf lobe presence of hawthorn (UPOV 2007)



**Figure 7.** Determination of lobe depth (UPOV 2007)

## RESULTS AND DISCUSSION

### Plant Features

We determined the plant form of the hawthorn genotypes were 64 genotypes tree, 28 genotypes semi-shrub and 9 genotype shrubs (Table 1). Bektaş et al. (2017) conducted a study on hawthorn genotypes in Malatya province in Turkey and stated that 29 of the 40 genotypes determined as tree plant form, 5 of them were in semi-shrubs form and 6 of them were shrubs.

The growth habit of the hawthorn genotypes used in our study were determined as vertical in 34 genotypes, spreading in 31 genotypes, semi-drooping in 16 genotypes, conical in 11 genotypes, drooping in 6 genotypes and falling in 3 genotypes (Table 1). Keles (2018) found that the growth habit of the hawthorn genotypes was spreading in 58 genotypes, vertical in 21 genotypes, semi-drooping in 16 genotypes, conical in 5 genotypes, and drooping in 4 genotypes in his study on hawthorn genotypes grown in Yozgat province of Turkey.

In present study, crown shape of the hawthorn genotypes were determined as 24 genotypes oval, 22 genotypes transversely elliptical, 19 genotypes circular, 18 genotypes inverted ovoid, 16 genotypes rectangular and 2 genotypes semi-circular (Table 1). Özderin (2014) reported that hawthorn genotypes found in western Anatolia had open and informal crown shape.

The thorn situation of the shoots of the hawthorn genotypes used in our study were also determined and we found that 56 genotypes had thorn and 45 genotypes thorn free. The number

of thorns was determined as 45 genotypes thornless, 31 genotypes had few numbers of thorn, 17 genotypes had medium thorn and 8 genotypes had many thorn (Table 1). Yanar et al. (2011) reported that hawthorn genotypes had medium thorn (10 genotypes), thornless (5 genotypes), many (3 genotypes) and few (3 genotypes) indicating similarities with our present study.

The highest plant height was determined as 9.2 m in 25C16 genotype while the lowest value was determined in 25C37 genotype as 1.5 m; tree diameter was the highest in 25C96 genotype as 8.05 m and was the lowest in 25C37 genotype as 1.2 m, respectively (Table 1).

The shoot length of hawthorn genotypes was the highest in 25C65 genotype (39.5 cm) whereas was the lowest in 25C90 genotype as 1.5 cm, respectively (Table 1). Çalışkan et al. (2018) reported that average shoot length of hawthorn genotypes grown in Hatay was 14.87 cm.

### Leaf Features

Leaf margins of the hawthorn genotypes were found as crenate in 34 genotypes, bicrenate in 31 genotypes, serrate in 24 genotypes and biserrate in 12 genotypes and all genotypes were grouped as lobed leaves. Beigmohamadi and Rahmani (2011) and Cengiz et al. (2011) reported that most hawthorn species have serrate leaf margins and the presence of lobes. Özderin (2014) found that among the *Crataegus* taxon with the highest leaf size and petiole length was *Crataegus pentagyna* subsp. *pentagyna* (leaf length 7 cm, leaf width 3.8 cm and leaf petiole length 3.0 cm) and the lowest values were observed in *Crataegus monogyna* subsp. *lasiocarpa* (leaf length 0.8 cm, leaf width 1.1 cm and petiole length 0.3 cm). Çalışkan et al. (2018) used a number of hawthorn genotypes in Hatay and they found that the leaf length was 6.72 cm, the leaf width was 4.84 cm, the petiole length was 1.46 cm, and the leaf area was 32.42 cm<sup>2</sup>. Keles (2018) found that the lobe depth of hawthorn leaves in the range of 0.81-2.21 mm.

The leaf length of the hawthorn genotypes used in our study was the highest in 25C66 genotype (64.61 mm) and was the lowest in 25C36 genotype (24.06 mm); leaf width was the highest in 25C66 genotype (62.75 mm) and lowest in 25C101 genotype (24.02 mm); leaf length/width ratio with the highest in 25C39 genotype (1.71) and lowest in 25C15 genotype (0.74); petiole length was the highest in 25C38 genotype (40.87 mm) and the lowest in 25C36 genotype (4.14 mm); leaf area was the highest in 25C57 genotype (20.75 cm<sup>2</sup>) and the lowest in 25C36 genotype (2.73 cm<sup>2</sup>); the lobe depth of the leaves was the highest in 25C13 genotype (34.05 mm) and the lowest in 25C101 genotype (8.03 mm), respectively.



## CONCLUSION

According to the results of the study, it was observed that the morphological diversity of the examined hawthorn genotypes was very high, the necessary importance was not given to the species, and therefore the genetic resources of the hawthorn species were rapidly lost in the Coruh Valley. If adequate precautions are not taken, it is inevitable that the hawthorn species will disappear completely within the next years. As a matter of fact, at the end of our study, hawthorn genotypes with superior fruit qualities should be preserved *ex-situ* and the future of hawthorn genetic resources in the Coruh Valley was guaranteed. It should be ensured that hawthorn, which is a rich biodiversity gene resource, is a valuable fruit species throughout our country, and more effective conservation strategies should be developed for species in danger of disappearing with agricultural policies implemented in our country.

## ACKNOWLEDGEMENTS

We would like to thank Atatürk University Scientific Research Projects Coordination Unit for financial and administrative support in the execution of this research.

## REFERENCES

- Beigmohamadi, M., & Rahmani, F. (2011). Genetic variation in hawthorn (*Crataegus* spp.) using RAPD markers. *African Journal of Biotechnology*, 10(37), 7131-7135.
- Bektaş, M., Bükücü, Ş. B., Özcan, A., Sütyemez, M., 2017. Akçadağ ve Hekimhan İlçelerinde yetişen Aliç (*Crataegus* Spp.) genotiplerinin bitki ve pomolojik özellikleri. *Türk Tarım ve Doğa Bilimleri Dergisi-Turkish Journal of Agricultural and Natural Sciences*, 4(4), 484-490.
- Cengiz, B., Sabaz, M., & Sarıbaş, M. (2011). The use of some natural *Crataegus* L.(Hawthorn) taxa from Western Black Sea Region of Turkey for landscape applications. *Fresenius Environmental Bulletin*, 20(3), 656-664.
- Çalışkan, O., Gündüz, K., Serçe, S., Toplu, C., Kamiloğlu, Ö., Şengül, M., and Ercişli, S., 2012. Phytochemical characterization of several hawthorn (*Crataegus* spp.) species sampled from the Eastern Mediterranean region of Turkey. *Pharmacognosy magazine*, 8(29), 16.
- Caliskan, O. (2015). Mediterranean Hawthorn Fruit (*Crataegus*) Species and Potential Usage. *The Mediterranean Diet an Evidence-Based Approach* (Eds. Preedy, V.R., Watson, R.R.). Elsevier Publishing, Chapter 55, 621-628.
- ÇALIŞKAN, O., GÜNDÜZ, K., & BAYAZIT, S. (2018). Investigation of Morphological, Biological and Fruit Quality Characteristics of Yellow Hawthorn Genotype (*Crataegus azarolus* L.). *Gaziosmanpaşa Üniversitesi Ziraat Fakültesi Dergisi*, 35(4), 69-74.
- Donmez, A.A., 2004. The genus *Crataegus* L. (Rosaceae) with special reference to hybridisation and biodiversity in Turkey. *Turkish J Bot* 28:29-37.
- Ercisli, S., 2004. A short review of the fruit germplasm resources of Turkey. *Genet Resour Crop Evol* 51:419-435.
- Keles, H. (2018). Selection morphological, biochemical and molecular characterization of hawthorn (*Crataegus* spp.) genetic resources from Yozgat province and districts. Atatürk University Graduate School of Natural and Applied Sciences, Ph.D. Thesis, 125p.
- Kültür, Ş. (2007). Medicinal plants used in Kırklareli province (Turkey). *Journal of ethnopharmacology*, 111(2), 341-364.

- Ljubuncic, P., Portnaya, I., Cogan, U., Azaizeh, H., & Bomzon, A. (2005). Antioxidant activity of *Crataegus aronia* aqueous extract used in traditional Arab medicine in Israel. *Journal of ethnopharmacology*, 101(1-3), 153-161.
- Meriçli, A. H., & Ergezen, K. (1994). Flavonoids of *Crataegus tanacetifolia* (Lam.) Pers. (Rosaceae), an endemic species from Turkey. *Scientia Pharmaceutica*, 62, 277-277.
- Miller, A.J., Schaal, B.A., 2006. Domestication and the distribution of genetic variation in wild and cultivated populations of the mesoamerican fruit tree *Spondias purpurea* L. (Anacardiaceae). *Molecular Ecology*, 15, 1467–1480.
- Ministry of Agriculture and Forest (2021). Noah's Ark National Biodiversity Database. Accessed on 15/04/2021.
- Nas M. N., 2012. Cultivation of hawthorn (*Crataegus* spp.): Opportunities and challenges. I. National Hawthorn Workshop, Malatya. pp 3-8.
- Özderin, S. (2014). Botanical and chemical properties of some hawthorn (*Crataegus* L. spp.) taxa natural distributed in western anatolia. Süleyman Demirel University Graduate School of Applied and Natural Sciences, Ph.D. Thesis, 173p.
- Ozyurek, M., Bener, M., Guclu, K., Donmez, A. A., Suzgec-Selcuk, S., Pirildar, S., ... & Apak, R. (2012). Evaluation of antioxidant activity of *Crataegus* species collected from different regions of Turkey.
- Sagbas, H. I., Ilhan, G., Ercisli, S., Anjum, M. A., & Holubec, V. (2021). Characterization of Oleaster-Leafed Pear (*Pyrus elaeagrifolia* Pall. subsp. *elaegrifolia*) Fruits in Turkey. *Agronomy*, 11(3), 430.
- UPOV (2007). Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability. Hawthorn, International Union for the Protection of New Varieties of Plants, Genova, Italy. [https://www.upov.int/edocs/mdocs/upov/en/tc\\_edc/2008/tg\\_hawth\\_proj\\_5\\_e.pdf](https://www.upov.int/edocs/mdocs/upov/en/tc_edc/2008/tg_hawth_proj_5_e.pdf)
- Yanar, M., Ercisli, S., Yilmaz, K.U., Sahiner, H., Taskin, T., Zengin, Y., Akgul, I., Celik, F., 2011. Morphological and chemical diversity among hawthorn (*Crataegus* spp.) genotypes from Turkey. *Scientific Research and Essays*, 6(1), 35-38.

**Table 1.** Tree and leaf characteristics of selected hawthorn genotypes

| Genotype Names | Plant Form | Growth Habit  | Crown Shape             | Plant Height (m) | Tree Diameter (m) | Presence of Thorn | Number of Thorn | Shoot Length (cm) | Leaf Margin Shapes | Leaf Lobe Presence | Leaf Length (mm) | Leaf Width (mm) | Leaf Length/Width Ratio | Leaf Area (cm <sup>2</sup> ) | Leaf Lobe Depth (mm) | Petiole Length (mm) |
|----------------|------------|---------------|-------------------------|------------------|-------------------|-------------------|-----------------|-------------------|--------------------|--------------------|------------------|-----------------|-------------------------|------------------------------|----------------------|---------------------|
| 25C01          | Tree       | Semi drooping | Transversely elliptical | 6.50             | 6.00              | Absent            | Thornless       | 10.05             | Crenate            | Present            | 45.86            | 38.80           | 1.18                    | 10.18                        | 22.10                | 16.04               |
| 25C02          | Semi-shrub | Conical       | Oval                    | 4.50             | 5.10              | Absent            | Thornless       | 11.98             | Bicrenate          | Present            | 44.15            | 45.18           | 0.98                    | 11.09                        | 20.55                | 8.76                |
| 25C03          | Shrub      | Semi drooping | Transversely elliptical | 4.18             | 5.13              | Absent            | Thornless       | 18.33             | Serrate            | Present            | 40.41            | 40.23           | 1.00                    | 9.57                         | 17.52                | 11.55               |
| 25C04          | Tree       | Spreading     | Circular                | 4.28             | 5.82              | Absent            | Thornless       | 9.25              | Crenate            | Present            | 45.53            | 46.73           | 0.97                    | 9.04                         | 22.96                | 14.59               |
| 25C05          | Semi-shrub | Spreading     | Transversely elliptical | 6.92             | 7.43              | Absent            | Thornless       | 12.73             | Serrate            | Present            | 48.98            | 41.86           | 1.17                    | 9.73                         | 17.12                | 9.87                |
| 25C06          | Semi-shrub | Spreading     | Circular                | 5.20             | 5.30              | Absent            | Thornless       | 21.25             | Crenate            | Present            | 41.03            | 41.53           | 0.99                    | 8.04                         | 18.46                | 14.58               |
| 25C07          | Tree       | Drooping      | Transversely elliptical | 5.30             | 5.12              | Absent            | Thornless       | 7.38              | Serrate            | Present            | 34.99            | 36.90           | 0.95                    | 7.86                         | 18.29                | 12.23               |
| 25C08          | Semi-shrub | Falling       | Transversely elliptical | 3.80             | 5.30              | Absent            | Thornless       | 12.05             | Bicrenate          | Present            | 36.60            | 42.72           | 0.86                    | 8.58                         | 17.77                | 14.55               |
| 25C09          | Tree       | Vertical      | Oval                    | 5.40             | 4.20              | Absent            | Thornless       | 3.05              | Crenate            | Present            | 39.06            | 32.11           | 1.22                    | 6.38                         | 15.88                | 10.65               |
| 25C10          | Tree       | Semi drooping | Transversely elliptical | 6.10             | 6.50              | Absent            | Thornless       | 7.43              | Serrate            | Present            | 42.80            | 35.66           | 1.20                    | 8.65                         | 18.12                | 17.10               |
| 25C11          | Shrub      | Vertical      | Oval                    | 3.20             | 2.91              | Present           | Few             | 3.55              | Serrate            | Present            | 39.56            | 32.25           | 1.23                    | 6.25                         | 24.44                | 9.84                |
| 25C12          | Shrub      | Vertical      | Oval                    | 1.70             | 1.82              | Present           | Few             | 7.45              | Biserrate          | Present            | 30.89            | 26.04           | 1.19                    | 4.80                         | 16.59                | 13.84               |
| 25C13          | Tree       | Vertical      | Oval                    | 3.80             | 2.20              | Absent            | Thornless       | 18.15             | Bicrenate          | Present            | 47.34            | 40.69           | 1.16                    | 9.49                         | 34.05                | 10.69               |
| 25C14          | Tree       | Vertical      | Oval                    | 4.20             | 2.36              | Present           | Medium          | 14.83             | Bicrenate          | Present            | 42.41            | 41.85           | 1.01                    | 11.50                        | 18.53                | 11.07               |
| 25C15          | Tree       | Conical       | Inverted ovoid          | 7.86             | 6.15              | Absent            | Thornless       | 6.03              | Bicrenate          | Present            | 43.23            | 58.73           | 0.74                    | 14.57                        | 24.17                | 13.84               |
| 25C16          | Tree       | Conical       | Inverted ovoid          | 9.20             | 6.96              | Absent            | Thornless       | 4.00              | Bicrenate          | Present            | 45.81            | 40.20           | 1.14                    | 9.65                         | 17.15                | 16.08               |
| 25C17          | Tree       | Vertical      | Oval                    | 8.70             | 5.68              | Present           | Few             | 6.05              | Serrate            | Present            | 46.23            | 41.16           | 1.12                    | 10.92                        | 20.17                | 15.13               |
| 25C18          | Shrub      | Drooping      | Transversely elliptical | 3.20             | 4.33              | Present           | Few             | 10.23             | Serrate            | Present            | 29.33            | 28.10           | 1.04                    | 3.85                         | 11.73                | 9.04                |
| 25C19          | Tree       | Spreading     | Circular                | 4.10             | 3.08              | Absent            | Thornless       | 10.25             | Biserrate          | Present            | 40.79            | 43.06           | 0.95                    | 11.57                        | 17.75                | 16.39               |
| 25C20          | Tree       | Semi drooping | Transversely elliptical | 6.20             | 5.44              | Absent            | Thornless       | 23.55             | Biserrate          | Present            | 44.32            | 46.07           | 0.96                    | 9.49                         | 18.59                | 11.37               |
| 25C21          | Semi-shrub | Semi drooping | Transversely elliptical | 2.11             | 2.60              | Present           | Medium          | 17.78             | Biserrate          | Present            | 30.93            | 27.19           | 1.14                    | 4.61                         | 17.85                | 14.23               |
| 25C22          | Tree       | Vertical      | Inverted ovoid          | 4.20             | 3.20              | Present           | Few             | 20.25             | Crenate            | Present            | 39.79            | 42.11           | 0.94                    | 10.89                        | 19.80                | 12.50               |



### 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021



|       |            |               |                         |      |      |         |           |       |           |         |       |       |      |       |       |       |
|-------|------------|---------------|-------------------------|------|------|---------|-----------|-------|-----------|---------|-------|-------|------|-------|-------|-------|
| 25C23 | Tree       | Spreading     | Transversely elliptical | 5.40 | 7.40 | Absent  | Thornless | 7.20  | Crenate   | Present | 40.81 | 32.19 | 1.27 | 7.55  | 15.44 | 11.51 |
| 25C24 | Tree       | Vertical      | Transversely elliptical | 4.50 | 7.52 | Absent  | Thornless | 17.88 | Crenate   | Present | 41.53 | 33.25 | 1.25 | 8.12  | 14.41 | 12.10 |
| 25C25 | Semi-shrub | Semi drooping | Transversely elliptical | 3.40 | 5.00 | Present | Many      | 16.88 | Bicrenate | Present | 29.59 | 26.17 | 1.13 | 7.85  | 15.34 | 20.33 |
| 25C26 | Tree       | Spreading     | Circular                | 3.40 | 5.80 | Present | Few       | 14.38 | Biserrate | Present | 44.52 | 38.38 | 1.16 | 11.48 | 27.75 | 14.08 |
| 25C27 | Semi-shrub | Semi drooping | Circular                | 3.15 | 4.80 | Present | Medium    | 23.60 | Biserrate | Present | 25.96 | 28.93 | 0.90 | 4.68  | 11.53 | 10.91 |
| 25C28 | Semi-shrub | Vertical      | Oval                    | 2.20 | 3.02 | Present | Medium    | 13.05 | Bicrenate | Present | 34.64 | 32.97 | 1.05 | 6.53  | 15.43 | 9.92  |
| 25C29 | Tree       | Spreading     | Circular                | 5.50 | 4.00 | Present | Few       | 17.50 | Crenate   | Present | 30.26 | 25.69 | 1.18 | 4.67  | 13.49 | 9.80  |
| 25C30 | Semi-shrub | Spreading     | Transversely elliptical | 3.30 | 5.85 | Present | Medium    | 10.35 | Bicrenate | Present | 44.51 | 38.15 | 1.17 | 11.51 | 26.95 | 14.19 |
| 25C31 | Semi-shrub | Vertical      | Oval                    | 5.20 | 6.12 | Present | Few       | 16.13 | Crenate   | Present | 46.89 | 41.13 | 1.14 | 11.13 | 13.24 | 4.28  |
| 25C32 | Tree       | Vertical      | Inverted ovoid          | 1.70 | 1.50 | Present | Many      | 7.25  | Serrate   | Present | 41.22 | 40.66 | 1.01 | 9.49  | 19.82 | 13.29 |
| 25C33 | Tree       | Spreading     | Inverted ovoid          | 4.10 | 4.80 | Present | Few       | 3.78  | Crenate   | Present | 45.32 | 33.83 | 1.34 | 9.56  | 20.48 | 13.62 |
| 25C34 | Semi-shrub | Vertical      | Circular                | 4.00 | 3.00 | Present | Medium    | 15.38 | Serrate   | Present | 42.62 | 42.72 | 1.00 | 9.08  | 21.30 | 23.32 |
| 25C35 | Semi-shrub | Vertical      | Oval                    | 3.70 | 3.70 | Present | Medium    | 15.60 | Biserrate | Present | 45.91 | 26.79 | 1.71 | 6.29  | 20.48 | 12.01 |
| 25C36 | Shrub      | Vertical      | Inverted ovoid          | 1.80 | 2.00 | Present | Medium    | 5.50  | Crenate   | Present | 24.06 | 26.98 | 0.89 | 2.73  | 11.88 | 4.14  |
| 25C37 | Shrub      | Vertical      | Oval                    | 1.50 | 1.20 | Present | Many      | 6.78  | Serrate   | Present | 40.52 | 39.76 | 1.02 | 9.50  | 19.70 | 13.51 |
| 25C38 | Tree       | Semi drooping | Inverted ovoid          | 6.70 | 5.40 | Present | Few       | 4.58  | Bicrenate | Present | 40.82 | 51.08 | 0.80 | 15.73 | 13.56 | 40.87 |
| 25C39 | Semi-shrub | Vertical      | Oval                    | 3.40 | 3.00 | Present | Few       | 3.83  | Crenate   | Present | 45.97 | 26.81 | 1.71 | 6.27  | 20.50 | 12.89 |
| 25C40 | Tree       | Vertical      | Oval                    | 6.50 | 4.50 | Present | Medium    | 13.50 | Serrate   | Present | 45.90 | 45.37 | 1.01 | 10.34 | 21.67 | 14.84 |
| 25C41 | Tree       | Spreading     | Rectangular             | 3.60 | 3.30 | Present | Medium    | 17.50 | Bicrenate | Present | 45.74 | 48.84 | 0.94 | 11.70 | 21.79 | 15.88 |
| 25C42 | Semi-shrub | Drooping      | Transversely elliptical | 3.70 | 4.15 | Absent  | Thornless | 17.19 | Serrate   | Present | 39.33 | 43.29 | 0.91 | 8.68  | 17.24 | 12.04 |
| 25C43 | Tree       | Spreading     | Rectangular             | 2.90 | 3.10 | Present | Few       | 14.25 | Bicrenate | Present | 36.01 | 30.81 | 1.17 | 4.63  | 16.07 | 8.92  |
| 25C44 | Shrub      | Drooping      | Transversely elliptical | 3.15 | 4.30 | Present | Many      | 13.68 | Crenate   | Present | 36.59 | 31.40 | 1.17 | 4.94  | 16.26 | 8.12  |
| 25C45 | Tree       | Vertical      | Oval                    | 3.40 | 3.08 | Present | Few       | 17.05 | Crenate   | Present | 31.49 | 34.88 | 0.90 | 6.27  | 9.79  | 9.26  |
| 25C46 | Tree       | Spreading     | Circular                | 4.90 | 4.60 | Present | Few       | 28.28 | Crenate   | Present | 50.69 | 40.75 | 1.24 | 14.46 | 21.99 | 27.15 |
| 25C47 | Tree       | Spreading     | Inverted ovoid          | 5.60 | 5.70 | Present | Medium    | 30.00 | Serrate   | Present | 57.20 | 56.40 | 1.01 | 14.23 | 26.76 | 11.93 |
| 25C48 | Tree       | Semi drooping | Circular                | 5.10 | 6.15 | Present | Few       | 18.68 | Bicrenate | Present | 45.63 | 42.14 | 1.08 | 7.81  | 22.50 | 15.88 |
| 25C49 | Tree       | Semi drooping | Rectangular             | 3.70 | 3.45 | Present | Many      | 15.38 | Serrate   | Present | 32.88 | 39.03 | 0.84 | 7.20  | 19.76 | 11.43 |
| 25C50 | Semi-shrub | Spreading     | Rectangular             | 2.70 | 4.10 | Present | Few       | 19.25 | Bicrenate | Present | 45.98 | 49.83 | 0.92 | 11.40 | 15.82 | 37.56 |



### 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021



|       |            |               |                         |      |      |         |           |       |           |         |       |       |      |       |       |       |
|-------|------------|---------------|-------------------------|------|------|---------|-----------|-------|-----------|---------|-------|-------|------|-------|-------|-------|
| 25C51 | Tree       | Spreading     | Oval                    | 2.70 | 3.70 | Present | Few       | 17.00 | Bicrenate | Present | 35.25 | 30.11 | 1.17 | 6.28  | 14.34 | 15.32 |
| 25C52 | Tree       | Spreading     | Semi-circular           | 5.50 | 5.50 | Present | Few       | 11.50 | Serrate   | Present | 43.03 | 36.47 | 1.18 | 7.31  | 18.85 | 11.94 |
| 25C53 | Tree       | Vertical      | Oval                    | 2.00 | 2.15 | Present | Medium    | 5.08  | Biserrate | Present | 34.31 | 32.82 | 1.05 | 6.60  | 15.35 | 9.58  |
| 25C54 | Tree       | Spreading     | Circular                | 3.00 | 3.10 | Present | Many      | 15.75 | Biserrate | Present | 37.40 | 32.64 | 1.15 | 7.02  | 13.14 | 8.94  |
| 25C55 | Tree       | Spreading     | Rectangular             | 2.70 | 2.20 | Present | Medium    | 8.90  | Biserrate | Present | 30.45 | 33.14 | 0.92 | 6.13  | 15.70 | 10.06 |
| 25C56 | Tree       | Spreading     | Rectangular             | 2.10 | 2.30 | Present | Medium    | 12.93 | Crenate   | Present | 34.40 | 30.24 | 1.14 | 4.65  | 15.16 | 7.14  |
| 25C57 | Tree       | Vertical      | Rectangular             | 3.60 | 2.20 | Present | Few       | 30.50 | Serrate   | Present | 54.03 | 58.17 | 0.93 | 20.75 | 21.74 | 32.30 |
| 25C58 | Tree       | Vertical      | Rectangular             | 7.30 | 5.20 | Present | Few       | 19.50 | Bicrenate | Present | 30.84 | 32.61 | 0.95 | 6.78  | 14.62 | 7.41  |
| 25C59 | Tree       | Spreading     | Rectangular             | 5.10 | 4.70 | Present | Medium    | 22.75 | Biserrate | Present | 43.54 | 33.68 | 1.29 | 10.19 | 13.09 | 18.04 |
| 25C60 | Tree       | Vertical      | Oval                    | 2.15 | 1.20 | Present | Many      | 9.50  | Serrate   | Present | 36.08 | 30.22 | 1.19 | 5.50  | 13.25 | 7.65  |
| 25C61 | Tree       | Vertical      | Oval                    | 5.80 | 4.10 | Absent  | Thornless | 19.25 | Crenate   | Present | 47.67 | 40.30 | 1.18 | 11.07 | 15.84 | 23.83 |
| 25C62 | Tree       | Vertical      | Rectangular             | 2.35 | 2.45 | Present | Few       | 21.25 | Bicrenate | Present | 46.16 | 42.11 | 1.10 | 10.34 | 18.08 | 24.44 |
| 25C63 | Tree       | Conical       | Oval                    | 3.70 | 2.35 | Present | Few       | 17.30 | Serrate   | Present | 41.49 | 42.31 | 0.98 | 12.04 | 30.03 | 19.74 |
| 25C64 | Semi-shrub | Conical       | Oval                    | 2.90 | 3.50 | Absent  | Thornless | 27.25 | Bicrenate | Present | 41.49 | 49.73 | 0.83 | 11.32 | 16.92 | 14.63 |
| 25C65 | Tree       | Spreading     | Inverted ovoid          | 2.80 | 3.10 | Present | Medium    | 39.50 | Serrate   | Present | 38.23 | 35.41 | 1.08 | 8.31  | 13.54 | 18.78 |
| 25C66 | Tree       | Semi drooping | Circular                | 3.90 | 4.95 | Absent  | Thornless | 6.75  | Serrate   | Present | 64.61 | 62.75 | 1.03 | 16.41 | 24.79 | 19.74 |
| 25C67 | Tree       | Vertical      | Inverted ovoid          | 4.60 | 5.70 | Absent  | Thornless | 6.00  | Bicrenate | Present | 45.33 | 44.71 | 1.01 | 10.71 | 21.08 | 14.93 |
| 25C68 | Tree       | Semi drooping | Inverted ovoid          | 6.90 | 6.80 | Absent  | Thornless | 11.75 | Bicrenate | Present | 50.96 | 42.20 | 1.21 | 11.55 | 16.28 | 12.21 |
| 25C69 | Tree       | Vertical      | Rectangular             | 4.10 | 3.90 | Absent  | Thornless | 15.50 | Crenate   | Present | 43.76 | 37.20 | 1.18 | 9.68  | 10.93 | 9.31  |
| 25C70 | Tree       | Conical       | Oval                    | 3.20 | 3.10 | Absent  | Thornless | 9.50  | Crenate   | Present | 53.58 | 44.99 | 1.19 | 12.30 | 19.24 | 18.23 |
| 25C71 | Semi-shrub | Semi drooping | Transversely elliptical | 3.43 | 5.90 | Absent  | Thornless | 18.25 | Serrate   | Present | 37.58 | 41.06 | 0.92 | 9.60  | 10.09 | 22.17 |
| 25C72 | Tree       | Semi drooping | Transversely elliptical | 3.40 | 5.10 | Absent  | Thornless | 8.00  | Crenate   | Present | 39.30 | 32.02 | 1.23 | 7.28  | 18.29 | 7.10  |
| 25C73 | Tree       | Vertical      | Inverted ovoid          | 3.90 | 2.85 | Absent  | Thornless | 21.00 | Crenate   | Present | 44.25 | 30.67 | 1.44 | 8.03  | 21.00 | 8.94  |
| 25C74 | Tree       | Conical       | Circular                | 5.80 | 3.80 | Absent  | Thornless | 7.75  | Serrate   | Present | 46.96 | 36.27 | 1.29 | 9.50  | 19.58 | 6.12  |
| 25C75 | Tree       | Spreading     | Circular                | 4.30 | 4.10 | Absent  | Thornless | 13.25 | Bicrenate | Present | 50.90 | 38.30 | 1.33 | 10.10 | 20.01 | 14.26 |
| 25C76 | Tree       | Vertical      | Oval                    | 5.20 | 4.10 | Present | Few       | 17.50 | Crenate   | Present | 46.61 | 35.86 | 1.30 | 6.79  | 21.06 | 13.04 |
| 25C77 | Semi-shrub | Drooping      | Transversely elliptical | 2.90 | 4.30 | Present | Medium    | 12.50 | Bicrenate | Present | 35.52 | 33.56 | 1.06 | 6.35  | 14.86 | 6.18  |



### 3<sup>rd</sup> International Symposium on Biodiversity Research

Erzurum, Turkey, 20 - 22 October 2021



|        |            |               |                         |      |      |         |           |       |           |         |       |       |      |       |       |       |
|--------|------------|---------------|-------------------------|------|------|---------|-----------|-------|-----------|---------|-------|-------|------|-------|-------|-------|
| 25C78  | Semi-shrub | Semi drooping | Circular                | 2.90 | 4.15 | Present | Many      | 14.00 | Bicrenate | Present | 50.94 | 43.37 | 1.17 | 12.23 | 20.13 | 26.58 |
| 25C79  | Tree       | Conical       | Inverted ovoid          | 5.70 | 3.55 | Absent  | Thornless | 19.50 | Bicrenate | Present | 45.20 | 54.04 | 0.84 | 14.89 | 26.87 | 8.04  |
| 25C80  | Semi-shrub | Spreading     | Transversely elliptical | 4.80 | 5.55 | Present | Few       | 13.25 | Serrate   | Present | 36.03 | 28.25 | 1.28 | 5.27  | 17.41 | 8.90  |
| 25C81  | Shrub      | Semi drooping | Semi-circular           | 3.05 | 3.05 | Present | Few       | 21.50 | Bicrenate | Present | 39.19 | 30.16 | 1.30 | 6.71  | 21.97 | 11.51 |
| 25C82  | Tree       | Spreading     | Circular                | 7.10 | 5.80 | Absent  | Thornless | 17.25 | Serrate   | Present | 39.17 | 31.78 | 1.23 | 5.93  | 11.48 | 5.94  |
| 25C83  | Semi-shrub | Spreading     | Circular                | 3.90 | 5.05 | Absent  | Thornless | 16.50 | Bicrenate | Present | 46.67 | 42.46 | 1.10 | 10.62 | 22.23 | 10.77 |
| 25C84  | Semi-shrub | Spreading     | Circular                | 3.10 | 3.20 | Present | Few       | 16.50 | Bicrenate | Present | 38.20 | 32.59 | 1.17 | 5.24  | 16.84 | 9.52  |
| 25C85  | Tree       | Conical       | Rectangular             | 3.93 | 3.50 | Present | Few       | 20.25 | Crenate   | Present | 36.33 | 29.49 | 1.23 | 5.11  | 15.24 | 11.62 |
| 25C86  | Tree       | Spreading     | Inverted ovoid          | 3.60 | 3.70 | Present | Few       | 23.50 | Crenate   | Present | 39.55 | 37.85 | 1.04 | 8.44  | 25.95 | 8.82  |
| 25C87  | Tree       | Vertical      | Rectangular             | 2.70 | 3.80 | Present | Few       | 16.25 | Crenate   | Present | 48.79 | 35.60 | 1.37 | 9.30  | 24.67 | 13.78 |
| 25C88  | Tree       | Vertical      | Inverted ovoid          | 5.50 | 5.50 | Absent  | Thornless | 11.75 | Bicrenate | Present | 39.14 | 29.08 | 1.35 | 6.14  | 12.00 | 7.16  |
| 25C89  | Tree       | Vertical      | Rectangular             | 8.10 | 3.05 | Absent  | Thornless | 20.25 | Bicrenate | Present | 43.54 | 31.84 | 1.37 | 7.38  | 18.10 | 12.28 |
| 25C90  | Tree       | Conical       | Inverted ovoid          | 7.30 | 4.40 | Absent  | Thornless | 1.50  | Crenate   | Present | 50.84 | 41.68 | 1.22 | 9.53  | 20.24 | 15.95 |
| 25C91  | Semi-shrub | Vertical      | Circular                | 4.50 | 3.80 | Absent  | Thornless | 14.25 | Crenate   | Present | 50.26 | 35.07 | 1.43 | 8.60  | 18.07 | 12.58 |
| 25C92  | Tree       | Falling       | Transversely elliptical | 8.50 | 5.90 | Absent  | Thornless | 8.00  | Crenate   | Present | 45.67 | 38.88 | 1.17 | 9.86  | 23.45 | 10.65 |
| 25C93  | Tree       | Conical       | Oval                    | 3.20 | 3.00 | Absent  | Thornless | 23.25 | Crenate   | Present | 52.77 | 40.24 | 1.31 | 9.89  | 17.71 | 8.83  |
| 25C94  | Semi-shrub | Spreading     | Circular                | 3.80 | 5.20 | Absent  | Thornless | 11.75 | Bicrenate | Present | 47.72 | 40.47 | 1.18 | 10.11 | 20.76 | 13.68 |
| 25C95  | Semi-shrub | Spreading     | Rectangular             | 6.15 | 4.05 | Present | Few       | 13.75 | Crenate   | Present | 38.89 | 34.52 | 1.13 | 6.49  | 15.87 | 6.79  |
| 25C96  | Shrub      | Falling       | Transversely elliptical | 6.15 | 8.05 | Absent  |           | 29.75 | Biserrate | Present | 47.60 | 42.56 | 1.12 | 14.47 | 23.16 | 22.44 |
| 25C97  | Semi-shrub | Drooping      | Transversely elliptical | 5.30 | 6.15 | Absent  | Thornless | 19.50 | Crenate   | Present | 56.43 | 48.11 | 1.17 | 14.70 | 23.55 | 7.10  |
| 25C98  | Tree       | Vertical      | Oval                    | 4.70 | 3.10 | Absent  | Thornless | 14.00 | Crenate   | Present | 43.96 | 44.12 | 1.00 | 9.59  | 20.96 | 11.44 |
| 25C99  | Tree       | Vertical      | Rectangular             | 3.50 | 3.10 | Absent  | Thornless | 17.00 | Crenate   | Present | 41.25 | 32.83 | 1.26 | 5.58  | 15.92 | 6.81  |
| 25C100 | Semi-shrub | Spreading     | Inverted ovoid          | 3.40 | 2.90 | Present | Few       | 11.75 | Crenate   | Present | 37.03 | 33.80 | 1.10 | 6.89  | 20.14 | 4.41  |
| 25C101 | Semi-shrub | Spreading     | Inverted ovoid          | 5.10 | 4.50 | Absent  | Thornless | 11.00 | Bicrenate | Present | 35.16 | 24.02 | 1.46 | 5.87  | 8.03  | 4.61  |

Oral Presentation

Thursday

Diversity of Plant Species, Systematics and Phylogeny-3

Comparison of ATR-FTIR Spectra on Two Endemic Species of *Asperula* L. (Rubiaceae)

Growing at the Same Substrate in Turkey

Ayşenur Kayabaş<sup>1\*</sup>, Ertan Yıldırım<sup>2</sup><sup>1</sup>Çankırı Karatekin University, Faculty of Science, Department of Biology, Çankırı, Turkey.<sup>2</sup>Gazi University, Faculty of Science, Department of Chemistry, Ankara, Turkey.

\*Corresponding author e-mail: aysenurkayabas@karatekin.edu.tr

**Abstract**

The substrate factor is very substantial for plant growth in gypsum habitats that host rare and specialized plant species. In extreme gypsum habitats, chemical limitations such as low nutrient concentrations, high sulphate (S) and calcium (Ca) content, along with physical limitations caused by the substrate factor, affect the plants. Here, a detailed ATR-FTIR (attenuated total reflection-fourier transform infrared) spectroscopic examination of two different *Asperula* L. (Rubiaceae) taxa growing in gypsum habitats was performed. The ATR-FTIR spectra of the vegetative and generative parts of *Asperula bornmuelleri* Velen. and *Asperula cankiriense* B. Şahin & Sağıroğlu were examined both within themselves and by comparing the plant parts of two different taxa. The specific chemical bands of the plant parts of *Asperula* taxa grown on the same extreme substrate were similar, but the band intensities of the root, stem, leaf, and flower (sepal, petal) parts of the same species and the band intensities between the two taxa differed. This reflects that two different *Asperula* taxa grown on gypsum substrate are similar in chemical diversity but differ in band intensities. As a result, in the ATR-FTIR analysis, the components of two plant species growing on the same substrate and the quantitative analysis of these components were made for the first time in this study, and the functional structures of the plants were determined. When the ATR-FTIR technique is evaluated in this context, it is thought that this study will shed light on future chemical component studies, as it has the potential to be used in both quantitative and qualitative analyzes of phytochemical components in plants, as well as being cheap and convenient.

**Keywords:** *Asperula*, endemic, fourier transform infrared spectroscopy, gypsum stress

Oral Presentation  
Thursday

Biodiversity, Landscape, Tourism-1, Environmental Toxicology-2

**Monitoring the Dynamics of the Area of Lake Azegza (Middle Atlas-Morocco) in the Context of Climate Change Using the Techniques of Space Remote Sensing.**Amal Raillani<sup>1\*</sup>, Lahsen Chillasse<sup>1</sup>, Mhamed Khaffou<sup>2</sup><sup>1</sup>Moulay Ismail University, Faculty of Sciences, Department of Biology, Meknes, Morocco.<sup>2</sup>Sultan Moulay Sliman University, Technology High School, Department of Environmental Engineering, Khenifra, Morocco.

\*Corresponding author e-mail: amaleddt@gmail.com

**Abstract**

Located in the Moroccan Middle Atlas, the Aguelmam Azegza Lake is a natural lake of karstic origin. This permanent depression about 25 m deep is mainly fed by the water table and snowmelt, in addition to springs gushing into the lake itself. In the context of climate change, and in order to understand the response of this aquatic ecosystem to these changes and to develop their possible impacts on the evolution of the lake's surface, our study aims to monitor the dynamics of the surface of Lake Aguelmam Azegza over the past fifty years, using Earth observation techniques and in situ climate data. The surface dynamics of Lake Azegza have been monitored using remote sensing methods. Landsat images from 1975 to 2018 were used. To achieve this, a set of Landsat images were acquired and processed. The boundary of the lake was mapped using Normalized Difference Water Index (NDWI) and Modified Normalized Difference Water Index (MNDWI) where the histogram threshold segmentation method was used to extract water pixels. The overall precision and Kappa coefficient were calculated to assess the accuracy of the results. The climate data series were subjected to statistical processing to define climate variability and its historical trends at the lake watershed scale. The results indicate an intense decreasing trend in the lake over the period 1975-1995 from 65.05 ha to 22.5 ha. Over the period 1995-2018, the results show a progressive increasing trend in the reference level, reaching 38 ha. The study attempts to investigate the probable causes of the dynamics of the lake surface using Earth observation techniques and in situ data, with the objective of giving strong scientific arguments that will be useful in the process of the preservation of mountain lakes.

**Keywords:** Aguelmam Azegza Lake, middle atlas, landsat imagery, water index, climate change



Oral Presentation

Thursday

Biodiversity, Landscape, Tourism-1, Environmental Toxicology-2

## Crop Raiding by Wildlife of The Neighbouring Conservation Area on Subsistence Homesteads in Northern Kwazulu-Natal Province, South Africa

Tlou Raphela<sup>1\*</sup>, Pillay Neville<sup>2</sup>

<sup>1</sup>Disaster Management Training and Education Centre for Africa, University of the Free State, Bloemfontein, South Africa.

<sup>2</sup>School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

\*Corresponding author e-mail: madeizen@gmail.com

### Abstract

Globally, human-wildlife conflict often arises from crop raiding. Therefore, there is a need to quantify crop damage by the suspected wildlife species around protected areas. We assessed and quantified crop damage by wildlife on subsistence farms on the edge of the Hluhluwe Game Reserve, northern KwaZulu-Natal, South Africa. Twenty farms were assessed monthly from April 2016 to March 2017, using direct observations of wildlife, detectable evidence of their consuming crops and remote camera trap footage of their presence. We recorded the animals involved in raiding, crops affected, and differences in the level of crop damage by season and farm proximity to the reserve boundary. Rodents, arthropods (mainly insects) and birds were found to feed on crops on the 20 farms, with rodents causing the highest levels of crop damage as compared to the other animals. Contrary to expectations, primates (vervet monkey *Chlorocebus pygerythrus* and chacma baboons *Papio ursinus*) were not identified as raiders during my study, since these species never left the reserve to raid farms. However, camera trap footage showed that both primate species engaged in feeding behaviour on the inside boundary edge of the reserve (close to farms) during the dry season. Maize (*Zea mays*) was the main affected crop throughout the study. The highest level of crop damage was during the dry season compared to the wet season. The distance of farms from the reserve was not a significant predictor of the level of crop damage in the farms sampled, contrary to the findings of other studies, which mentioned that crop raiding decreases away from the protected area boundary. Using systematic trapping, crop assessment and observation, our study showed that small rather than larger animals from the neighbouring conservation area were the main crop raiders during sampling period and that maize was the most affected crop, especially during the dry season.

**Keywords:** Camera trap survey, crop raiding, human-wildlife conflict, primates, rodents

Oral Presentation

Thursday

Biodiversity, Landscape, Tourism-1, Environmental Toxicology-2

**Determination of The Acute Effects of Olive Mill Wastewater on *Potamopyrgus Antipodarum*, *Melanopsis Buccinoidea* and *Theodoxus Sp.* (Gastropoda: Tetaidae: Melanopsidae: Neritidae)**

Deniz Anıl Odabaşı<sup>1\*</sup>, Aytuğ Zilifli<sup>2</sup>, Sevdan Yılmaz<sup>3</sup>

<sup>1\*</sup>Department of Marine and Inland Water Sciences, Faculty of Marine Science and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

<sup>2</sup>School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

<sup>3</sup>Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart University, Çanakkale, Turkey

### Abstract

Olive mill wastewater (OMW), which causes environmental problems in aquatic ecosystems in our country and especially in Mediterranean countries, has negative effects on aquatic organisms. In this study, acute effects of OMW were experimentally investigated on three freshwater snails; *Potamopyrgus antipodarum*, *Melanopsis buccinoidea*, and *Theodoxus sp.* Freshwater gastropods selected as the experiment organism were transferred from their natural habitat to the laboratory and their adaptations were provided as separate taxa. OMW that was used as the active substance in various ratios for this experiment was obtained from a company that produces olive oil in Çanakkale Province. To determine the acute effects of OMW on the target organisms, OMW was introduced in different ratios into the test tanks which have twenty individuals. According to the findings, LC50 values of the OMW on *Potamopyrgus antipodarum* (for 96 hours), *Melanopsis buccinoidea* (for 48 hours), and *Theodoxus sp.* (for 48 hours) were calculated 9.51%, 6.40%, and 5.08%, respectively.

**Keywords:** Olive mill wastewater, toxicity, acute effects, Gastropoda, freshwater

Oral Presentation

Thursday

Biodiversity, Landscape, Tourism-1, Environmental Toxicology-2

***In Vivo* Biotoxic Effects of Synacryl Black Xfdl Textile Dye on Larval Viability and  
Lifespan in *Drosophila melanogaster* Oregon-R**

Emine Öztürk<sup>1\*</sup>, Handan Uysal<sup>1</sup>

<sup>1</sup>Atatürk University, Faculty of Science, Department of Biology, 25240, Erzurum, Turkey

emine.ozturk@avrasya.edu.tr, hauysal@atauni.edu.tr

\*Corresponding author e-mail: hauysal@atauni.edu.tr  
(ORCID: 0000-0002-6092-9212, 0000-0002-4290-8223)

### Abstract

Synthetic dyestuffs are produced in many countries, approximately 10,000 types and 700,000 tons per year, and are used in many areas such as textile, cosmetics, food, automotive, medicine and furniture. The textile industry also constitutes an important part of the entire industrial waste amount (1/5) with the use and discharge of dyestuffs. The flow of waste water from the textile industry to agricultural areas causes the soil pores to become clogged and the yield decreases, and its flow to aquatic areas causes drinking water to become unsuitable for human consumption. In this study, the biotoxic effect of Synacryl Black XFDL (SBXFDL), used as a textile dye, on larval viability and lifespan in female and male populations in *Drosophila melanogaster* Oregon-R wild strain was investigated *in vivo*. Standard *Drosophila* Medium (SDM) prepared with distilled water was used for the control groups. Different doses (10, 20, 40, 60 and 80 ppm) of SBXFDL were added to the SDM for the treatment groups. In order to evaluate the larval viability, the development of the 3<sup>rd</sup> stage larvae in the application groups was followed and it was observed that the larval viability decreased significantly ( $p<0.05$ ). In the longevity study, adult individuals of the same age were placed in the media prepared with different doses of SBXFDL (10, 20, 40, 60 and 80 ppm) and their lifespans were followed. It was determined that both the maximum and mean life span of *D. melanogaster* were shortened depending on the increasing dose in male and female populations of Oregon-R wild strain. When the data belonging to the application groups were compared with the control groups, the difference between them was found to be statistically significant at the  $p<0.05$  level.

**Keywords:** Synthetic dye, larval viability, lifetime, biotoxic

## INTRODUCTION

Dyestuffs are a colorful danger that has been in our lives from past to present, as it is dyeing wool and cotton, as well as clothing and upholstery. Dyestuffs obtained from natural sources in ancient times began to be produced synthetically, as tar and petroleum-derived, with the industrial revolution. Textile dyes, which are a group of dyestuffs, are produced mostly in the USA, China, India and the Middle East countries, approximately  $7 \times 10^5$  tons per year. These paints are used in many fields such as printing, automotive, machinery, construction, glass/porcelain, medicine and cosmetics, especially in the textile industry (Kurbanova *et al.*, 1998). In the analyzes made, it has been determined that there are dyestuffs and chemicals that help dyeing, especially in the wastewater of the textile industry (de Oliveira *et al.*, 2016). Between 2% and 50% of the dyes used are given to the receiving environment and these rates constitute 1/5 of the total wastewater pollution in the world. Wastes from the textile industry are left to aquatic ecosystems such as drinking water channels, rivers, lakes, seas, and land ecosystems such as fields. The dyes in the wastes increase the turbidity in aquatic ecosystems and give the water a bad appearance and odor. Mutagenicity tests with wastewater samples taken from areas exposed to this type of pollution have shown that textile industry wastes can pose a moderate risk in different living groups (Mathur *et al.*, 2005). The dyestuffs in the aquatic environment are separated into their components with the help of crustaceans and fish, and the toxic/genotoxic substances that are released affect organs such as liver, gills, skin and the systems associated with these organs by bioaccumulation. Humans are another group most affected by these toxic components through the food chain (Şenel *et al.*, 2012). In this study, the biotoxic effects of Synacryl Black XFDL (SBXF DL) textile dye on larval viability and lifespan in Oregon-R strain of *Drosophila melanogaster* were investigated.

## MATERIALS AND METHODS

Synacryl Black XFDL (SBXF DL) dye at different concentrations (10, 20, 40, 60 and 80 ppm) was added to SDM and treatment groups were formed. Control groups were prepared using distilled water only. For larval viability test, ♂10 X ♀10 cross of *D.melanogaster* Oregon-R strain was made and 3<sup>rd</sup> stage larvae were collected. Then, 100 3<sup>rd</sup> stage larvae were placed in the media of the control and application groups and the daily development of the larvae was followed. For the *in vivo* longevity test, controlled crossovers (P: ♂10 X 10♀) were also made. Male and female individuals of the same age, 1-3 days old, belonging to the F1 generation were collected separately for three days and every four hours by making male-female distinction. 100 ♀ and 100 ♂

individuals were placed separately in the control groups and application containing SBXFDL dye at different concentrations (10, 20, 40, 60 and 80 ppm). All individuals in the treatment groups were transferred to fresh medium suitable for their initial concentrations twice a week. The number of individuals who died during this transfer was also determined. Both larval viability and longevity experiments were carried out in heated-cooled incubators at 25±1 °C, 60% relative humidity and continuous dark conditions. All experiments were performed in triplicate. The results of the application and control groups of larval mortality were compared with the One-Way ANOVA test. Tukey one-way variance and Duncan multi-way comparison test were used to compare the maximum and mean lifespan data obtained from the longevity experiments.

## RESULTS

According to the data obtained from the study, SBXFDL textile dye decreased larval viability in the Oregon-R wild strain of *D.melanogaster* due to dose increase and chronic application in all application groups (Table 1). The mean larval viability in the control group (no.1) was 96.33±0.88/larva. This value was determined as 67.33±1.20/larva at 10 ppm (no.2) SBXFDL application group and 45.33±1.45/larva at 80 ppm (no.6). The decrease in larval viability observed due to dose increase in all SBXFDL application groups (10-80 ppm) was found to be statistically significant at the  $p<0.05$  level (Table 1).

**Table 1.** Statistical evaluations of larval viability and larval mortality values in 3<sup>rd</sup> stage larvae of *D.melanogaster* exposed to SBXFDL textile dye.

| Application groups | N   | Synacryl Black XFDL (SBXFDL) |     |     | Σ viability and mortality (%) | Mean±SE                   | P-value |
|--------------------|-----|------------------------------|-----|-----|-------------------------------|---------------------------|---------|
|                    |     | (1)                          | (2) | (3) |                               |                           |         |
| Control (SDM no.1) | 100 | 98                           | 96  | 95  | 96,3 (%3,7)                   | 96,33±0,88 <sup>a</sup>   |         |
| SDM+10 ppm (no.2)  | 100 | 67                           | 64  | 68  | 67,3 (%36,7)                  | 67,33±1,20 <sup>b</sup>   | <0,05   |
| SDM+20 ppm (no.3)  | 100 | 60                           | 62  | 59  | 60,3 (%39,7)                  | 60,33±0,88 <sup>b,c</sup> | <0,05   |
| SDM+40 ppm (no.4)  | 100 | 54                           | 59  | 58  | 57,0 (%43,0)                  | 57,00±1,52 <sup>c</sup>   | <0,05   |
| SDM+60 ppm (no.5)  | 100 | 53                           | 54  | 50  | 52,3 (%47,7)                  | 52,33±1,20 <sup>c,d</sup> | <0,05   |
| SDM+80 ppm (no.6)  | 100 | 43                           | 45  | 48  | 45,3 (%54,7)                  | 45,33±1,45 <sup>d</sup>   | <0,05   |

N: Number of larvae, \* The difference between the values given with different letters is significant at the <0.05 level.

SBXFDL textile dye also affected longevity in male and female populations of the Oregon-R wild strain of *D. melanogaster*. In the control group, the maximum lifespan was 89 days in the ♀ population and 80 days in the ♂ population. Maximum lifespan was determined as 73 and 56 days in the ♀ population and 66 and 49 days in the ♂ population, respectively, in the lowest and highest SBXFDL application groups (10-80 ppm) (Table 2). In the present study, the mean lifespans of

male and female populations were also determined. The mean lifespan of the control group was  $60.97 \pm 1.57$ /days in the ♀ population, and  $51.60 \pm 1.61$ /days in the ♂ population. In the lowest and highest SBXFDL application groups (10-80 ppm), the mean lifespan for the ♀ population was  $45.90 \pm 1.38$  and  $31.71 \pm 1.17$ /days, respectively; It was calculated as  $43.45 \pm 1.18$  and  $28.37 \pm 1.19$ /days for the ♂ population (Table 2). The mean lifespan data obtained from the control and treatment groups were compared with each other statistically, and the difference between both populations was found to be statistically significant ( $p < 0.05$ ). According to the mean lifespan data, the regression level was calculated as  $R = -0.549$  for ♀ and  $R = -0.514$  for ♂.

**Table 2.** Comparison of the effects of SBXFDL on maximum and mean longevity in female and male populations of *D.melanogaster*.

| Synacryl Black XFDL (SBXFDL) |     |              |                  |  |     |              |                  |              |  |
|------------------------------|-----|--------------|------------------|--|-----|--------------|------------------|--------------|--|
|                              |     | ♀♀           |                  |  |     | ♂♂           |                  |              |  |
| Application groups           | N   | ML           | Mean lifespan±SE | P-value                                    | N   | ML           | Mean lifespan±SE | P-value      |  |
| Control (no 1)               | 100 | 89           | $60,97 \pm 1,57$ |  | 100 | 80           | $51,60 \pm 1,61$ |              |  |
| 10 ppm (no 2)                | 100 | 73           | $45,90 \pm 1,38$ | 1-2,3,4,5,6*<br>2-5,6*<br>3-5,6*<br>4-5,6* | 100 | 66           | $43,45 \pm 1,18$ | 1-2,3,4,5,6* |  |
| 20 ppm (no 3)                | 100 | 70           | $45,26 \pm 1,42$ |  | 100 | 63           | $39,69 \pm 1,23$ | 2-4,5,6*     |  |
| 40 ppm (no 4)                | 100 | 66           | $42,09 \pm 1,32$ |  | 100 | 60           | $37,79 \pm 1,08$ | 3-5,6*       |  |
| 60 ppm (no 5)                | 100 | 63           | $33,44 \pm 1,24$ |  | 100 | 53           | $30,91 \pm 1,22$ | 4-5,6*       |  |
| 80 ppm (no 6)                | 100 | 56           | $31,71 \pm 1,17$ |  | 100 | 49           | $28,37 \pm 1,19$ |              |  |
| Regression Level             |     | $R = -0,549$ |                  |  |     | $R = -0,514$ |                  |              |  |

N: Number of individuals, ML: Maximum lifespan, \*The difference between the values given in the same column is significant at the  $< 0.05$  level.

## DISCUSSION

SBXFDL dye, which is one of the water-soluble cationic dyestuffs that is frequently used in the textile industry, decreased larval viability and increased mortality in the Oregon-R wild strain of *D.melanogaster* (Table 1). In the larvae that were active and willing to feed, first immobility and intense pigmentation were observed, followed by mass death. Malformations such as deformed wings, reduction in total body size and incomplete formation in the thorax segments have been observed in adult individuals who can complete the metamorphosis. In addition, it was also determined that this dye shortened the maximum and mean lifespan in female and male individuals of the same strain (Table 2). Lifespan shortening has also been considered as population aging. In the literature, there are studies related to the effects of textile dyes on different organisms. In a study, it was determined that some textile dyes cause malformations in *Xenopus leavis* embryos (Birhanlı and Özmen, 2005). According to Hernandez-Zamora and Martinez-Jeronimo (2019), Congo Red textile dye prevents egg hatching in *Danio rerio* and causes cardiac and skeletal anomalies in developing adults. Reactive Blue 203 (RB203) and Maxillon Blue 5G textile dyes

also caused developmental anomalies such as microphthalmia, pericardial edema and curved body structure and genotoxicity in *D. rerio* embryos (Köktürk *et al.*, 2021). In previous studies, it was determined that different textile dyes both reduced the survival rate (Şahin and Türkoğlu, 2014) and stimulated somatic mutations in *D. melanogaster* (Vogel and Nivard, 1993; Eroğlu Doğan 2002; Özata 2006). Direct Black 38 (DB38) and Reactive Blue 15 (RB15) dyes also caused DNA damage and oxidative damage in *Daphnia magna* (de Olivera *et al.*, 2018). Dyes are organic compounds with complex chemical structure. These substances can react with many disinfectants to form carcinogenic products (de Oliveira *et al.*, 2016). The World Health Organization has defined the degradation products of textile dyes such as benzidine, phenylenediamine, aniline, aromatic amine as toxic and carcinogenic (Lourenco *et al.*, 2001).

In our opinion, biodegradation products of dyes can induce malformations by causing homeotic gene or regulatory gene mutations as "genomic destabilizing agents". In addition, the increase in mortality rates, especially in high-dose applications, suggests the possibility of mutations in vital genes. The fact that somatic mutations have been observed in different studies confirms the "Mutation Accumulation Theory" of aging. Again, the fact that biodegradation products cause oxidative damage is compatible with the "Free Radical Theory of Aging" of aging. Decomposition products of textile dyes can cause damage by binding to bases in DNA. The increase of hydroxyl and superoxide radicals and reactive oxygen species such as hydrogen peroxide can affect the structure of amino acids, causing misfolding and changes in protein conformations (Ulian *et al.*, 2013). The presence of products such as malondialdehyde, protein carbonyl and 8-hydroxyguanine, which are released by the effects of free radicals, may cause premature death and population aging in organisms, as in this study.

## ACKNOWLEDGEMENT

This study was produced from the master thesis prepared by the first author under the supervision of the second author.

## REFERENCES

- Birhanlı, A., and Özmen, M. (2005). Evaluation of the toxicity and teratogenicity of six commercial textile dyes using the frog embryo teratogenesis assay–*Xenopus*. *Drug and Chemical Toxicology*, 28(1), 51-65.
- de Oliveira, G. A. R., de Lapuente, J., Teixidó, E., Porredón, C., Borràs, M., and de Oliveira, D. P. (2016). Textile dyes induce toxicity on zebrafish early life stages. *Environmental Toxicology and Chemistry*, 35(2), 429-434.
- de Oliveira, G. A. R., Leme, D. M., de Lapuente, J., Brito, L. B., Porredón, C., de Brito Rodrigues, L., Brull, N., Serret, J. T., Borràs, M., Disner, G. R., Cestari, M. M., and de Oliveira, D. P. (2018). A test battery for assessing the ecotoxic effects of textile dyes. *Chemico-Biological Interactions*, 291, 171-179.
- Eroğlu Doğan, E. (2002). Bazı Astrazon Grubu Tekstil Boyalarının Genotoksik Etkisinin *Drosophila melanogaster*

- Somatik Mutasyon ve Rekombinasyon Testi (SMART) ile Araştırılması. Yüksek Lisans Tezi, İnönü Üniversitesi Fen Bilimleri Enstitüsü, Malatya.
- Hernández-Zamora, M., and Martínez-Jerónimo, F. (2019). Congo red dye diversely affects organisms of different trophic levels: a comparative study with microalgae, cladocerans, and zebrafish embryos. *Environmental Science and Pollution Research*, 26(12), 11743-11755.
- Köktürk, M., Altındağ, F., Ozhan, G., Çalimli, M. H., and Nas, M. S. (2021). Textile dyes Maxilon blue 5G and Reactive blue 203 induce acute toxicity and DNA damage during embryonic development of *Danio rerio*. *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 242, 108947.
- Kurbanova, R., Okudan, A., Mirzaoglu, R., Kurbanov, S., Karatas, I., Ersöz, M., Özcan, E., Ahmedova, G., and Pamuk, V. (1998). Effects of the functional groups of polystyrene on its adhesion improvement and corrosion resistance. *Journal of Adhesion Science and Technology*, 12(9), 947-955.
- Mathur, N., Bhatnagar, P., Nagar, P., and Bijarnia, M. K. (2005). Mutagenicity assessment of effluents from textile/dye industries of Sanganer, Jaipur (India): A case study. *Ecotoxicology and Environmental Safety*, 61(1), 105-113.
- Özata, L. (2006). Bazı Tekstil Boyalarının *Drosophila melanogaster* Üzerine Toksik ve Genotoksik Etkilerinin Araştırılması. Doktora Tezi, İnönü Üniversitesi Fen Bilimleri Enstitüsü, Malatya.
- Şahin, N., ve Türkoğlu, Ş. (2014). Bazı tekstil boyaalarının *Drosophila melanogaster*'de ömür uzunluğu, yaşama yüzdesi ve yavru birey sayısına etkileri. *Cumhuriyet Üniversitesi, Fen Edebiyat Fakültesi, Fen Bilimleri Dergisi*, 35(4), 73-93.
- Şenel, U., Sur, H. I., ve Demirtas, M. (2012). Tekstil Endüstrisinde Kullanılan Bazı Sentetik Reaktif Boyarmaddelerin Mutajenik Etkisinin Umu-Test İle Araştırılması. *Ekoloji*, 21(85), 49-56.
- Ulian, G., Valdrè, G., Corno, M., and Ugliengo, P. (2013). The vibrational features of hydroxylapatite and type a carbonated apatite: A first principle contribution. *American Mineralogist*, 98(4), 752-759.
- Vogel, E. W., and Nivard, M. J. (1993). Performance of 181 chemicals in a *Drosophila* assay predominantly monitoring interchromosomal mitotic recombination. *Mutagenesis*, 8(1), 57-81.



Oral Presentation

Thursday

Diversity of Animal species, Systematics and Phylogeny-3

**New Mite (Acari: Erythraeoidea) Records from Turkey**İbrahim Karakurt<sup>1\*</sup>, Sevgi Sevsay<sup>2</sup>

<sup>1</sup>Department of Home Care, Vocational School of Health Services, Erzincan Binali Yıldırım University, Erzincan, Turkey

<sup>2</sup>Department of Biology, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University, Erzincan, Turkey

\*Corresponding author e-mail: [ibrahim.karakurt@erzincan.edu.tr](mailto:ibrahim.karakurt@erzincan.edu.tr)

**Abstract**

The superfamily Erythraeoidea Grandjean, 1947 is a group that is very rich in terms of species diversity and has a wide distribution worldwide. Erythraeoidea includes two large families: Erythraeidae Robineau-Desvoidy, 1828 and Smarididae Vitzthum, 1929. The number of erythraeoid mites recorded from Turkey is relatively low. This study is the first report on mites of the superfamily Erythraeoidea living in Bayburt Province (Turkey). The mite specimens were collected from Bayburt Province, Turkey. The samples were caught with extraction in Berlese funnels using % 70 ethanol containers. Examined material was preserved in 70% ethyl alcohol and cleaned in 9% KOH solution. The specimens were fixed on slides in Hoyer's medium. As a result of the examinations, two erythraeoid species were identified: *Leptus (L.) molochinus* (C.L. Koch, 1837) (adult) and *Hirstiosoma ampulligera* (Berlese, 1887) (adult). *Leptus (L.) molochinus* was recorded as the first adult species for the genus in Turkey. Also, *Hirstiosoma ampulligera* was recorded as the first species for the genus in Turkey. In the present work, it is aimed to contribute to the knowledge on distribution of erythraeoid mites.

**Keywords:** Acarology, parasitengona, new record, distribution

**Acknowledgement:** This work was mainly funded by Scientific Research Project (BAP) number FEN-A-140613-0026 (Scientific Research Department of Erzincan Binali Yıldırım University).

Oral Presentation

Thursday

Diversity of Animal species, Systematics and Phylogeny-3

#### Determination of The Chromosome Number of The *Trombidium holosericeum* for The First Time

Rümeysa Karagaç<sup>1\*</sup>, Halil Erhan Eroğlu<sup>2</sup>, Evren Buğa<sup>3</sup>, Sevgi Sevsay<sup>4</sup>

<sup>1</sup>Institute of Science, Department of Biology, Erzincan Binali Yıldırım University, Turkey.

<sup>2</sup>Faculty of Science and Literature, Department of Biology, Yozgat Bozok University, Turkey.

<sup>3</sup>İliç Dursun Yıldırım Vocational School, Medical Laboratory Techniques Program, Erzincan Binali Yıldırım University, Turkey

<sup>4</sup>Faculty of Science and Literature, Department of Biology, Erzincan Binali Yıldırım University, Turkey

\*Corresponding author e-mail: r.karagac2515@gmail.com

#### Abstract

Cytogenetic data are available for some mites in the suborders, Mesostigmata, Astigmata, Cryptostigmata and Prostigmata, but no information is available for mites of the family Trombidiidae. To date, no study has been conducted on the chromosome numbers of the Trombidoidea superfamily. In this study, *Trombidium holosericeum* of belonging to trombidiid mites, which has been studied morphologically and taxonomically, but no cytologically, was examined, this chromosome numbers, monoploid idiograms and chromosomal measurements were made. The cytogenetic protocol modified by Imai et. al. (1988) and Gokhman Quicke (1995) was used as a method for this study. All dissections were performed in a small drop of hypotonic sodium citrate solution (1 g Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>C<sub>7</sub>.2H<sub>2</sub>O in 100 ml distilled water). Tissues were placed in a colchicine solution (% 0.01 mg in 100 ml Earle's Minimum Essential Medium) and incubated at room temperature for 10-20 min. Tissues were transferred into a small drop of %45 acetic acid on a siliconized cover slip for 20-30 s. A drop of lacto-acetoorcein [1-part concentrated orcein stain with 3 parts (1:1) lacto-acetic acid] was then added and mixed with the fixative. A clean slide was placed onto the siliconized cover slip and the preparation was air-dried. The mitotic chromosomes of *T. holosericeum* was found to be 2n=12. *T. holosericeum* has a total haploid chromosome length of 8.31 µm, an average chromosome length of 1.38 µm. Chromosome number studies on mites found a wide variety of chromosome numbers. In general, mites and ticks have been reported to have 2n=2 to 36 chromosomes. Comparison could not be made because there is no other study on trombidioid.

**Keywords:** Actinotrichida, cytogenetic, karyotype, Trombidiidae

**Acknowledgement:** This research was supported by Erzincan Binali Yıldırım University Scientific Projects Coordinatorship with the project number FYL-2020-748.

Oral Presentation

Thursday

Diversity of Animal species, Systematics and Phylogeny-3

**Parasitism Relationship of Trombidioidea Mites with Spiders**Evren Buğ̃a<sup>1\*</sup>, Sevgi Sevsay<sup>2</sup><sup>\*1</sup>Medical Laboratory Techniques Program, İliç Dursun Yıldırım Vocational School, Erzincan Binali Yıldırım University, Erzincan, Turkey<sup>2</sup>Biology Department, Sciences and Arts Faculty, Erzincan Binali Yıldırım University, Erzincan, Turkey\*Corresponding author e-mail: [evren.buga@erzincan.edu.tr](mailto:evren.buga@erzincan.edu.tr)**Abstract**

Parasitengona is one of the largest and most diverse mite groups. These mites are ectoparasites on different arthropods when they are in larva stages, and predatory in their active, postlarval stages (adults and deutonymphs). Trombidioidea has three active life stages: larva, deutonymph, and adult. Of these, deutonymph and adult stages are four-legged and feed as a predator. Larvae are three-legged and obligate parasites. After the larvae hatch, they seek an arthropod host to absorb bodily fluids. They prefer members of nearly all Insecta orders and many Chelicerata as hosts and feed on them. The activity periods of the mite species (ovulation and hatching periods, which are very variable according to the species) and the preferred host period (molting, wing formation, etc.) determine the parasite-host relationships. When the larvae complete their feeding, their body volume increases, then they separate themselves from the host and move on to the next stage. Since they are transported by the host during the feeding process, their habitat diversity increases. In case of attachment of too many larvae, it has the potential to negatively affect the vital activity of the host. This study is an evaluation of the host spider species preferred by thrombidid mite larvae, published papers and our studies. As a result of the studies carried out; To date, 17 spider families have been shown to be parasitized by 17 different species of trombidoid mites. In addition, in eight of these studies, the mites found on spiders were given as a new record for the scientific world, and the last record from our country is *Trombidium demirsoyi* Sevsay & Buğ̃a 2020. At the same time, in this study, a parasitism record of the spider family Zodariidae was given for the first time by a mite. These studies are very important in biological control and biodiversity due to the effects and relationships of mites on spiders.

**Keywords:** Acari, Araneae, association, parasitism, Trombidioidea

Oral Presentation

Thursday

Diversity of Animal species, Systematics and Phylogeny-3

### New Locality Records of Trombidoid Mites (Acari: Prostigmata) from Sansa George (Turkey)

Evren Buğ<sup>1\*</sup>, Sevgi Sevsay<sup>2</sup>

<sup>\*1</sup>Medical Laboratory Techniques Program, İliç Dursun Yıldırım Vocational School, Erzincan Binali Yıldırım University, Erzincan, Turkey

<sup>2</sup>Biology Department, Sciences and Arts Faculty, Erzincan Binali Yıldırım University, Erzincan, Turkey

\*Corresponding author e-mail: evren.buga@erzincan.edu.tr

#### Abstract

Trombidoid mites, also known as velvet mites, have a very important place in the food chain that have adapted to different habitats. Habitat preferences of velvet mites vary at the family level. Of the 14 families identified to date, these families include groups that prefer different habitats; there are species that prefer a fully aquatic habitat, semi-aquatic, arid or a special plant habitat. Body structures, target prey, host preferences and distribution possibilities are effective in these habitat preferences. All these vary at the family level. For all these reasons, velvet mites are found in many different habitats, especially in places where human destruction is low. In our country, research on this living group has been gaining momentum in recent years and new locations of known species have been introduced to the scientific world with new species definitions.

The aim of this study was to evaluate the trombidoid mites collected from Sansa George (Erzincan/Turkey) between the years of 2018-2019 and to provide information about zoogeographic and working area distribution, and habitats of these species. In this context, mossy, grassy soil and different debris samples from different localities were placed in plastic bags and brought to the laboratory. The brought samples were placed in the sorting device consisting of Berlese funnels. In addition, live animals were collected in nature by hand and with the help of an aspirator. These collected specimens were put into living bottles and kept waiting for the mite to spawn. The descriptions of these species were made and detailed photographs of the identification characters were taken with an Olympus BX63 microscope. As a result of this study, a total of 20 taxa belonging to 5 families were recorded for the first time from the new localities, Sansa George and its immediate surroundings.

**Keywords:** Acari, new locality, Sansa George, Trombidioidea, Turkey

**Acknowledgement:** This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) with project number 217Z184.

Oral Presentation

Thursday

Aquatic (Marine and Freshwater) Biodiversity-1

**Contribution to the Water Mite Fauna of Bingöl Province, Turkey (Acari, Hydrachnidia)**Yunus Esen<sup>1\*</sup><sup>1\*</sup>Solhan Vocational School of Health Services, Bingöl University, Bingöl, Turkey.**Abstract**

This study provides new records of water mites from running waters of Bingöl Province (eastern Turkey) and an updated list of Turkish provinces. Seven species i.e. *Panisopsis setipes* (Viets, 1911), *P. thori* (Walter, 1907), *Thyopsis cancellata* (Protz, 1896), *Tadjicothyas connexa schwoerbelii* Oezkan, 1988, *Nilotonia vietsi* Bader & Sepasgozarian, 1980, *Lebertia sefvei* Walter, 1911 and *Atractides (Polymegapus) persica* Pešić & Asadi, 2010 have been registered as new for the water mite fauna of Bingöl Province. Including the new data, the total number of taxa recorded from Bingöl Province tallies 149 species in 20 families. Bingöl (149 species), Isparta (119), Erzurum (102), Burdur (81), Afyon (80), Elazığ (78) and Erzincan (77) Provinces are richest in number of species.

**Keywords:** Water mite, fauna, Acari: Hydrachnidia, Bingöl province, Turkey

Oral Presentation

Thursday

Aquatic (Marine and Freshwater) Biodiversity-1

### Comparison of Distribution Altitudes of Some Helophoridae, Hydrochidae and Hydrophilidae Species in Turkey

Serhat Özcan<sup>1\*</sup>, Numan Yıldız<sup>1</sup>, Ahmet Polat<sup>1</sup>, Ümit İncekara<sup>1</sup><sup>1</sup>Ataturk University, Faculty of Science, Department of Biology, Erzurum.

\*Corresponding author e-mail: s.ozcn@hotmail.com

#### Abstract

In this study, altitude values of Helophoridae, Hydrochidae and Hydrophilidae species collected from Erzurum Marshes, Erzurum Geological Formations and Muş Hamurpet (Akdoğan) Lake were compared with the altitude data recorded throughout Turkey.

From the research area; total of 32 taxa were identified, 13 species from Helophoridae, 1 species from Hydrochidae, 17 species and 1 subspecies from Hydrophilidae. The altitudes of the locations where these taxa had collected were determined and compared with the previously recorded data. Species outside the distribution range were determined and their distribution in Turkey was revised.

**Keywords:** Helophoridae, Hydrochidae, Hydrophilidae, altitude, Turkey

#### INTRODUCTION

Represented by 201 species worldwide, Helophoridae has a very wide living area (Balfour-Browne, 1958; Angus, 1969, 1970a, 1970b, 1971a, 1971b, 1983, 1984, 1985a, 1985b, 1988, 1992, 1996, 1998; Smetana, 1985, 1988; Hansen, 1987; İncekara et al., 2004a). 156 of them were recorded from Palearctic, (Angus, 1984, 1985a, 1992; Taşar, 2018), 41 Nearctic (Smetana, 1985; Hansen, 1987) and only four species were recorded from the Ethiopian region (Angus, 1992). 52 species are known in Turkey (Polat et al., 2021).

Helophoridae species have been observed to spread in sand or mud at the water's edge, in slow flowing streams, and often among wet algae and aquatic plants. (Smetana, 1985).

Hydrochidae, which can be found in all zoogeographic regions, is represented by a single genus and 87 species. There are only eight species of this family known both in Turkey and Europe

(Hansen, 1991; Hebauer, 1994; İncekara, 2004; İncekara et al., 2004b; Darılmaz and İncekara, 2011; Taşar 2017; Polat et al., 2021).

Hydrochidae usually live in stagnant or slow-flowing waters by clinging to aquatic plants (Shepard and Chaboo, 2015).

Hydrophilidae, which spreads all over the world, is represented by 172 genera and 2932 species. Species found in Turkey are more similar to Asian fauna (Kosswing, 1995; Mart et al., 2014; Taşar, 2018). 108 species are known from Turkey (Polat et al., 2021).

Hydrophilidae live in shallow and usually stagnant waters. Larvae and adults are in the same localities. Some aquatic species inhabit vertical surfaces with sheet flow, such as in seeps and splash zones near waterfalls. Some have also been reported to be found in manure, carrion and rotting vegetation (Shepard and Chaboo, 2015).

Turkey is located in a very important zoogeographically region. It has quite different heights ranging from 0 m to 5137 m. Due to the presence of wetlands in almost every region between these altitudes, it is quite possible to encounter aquatic insects.

Erzurum marshes are 12.1 km<sup>2</sup> and their altitude is between 1750-1760 m., Erzurum Geological Formations is a region with many geologically formed lakes exceeding 1464 ha and its altitude is between 2650-2670 m. Hamurpet Lakes (Akdoğan Lake) is in the form of two lakes, known as Small and Great Hamurpet, whose lake basin is located within the borders of Varto district of Muş province. The circumference of Small Hamurpet lake is approximately 5.7 km and its area is 1.60 km<sup>2</sup>. The altitude of Great Hamurpet lake is approximately 2149 m and its depth is 21 m. The circumference of Great Hamurpet Lake is approximately 19.24 km and its area is approximately 11.31 km<sup>2</sup> (ÜNİDAP, 2016).

In this study, the samples collected from Erzurum Marshes at 1750 m, Hamurpet Lake at 2150 m and Erzurum Geological Formations at 2670 m were compared with the data of Turkey. The results are presented as a table (Table 1.).

## **MATERIALS AND METHODS**

The samples were collected from near the shore of Erzurum Marshes, Erzurum Geological Formations and Muş Hamurpet Lake, through slow flowing water, grassy areas where aquatic insects can live, and places where vegetative decay is high. Collected samples were taken to the laboratory after treatment with ethyl acetate.

Aedeagophores were soaked for 1-2 hours in 10% KOH solution to separate the muscle tissue around the chitin structure. They were then placed on a slide with a drop of glycerin. Measurements

were made on Nikon SMZ1500 stereomicroscope by drawing aedeagophore shapes. Photographs of the common and distinctive features of the species were taken with the Leica DFC295 brand macroscope. Results were compared with previous Turkish literature.



**Figure 1.** The location of the research area on the map of Turkey.

## RESULTS

**Table 2.** The localities where the samples were collected and the lowest and highest altitudes in the literature

|   | Erzurum Geological Formations | Muş Hamurpet Lake | Erzurum Marshes | Lowest Altitude | Highest Altitude |
|---|-------------------------------|-------------------|-----------------|-----------------|------------------|
| <i>Helophorus micans</i> Falderman, 1835      | 2650-2670 m                   | -                 | -               | Samsun 0 m      | Bayburt 2409 m   |
| <i>Helophorus aquaticus</i> (Linnaeus, 1758)  | 2650-2670 m                   | 2149 m            | 1750-1760 m     | Samsun 0 m      | Gümüşhane 2582 m |
| <i>Helophorus grandis</i> (Illiger, 1798)     | -                             | 2149 m            | -               | Aydın 36 m      | Denizli 1885 m   |
| <i>Helophorus discrepans</i> Rey, 1885        | 2650-2670 m                   | 2149 m            | -               | Hatay 115 m     | Gümüşhane 2582 m |
| <i>Helophorus hilaris</i> Sharp, 1916         | 2650-2670 m                   | 2149 m            | 1750-1760 m     | Samsun 0 m      | Bitlis 2232 m    |
| <i>Helophorus lapponicus</i> Thomson, 1853    | 2650-2670 m                   | 2149 m            | 1750-1760 m     | Samsun 0 m      | Gümüşhane 2453 m |
| <i>Helophorus longitarsis</i> Wollaston, 1864 | 2650-2670 m                   | 2149 m            | -               | Aydın 60 m      | Gümüşhane 2453 m |
| <i>Helophorus similis</i> (Kuwert, 1887)      | -                             | -                 | 1750-1760 m     | Kayseri 1072 m  | Erzincan 2500 m  |
| <i>Helophorus arvernensis</i> (Mulsant, 1846) |                               |                   | 1750-1760 m     | Samsun 0 m      | Van 1891 m       |
| <i>Helophorus brevipalpis</i> Bedel, 1881     | 2650-2670 m                   | -                 | 1750-1760 m     | Samsun 0 m      | Bitlis 2173 m    |
| <i>Helophorus daedalus</i> d'Orchymont, 1932  | 2650-2670 m                   | -                 | -               | Samsun 0 m      | Gümüşhane 2582 m |



|   |             |        |             |                 |                  |
|---|-------------|--------|-------------|-----------------|------------------|
| <i>Helophorus montenegrinus</i> Kuwert, 1885      | 2650-2670 m | -      | 1750-1760 m | Ordu 4 m        | Van 2050 m       |
| <i>Helophorus terminassianae</i> (Angus, 1984).   | -           | -      | 1750-1760 m | Samsun 0 m      | Erzurum 1750 m   |
| <i>Hydrochus flavipennis</i> Küster, 1852         | -           | 2149 m | -           | Zonguldak 60 m  | Kütahya 1309 m   |
| <i>Paracymus chalceolus</i> (Solsky, 1874)        | -           | -      | 1750-1760 m | Hatay 22 m      | Van 2066 m       |
| <i>Berosus signaticollis</i> (Charpentier, 1825)  | -           | 2149 m | -           | Samsun 0 m      | Artvin 1640 m    |
| <i>Berosus asiaticus</i> (Kuwert, 1888)           | -           | -      | 1750-1760 m | Muş 1486 m      | Bitlis 1658 m    |
| <i>Berosus fulvus</i> (Kuwert, 1888)              | -           | -      | 1750-1760 m | Burdur 845 m    | Van 2055 m       |
| <i>Berosus guttalis</i> (Rey, 1883)               | -           | 2149 m | -           | Bitlis 1286 m   | Van 2055 m       |
| <i>Enochrus bicolor</i> (Fabricius, 1792)         | -           | 2149 m | 1750-1760 m | İzmir 13 m      | Van 2057 m       |
| <i>Enochrus fuscipennis</i> (Thomson, 1884)       | -           | 2149 m | -           | Hatay 0 m       | Bayburt 2409 m   |
| <i>Enochrus quadripunctatus</i> (Herbst, 1797)    | -           | 2149 m | -           | Hatay 0 m       | Bitlis 2250 m    |
| <i>Helochaeres obscurus</i> (Müller, 1776)        | -           | 2149 m | -           | Samsun 0 m      | Ankara 1880 m    |
| <i>Hydrobius fuscipes</i> (Linnaeus, 1758)        | -           | 2149 m | -           | Samsun 0 m      | Gümüşhane 2582 m |
| <i>Hydrochara dichroma</i> (Fairmaire, 1892)      | 2650-2670 m | 2149 m | -           | Samsun 0 m      | Bayburt 2324 m   |
| <i>Laccobius bipunctatus</i> (Fabricius, 1775)    | 2650-2670 m | 2149 m | -           | Trabzon 7 m     | Bayburt 2409 m   |
| <i>Laccobius obscuratus aegaeus</i> Gentili, 1974 | 2650-2670 m | -      | -           | Trabzon 7 m     | Rize 2600 m      |
| <i>Laccobius sulcatulus</i> Reitter, 1909         | 2650-2670 m | -      | -           | Samsun 823 m    | Bayburt 2267 m   |
| <i>Laccobius syriacus</i> Guillebeau, 1896        | 2650-2670 m | 2149 m | -           | Samsun 0 m      | Van 2600 m       |
| <i>Coelostoma orbiculare</i> (Fabricius, 1775)    | -           | -      | 1750-1760 m | Samsun 0 m      | Bayburt 2267 m   |
| <i>Cercyon quisquilius</i> (Linnaeus, 1760)       | -           | -      | 1750-1760 m | Çanakkale 279 m | Mersin 1600 m    |
| <i>Cercyon tristis</i> (Illiger, 1801)            | -           | -      | 1750-1760 m | Kayseri 1416 m  | Kayseri 1416 m   |

A total of 32 species were identified from the research areas and listed as a table. The altitudes in the literature of these species, whose distribution in Turkey are given below, and the altitudes we obtained in our study are compared in Table 1. In the literature up to now, Helophoridae, Hydrochidae and Hydrophilidae species were known to spread between 0-2600 m in Turkey. When the data of 32 collected species are examined, it is seen that 10 species from Helophoridae, 1 species from Hydrochidae and 12 species from Hydrophilidae, totally 23 species were found at higher altitudes than the altitudes given so far and added to the literature. With this study, living

altitudes of Helophoridae, Hydrochidae and Hydrophilidae families were revised.

Order **COLEOPTERA**

Suborder **POLYPHAGA**

Superfamily **HYDROPHILOIDEA**

Family **HELOPHORIDAE**

Genus *Helophorus* Fabricius, 1775

Subgenus *Eutrichelophorus* Sharp, 1915

*Helophorus micans* (Falderman, 1835)

**Distribution in Turkey:** Adana, Adıyaman, Afyon, Ağrı, Aksaray, Ankara, Aydın, Balıkesir, Batman, Bayburt, Burdur, Çanakkale, Çorum, Denizli, Diyarbakır, Elazığ, Erzurum, Giresun, Hakkâri, Hatay, Isparta, İzmir, Kahramanmaraş, Kars, Kayseri, Kütahya, Malatya, Manisa, Mardin, Mersin, Muş, Samsun, Şanlıurfa, Tokat, Trabzon, Van (İncekara et al., 2009a; Mart et al., 2010; Taşar, 2011; Polat et al., 2021).

Subgenus *Helophorus* Fabricius, 1775

*Helophorus aquaticus* (Linnaeus, 1758)

**Distribution in Turkey:** Adana, Adıyaman, Afyon, Aksaray, Ankara, Aydın, Batman, Bayburt, Bilecik, Bingöl, Bitlis, Bolu, Burdur, Bursa, Çorum, Denizli, Diyarbakır, Elazığ, Erzurum, Giresun, Gümüşhane, Hakkâri, Isparta, Mersin, İstanbul, Kahramanmaraş, Kars, Kastamonu, Kayseri, Kırklareli, Kütahya, Mardin, Muş, Ordu, Sakarya, Samsun, Sinop, Şanlıurfa, Şırnak, Uşak, Van (İncekara et al., 2009a; Mart et al., 2010; Taşar, 2011; Polat et al., 2021).

*Helophorus grandis* (Illiger, 1798)

**Distribution in Turkey:** Adıyaman, Antalya, Aydın, Batman, Bitlis, Burdur, Denizli, Diyarbakır, Elazığ, İzmir, Kahramanmaraş, Manisa, Mardin, Şanlıurfa, Tokat, Van (Taşar, 2011; Topkara and Ustaoglu, 2015; Akunal and Aslan, 2017; Polat et al., 2021).

Subgenus *Rhopalohelophorus* Kuwert, 1886

*Helophorus discrepans* Rey, 1885

**Distribution in Turkey:** Afyon, Ağrı, Ankara, Antalya, Artvin, Bayburt, Bitlis, Bolu, Çorum, Denizli, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hakkâri, Kahramanmaraş, Kars,

Kayseri, Kütahya, Muş, Ordu, Tokat, Trabzon, Uşak, Van, Yozgat (Mart et al., 2010; Taşar, 2011; Bektaş et al., 2019; Polat et al., 2021).

*Helophorus hilaris* Sharp, 1916

**Distribution in Turkey:** Adıyaman, Ağrı, Aydın, Batman, Bayburt, Bitlis, Burdur, Diyarbakır, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hakkâri, Kahramanmaraş, Kars, Kayseri, Mardin, Muş, Ordu, Samsun, Şanlıurfa, Şırnak, Tokat, Van (Polat et al., 2010, 2021; Taşar, 2011).

*Helophorus lapponicus* Thomson, 1853

**Distribution in Turkey:** Afyon, Ardahan, Bayburt, Bitlis, Erzincan, Erzurum, Gümüşhane, Kars, Kütahya, Muş, Ordu, Samsun, Tokat, Trabzon, Van (Mart et al., 2010; Polat et al., 2010, 2021; Taşar, 2011).

*Helophorus longitarsis* Wollaston, 1864

**Distribution in Turkey:** Afyon, Aksaray, Ankara, Aydın, Balıkesir, Burdur, Denizli, Erzincan, Gümüşhane, Isparta, Kahramanmaraş, Kayseri, Kütahya, Muş, Ordu, Van (Kıyak et al., 2006; Mart et al., 2010; Taşar, 2011; Polat et al., 2021).

*Helophorus similis* Kuwert, 1887

**Distribution in Turkey:** Erzincan, Erzurum, Kayseri (İncekara et al., 2004a, 2010; Polat et al., 2021)

Subgenus *Atracthelophorus* Kuwert, 1886

*Helophorus arvernicus* Mulsant, 1846

**Distribution in Turkey:** Bitlis, Çorum, Diyarbakır, Erzincan, Erzurum, Gümüşhane, Kars, Kayseri, Muş, Samsun, Tokat, Van (Polat et al., 2010, 2021; Taşar, 2011).

*Helophorus brevialpis brevialpis* Bedel, 1881

**Distribution in Turkey:** Adıyaman, Ağrı, Afyon, Aksaray, Ankara, Antalya, Artvin, Aydın, Balıkesir, Batman, Bayburt, Bitlis, Burdur, Bursa, Çorum, Denizli, Diyarbakır, Erzincan, Erzurum, Giresun, Gümüşhane Isparta, İstanbul, İzmir, Kahramanmaraş, Kars, Kastamonu,

Kayseri, Kırklareli, Kütahya, Manisa, Muğla, Muş, Niğde, Ordu, Sakarya, Samsun, Sinop, Şanlıurfa, Trabzon, Uşak, Van Zonguldak (İncekara et al., 2009a; Topkara and Balık, 2010; Taşar, 2011; Polat et al., 2021).

*Helophorus daedalus* d'Orchymont, 1932

**Distribution in Turkey:** Adıyaman, Afyon, Ankara, Bayburt, Bitlis, Bolu, Burdur, Çorum, Denizli, Diyarbakır, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hakkâri, Isparta, İzmir, Kahramanmaraş, Kayseri, Kütahya, Muş, Ordu, Samsun, Şırnak, Tokat, Uşak, Van (Mart et al., 2010; Polat et al., 2010, 2021; Topkara and Balık, 2010; Taşar, 2011).

*Helophorus montenegrinus* Kuwert, 1885

**Distribution in Turkey:** Ankara, Balıkesir, Bolu, Burdur, Bursa, Elazığ, Giresun, Isparta, Mersin, İstanbul, İzmir, Kahramanmaraş, Kastamonu, Kırklareli, Kütahya, Ordu, Samsun, Sinop, Tokat, Trabzon, Van (Mart et al., 2010; Taşar, 2011; Polat et al., 2021).

Subgenus *Transithelophorus* Angus, 1970

*Helophorus terminassianae* Angus, 1984

**Distribution in Turkey:** Çorum, Erzurum, İzmir, Konya, Muş, Samsun, Tokat (Mart and Erman, 2001; İncekara et al., 2009a; Taşar, 2011; Polat et al., 2021).

Family **HYDROCHIDAE**

Genus *Hydrochus* Leach, 1817

*Hydrochus flavipennis* Kuster, 1852

**Distribution in Turkey:** Adıyaman, Afyon, Bingöl, Denizli, Diyarbakır, Erzurum, Kahramanmaraş, Kütahya, Şanlıurfa, Tokat, Van, Zonguldak (Topkara and Balık, 2010; Darılmaz and Kıyak, 2018; Polat et al., 2021).

Family **HYDROPHILIDAE**

Subfamily **HYDROPHILINAE** Latreille, 1802

Genus *Paracymus* Thomson, 1867

*Paracymus chalceolus* (Solsky, 1874)

**Distribution in Turkey:** Adıyaman, Bayburt, Bingöl, Bitlis, Diyarbakır, Elazığ, Hakkâri, Hatay,

Muş, Van (Türken, 2011; Mart et al., 2014; Polat et al., 2021).

Genus *Berosus* Leach, 1817

Subgenus *Berosus* Leach, 1817

*Berosus signaticollis* (Charpentier, 1825)

**Distribution in Turkey:** Afyon, Amasya, Ankara, Antalya, Artvin, Aydın, Bayburt, Bingöl, Denizli, Elazığ, Erzincan, Erzurum, Hatay, Isparta, İzmir, Kars, Kastamonu, Kayseri, Ordu, Rize, Samsun, Sivas, Tokat (İncekara et al., 2009a, 2011; Polat et al., 2021).

Subgenus *Enoplurus* Hope, 1838

*Berosus asiaticus* Kuwert, 1888

Distribution in Turkey: Bitlis, Muş, Van (İncekara et al., 2011; Taşar et al., 2012; Polat et al., 2021).

*Berosus fulvus* Kuwert, 1888

**Distribution in Turkey:** Burdur, Van (Schödl, 1991; Türken, 2011; Taşar et al., 2012; Polat et al., 2021).

*Berosus guttalis* Rey, 1883

Distribution in Turkey: Bitlis, Sivas, Van (İncekara et al., 2011; Türken, 2011; Taşar et al., 2012; Polat et al., 2021).

Genus *Enochrus* Thomson, 1859

Subgenus *Lumetus* Zaitzev, 1908

*Enochrus bicolor* (Fabricius, 1792)

**Distribution in Turkey:** Adıyaman, Afyon, Aksaray, Ankara, Antalya, Aydın, Balıkesir, Bitlis, Burdur, Çanakkale, Denizli, Diyarbakır, Edirne, Elazığ, Erzincan, Mersin, İzmir, Kars, Kayseri, Kırşehir, Kütahya, Malatya, Manisa, Muş, Ordu, Sivas, Şanlıurfa, Uşak, Van (Aydoğan, 2011; Türken, 2011; Akünal and Aslan, 2017; Polat et al., 2021).

*Enochrus fuscipennis* (Thomson, 1884)

**Distribution in Turkey:** Afyon, Aksaray, Ankara, Artvin, Aydın, Balıkesir, Bayburt, Bingöl,

Bitlis, Bolu, Burdur, Bursa, Çanakkale, Çorum, Denizli, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hatay, Hakkâri, Isparta, İzmir, Kars, Kayseri, Kütahya, Malatya, Manisa, Muş, Ordu, Rize, Sivas, Uşak, Van (Bektaş et al., 2019; Mart, 2009; Topkara and Balık, 2010; Aydoğan, 2011; Polat et al., 2021).

*Enochrus quadripunctatus* (Herbst, 1797)

**Distribution in Turkey:** Adıyaman, Ankara, Antalya, Batman, Bingöl, Bitlis; Denizli, Diyarbakır, Edirne, Elazığ, Isparta, İzmir, Kars, Malatya, Manisa, Mardin, Muş; Ordu, Sivas, Şanlıurfa, Van (Bektaş et al., 2019; Topkara and Balık, 2010; Aydoğan, 2011; Polat et al., 2021).

Genus *Helochares* Mulsant, 1844

Subgenus *Helochares* Mulsant, 1844

*Helochares obscurus* (O. F. Müller, 1776)

**Distribution in Turkey:** Adana, Adıyaman, Afyon, Ankara, Balıkesir, Bayburt, Bingöl, Burdur, Bursa, Çanakkale, Denizli, Diyarbakır, Elazığ, Giresun, Hatay, Isparta, İzmir, Kahramanmaraş, Kayseri, Kütahya, Mardin, Sakarya, Samsun, Sivas, Şanlıurfa, Ordu (İncekara et al., 2009a; Hızarcıoğlu et al., 2010; Polat et al., 2021).

Genus *Hydrobius* Leach, 1815

*Hydrobius fuscipes* (Linnaeus, 1758)

**Distribution in Turkey:** Adıyaman, Afyon, Ankara, Artvin, Batman, Bayburt, Bilecik, Bingöl, Bitlis, Burdur, Çorum, Denizli, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hakkâri, Hatay, Isparta, İzmir, Kütahya, Mersin, Muş, İzmir, Kars, Kayseri, Konya, Ordu, Rize, Samsun, Sivas, Tokat, Trabzon, Van (İncekara et al., 2009a; Mart, 2009; Aydoğan, 2011; Türken, 2011; Polat et al., 2021).

Genus *Hydrochara* Berthold, 1827

*Hydrochara dichroma* (Fairmaire, 1892)

**Distribution in Turkey:** Adana, Adıyaman, Afyon, Ankara, Amasya, Balıkesir, Batman, Bayburt, Bingöl, Çanakkale, Denizli, Diyarbakır, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hakkâri, Hatay, İstanbul, İzmir, Kars, Kayseri, Kütahya, Muş, Ordu, Rize, Samsun, Sivas, Şanlıurfa, Tokat, Trabzon, Van (İncekara et al., 2009a, 2009b; Aydoğan, 2011; Türken, 2011;

Polat et al., 2021).

Genus *Laccobius* Erichson, 1837

Subgenus *Dimorpholaccobius* Zaitzev, 1938

*Laccobius bipunctatus* (Fabricius, 1775)

**Distribution in Turkey:** Adıyaman, Afyon, Artvin, Batman, Bayburt, Bingöl, Bitlis, Bolu, Çorum, Diyarbakır, Elazığ, Erzurum, Giresun, Gümüşhane, Isparta, Kars, Kastamonu, Kütahya, Muş, Ordu, Sivas, Şanlıurfa, Trabzon, Van (Karaman, 2007; Mart, 2009; Aydoğan, 2011; Türken, 2011; Polat et al., 2021).

*Laccobius obscuratus aegaeus* Gentili, 1974

**Distribution in Turkey:** Adana, Adıyaman, Afyon, Ankara, Antalya, Artvin, Aydın, Balıkesir, Bayburt, Bilecik, Bingöl, Bitlis, Bolu, Burdur, Bursa, Çanakkale, Çorum, Denizli, Elazığ, Erzincan, Erzurum, Giresun, Gümüşhane, Hatay, Isparta, Mersin, İstanbul, İzmir, Kastamonu, Kayseri, Kırklareli, Kocaeli, Konya, Kütahya, Manisa, Muğla, Muş, Niğde, Ordu, Osmaniye, Rize, Samsun, Sinop, Sivas, Tokat, Trabzon, Uşak, Van (Gentili, 2000; Karaman, 2007; Aydoğan, 2011; Polat et al., 2021).

*Laccobius sulcatulus* Reitter, 1909

**Distribution in Turkey:** Afyon, Amasya, Ankara, Antalya, Bayburt, Bingöl, Bitlis, Burdur, Denizli, Diyarbakır, Erzincan, Erzurum, Gümüşhane, Isparta, Kahramanmaraş, Kars, Kayseri, Konya, Kütahya, Manisa, Muş, Samsun, Sivas, Uşak, Van (Mart, 2009; Polat et al., 2010, 2021; Aydoğan, 2011).

*Laccobius syriacus* Guillebeau, 1896

**Distribution in Turkey:** Adana, Adıyaman, Afyon, Aksaray, Ankara, Antalya, Artvin, Aydın, Balıkesir, Batman, Bayburt, Bilecik, Bingöl, Bitlis, Bolu, Burdur, Bursa, Çorum, Denizli, Diyarbakır, Edirne, Elazığ, Gaziantep, Giresun, Gümüşhane, Erzincan, Erzurum, Hakkâri, Hatay, Isparta, Mersin, İzmir, Kahramanmaraş, Kars, Kastamonu, Kayseri, Konya, Kütahya, Malatya, Manisa, Mardin, Muğla, Muş, Ordu, Osmaniye, Rize, Sakarya, Samsun, Sinop, Sivas, Şanlıurfa, Tokat, Trabzon, Uşak, Van (Gentili, 2000; İncekara et al., 2009a; Aydoğan, 2011; Polat et al., 2021).

Subfamily **SPHAERIDIINAE** Latreille, 1802

Genus *Coelostoma* Brullé, 1835

Subgenus *Coelostoma* Brullé, 1835

*Coelostoma orbiculare* (Fabricius, 1775)

**Distribution in Turkey:** Adıyaman, Afyon, Ankara, Antalya, Artvin, Bayburt, Bingöl, Bitlis, Burdur, Bursa, Çanakkale, Çorum, Denizli, Diyarbakır, Elazığ, Erzurum, Giresun, Gümüşhane, Isparta, Manisa, Mersin, Muş, Kars, Kayseri, Kütahya, Ordu, Samsun, Sivas, Şanlıurfa, Tokat, Trabzon, Van (İncekara et al., 2009a; Mart, 2009; Aydoğan, 2011; Polat et al., 2021).

Genus *Cercyon* Leach, 1817

Subgenus *Cercyon* Leach, 1817

*Cercyon quisquilius* (Linnaeus, 1760)

**Distribution in Turkey:** Adana, Çanakkale, Mersin (Peyron, 1858; D'Orchymont, 1940, Darılmaz and İncekara, 2011; Polat et al., 2021).

*Cercyon tristis* (Illiger, 1801)

**Distribution in Turkey:** Kayseri (İncekara et al., 2010; Polat et al., 2021)

## DISCUSSION

It is known that the altitudes of the families that are the subject of the study in Turkey generally vary between 0 m and 2600 m. It is seen that 9 of the 32 species (*Helophorus similis*, *H. arvernensis*, *H. terminassianae*, *Paracymus chalceolus*, *Berosus fulvus*, *Enochrus fuscipennis*, *E. quadripunctatus*, *Hydrobius fuscipes*, *Coelostoma orbiculare*) collected from the research areas are located in the altitude ranges given from Turkey, and 23 species (*Helophorus micans*, *H. aquaticus*, *H. grandis*, *H. discrepans*, *H. hilaris*, *H. lapponicus*, *H. longitarsis*, *H. brevipalpis*, *H. aedalus*, *H. montenegrinus*, *Hydrochus flavipennis*, *Berosus signaticollis*, *B. asiaticus*, *B. guttalis*, *Enochrus bicolor*, *Helochares obscurus*, *Hydrochara dichroma*, *Laccobius bipunctatus*, *Laccobius obscuratus aegaeus*, *L. sulcatulus*, *L. syriacus*, *Cercyon quisquilius*, *C. tristis*) are collected from higher localities than the altitudes given in the literature. With this study, it was determined that Helophoridae, Hydrochidae and Hydrophilidae families living in Turkey can be found at altitudes higher than 2600 m.



## CONCLUSIONS

In this study; the lowest and highest altitudes of the species belonging to the families of Helophoridae, Hydrochidae and Hydrophilidae, which spread from sea level to the peaks of high mountains in Turkey, are presented comparatively. Samples were collected between May 2016 and October 2017. 500 specimens, 304 male and 196 females, collected from various localities from Erzurum Marshes, Erzurum Geological Formations and Muş Hamurpet Lake were evaluated and 32 species were identified. In studies conducted in Turkey, some species could not be detected at high altitudes. With this study; it has been revealed that the species belonging to the mentioned families can be distributed in a much wider altitude range than the known altitude values.

## ACKNOWLEDGEMENTS

This study was supported by Ataturk University with B.A.P No. 2016/143 and 2016/144, and were carried out in the Department of Biology, Faculty of Science, Atatürk University.

## REFERENCES

- Angus RB (1969). Revisional notes on *Helophorus* F. (Col., Hydrophilidae) 1.- General Introduction and some species resembling *H. minutus* F. *Entomologist's Monthly Magazine* 105: 1-24.
- Angus RB (1970a). A revision of the beetles of the genus *Helophorus* F. (Coleoptera: Hydrophilidae), subgenera *Orphelophorus* d'Orchymont, *Gephelophorus* Sharp and *Meghelophorus* Kuwert. *Acta Zoologica Fennica* 129: 1-62.
- Angus RB (1970b). Revisional studies on east palearctic and some nearctic species of *Helophorus* F. (Coleoptera: Hydrophilidae). *Acta Zoologica Academiae Scientiarum Hungaricae* 16: 249-290.
- Angus RB (1971a). Revisional notes on *Helophorus* F. (Col., Hydrophilidae) 2.-The complex round *H. flavipes* F. *Entomologist's Monthly Magazine* 106: 129-148.
- Angus RB (1971b). Revisional notes on *Helophorus* F. (Col., Hydrophilidae) 3. Species resembling *H. strigifrons* Thoms. and some further notes on species resembling *H. minutus* F. *Entomologist's Monthly Magazine* 106: 238-256.
- Angus RB (1983). Separation of *Helophorus grandis*, *maritimus* and *occidentalis* sp. n. (Coleoptera: Hydrophilidae) by banded chromosome analysis. *Systematic Entomology* 8: 1-13.
- Angus RB (1984). Towards a revision of the palearctic species of *Helophorus* F. (Coleoptera: Hydrophilidae) I. *Entomological Review* 63 (3): 89-119.
- Angus RB (1985a). A new species of *Helophorus* (Coleoptera: Hydrophilidae) from Mongolia. Results of the Mongolian-German biological expeditions since 1962. *Mitteilungen aus dem Zoologischen Museum Berlin* 61: 163-164.
- Angus RB (1985b). A new species of *Helophorus* F. (Col., Hydrophilidae) from northern Spain. *Entomologist's Monthly Magazine* 121: 89-90.
- Angus RB (1988). Notes on the *Helophorus* (Coleoptera: Hydrophilidae) occurring in Turkey, Iran and neighboring territories. *Revue suisse de Zoologie* 95 (1): 209-248.
- Angus RB (ed.) (1992). *Süsswasserfauna von Mitteleuropa (Insecta: Coleoptera: Hydrophilidae: Helophorinae)*, Gustav Fischer Verlag, Jena, 144 p.
- Angus RB (1996). A re-evaluation of the *Helophorus flavipes* group of species (Coleoptera: Hydrophilidae), based on chromosomal analysis, larva and biology. *Nouvelle Revue d'Entomologie (N.S.)* 13: 111-122.
- Angus RB (1998). A New Turkish *Helophorus*, with notes on *H. griseus* Herbstand *H. montanus* d'Orchymont (Col., Hydrophiloidea). *The Entomologist's Monthly Magazine* 134: 5-9.
- Akünel AY & Aslan EG (2017). Aquatic Beetles (Coleoptera: Hydrophilidae, Helophoridae) of İzmir, Manisa and Aydın Provinces (Turkey) with New Locality Records for the Aegean Region. *Turkish Journal of Fisheries and Aquatic Sciences* 17: 777-785.

- Aydođan Z (2011). Bitlis ve Muş İlleri Hydrophilidae (Coleoptera) faunasının araştırılması. Yüksek Lisans tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum.
- Balfour-Browne F (1958). *British Water Beetles III*, Ray Society, London, 210 p.
- Bektaş M, Taşar GE, İncekara Ü & Polat A (2019). A faunistic study on aquatic Coleoptera of the Eastern Mediterranean Region of Turkey. *Munis Entomology and Zoology* 14 (2): 478-488.
- Darılmaz MC & İncekara Ü (2011). Checklist of Hydrophiloidea of Turkey (Coleoptera: Polyphaga). *Journal of Natural History* 45 (11): 685-735.
- Darılmaz MC. & Kıyak S (2018). Research of aquatic Coleoptera fauna of the inner Western Anatolia, Part - II (Coleoptera: Helophoridae, Hydrochidae and Hydrophilidae). *Munis Entomology and Zoology* 13 (1): 58-69.
- D'Orchymont A (1940). Palpicornia de Chypre. Voyage de M.A. Ball (Octobre-Novembre 1932). *Mémoires du Musée Royale d'Histoire Naturelle de Belgique* 19: 1-35.
- Gentili E (2000). Distribuzione del genere *Laccobius* (Coleoptera, Hydrophilidae) in Anatolia e problemi relativi. *Biogeographia* 21: 173-214.
- Hansen M (1987). The Hydrophilidae (Coleoptera) of Fennoscandia and Denmark. *Fauna Entomologica Scandinavica* 18: 1-253.
- Hansen M (1991). *The Hydrophiloid Beetles. Phylogeny, Classification and a revision of the genera (Coleoptera; Hydrophiloidea)*, Biologiske Skrifter 40, The Royal Danish Academy of Science and Letters, Copenhagen, 368 p.
- Hebauer F (1994). The Hydrophilidae of Israel and Sinai (Coleoptera: Hydrophilidae). *Zoology in the Middle East* 10: 74-137.
- Hızarcıođlu R, Kıyak S & Darılmaz MC (2010). Some aquatic Coleoptera from Ankara province, Turkey. *Munis Entomology and Zoology* 5 (1): 278-282.
- İncekara Ü (2004). Erzincan İli Hydrophilidae, Helophoridae ve Hydrochidae (Coleoptera) türleri üzerine sistematik araştırmalar. Doktora Tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum.
- İncekara Ü, Mart A & Erman O (2004a). Distribution of Turkish *Helophorus* Fabricius, 1775 (Coleoptera, Helophoridae) I. Subgenus *Rhopalhelophorus*, with two new records. *Journal of the Entomological Research Society* 6 (2): 51-62.
- İncekara Ü, Mart A & Erman O (2004b). Two new records of Hydrochidae (Coleoptera) species from Turkey, with some ecological notes. *Turkish Journal of Zoology* 28: 213-216.
- İncekara Ü, Darılmaz MC, Mart A, Polat A & Karaca H (2009a). Faunistic study on two sisters plain (Bafra and Çarşamba) aquatic Coleoptera fauna in Turkey: two similar geography but rather different fauna, with a new record. *Munis Entomology and Zoology* 4 (1): 125-138.
- İncekara Ü, Mart A, Polat A & Karaca H (2009b). Turkish Hydrophilidae (Coleoptera) III. genus *Hydrochara* Berthold 1827 with the description of a new species, *Hydrochara major* sp. n. *Turkish Journal of Zoology* 33 (3): 315-319.
- İncekara Ü, Polat A, Darılmaz MC, Mart A & Taşar GE (2010). Aquatic Coleoptera fauna of Ramsar site Sultan Sazlığı (Kayseri Turkey) and its surroundings: with new distribution records of four species from the southern limit of its ranges. *Archives of Biological Science Belgrade* 62 (4): 1181-1191.
- İncekara Ü, Mart A, Polat A, Aydođan Z, Türken H, Taşar GE & Bayram S (2011). Studies on Turkish Hydrophilidae (Coleoptera) IV. Genus *Berosus* Leach, 1817 with description of a new species: *Berosus dentalis* sp. n. *Turkish Journal of Entomology* 35 (2): 231-244.
- Karaman B (2007). Trabzon İli Sucul Coleoptera (Insecta) faunası. Yüksek Lisans tezi, Gazi Üniversitesi Fen Bilimleri Enstitüsü, Ankara.
- Kıyak S, Canbulat S, Salur A & Darılmaz MC (2006). Additional notes on aquatic Coleoptera fauna of Turkey with a new record (Helophoridae, Hydrophilidae). *Munis Entomology and Zoology* 1 (2): 273-278.
- Kosswing C (1995). Zoogeography of the near east. *Systematic Zoology* 4 (1-4): 48-96.
- Mart A & Erman O (2001). A study on *Helophorus* Fabricius, 1775 (Coleoptera: Hydrophilidae) species. *Turkish Journal of Zoology* 25 (1): 35-40.
- Mart A (2009). Water scavenger beetles (Coleoptera: Hydrophilidae) provinces of Central Black Sea Region of Turkey. *Journal of the Entomological Research Society* 11 (1): 47-70.
- Mart A, İncekara Ü & Karaca H (2010). Faunistic study of the aquatic beetles (Coleoptera: Helophoridae) provinces (Bayburt, Giresun, Gümüşhane, Ordu and Trabzon) of Turkey. *Turkish Journal of Zoology* 34 (4): 509-521.
- Mart A, Aydođan A & Fırat Z (2014). A contribution on zoogeographical distribution of Hydrophilidae species in Turkey. *Munis Entomology and Zoology* 9 (2): 842-847.
- Peyron E (1858). Catalogue des coléoptères des environs de Tarsous (Caramanie). *Annales de la Société Entomologique de France* 6 (3): 353-434.

- Polat A, İncekara Ü & Mart A (2010). A faunistic study on the Helophoridae, Hydrophilidae and Hydrochidae (Coleoptera) in Samsun and Tokat provinces (Turkey). *Turkiye Entomoloji Dergisi* 34 (2): 227-239.
- Polat A, Darılmaz MC & İncekara Ü (2021). An annotated checklist of the Hydrophiloidea (Coleoptera) of Turkey. *Munis Entomology and Zoology* 16 (1): 151-178.
- Shepard WD & Chaboo CS (2015). Beetles (Coleoptera) of Peru: A Survey of the Families. Epimetopidae, Hydrochidae, Hydrophilidae (Hydrophiloidea). *Journal of the Kansas Entomological Society* 88 (2):169-172.
- Schödl S (1991). Revision der Gattung *Berosus* Leach 1. Teil: Die palaarktischen Arten der Untergattung *Enoplurus* (Coleoptera: Hydrophilidae). *Koleopterol Rundsch* 61: 111-135.
- Smetana A (1985). Revision of the subfamily Helophorinae of the Nearctic region (Coleoptera: Hydrophilidae). *Memoirs of the Entomological Society of Canada* 131: 1-151.
- Smetana A (1988). Review of the family Hydrophilidae of Canada and Alaska (Coleoptera). *Memoirs of the Entomological Society of Canada* 142: 1-316.
- Taşar GE (2011). Van Gölü Havzası Helophoridae (Coleoptera) faunasının araştırılması. Doktora tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum.
- Taşar GE (2017). *Hydrochus adiyamanensis* sp. n. from Adiyaman Province in south-eastern Turkey (Coleoptera: Hydrochidae). *Zoology in the Middle East* 63: 1-6.
- Taşar GE (2018). Contributions to the knowledge of Aquatic Coleoptera Fauna (Dryopidae, Helophoridae, Heteroceridae, Hydrochidae, Hydrophilidae, Gyridae, Halplidae and Noteridae) of Diyarbakır, Mardin and Batman Provinces. *Turkish Journal of Fisheries and Aquatic Sciences* 18: 927-936.
- Taşar GE, Erman O, Polat A & İncekara Ü (2012). Phoresy on the aquatic Coleoptera: Helophoridae and Hydrophilidae species in Lake Van basin. Turkey. *Munis Entomology and Zoology* 7 (2): 867-869.
- Topkara ET & Balık S (2010). Contribution to the Knowledge on Distribution of the Aquatic Beetles (Ordo: Coleoptera) in the Western Black Sea Region and Its Environs of Turkey. *Turkish Journal of Fisheries and Aquatic Sciences* 10: 323-332.
- Topkara ET & Ustaoglu MR (2015). Kartal Gölü'nün (Denizli) sucul Coleoptera ve sucul-yarisucul Heteroptera (Insecta) faunası üzerine bir çalışma ve ekolojik notlar. *Ege Journal of Fisheries and Aquatic Sciences* 32 (1): 45-50.
- Türken H (2011). Van İli Hydrophilidae (Coleoptera) faunasının araştırılması. Yüksek Lisans tezi, Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum.
- ÜNİDAP, 2016. *Uluslararası Bölgesel Kalkınma Konferansı Bildiriler Kitabı*. Mega ofset, Erzurum, 300 s.

Oral Presentation

Thursday

Aquatic (Marine and Freshwater) Biodiversity-1

**Changes in the Blood Cells of the *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Ranidae) Living in Different Streams in the Çanakkale**

Begüm Boran<sup>1\*</sup>, Çiğdem Gül<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies,

Department of Biology, Çanakkale, Turkey

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, 17100, Çanakkale, Turkey.

\*Corresponding author e-mail: begumboran@hotmail.com

**Abstract**

Amphibians are considered to be one of the groups that have an important role in monitoring wetlands due to their highly permeable skins and life cycles. The body size (snout-vent length) and blood cell counts and measurements (erythrocyte count, erythrocyte size, leukocyte count, differential blood formula and nuclear abnormalities) of 12 individuals belonging to the *Pelophylax ridibundus* (Marsh Frog) species living in 3 different regions in Çanakkale were determined and whether some parameters (pH, dissolved oxygen, temperature) of different water qualities caused changes in the blood cells of this species were identified. Water parameters (pH, dissolved oxygen, temperature) from three localities (Sarıçay, Atikhisar, Yeniköy) were measured and their water quality was determined. Hematological analyzes were performed to determine the erythrocyte and leukocyte count, erythrocyte sizes, leukocyte types and nuclear abnormalities. According to the physicochemical analysis of water samples, it was determined that Sarıçay was in fourth class water quality, Atikhisar and Yeniköy were in first- or second-class water quality. In the *Pelophylax ridibundus* species, it was determined that there was a significant difference in erythrocyte counts between Sarıçay-Atikhisar and Atikhisar-Yeniköy localities, but the same result could not be obtained for leukocyte counts. A statistically negative correlation was found between body size (snout-vent length) and nucleus size. When erythrocyte size and differential blood formula (leukocyte formula) were compared between localities, it was determined that the erythrocyte sizes in Sarıçay which has a low water quality were larger than other localities and the number of basophils was higher in comparison to other localities. When micronuclei and other nuclear abnormality percentages were examined, it was determined that the percentage of abnormality in Sarıçay was higher than the other localities.

**Keywords:** *Pelophylax ridibundus*, hematology, micronucleus, Çanakkale

## INTRODUCTION

Amphibians are considered to be one of the groups that have an important role in monitoring wetlands due to their highly permeable skins and life cycles. Anthropogenic pollutants act directly on the hematology of vertebrates, leading to some changes in the cell form and function of both erythrocytes and leukocytes (Beynon et al., 1992; Browne, 2004). Hematological parameters, are quite important to develop precautions that serve as an early warning signals to determine the environmental risks and health effects of potentially toxic chemicals in contaminated areas (Salinas et al., 2015; Pollo et al., 2016; Zhelev et al., 2017).

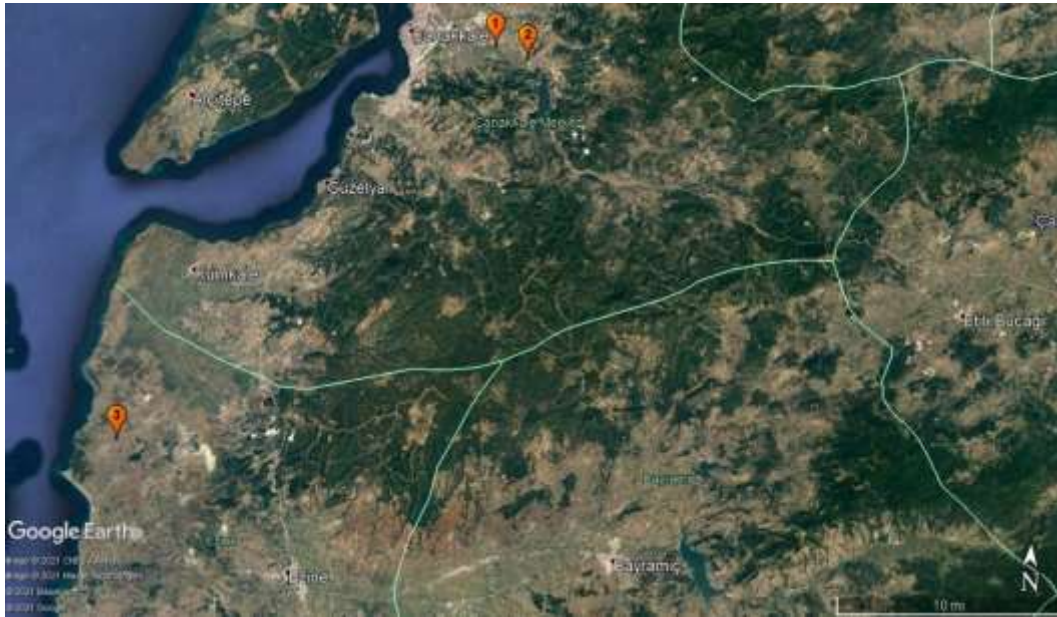
The blood parameters of amphibians are sensitive to many pollutants, which make them a good bioindicator (Cabagna et al., 2005; Teixeira et al., 2012; Carvalho et al., 2016; Medina et al., 2016; Zhelev et al., 2017). The hematological parameters of tailless frogs are sensitive to substances with toxic properties, and by studying the hematological parameters it becomes possible to understand the effects of these substances on the environment (Salinas et al., 2015; Pollo et al., 2016; Şahin, 2019). *Pelophylax ridibundus* Pallas, 1771 is a marsh frog species that is widespread in Central Europe, Western Asia, and also in Turkey (Baran et al., 2012). In comparison with other aquatic vertebrates, since the *P. ridibundus* species spends all its life stages dependent on water, it is seen as a more useful bioindicator for assessing environmental risks (Marques et al., 2009; Zhelev et al., 2013; Şişman et al., 2021).

The purpose of this study is to determine whether the blood parameters (erythrocyte count and size, leukocyte count and types, nuclear abnormalities) of *P. ridibundus* species that lives in 3 different localities of Çanakkale province with different water quality are affected by different water qualities.

## MATERIALS AND METHODS

12 individuals belonging to the *P. ridibundus* species used in this study were collected from 3 different localities with the help of scoops. The necessary permissions have been obtained from the Ethics Committee of Animal Experiments of Çanakkale Onsekiz Mart University (decision no: 2021/01-06) for the studies that carried out.

The studied localities were selected as Sariçay (Locality 1), Atikhisar Dam (Locality 2) located in city center and Yeniköy village attached to Ezine district of Çanakkale province (Figure 1).

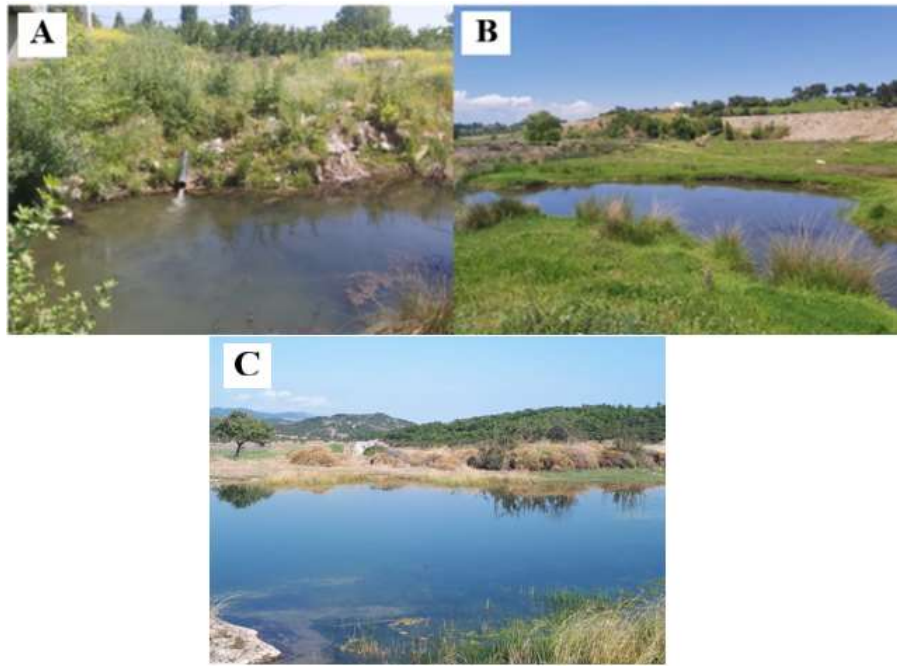


**Figure 1.** Studied localities in Çanakkale province (1: Sarıçay, 2: Atikhisar Dam, 3: Yeniköy).

As a result of some studies conducted to detect pollution in Sarıçay and Atikhisar Dam; it was determined that Atikhisar Dam was in water quality class I and Sarıçay was in water quality class II or III in terms of pesticide concentrations. And it was determined that Sarıçay was polluted by nutrients ( $\text{NO}_2$ ,  $\text{NO}_3$ ,  $\text{NH}_4$ ,  $\text{PO}_4$ , Org.  $\text{PO}_4$ ,  $\text{SiO}_2$ ), alkaline earth metals (Ca, Mg) and metals (Fe, Ni, Zn, Cu) (Odabaşı, 2005; Kaya, 2007). According to the literature and dissolved oxygen, pH and temperature parameters that measured with the Hach HQ40d brand ecological kit, based on the data in the Water Pollution Control Regulation (SKKY) (Table 1), the Sarıçay (Locality 1) was determined as polluted locality; the Atikhisar Dam (Locality 2) and Yeniköy (Locality 3) were determined as unpolluted localities (Figure 2).

**Table 1.** Some water quality classes according to SKKY (Water Pollution Control Regulation) (SKKY, 2004).

|                                | WATER QUALITY CLASSES |         |         |              |
|--------------------------------|-----------------------|---------|---------|--------------|
|                                | I                     | II      | III     | IV           |
| <b>pH</b>                      | 6.5-8.5               | 6.5-8.5 | 6.0-9.0 | <6.0 or >9.0 |
| <b>Dissolved Oxygen (mg/L)</b> | 8                     | 6       | 3       | <3           |
| <b>Temperature (°C)</b>        | 25                    | 25      | 30      | >30          |



**Figure 2.** Photographs of studied localities A) Sariçay (Locality 1) B) Atikhisar Dam (Locality 2) C) Yeniköy, Ezine (Locality 3).

A total of 1 ml of blood was taken from the middle abdominal veins of the individuals taken to the laboratory for the hematological analyzes with the help of a 5 ml syringe with a diameter of 21-gauge needle on the same day (Wright and Whitaker, 2001; Ballard and Cheek, 2003; Thrall et al., 2004). Approximately 4-5 blood smears were prepared from each individual with blood samples taken for hematological analysis. For the erythrocyte measurement and leukocyte formula, the blood smears were stained with Wright's stain and for the detection of nuclear abnormalities with Giemsa stain. Prepared blood smears were examined under the Olympus CX-21 microscope.

The erythrocytes and leukocytes were counted manually by the Neubauer hemocytometer. For erythrocytes Hayem's solution and for leukocytes Turk's solution were used as a dilution solution (Tanyer, 1985).

Four measurement were taken from 40 randomly selected erythrocytes from each blood smear by using Olympus 1-15X micrometric ocular: Erythrocyte Length (EL) and Erythrocyte Width (EW), Nucleus Length (NL) and Nucleus Width (NW) (at 1000x magnification). The shapes of erythrocytes and nuclei were determined by the EL/EW and NL/NW ratios, and the shape of the nucleus/cytoplasm was determined by the NS/ES ratio. Erythrocyte size (ES) and nucleus size (NS) were calculated mathematically in accordance with the results obtained from the measurements (Atatür et al., 1999). Also, leukocyte formula was created from the blood smears of each individual (Tanyer, 1985).

Micronucleus was defined according to the criteria as follows:

- a) MN should be less than one-third of the main nucleus.
- b) MN should not be in contact with the main nucleus.
- c) MN should be the same color and density as the main nucleus and should not be refractive (Heddle and Countryman, 1976; Fenech, 2000; Çördük et al., 2018).

Other nuclear abnormalities such as kidney-shaped nucleus, lobbed nucleus, notched nucleus, blebbed nucleus and binucleated cells were also detected by counting 1000 erythrocytes in the blood smears (at 1000x magnification).

The standard values of the obtained data were evaluated by using Microsoft Excel, IBM SPSS Statistics 20 and R Project for Statistical Computing programs. Pearson Correlation Test and non-parametric Mann-Whitney U Test, were used to compare the data of blood cells according to stations and body size. In all cases,  $p \leq 0.05$  value was considered statistically significant.

## RESULTS

In order to determine the water quality in the studied localities, some water parameters were measured and classified according to the water quality classes determined by the SKKY. In the view of the pH, dissolved oxygen and temperature values, it can be said that Sarıçay was included in class IV, Atıkhisar and Yeniköy were included in class I-II (Table 2).

**Table 2.** Physicochemical parameters of the water samples taken from studied 3 different localities.

|                                    | SARIÇAY<br>CLASS IV | ATIKHİSAR<br>CLASS I-II | YENİKÖY<br>CLASS I-II |
|------------------------------------|---------------------|-------------------------|-----------------------|
| <b>pH</b>                          | 5.32                | 6.82                    | 6.99                  |
| <b>Dissolved Oxygen<br/>(mg/L)</b> | 1.08                | 12.13                   | 10.81                 |
| <b>Temperature (°C)</b>            | 21                  | 28                      | 23.7                  |

Body size (snout vent length) were measured and hematological analyzes were performed to examine blood cells of 12 *P. ridibundus* individuals collected from 3 different localities in Çanakkale province. Descriptive statistics of obtained measurements are given in Table 3 in detail.

**Table 3.** The descriptive statistics results of body size (snout vent length), blood cell counts and measurements of *Pelophylax ridibundus* species in three localities.

| SARIÇAY                     |   |         |         |           |         |          |
|-----------------------------|---|---------|---------|-----------|---------|----------|
|                             | N | Minimum | Maximum | Mean      | SE      | SD       |
| <b>SVL (mm)</b>             | 4 | 35.93   | 80.55   | 52.64     | 1.41    | 17.94    |
| <b>EC (1mm<sup>3</sup>)</b> | 4 | 240000  | 265000  | 250000.00 | 741.832 | 9383.513 |



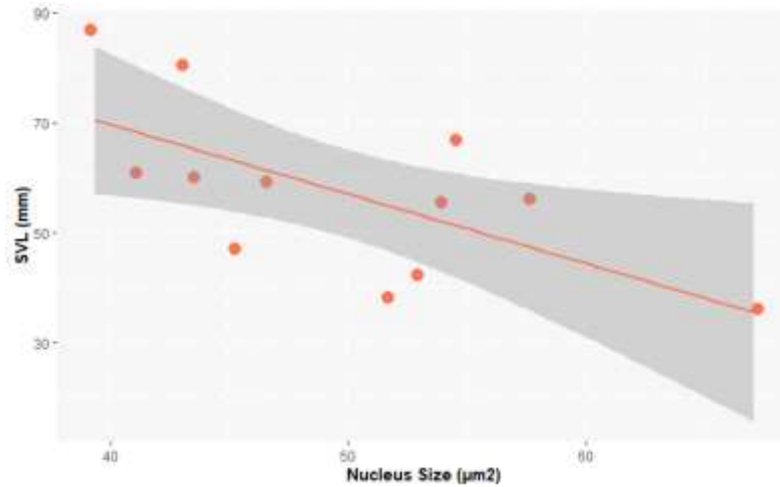
|                        |          |                |                |             |           |           |
|------------------------|----------|----------------|----------------|-------------|-----------|-----------|
| LC (1mm <sup>3</sup> ) | 4        | 3600           | 4150           | 3850.00     | 18.386    | 232.568   |
| EL (µm)                | 4        | 18.5           | 31.0           | 24.87       | 0.19      | 2.47      |
| EW (µm)                | 4        | 10.0           | 161.5          | 16.06       | 0.92      | 11.75     |
| NL (µm)                | 4        | 8.0            | 14.0           | 10.61       | 0.10      | 1.30      |
| NW (µm)                | 4        | 5.0            | 9.0            | 6.53        | 0.07      | 0.93      |
| ES (µm <sup>2</sup> )  | 4        | 188.40         | 3739.93        | 317.76      | 21.95     | 277.70    |
| NS (µm <sup>2</sup> )  | 4        | 33.36          | 91.84          | 54.79       | 0.95      | 12.04     |
| EL/EW (µm)             | 4        | 0.18           | 2.45           | 1.65        | 0.02      | 0.29      |
| NL/NW (µm)             | 4        | 1.11           | 2.54           | 1.64        | 0.01      | 0.25      |
| ES/NS (µm)             | 4        | 3.28           | 56.71          | 5.86        | 0.32      | 4.17      |
| Lym (%)                | 4        | 70             | 74             | 71.50       | 0.11      | 1.50      |
| Mono (%)               | 4        | 10             | 20             | 14.50       | 0.30      | 3.85      |
| Eos (%)                | 4        | 2              | 7              | 5.00        | 0.14      | 1.87      |
| Neut (%)               | 4        | 1              | 2              | 1.75        | 0.03      | 0.43      |
| Baso (%)               | 4        | 2              | 13             | 7.25        | 0.35      | 4.45      |
| <b>ATIKHISAR</b>       |          |                |                |             |           |           |
|                        | <b>N</b> | <b>Minimum</b> | <b>Maximum</b> | <b>Mean</b> | <b>SE</b> | <b>SD</b> |
| SVL (mm)               | 4        | 47.10          | 86.79          | 63.27       | 1.15      | 14.55     |
| EC (1mm <sup>3</sup> ) | 4        | 304000         | 305000         | 304562.50   | 29.323    | 370.916   |
| LC (1mm <sup>3</sup> ) | 4        | 3500           | 3650           | 3575.00     | 4.433     | 56.077    |
| EL (µm)                | 4        | 18.0           | 27.0           | 21.92       | 0.13      | 1.70      |
| EW (µm)                | 4        | 11.0           | 19.0           | 13.90       | 0.12      | 1.63      |
| NL (µm)                | 4        | 8.0            | 12.5           | 10.02       | 0.07      | 0.98      |
| NW (µm)                | 4        | 4.0            | 8.0            | 5.54        | 0.04      | 0.61      |
| ES (µm <sup>2</sup> )  | 4        | 162.49         | 374.44         | 239.74      | 3.01      | 38.14     |
| NS (µm <sup>2</sup> )  | 4        | 29.83          | 75.36          | 43.66       | 0.55      | 6.98      |
| EL/EW (µm)             | 4        | 1.22           | 2.18           | 1.59        | 0.01      | 0.20      |
| NL/NW (µm)             | 4        | 1.28           | 2.44           | 1.82        | 0.01      | 0.24      |
| ES/NS (µm)             | 4        | 3.22           | 8.33           | 5.54        | 0.06      | 0.78      |
| Lym (%)                | 4        | 54             | 86             | 69.25       | 0.93      | 11.81     |
| Mono (%)               | 4        | 7              | 20             | 12.50       | 0.39      | 5.04      |
| Eos (%)                | 4        | 2              | 27             | 14.25       | 0.70      | 8.89      |
| Neut (%)               | 4        | 2              | 4              | 3.00        | 0.05      | 0.70      |
| Baso (%)               | 4        | 0              | 2              | 1.00        | 0.05      | 0.70      |
| <b>YENIKÖY</b>         |          |                |                |             |           |           |
|                        | <b>N</b> | <b>Minimum</b> | <b>Maximum</b> | <b>Mean</b> | <b>SE</b> | <b>SD</b> |
| SVL (mm)               | 4        | 42.31          | 66.84          | 56.39       | 0.71      | 9.09      |

|                             |   |        |        |           |          |           |
|-----------------------------|---|--------|--------|-----------|----------|-----------|
| <b>EC (1mm<sup>3</sup>)</b> | 4 | 223000 | 260000 | 236500.00 | 1113.807 | 14088.668 |
| <b>LC (1mm<sup>3</sup>)</b> | 4 | 2900   | 3650   | 3325.00   | 22.518   | 284.837   |
| <b>EL (µm)</b>              | 4 | 21.0   | 31.0   | 24.78     | 0.14     | 1.82      |
| <b>EW (µm)</b>              | 4 | 11.5   | 20.0   | 15.38     | 0.17     | 2.18      |
| <b>NL (µm)</b>              | 4 | 8.0    | 14.5   | 10.40     | 0.08     | 1.13      |
| <b>NW (µm)</b>              | 4 | 5.0    | 8.0    | 6.15      | 0.05     | 0.66      |
| <b>ES (µm<sup>2</sup>)</b>  | 4 | 197.82 | 462.36 | 300.88    | 4.53     | 57.30     |
| <b>NS (µm<sup>2</sup>)</b>  | 4 | 33.36  | 75.36  | 50.51     | 0.68     | 8.71      |
| <b>EL/EW (µm)</b>           | 4 | 1.26   | 2.25   | 1.63      | 0.01     | 0.20      |
| <b>NL/NW (µm)</b>           | 4 | 1.12   | 2.41   | 1.70      | 0.01     | 0.21      |
| <b>ES/NS (µm)</b>           | 4 | 3.54   | 8.33   | 5.98      | 0.05     | 0.71      |
| <b>Lym (%)</b>              | 4 | 72     | 82     | 75.75     | 0.30     | 3.90      |
| <b>Mono (%)</b>             | 4 | 2      | 19     | 11.50     | 0.48     | 6.12      |
| <b>Eos (%)</b>              | 4 | 2      | 15     | 9.50      | 0.39     | 5.04      |
| <b>Neut (%)</b>             | 4 | 0      | 4      | 1.75      | 0.11     | 1.48      |
| <b>Baso (%)</b>             | 4 | 1      | 2      | 1.50      | 0.04     | 0.50      |

(N: Sample Number, SE: Standart Error, SD: Standart Deviation EC: Erythrocyte Count, LC: Leukocyte Count, EL: Erythrocyte Length, EW: Erythrocyte Width, NL: Nucleus Length, NL: Nucleus Width, ES: Erythrocyte Size, NS: Nucleus Size, EL/EW: Erythrocyte Length/Erythrocyte Width, NL/NW: Nucleus Length/Nucleus Width, ES/NS: Erythrocyte Size/Nucleus Size, Lym: Lymphocyte, Mono: Monocyte, Eos: Eosinophil, Neut: Neutrophil, Baso: Basophil).

A statistically significant correlation has not been found between body sizes and the erythrocyte and leukocyte count ( $P \geq 0,05$ ). When the erythrocyte counts were compared according to the localities, it was found that there was a statistically significant difference between the Sarıçay-Atikhisar localities (U: 0.000; W: 10.000; Z: -2.309; p: 0.021) and the Atikhisar-Yeniköy localities (U: 0.000; W: 10.000; Z: -2.309; p: 0.021). But, it was found that there was no statistically significant difference when the leukocyte counts were compared according to the localities ( $P \geq 0,05$ ). Erythrocyte counts were determined as on average 250000 1mm<sup>3</sup> in Sarıçay which is included in class IV, on average 304562 1mm<sup>3</sup> in Atikhisar and on average 236500 1mm<sup>3</sup> in Yeniköy which are included in class I-II.

Whether there is a correlation between body sizes and erythrocyte and nucleus sizes was determined by the Pearson Correlation test, it was found that there was a negative correlation only in nucleus sizes between 3 localities, and the nucleus size decreases as the body size increases ( $r = -0.649$ ,  $p = 0.023$ ) (Figure 3). A statistically significant correlation has not been found between body size and other measurements of erythrocytes ( $P \geq 0,05$ ).



**Figure 3.** Scatter point graph of the correlation between body size (snout vent length) and nucleus size.

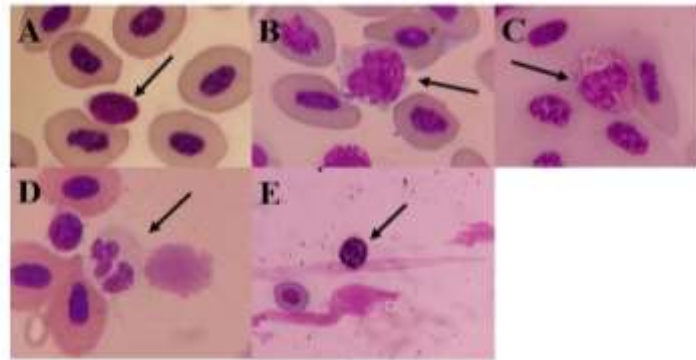
When the measurements of erythrocytes were compared according to the localities, it was found that there was a statistical difference in erythrocyte size only between Sarıçay-Atikhisar localities (U: 0.000; W: 10.000; Z: -2.309; p: 0.021). It was found that the erythrocyte sizes were on average 317.76 µm<sup>2</sup> in Sarıçay that has low levels of dissolved oxygen (1.08 mg/L), while in Atikhisar that has higher levels of dissolved oxygen (12.13 mg/L) were on average 239.74 µm<sup>2</sup>. It was determined that the erythrocyte sizes were larger in Sarıçay, which is considered as polluted locality, than in unpolluted localities. And it was determined that other measurements of erythrocytes do not differ statistically between localities ( $P \geq 0,05$ ) (Figure 4).



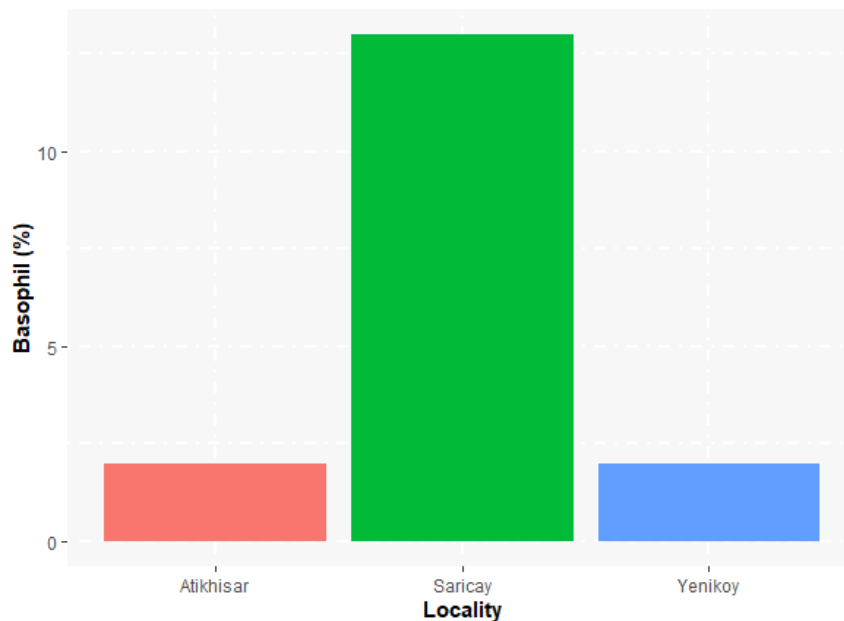
**Figure 4.** Bar graph of the comparison of erythrocyte measurements between 2 localities.

When the percentages of leukocytes were compared according to the localities, it was determined that there was a difference in the basophil percentage between the Sarıçay-Atikhisar localities (U:1.500; W:10.500; Z: -2.191; p: 0.028) and between Sarıçay-Yeniköy localities (U:1.000; W:11.000; Z: -2.084; p:0.037).

No statistically significant difference was detected between Sarıçay with Atikhisar and Yeniköy localities in terms of other types of leukocytes ( $P \geq 0,05$ ) (Figure 5). But it has been determined that there was a statistically significant difference in the basophil percentage between Sarıçay which is included class IV with Atikhisar and Yeniköy which are included in class I-II. It was determined that the percentage of basophils that seen in Sarıçay individuals was higher than the other two localities (Figure 6).

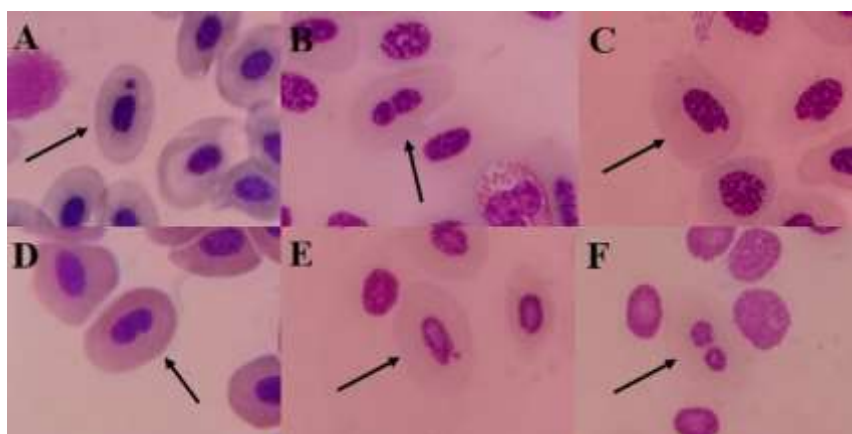


**Figure 5.** The leukocyte types of *Pelophylax ridibundus*; Lymphocyte (A), Monocyte (B), Eosinophil (C), Neutrophil (D), Basophil (E).



**Figure 6.** Bar graph of the percentages of basophil that found significant difference between the localities.

The frequency of micronucleus and other nuclear abnormalities in erythrocytes were measured (Figure 7) from the blood smears by counting 1000 erythrocytes and the statistical data is shown in Table 4 in detail. When the total percentage of nuclear abnormalities that measured in erythrocytes was compared between the localities, it was found that there was a statistical difference between Sarıçay-Atikhisar localities (U: 1.000; W: 11.000; Z: -2.033; p: 0.042) and Sarıçay-Yenikoy localities (U: 0.000; W: 10.000; Z: -2.309; p: 0.021). According to the obtained results, it was determined that the percentages of micronucleus and other nuclear abnormalities were higher in the Sarıçay that is more polluted than the other two localities (Table 4). The lowest percentage of total nuclear abnormalities was found in the Yeniköy locality that has not seen micronucleus, kidney-shaped nucleus and binucleated cells in the blood smears. Micronucleus and binucleated cells has not seen also in the blood smears from Atikhisar locality.



**Figure 7.** Micronucleus and other nuclear abnormalities of *Pelophylax ridibundus* species. Micronucleus (A), Lobbed Nucleus (B), Notched Nucleus (C), Kidney-shaped Nucleus (D), Blebbed Nucleus (E), Binucleated Cell (F).

**Table 4.** Mean and standard deviation values of micronucleus and other nuclear abnormality percentages (%) that measured in erythrocytes.

|  | SARIÇAY             | ATIKHİSAR           | YENİKÖY             |
|--|---------------------|---------------------|---------------------|
| <b>Micronuclei (%)</b>                 | 0.050±0.0502        | 0±0.00              | 0±0.00              |
| <b>Lobbed nuclei (%)</b>               | 0.200±0.2925        | 0.050±0.0502        | 0.075±0.0832        |
| <b>Notched nuclei (%)</b>              | 2.125±0.6239        | 1.050±0.4401        | 0.875±0.2495        |
| <b>Kidney-shaped nuclei (%)</b>        | 0.250±0.2881        | 0.125±0.1093        | 0±0.00              |
| <b>Blebbed nuclei (%)</b>              | 3.100±2.0050        | 0.825±0.4451        | 0.975±0.2689        |
| <b>Binucleated cell (%)</b>            | 0.025±0.0434        | 0±0.00              | 0±0.00              |
| <b>Total nuclear abnormalities (%)</b> | <b>5.750±2.4101</b> | <b>2.050±0.8986</b> | <b>1.925±0.3778</b> |

No statistical correlation has been found between the total nuclear abnormalities determined in erythrocytes and body size (snout vent length) ( $P \geq 0,05$ ).

## DISCUSSION

Streams and their sources are under threat as a result of increasing pollution day by day (Kaya, 2007). It is observed that the impact of environmental pollution on the living conditions of aquatic organisms is increasing (Romanova and Egorikhina, 2006). Interactions between biotic and abiotic factors such as environmental pollutants, pH changes, habitat fragmentation, wastewater discharge and recreational water use negatively affect amphibia and reptile species (Croteau et al., 2008). In all the studies that conducted on Sariçay, it has been reported that in the areas near to the city center, water is generally more polluted as physically, chemically and biologically, and the pollution levels decrease from the city center towards to the Atikhisar Dam (Ilgar, 2000; Odabaşı, 2005; Akbulut et al., 2006; Kaya, 2007). Hypoxia caused by a decrease in dissolved oxygen levels in aquatic ecosystems can be caused by biological and chemical wastes of human origin (Keleştemur, 2012). However, in recent studies, it has been reported that hypoxia is accompanied by low pH values. Therefore, many organisms exposed to hypoxic stress also need to cope with low pH values, which is called acidification (Gobler and Bauman, 2016; Tomasetti and Gobler, 2020). According to some water parameters that measured, Sariçay was classified as class IV, Atikhisar and Yeniköy were classified as class I-II in respect to water quality (SKKY, 2004). When the erythrocyte and leukocyte count of *P. ridibundus* species living in 3 localities (Sariçay, Atikhisar, Yeniköy) with different water parameters located in Çanakkale province were examined and it was determined that the results of blood counts did not correlate with body size (snout vent length). When the results of the blood counts were compared between the localities it was concluded that there was a statistical difference in the number of erythrocytes between the Sariçay-Atikhisar localities and the Atikhisar-Yeniköy localities. It was found that the number of leukocytes were not statistically different between the localities. Zhelev et al., 2013, found that erythrocyte counts increased in polluted areas, but these results do not show similarity with the results that we obtained. Fierascu et al., 2018, reported that erythrocytopenia and leukocytopenia could be seen in individuals exposed to the pesticide. In this study when Sariçay-Atikhisar localities were compared, it was determined that the number of erythrocytes was lower in the polluted area, so that the results are in accordance with this information in the literature. When the results of the comparison of erythrocyte measurements and body size (snout vent length) were examined; it was determined that there was a negative correlation between the body size (snout vent length) and only in nucleus size in 3 three localities, but there were not any correlations in other erythrocyte measurements. However, study that compare body size and nucleus size has not

been found. When the results of comparison of erythrocyte measurements according to the 3 localities were examined, it was found that there was a statistical difference in only in the erythrocyte size between Sarıçay and Atikhisar. It was determined that other measurements of erythrocytes were not show statistically difference between the localities. It is believed that the erythrocyte size undergoes adaptation to increase oxygen uptake capacity due to low oxygen levels in Sarıçay that included in class IV in terms of some water parameters. Zhelev et al., 2017, found that the EL, EW and ES measurements were increased in the erythrocytes of *P. ridibundus* species living in a polluted stream with household wastes. The results of our study are in accordance with this study. When the comparison of measurements of the percentage of leukocytes between 3 localities were examined; it was found that there was a significant difference between Sarıçay-Atikhisar and between Sarıçay-Yeniköy. There was not a significant difference between the localities in terms of other leukocyte types. In the view of the information obtained from the previous studies, it can conclude that the percentage of basophils has increased in highly contaminated areas (Sils, 2008; Zhelev et al., 2013). When the percentages of micronucleus and other nuclear abnormalities were examined, it was determined that there was a statistically significant difference between Sarıçay-Atikhisar and Sarıçay-Yeniköy localities in terms of the percentage of total nuclear abnormalities. And it was found that the highest percentage of the total nuclear abnormalities was in Sarıçay and the lowest was in Yeniköy. Çördük et al., 2018, reported a high correlation between heavy metal concentrations in a stream that contaminated with heavy metals and the percentages of nuclear abnormalities in the *P. ridibundus* species. According to Şişman et al., 2015, the formation of micronucleus in polluted areas in the *P. ridibundus* species increases significantly in comparison with control areas. The results of our study show similarity to the previous studies.

## CONCLUSIONS

As a result of this study, due to the pollution occurring in and around the Sarıçay, it has been established that there were some changes in the number and size of blood cells, leukocyte types and nuclear abnormalities of the *P. ridibundus* species. Thus, it has been concluded that the morphological characteristics and counts of blood cells of the *P. ridibundus* species may play a bioindicator role in determining the environmental pollution.

## ACKNOWLEDGEMENTS

This study is a part of Master's Thesis titled "Sarıçay (Çanakkale)'daki Çevresel Parametrelerin *Pelophylax ridibundus* ve *Mauremys rivulata* Türleri Üzerinde Hematolojik ve Genotoksikolojik Etkilerinin Belirlenmesi", Çanakkale Onsekiz Mart University, School of Graduate Studies.

## REFERENCES

- Akbulut M, Odabaşı SS, Odabaşı DA & Çelik EŞ (2006). Çanakkale İli'nin Önemli İçsuları ve Kirlenici Kaynakları. *Su Ürünleri Dergisi*, 23(1), pp.9-15.
- Atatür MK, Arıkan H & Çevik İE (1999). Erythrocyte sizes of some anurans from Turkey. *Turkish Journal of Zoology*, 23, 111–114.
- Ballard BM & Cheek R (eds.) (2003). *Exotic animal medicine for the veterinary technician*. Wiley and Sons /Blackwell Publishing, New York.
- Baran İ, Ilgaz Ç, Avcı A, Kumlutaş Y & Olgun K (2012). *Türkiye Amfibi ve Sürüngenleri*. Tübitak Popüler Bilim Kitapları, 1-204.
- Beynon PH, Lawton MPC & Cooper JE (1992). *Manual of reptiles*. British Small Animal Veterinary Association, Cheltenham. pp 228.
- Browne CL (2004). Impacts of Urbanisation and Metal Pollution on Freshwater Turtles. School of Biological Sciences, Faculty of Science, University of Sydney.
- Gobler CJ, & Baumann H (2016). Hypoxia and acidification in ocean ecosystems: coupled dynamics and effects on marine life. *Biology letters*. 12(5), 20150976.
- Cabagna MC, Lajmanovich RC, Stringhini G, SanchezHernandez JC & Peltzer PM (2005). Hematological parameters of health status in the common toad *Bufo arenarum* in agroecosystems of Santa Fe province, Argentina, *Applied Herpetology*, 2, 373–380.
- Carvalho CS, Utsunomiya HSM, Pasquoto T, Lima R, Costa MJ & Fernandes MN (2016) Blood cell responses and metallothionein in the liver, kidney and muscles of bullfrog tadpoles, *Lithobates catesbeianus*, following exposure to different metals. *Environ Pollut* 1–8. doi:10.1016/j. envpol.2016.12.012
- Croteau MC, Hogan N, Gibson JC, Lean D, Trudeau VL (2008). Toxicological threats to amphibians and reptiles in urban environments. *Urban herpetology, Herpetological Conservation*, 3, 197-209.
- Çördük N, Hacıoğlu-Doğru N, Gül Ç & Tosunoğlu M (2018). Monitoring of micronuclei and nuclear abnormalities in *Pelophylax ridibundus* erythrocytes from the Biga Stream (Canakkale, Turkey). *Fresenius Environmental Bulletin*, 27(1), pp.147-153.
- Fenech M (2000). The in vitro micronucleus technique. *Mutation Research*, 455, 81–95.
- Fierascu I, Paunescu A, Soare LC, Fierascu RC & Ponepal MC (2018). The influence of six pesticides on physiological indices of *Pelophylax ridibundus* (Pallas, 1771). *Bulletin of environmental contamination and toxicology*, 100(3), 376-383.
- Heddle JA & Countryman RI (1976). The production of micronuclei from chromosome aberration in irradiated cultures of human lymphocytes. *Mutat Res*. 41, 321-332.
- İlgar R (2000). A Geographical Investigation Of Çanakkale Straits and Their Around Ecosystem (in Turkish). Doktora Tezi, İstanbul Üniv. Deniz Bilimleri ve İşletmeciliği Enstitüsü, Denizel Çevre Ana Bilim Dalı, Deniz ve Kıyı Koruma Bilim Dalı, İstanbul, 153.
- Kaya H (2007). Atıkhisar Barajı ve Sarıçay'da pestisit ve evsel kirliliğin araştırılması. Yüksek Lisans Tezi. Çanakkale Onsekiz Mart Üniversitesi, Çanakkale.
- Keleştemur GT (2012). Hipoksik Suların Balıklar Üzerinde Oluşturduğu Fizyolojik Etkiler. *Türk Bilimsel Derlemeler Dergisi*, 5(1), 87-90.
- Marques SM, Antunes SC, Pissarra H, Pereira ML, Gonçaves F & Pereira R (2009). Histopathological changes and erythrocytic nuclear abnormalities in Iberian green frogs (*Rana perezi Seoane*) from a uranium mine pond. *Aquatic Toxicology*, 91, 187–195.
- Medina MF, González ME, Klyver SMR & Odstrcil IMA (2016). Histopathological and biochemical changes in the liver, kidney, and blood of amphibians intoxicated with cadmium. *Turkish Journal of Biology*, 40(1), 229-238.
- Odabaşı SS (2005). Water Quality Resaerach On Sarıçay Stream In Çanakkale Region (in Turkish). Yüksek Lisans Tezi. ÇOMÜ. Fen Bilim. Enst. Çanakkale. 68 s.
- Pollo F, Grenat P, Otero M, Salas N & Martino A (2016). Assessment in situ of genotoxicity in tadpoles and adults of frog *Hypsiboas cordobae* (Barrio 1965) inhabiting aquatic ecosystems associated to fluorite mine. *Ecotoxicol Environ Saf* 133:466–474.
- Romanova EB & Egorikhina MN (2006). Changes in hematological parameters of *Rana* frogs in a transformed urban



- environment, *Russian Journal of Ecology*, 37(3), 188-192.
- Salinas Z, Salas N, Baraquet M & Martino A (2015). Biomarcadores hematológicos del sapo común *Bufo (Rhinella) arenarum* en ecosistemas alterados de la provincia de Córdoba hematologic biomarkers of the common toad *Bufo arenarum* in alteredecosystem of Córdoba province. *Acta Toxicol Argent* 23(1):25–35
- Sils EA (2008). Specific of amphibian (genus *Rana*) peripheral blood leucogram under condition of anthropogenic load. – In: Ananjeva N. B. (Ed.): *The Problems of Herpetology*. Saint-Petersburg, *Russian Collection publishing*, 369-374. (In Russian).
- SKKY (2004). Su Kirliliği Kontrolü Yönetmeliği. 31.12.2004 Tarih ve 25687 Sayılı Resmi Gazete. Ankara.
- Şahin E (2019). Gediz Nehri'nde yaşayan *Pelophylax bedriagae*'nin (Camerano, 1882) (Amphibia: Ranidae) eritrosit morfolojisi ve sayısında kirliliğe bağlı değişimin araştırılması. Yüksek Lisans Tezi. Ege Üniversitesi, Fen Bilimleri Enstitüsü, İzmir.
- Şişman T, Aşkın H, Türkez H, Özkan H, İncekara Ü & Çolak S (2015). Determination of Nuclear Abnormalities in Peripheral Erythrocytes of the Frog *Pelophylax ridibundus* (Anura: Ranidae) sampled from Karasu River Basin (Turkey) for Pollution Impacts. *LIMNOFISH-Journal of Limnology and Freshwater Fisheries Research* 1(2): 75-81 (2015)
- Şişman T, Alnoaimi F & Dane H (2021). Histopathologic and genotoxic effects of deltamethrin on marsh frog, *Pelophylax ridibundus* (Anura: Ranidae). *Environmental Science and Pollution Research*, 28(3), 3331-3343.
- Tanyer G (1985). *Hematology and Laboratory*. Ayyıldız Matbaası A.ş. Ankara.
- Teixeira PC, Dias DC, Rocha GC, Antonucci AM, França FM, Marcantonio AS, ... & Ferreira CM (2012). Profile of cortisol, glycaemia, and blood parameters of American Bullfrog tadpoles *Lithobates catesbeianus* exposed to density and hypoxia stressors. *Pesquisa Veterinária Brasileira*, 32, 91-98.
- Thrall A, Baker DC, Campbell TW, DeNicola D, Feetman MJ, Lassen ED, Rebar A & Weiser G (2004). Veterinary hematology and clinical chemistry. Philadelphia: *Blackwell Publishing*. 776 p.
- Tomasetti SJ & Gobler CJ (2020). Dissolved oxygen and pH criteria leave fisheries at risk. *Science*, 368(6489), 372-373.
- Wright KN & Whitaker BR (2001). *Amphibian Medicine and Captive Husbandry*. Malabar, *Krieger Publishing Company*. 570 pp.
- Zhelev Z, Popgeorgiev G, Ivanov I & Boyadzhiev P (2017). Changes of erythrocyte-metric parameters in *Pelophylax ridibundus* (Amphibia: Anura: Ranidae) inhabiting water bodies with different types of anthropogenic pollution in Southern Bulgaria. *Environmental Science and Pollution Research*, 24(21), 17920-17934.
- Zhelev ZM, Popgeorgiev GS & Angelov MV (2013). Investigating the changes in the morphological content of the blood of *Pelophylax ridibundus* (Amphibia: Ranidae) as a result of anthropogenic pollution and its use as an environmental bioindicator. *Acta Zoologica Bulgarica*, 65(2), pp.187-196.

Oral Presentation

Thursday

Aquatic (Marine and Freshwater) Biodiversity-1

#### Isolation and Molecular Characterization of Bacteria from Intestinal Flora of Some Aquatic Beetles (Coleoptera: Hydrophilidae)

Ahmet Polat<sup>1</sup>, Ayşenur Yazıcı<sup>2</sup>, A.Muhammet Çorapçı<sup>2</sup>, Serkan Örtücü<sup>2\*</sup>, Mesut Taşkın<sup>3</sup>, Ümit İncekara<sup>1,2</sup>

<sup>1</sup>Atatürk University, Faculty of Sciences, Department of Biology, Erzurum, Turkey.

<sup>2</sup>Erzurum Technical University, Department of Molecular Biology and Genetics, Erzurum, Turkey.

<sup>3</sup>Atatürk University, Department of Molecular Biology and Genetics, Erzurum, Turkey.

\*Corresponding author e-mail: serkanortucu@gmail.com

#### Abstract

The water scavenger beetles (Hydrophilidae) are the largest group of their superfamily which are often abundant in aquatic habitats. Insect guts contain many microbial species that affect their development and ecology. The intestinal flora and their genomes were recognized as a major genetic resource for biotechnology. Most of the studies were focused on investigating the intestinal flora for terrestrial insects. There is limited knowledge in the aquatic beetles bacterial flora. This study was conducted to isolate bacteria from the intestinal system of Hydrophilidae for possible use as new sources for biotechnological products. For this purpose, beetles were collected from Erzurum, Turkey in July-September 2020. To determine the intestinal flora, the surface of beetles was sterilized and dissected under aseptic conditions. The gut samples were homogenized and suspensions were spread on nutrient agar. The molecular characterization was performed by carrying out sequencing of 16S region. The sequences were compared to all known sequences in the GenBank for confirmation of their identity. A total five different species belonging to Hydrophilidae were obtained; *Berosus luridus*, *Hydrochara dichroma*, *Laccobius syriacus*, *Enochrus fuscipennis* and *Hydrobius fuscipes*. In the preliminary study, we describe the bacterial diversity of the intestinal system from five Hydrophilidae members. As a result, ten different bacterial species belonging to *Klebsiella pneumoniae*, *Serratia fonticola*, *Bacillus pumilus*, *Acinetobacter radioresistens*, *Carnobacterium divergens*, *Paenibacillus amylolyticus*, *Pseudomonas helmanticensis*, *Hafnia paralvei*, *Exiguobacterium mexicanum* and *Aeromonas rivuli* were obtained. A high bacterial diversity was found in the gut of *B. luridus*. This diversity may be due to its larger size compared to other studied beetles. *S. fonticola* was the most common bacteria isolated from intestinal flora except for *B. luridus*. These locally isolated bacteria may be the subject to new sources for industrial products in terms of production of some biotechnological products.

**Keywords:** Hydrophilidae, intestinal flora, biotechnology

**Acknowledgement:** This work was supported by the Erzurum Technical University Research Foundation (ETU-BAP: 2020/013).

Oral Presentation

Thursday

Conservation Biology, Policy and Strategies &amp; Protected

## Development of Microplastic Pollution Awareness Scale for Prospective Science and Biology Teachers

Tuğçe Güleşir<sup>1\*</sup>, Ali Gül<sup>2</sup>

<sup>1</sup>Necmettin Erbakan University, Ahmet Keleşoğlu Faculty of Education, Department of Mathematics and Science Education, Division of Biology Education, Konya, Turkey.

<sup>2</sup>Gazi University, Gazi Education Faculty, Department of Mathematics and Science Education, Division of Biology Education, Ankara, Turkey.

\*Corresponding author e-mail: tugce.gulesir@erbakan.edu.tr

### Abstract

Microplastic pollution is one of the current important environmental problems. In the literature review, the lack of educational studies on microplastic pollution attracted attention and no measurement tool for microplastic pollution was found. Therefore, in this the current study; it was aimed to develop a microplastic pollution awareness scale for science and biology teacher candidates and to determine the awareness levels of teacher candidates in terms of different variables. In this study, in which the survey (descriptive, survey) model, one of the quantitative research methods, was used, the sample group; It consists of 586 science and biology teacher candidates studying at different universities in the spring semester of the 2019-2020 academic year. After the scale items were created, expert opinions were taken and the scale was applied. The data obtained from the application were subjected to exploratory factor analysis (EFA) and the structure obtained was tested with confirmatory factor analysis (CFA). The general reliability coefficient of the scale was determined as .81. The "Microplastic Pollution Awareness Scale", whose validity and reliability analyses have been completed, has become a 3-factor scale consisting of a total of 14 items, 5 of which are negative and 9 of which are positive. The Likert scale consists of "No", "I have no idea" and "Yes" options. Finally; the prepared scale was applied to the sample group and the significance of the total scores of the teacher candidates from Microplastic Pollution Awareness Scale was tested according to gender, grade level, the status of taking environmental courses and academic grade point average. Determining the current awareness is important in terms of planning and implementing the necessary training. For this reason, it is thought that the study will contribute to the literature and may lead to similar studies in the future.

**Keywords:** Microplastic, microplastic pollution, awareness

Oral Presentation

Thursday

Conservation Biology, Policy and Strategies & Protected

***Ex-Situ* Conservation Strategies for *Antrodia cinnamomea*: An Endemic Medicinal  
Mushroom in Taiwan**

K.J. Senthil Kumar<sup>1\*</sup>, Büşra Albayrak<sup>2</sup>, Büşra Yazıcılar<sup>2</sup>, Merve Şimşek Geyik<sup>2</sup>

<sup>1</sup>Bachelor Program of Biotechnology, National Chung Hsing University, Taichung, Taiwan

<sup>2</sup>Department of Molecular Biology and Genetics, Erzurum Technical University, 25050 Erzurum, Turkey.

**Abstract**

*Antrodia cinnamomea* (Syn. *Antrodia camphorata*; *Taiwanofungus camphoratus*) is a medicinal mushroom endemic to Taiwan. In Traditional Chinese Medicine (TCM), it is known as “Niu Cheng Zhi” and regarded as “*National Treasure of Taiwan*”. Over a millennium, *A. cinnamomea* is traditionally used for treating various human ailments, including food poisoning, drug intoxication, diarrhea, abdominal pain, hypertension, skin irritation, inflammation, and cancer. Accumulating scientific evidence (more than 500 research articles within two decades) revealed that *Antrodia cinnamomea* possesses various therapeutic effects including, hepatoprotection, neuroprotection, anti-oxidant, anti-inflammation, anti-hypertensive, anti-hyperlipidemic, anti-metastatic, and anti-cancer. Recently, this mushroom is attracted by pharmaceutical and nutraceutical industries due to its unique bioactive components including, triterpenoids, polysaccharides, benzenoids, benzoquinone derivatives, and maleic/succinic acid derivatives. This endemic fungus grows the inner sap of the age-old bull Camphor tree *Cinnamomum kanehira* Hay (Lauraceae). Until a decade ago, to obtain this fungus from the natural source, the only option is to cut down the host species, which eventually accelerates species loss. To protect the biodiversity of the host species (*Cinnamomum kanehira*), Taiwan Forestry Bureau made policies to stop the wild extraction of *A. cinnamomea* and urged to develop of an alternative way to propagate this medicinal fungus. After continuous efforts, scientists developed several *in vitro* and *ex-situ* culture techniques, including cut-log cultivation, solid-state cultivation, liquid fermentation, and petri dish culture for the mass production of this fungus. After establishment of these techniques, wild extraction of *A. cinnamomea* was put an end.

**Keywords:** *Antrodia cinnamomea*, *Cinnamomum kanehira*, endemic mushroom

Oral Presentation

Thursday

Effects of Biodiversity to Human Health-1

**Antioxidant Capacity and Phenolic Composition of *Gagea chanae* Grossh. and *Scilla siberica* Haw.**

Bilge Aydın<sup>1\*</sup>, Enes Tekman<sup>2,3</sup>, Hafize Yuca<sup>4</sup>, Songül Karakaya<sup>2</sup>, Zühal Güvenalp<sup>4</sup>

<sup>1</sup>Erzincan Binali Yıldırım University, Faculty of Pharmacy, Department of Pharmacognosy, Erzincan, Turkey.

<sup>2</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Erzurum, Turkey.

<sup>3</sup>Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey.

<sup>4</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, Turkey.

\*Corresponding author e-mail: bilgeakcil03@hotmail.com, bilge.akcil@erzincan.edu.tr

**Abstract**

Free radicals are toxic byproducts of aerobic metabolism. Antioxidants are very important because they destroy free radicals. Disruption of the oxidant/antioxidant balance in the body in favor of oxidants causes many diseases. Plants may show strong antioxidant effects due to the polyphenolic compounds they contain and may be protective against many chronic diseases. The aim of our study is to evaluate and compare these plants in terms of antioxidant effect and total phenolic compound. These species were collected from Erzurum and dried corms, leaves and flowers extracted with methanol. These extracts were evaluated for their antioxidant capacities by DDPH and ABTS methods, and phenolic content using Folin-Ciocalteu's reagent (FCR). In the ABTS<sup>++</sup> scavenging activity of *Gagea chanae* and *Scilla siberica*,  $\alpha$ -tocopherol (TK) was used as standard. The extracts of *G. chanae*, flower extract (F) showed highest activity compared to corm (C) and leaf (L) extract [(TK)87.6>(F)29.6>(L)26.5>(C)12.1%; at 32,5  $\mu$ g/ml]. The extracts of *S. siberica*, leaf extract (L) showed highest activity compared to corm (C) and flower (F) extract [(TK)90.1>(L)24.5>(F)18.5>(C)7.7%; at 40  $\mu$ g/ml]. The results of the total phenolic compound was similar to the results of the ABTS<sup>++</sup> tests. Total phenolic compound test results for *G. chanae* [(F)50.7>(L)47.6>(C)42.1  $\mu$ g GAE/ mg extract]. Total phenolic compound test results for *S. siberica* [(L)53.5>(F)43.8>(C)38.0  $\mu$ g GAE/ mg extract]. DPPH• scavenging activity was not found within the limits determined for *G. chanae*. DPPH• scavenging activity test results for *S. siberica* [(TK)91.9>(L)7.0>(F)5.2>(C)2.7%; at 100  $\mu$ g/ml]. In a conclusion; our results should be useful in future studies about these plants.

**Keywords:** *Gagea*, *Scilla*, abts, dpph, fcr

**Acknowledgement:** Bilge Aydın would like to acknowledge the scholarship during her postgraduate program provided by the Turkish Scientific and Technical Research Council (TUBITAK).

Oral Presentation

Thursday

Effects of Biodiversity to Human Health-1

### ***In vitro* Evaluation of Antidiabetic Activity of *Colchicum speciosum* Different Parts and Their Anatomical Properties**

Hafize Yuca<sup>1,2\*</sup>

<sup>1</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, Turkey.

<sup>2</sup>Ataturk University, Medicinal and Aromatic Plant and Drug Research Center, Erzurum, Turkey.

\*Corresponding author e-mail: hafize.yuca@atauni.edu.tr

#### **Abstract**

*Colchicum speciosum* (Colchicaceae), named as “Vargit, Acı Çiğdem” in Turkey. Previous studies showed that bulbs and seeds have colchicine with its derivatives and is used for treatment of gout and thalassemia. Aim of our study is evaluating of *in vitro* antidiabetic activity of extracts prepared from different parts of plant and making anatomical examination of these parts. Methanol extracts were prepared from corm, leaf, and flower of plant with maceration. Enzymes inhibitory effects of extracts were determined. Sections were taken manually from plant parts in 70 % alcohol, and prepared with Sartur reagent for anatomical examinations. Corm extract exhibited  $\alpha$ -glucosidase inhibitory activity with an IC<sub>50</sub> value of 21039  $\mu$ g/mL compared to positive control acarbose (IC<sub>50</sub> = 4738  $\mu$ g/mL), as well as no inhibition against  $\alpha$ -amylase. Leaf and flower extracts showed no inhibition against both enzymes. In corm cross sections, epidermis is composed of a single row of cells in a square shape, closely arranged. Transmission bundles are larger and more numerous at center. In leaf cross section; below upper and lower epidermis cells are several rows of palisade parenchyma and sponge parenchyma and vascular bundles between them. Cells of upper and lower epidermis layers are similar. Tepal epidermis layer resembles leaf epidermis, is oblong in shape and larger. Ovary with 3 loculus, abundant starch cells, stomata are flush with epidermis cells and few in number. It is the first study of *in vitro* evaluation of antidiabetic activity of *C. speciosum* different parts.

**Keywords:** *Colchicum speciosum*, anatomy, antidiabet, enzyme inhibition

Oral Presentation

Thursday

Effects of Biodiversity to Human Health-1

***α*-Glucosidase and *α*-Amylase Inhibitory Potential of *Paliurus spina-christi* Mill. and Its  
Main Compounds**

Hafize Yuca<sup>1,2\*</sup>, Hilal Özbek<sup>1,2</sup>, L. Ömür Demirezer<sup>3</sup>, Zühal Güvenalp<sup>1,2</sup>

<sup>1</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmacognosy, Erzurum, Turkey.

<sup>2</sup>Ataturk University, Medicinal and Aromatic Plant and Drug Research Center, Erzurum, Turkey.

<sup>3</sup>Hacettepe University, Faculty of Pharmacy, Department of Pharmacognosy, Ankara, Turkey.

\*Corresponding author e-mail: hafize.yuca@atauni.edu.tr

**Abstract**

Type II diabetes mellitus is a common disease in the world and characterized by hyperglycemia. Prevention of diabetes by reducing hyperglycemia depends on the inhibition of  $\alpha$ -amylase and  $\alpha$ -glucosidase enzymes. In our study, the antidiabetic profiles of *Paliurus spina-christi* phytochemicals and extracts were investigated. The plant is used in folk medicine in Turkey because of antidiabetic effect.  $\alpha$ -Amylase and  $\alpha$ -glucosidase inhibitory effect studies were conducted to prove this effect. The *n*-hexane extract (IC<sub>50</sub> = 445.7  $\mu$ g/mL) possessed potent inhibitory activity against  $\alpha$ -glucosidase enzyme than that of acarbose (IC<sub>50</sub> = 4212.6  $\mu$ g/mL), unlike their slight/no inhibition on  $\alpha$ -amylase. Phytochemical investigation of the *n*-hexane extract of the fruits of *Paliurus spina-christi* Mill., Rhamnaceae led to the isolation of triterpenes, betulin, betulinic acid, lupeol and a sterol,  $\beta$ -sitosterol. The structures of compounds were elucidated by extensive 1D- and 2D-NMR spectroscopic analysis and comparison with the relevant literature. Betulin, betulinic acid and lupeol are reported for the first time from this species. All isolated compounds, especially betulin and betulinic acid mixture (IC<sub>50</sub> = 247  $\mu$ M) showed higher  $\alpha$ -glucosidase inhibitory activity than acarbose (IC<sub>50</sub> = 6517  $\mu$ M). As with all extracts, the compounds were also found to be ineffective against the  $\alpha$ -amylase enzyme.

**Keywords:** *Paliurus spina-christi*, betulin, betulinic acid,  $\alpha$ -glucosidase inhibition

**Acknowledgement:** This work was supported by The Scientific and Technological Research Council of Turkey (TÜBİTAK) 3001 – Starting R&D Projects Funding Program (No. 217S206).

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Useful Plants of Mountain Xerophytic Communities of The Lesser Caucasus (Within  
Azerbaijan)**Cabbarov M.<sup>1\*</sup>, Nebiyeva F.X.<sup>2</sup>, Ibrahimov A.S.<sup>2</sup>, Atamov V.V.<sup>3</sup><sup>1</sup>Baku State University, Faculty of Biology, Chair of Botany, Baku, Azerbaijan<sup>2</sup>ANAS, Merdekan Institute of Dendrology, Merdekan, Azerbaijan<sup>3</sup>Recep Tayyip Erdogan University, Department of Biology, 53100, Rize, Turkey**Abstract**

Plants show diversity according to their beneficial properties. Within this diversity, there are feed, water, medicinal, spice and essential oil plants and their subgroups. Plants have been used for cosmetics, perfumery, nutrition and therapeutic purposes for thousands of years. It is an indispensable part of the aromatic industry, which has recently been used in the home and pharmaceutical industry. The main purpose of this study was to examine the floristic properties with beneficial properties of mountain-xerophyte plant communities of Kichik Qafgaz (within the borders of Azerbaijan). Herbarium samples collected during the supply studies carried out in Talysh and Nakhchivan Autonomous Republic of Azerbaijan in 2017-2021 vegetation periods constituted the material of the research. Samples Flora of Azerbaijan (8 volumes)

Findings: Mountain-xerophyte flora elements were systematically grouped according to beneficial groups in the investigated areas. The vegetation of the region is richer with 420 (29.83%) forage plants, 280 (19.87%) with ornamental properties, 220 (15.63%) etheric essential oils with medicinal properties, 125 (8%) next. It has been revealed that there are .88 species of vitamin-containing and tragacanth plants, 86 (6.11%), honeyveran 56 (3.98%), and 50 food (3.55%) plants. Among the plant taxa, 93 (6.51%) resin and rubber content and 78 (5.54%) poisonous plants were also determined.

**Keywords:** Useful plants, mountain, lesser caucasus, Azerbaijan

**Acknowledgement:** This work was supported by Research Fund of the Atatürk University (FBA-2018-6832).



Oral Presentation

Thursday

Effects of Biodiversity to Human Health-1

### Antimicrobial Activity of Different Parts of *Gagea chanae* Grossh. and *Scilla siberica* Haw.

Enes Tekman<sup>1,2\*</sup>, Songül Karakaya<sup>1</sup>, Gamze Goger<sup>3</sup>

<sup>1</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Erzurum, Turkey.

<sup>2</sup>Ankara University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Ankara, Turkey.

<sup>3</sup>Trakya University, Faculty of Pharmacy, Department of Pharmacognosy, Edirne, Turkey.

\*Corresponding author e-mail: tekmanenes@gmail.com

#### Abstract

*Scilla siberica* Haw. has known as Siberian Squill, grow in Russia, Asia Minor. It is a perennial bulbous plant and has a short time of vegetation period from February to May. *Gagea* genus is represented by almost 280 to 300 species worldwide. In Flora of Turkey, this genus contained 25 species. *Gagea chanae* Grossh. and *S. siberica* were collected from Erzurum and dried corms, leaves and flowers were macerated with methanol. Antimicrobial activities were evaluated by microdilution method with some modifications against *E. coli* ATCC 8739, *S. aureus* ATCC 6538, *B. subtilis* ATCC 19659, *C. albicans* ATCC 10231, *C. krusei* ATCC 14243, *C. tropicalis* ATCC 750. *S. siberica* flower and leaf extracts showed best antimicrobial activity against *Candida krusei* ATCC 14243 with MIC =320 µg/mL. All of the extracts showed same antimicrobial activity against *Escherichia coli* ATCC 8739, *Bacillus subtilis* ATCC 19659 and *Candida albicans* ATCC 10231 with range of MIC=1280-2560 µg/mL. *Gagea chanae* corm extract showed best antimicrobial activity against *Candida tropicalis* ATCC 750, *Candida krusei* ATCC 14243, *Candida albicans* ATCC 10231 with MIC =640 µg/mL values and the leaf extract was also found effective against *Candida albicans* ATCC 10231 with MIC= 640 µg/mL. These findings should be useful in future investigations about these species with different microorganisms for antimicrobial evaluation.

**Keywords:** Antimicrobial, *Gagea chanae*, mic, *Scilla siberica*

Oral Presentation  
Thursday  
Population Ecology

**Bioactivity of Essential Oil of *Artemisia Herba Alba* and Its Effects on *Culex Pipiens*  
(Diptera; Culicidae)**

Salma Kaoutar Abdelali<sup>1\*</sup>, Karim Souttou<sup>2</sup>, Linda Aissaoui<sup>1</sup>

<sup>1</sup>Research laboratory on the improvement and development of animal and plant production, Department of animal biology and physiology, Ferhat Abbas University of Setif, Algeria

<sup>2</sup>Laboratory for Exploration and Valorization of Steppe Ecosystems, Department of Biology, Faculty of Natural and Life Sciences, University of Ziane Achour, Djelfa, B.P. 17000, Djelfa, Algeria.

\*Corresponding author e-mail: Abdelali.selmakaoutar@gmail.com

**Abstract**

*Aretemisia herba alba* Asso (*A. herba alba*) (Asteraceae) is widely used in herbal medicine it has a real mine of natural molecules like davanone which is a very interesting product on the international market. The present research proposes a method for controlling the pre-imaginary stages of *Culex pipiens* (L4 and pupae) based on essential oil of *Artemisia herba alba*. The aerial part of this plant was extracted by hydrodistillation which gave a yield of 1.5. Then it was analyzed by gas chromatography coupled to the mass spectrometry (CPG / SM) for the determination of its chemical composition. The results of the analysis showed that the oil of *A. herba alba* is a davanone chemotype which consists mainly of davanone (48.8%).

Three concentrations (1µl/ml, 5µl/ml and 10µl/ml) are prepared and directly tested on larvae (L4) and pupae. The results show that the essential oils have an important larvicidal and pupicidal activity. This efficiency is expressed by the calculated toxicological parameters which are successively LC50 and LC90, for larvae 3.278 µl/ml and 7.573 µl/ml, and for pupae 1.213 µl/ml and 2.288 µl/ml.

**Keywords:** Essential oil, larvicidal, pupicidal, *Culex pipiens*

**INTRODUCTION**

The control of immature mosquitoes considered as an advantageous means for the prevention of the transmission of vector diseases, because the larvae are usually concentrated, relatively

immobile and occupy minimal habitat compared to adults (IMBAHALE et al., 2011) The widespread use of chemical insecticides has developed disadvantages due to their persistent nature and the presence of residues in various environments and in food (AIR PARIF, 2016).

Today, in order to preserve the health of non-target populations, it is necessary to focus on natural compounds from plants (HABBACHI et al., 2013). by exploiting their capacity to produce secondary metabolites which can be included in the industry of new bioinsecticides (ACHEUK, 2017). *Artemisia herba alba* is a silvery perennial dwarf shrub that grows in arid areas and semi-arid climates. With rapid growth in dry and hot climates and in muddy areas (TILAOUI et al., 2015). In Algeria it represents an important fodder resource (BELHATTAB et al., 2014). The essential oil of this herb has antioxidant, disinfectant, antibacterial, antileishmanial, anthelmintic, nematicide and antispasmodic properties (ABU-DARWISH et al., 2015). In Algeria, the studies on the insecticidal activity of plant extracts against the mosquito larva are very limited (BENHISSEN et al., 2018) but in recent years has started to develop, through a multitude of recent works (HABBACHI et al., 2013; BELHATTAB et al., 2014; MERABTI et al., 2015; ACHEUK et al., 2017; MERABTI et al., 2017; MATOUG et al., 2017; BENHISSEN et al., 2018)

This study is therefore oriented towards biological control by the use of active natural substances, non-polluting and used in a less harmful and more reasoned fight, by developing an extract that is the least expensive and the most effective possible. However, our choice fell on the essential oil of *Artemisia herba alba* and this in order to evaluate its toxic activities on the larvae of the fourth stage and the pupae of *Culex pipiens*.

## MATERIALS AND METHODS

### Insect

*Culex pipiens* are completely metamorphic insects; they pass successively through very different stages: egg, larva, nymph then adult (imago) (DELAUNAY et al., 2001). *Culex* females lay their eggs in the form of rafts (MICHAELAKIS et al., 2005) The cycle breaks down in two phases: an aquatic phase for the first three stages, and an aerial phase for the last stage. Under optimal conditions, the cycle lasts from 10 to 14 days (RESSEGUIER, 2011) *Culex pipiens* larvae are found in the most diverse roosts in urban and peri-urban environments, especially those rich in organic matter (JIAFENG et al., 2011).

### Mosquito Rearing

In the laboratory, the captured larvae are sorted by larval stage and then transferred to containers

for rearing in cages (20 x 20 x 20 cm) at a temperature of  $25 \pm 2$  ° C, humidity of  $75 \pm 10\%$  and a scotophase 12 hours. A mixture of biscuit and dry yeast ensures the nutrition of the larvae (REHIMI & SOLTANI, 1999). Only the larvae having reached the fourth stage are the subject of a reliable identification with the help of the identification software of the Culicidae of Mediterranean Africa (BRHUNES et al., 1999) While the adults feed on raspberry and cotton swabs soaked in sugar water, However the blood meal, essential for the laying was provided by the introduction of a Petri dish containing about 5 ml of blood of horse mixed with heparin (anticoagulant) (COUZIN, 2006)

### Plant Material

The plant material used in this study consists of the aerial part of *Artemisia herba alba* its identification is made by Mr. BRAGUE A., Principal Forest Inspector at the National Institute of Forest Research of the province of Djelfa, harvested in May from the Medjbara (34° 30' N, 3° 28' E) region in Djelfa (Figure 1). After recovery of the plant, the aerial part was well cleaned. The drying was carried out naturally, protected from light and humidity, at room temperature (around 24 °C), for 15 days, in order to preserve the integrity of the molecules as much as possible.



**Figure 1.** *Artemisia herba alba*

## Extraction

The essential oil was obtained after 4 main stages, hydrodistillation, liquid-liquid extraction, elimination of water and elimination of solvent.

**Hydrodistillation:** A quantity of 50 g of the dried plant previously cut is introduced into a balloon of 1000 ml, then a quantity of 500 ml of distilled water is transferred and the whole is stirred. The balloon is then placed in a hydro-distillation assembly using a Clevenger type device (CLEVANGER, 1928) according to the recommendations of the Hellenic Pharmacopoeia (HELLENIC PHARMACOPOEI, 2002).

**Liquid-liquid extraction:** The distillate is put in a separatory funnel, then the solvent is added and the funnel is closed, vigorous stirring is practiced for a time necessary to establish a concentration equilibrium between the two phases and degassed, after it is fixed on a support with the removal of the cover. At the end, each phase is collected in an appropriate container (ABE, 2010).

**Removal of water:** To remove all traces of water, the organic phase is dried by adding a few grams of anhydrous magnesium sulfate MgSo<sub>4</sub>, then filtered using filter paper (FEKNOUS et al., 2014).

**Removal of the solvent:** The liquid obtained in the previous step is poured into an appropriate flask, then fixed to a rotary evaporator to carry out a simple distillation under reduced pressure with a temperature of 37 ° C (MECQUENEM et al., 2018) The oil obtained is stored in sterile glass bottles hermetically sealed, protected from light and at a temperature of 4 °C.

## Extraction Efficiency of Essential Oil

The extraction yield is calculated by the following formula (FALLEH *et al.* 2008):

$$R (\%) = (M_{\text{ext}} / M_{\text{éch.}}) * 100$$

$$R = 3/200 = 1.5\%$$

R is the yield in%.

M<sub>ext</sub> is the mass of the extract after evaporation of the solvent in g.

M<sub>éch</sub> is the dry mass of the plant sample in g.

## Chemical Analysis

The chemical composition of the essential oil was analyzed by gas chromatography coupled with mass spectrometry (CG/MS), which allows both a qualitative and quantitative determination of the majority compounds part of the sample (2-5 µl) was transferred to a GC vial, diluted in hexane (1-2 ml), then sealed with a high-performance septum (DELAZAR et al., 2004).

The identification of the constituents was carried out by coupling of a Chromatograph in gas phase

of the Clarus 680 Perkin Elmer type coupled to the Clarus SQ 8 mass spectrometer. The Rtx-5MS in fused silica (30 mx 0.25 mm ID, 0.25 pm df, RESTEK, USA) is directly coupled to the mass spectrometer (DELAZAR et al., 2004).

The carrier gas was helium (1 ml / min). The program used was 2 min isothermal at 60 ° C, then 3° C / min at 160 ° C, then 6 ° C / min at 240 ° C for 2 min. The temperature of the injection port was 250 ° C and the detector temperature 240 ° C. The ionization of components of the sample was performed in EI mode (70 eV). MS scan range was going from 30 to 300 amu (DELAZAR et al., 2004). The individual constituents were identified by comparing their mass spectra to spectra stored in the NIST / EPA / NIH mass spectral database. Version 2.0 g, version of May 19, 2011.

### **Treatment**

The sensitivity tests were carried out in accordance with the protocol recommended by the World Health Organization, adopted to test the sensitivity of the larvae towards insecticides used in control campaigns (WHO, 2005) This test is carried out on 2 larval stages, the larvae of the 4th stage and the pupae of *Culex pipiens*. Preliminary tests with different doses are carried out, in order to select a range of concentrations before starting the toxicity test. Three dilutions of 10% = 1µl/ml, 50% = 5µl/ml and 100% = 10µl/ml were prepared from the initial extract (1% stock solution). A total of 15 individuals (larvae/pupae) were sampled using a Pasteur pipette and placed in goblets, each containing 99 ml of water, then adding a milliliter of each solution thus diluted in the goblets previously prepared. The same number of individuals was placed in a control cup containing 100 ml of water. Three repetitions were performed for each dilution as well as for the control. Mortality rates were assessed after 24, 48 and 72 hours.

### **Statistical Analysis**

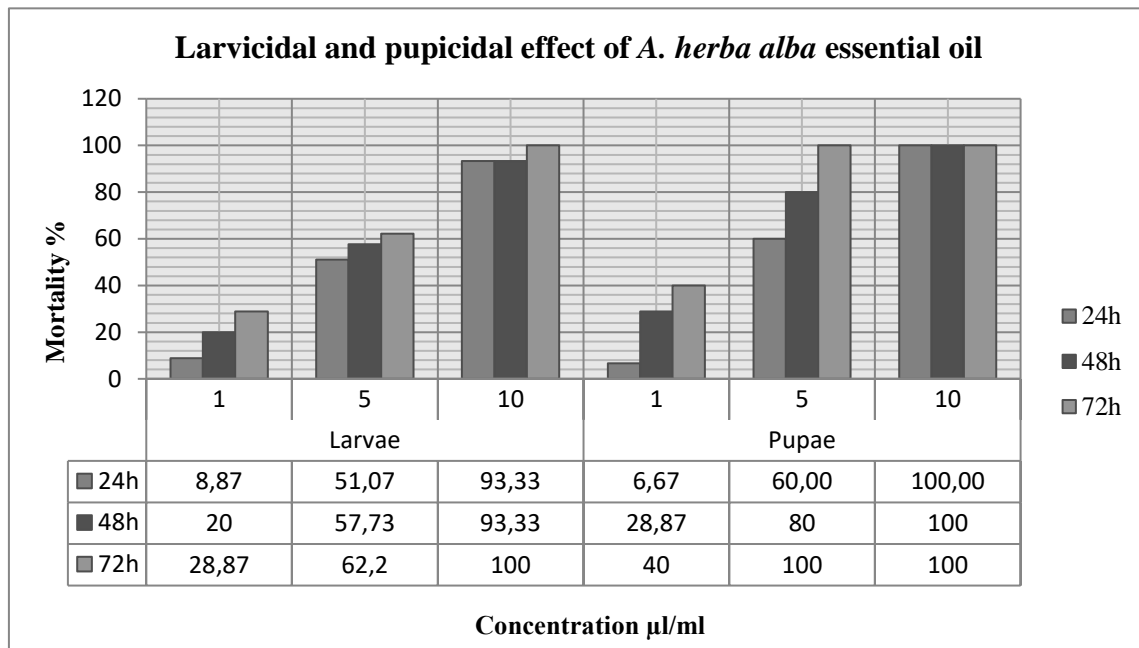
The mortality values obtained for the two stages in various concentrations were considered as means. The exploitation of these results was subjected by probit analysis (Finney, 1971) to calculate the lethal concentrations and lethal times (LC50% LC90%, LT50% and LT 90%). This analysis was performed using the IBM SPSS Statistics program<sup>23</sup> on Windows.

## **RESULTS**

### **The Effect of *A. Herba Alba* on The Mortality of *C. Papiens***

The two stages of *C. pipiens* are sensitive to *A. herba alba*. This sensitivity is reflected by higher or lower mortality rates depending on the concentrations used, and especially according to the time

of exposure to the extract (Figure 3). In the fourth stage of larvae the mortality rate ranges between 8.87% and 28.87 % for the lowest concentration (1  $\mu\text{l/ml}$ ) while it reaches 100% when the larvae are exposed to the highest concentration (10  $\mu\text{l/ml}$ ) after 48h. In the pupae the mortality rate ranges between 6.67% and 40 % for the lowest concentration (1  $\mu\text{l/ml}$ ) while it reaches 100% when the pupae are exposed to the medium concentration (5  $\mu\text{l/ml}$ ) after 72h.



**Figure 3:** Evolution of mortality rate% in the larvae and pupae of *Culex pipiens* treated with the different doses of *A. herba alba* essential oil

### Toxicological Parameters of *A. herba alba*

The results also show that there is a strong positive correlation between recorded mortality rates and the exposure time and/or the concentration of the extract used against mosquitoes (Table 1 and 2). To ensure a 50% mortality of the fourth stage of larvae after 24h, the concentration of *A. herba alba* must be equal to 5.081 $\mu\text{l/ml}$ , on the contrary, 9.128  $\mu\text{l/ml}$  of *A. herba alba* insures the mortality of 90% (Table 1A).

After 48h, the calculations show that the LC50% is 4.241 $\mu\text{l/ml}$ , while the LC90% is 9.166 $\mu\text{l/ml}$ .

After 72h of treatment, the LC50% is 3.278  $\mu\text{l/ml}$  and the LC90% is 7.573  $\mu\text{l/ml}$ .

On the lethal times, the concentration 1  $\mu\text{l/ml}$  of *A. herba alba* can eliminate 50% of the population of *C. pipiens* in the 4.37 day and 90% during 7.70 days of treatment (Table 1B). When 5 $\mu\text{l/ml}$  of *A. herba alba* extract is applied, LT50% is 0.75 days, while the LT90% is 9.77 days.

**Table 1.** Toxicological parameters of *A. herba alba* essential oil in larvae treated with *C. pipiens* (A: exposed time; B: used concentration)

| <b>A</b>              |                     |                        |                     |
|-----------------------|---------------------|------------------------|---------------------|
| Time (hours)          | <b>24</b>           | <b>48</b>              | <b>72</b>           |
| Regression line       | $Y = -1,62 + 0.32x$ | $Y = -1,1 + 0.26x$     | $Y = -0.77 + 0.22x$ |
| LC 50% (µl/ml)        | 5.081               | 4.241                  | 3.278               |
| LC 90% (µl/ml)        | 9.128               | 9.166                  | 7.573               |
| <b>B</b>              |                     |                        |                     |
| Concentration (µl/ml) | <b>1</b>            | <b>5</b>               | <b>10</b>           |
| Regression line       | $Y = -1.71 + 0.02x$ | $Y = -0.11 + 5.92E-3x$ | -                   |
| LT50% (hours)         | 104.910             | 18.025                 | -                   |
| LT90% (hours)         | 184.869             | 234.389                | -                   |

To ensure a 50% mortality of the pupae after 24h, the concentration of *A. herba alba* must be equal to 4.356 µl/ml, on the contrary, 7.110 µl/ml of *A. herba alba* insures the mortality of 90% (Table 2A). After 48h, the calculations show that the LC50% is 2.579µl/ml, while the LC90% is of 6.075 µl/ml. After 72h of treatment, the LC50% is 1,213 µl/ml and the LC90% is 2,288 µl/ml. On the lethal times, the concentration 1 µl of *A. herba alba* can eliminate 50% of the population of *C. pipiens* in the 3.3 days and 90% during 5.52 days of treatment (Table 2B). When 5 µl/ml of *A. herba alba* extract is applied, LT50% is 0.82 days, while the LT90% is 0.99 days.

**Table 2.** Toxicological parameters of *A. herba alba* essential oil in pupae treated with *C. pipiens* (A: exposed time; B: used concentration)

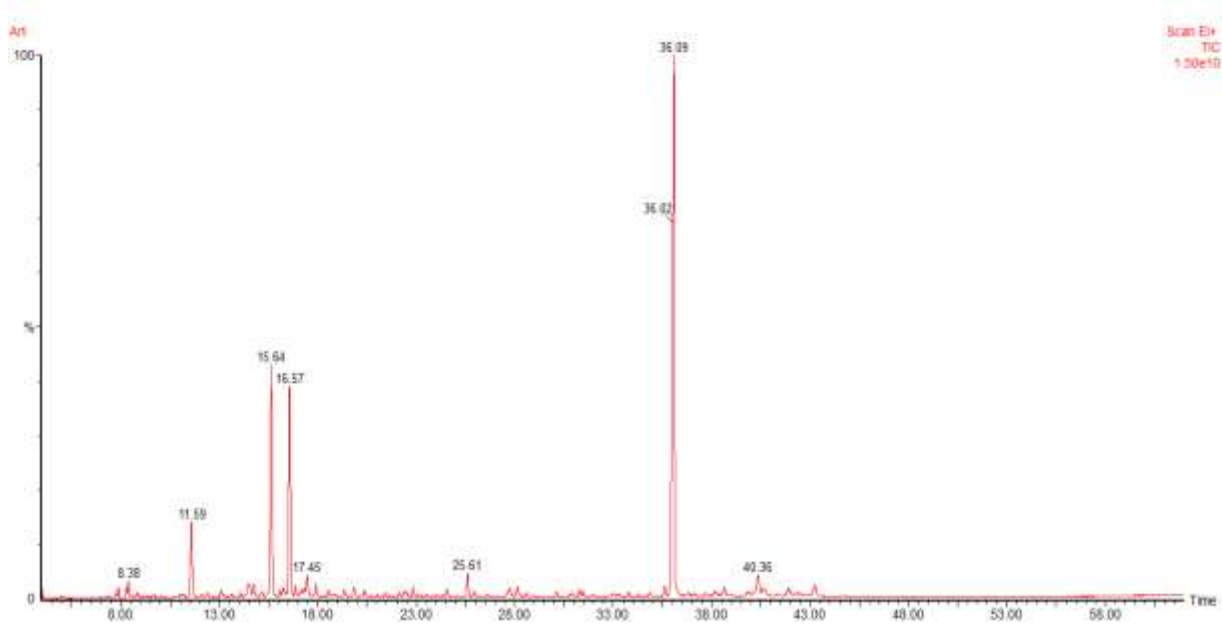
| <b>A</b>              |                     |                     |           |
|-----------------------|---------------------|---------------------|-----------|
| Time (hours)          | <b>24</b>           | <b>48</b>           | <b>72</b> |
| Regression line       | $Y = -1.94 + 0.44x$ | $Y = -0.91 + 0.35x$ | -         |
| LC 50% (µl/ml)        | 4.356               | 2.579               | 1.213     |
| LC 90% (µl/ml)        | 7.110               | 6.075               | 2.288     |
| <b>B</b>              |                     |                     |           |
| Concentration (µl/ml) | <b>1</b>            | <b>5</b>            | <b>10</b> |
| Regression line       | $Y = -2.02 + 0.03x$ | $Y = -0.33 + 0.02x$ | -         |
| LT50% (hours)         | 79.077              | 19.693              | -         |
| LT90% (hours)         | 132.479             | 53.257              | -         |

### Chemical analysis

Twenty-nine main molecules were extracted within forty minutes, we note that the large proportions were monopolized for the Davanone molecule by 48.84%, which is approximately



half, followed by chrysanthenone with 15.97%, then by camphor with 14.84%, then the remaining proportions from 0.04% to 5.69%.



**Figure 2.** Chromatographic profile of *A. herba alba* essential oil analyzed by CG/SM

**Table 3.** Main chemical compounds (%) of *A. herba alba* essential oil analyzed by the CG/SM

| Ret. Time | Compound Name                                     | Percentage% |
|-----------|---|-------------|
| 13,604    | $\alpha$ -Pinene                                  | 0,04        |
| 16,65     | Camphene  | 1,34        |
| 17,575    | 2(5H) -Furanone, 5,5-dimethyl-                    | 0,42        |
| 9,691     | $\beta$ -Myrcene                                  | 0,16        |
| 10,031    | o-Cymene  | 0,10        |
| 11,196    | Cyclohexene, 1-methyl-5-(1-methylethenyl) -, (R)- | 0,28        |
| 11,591    | Eucalyptol  | 5,69        |
| 12,112    | 2(3H) -Furanone, 5-ethenyldihydro-5-methyl-       | 0,21        |

|        |  |       |
|--------|--|-------|
| 13,647 | 1,5-Heptadien-4-ol, 3,3,6-trimethyl-                             | 0,20  |
| 14,743 | Bicyclo [3.1.0] hexan-3-one, 4-methyl-1-(1-methylethyl)-         | 0,71  |
| 15,173 | Thujone  | 0,47  |
| 15,643 | Chrysanthenone   | 15,97 |
| 16,083 | Cyclohexane, 2-ethenyl-1,1-dimethyl-3-methylene-                 | 0,37  |
| 16,253 | Isopinocarveol   | 0,70  |
| 16,568 | Camphor  | 14,84 |
| 16,868 | Cis-p-mentha-1(7),8-dien-2-ol                                    | 0,63  |
| 17,329 | Pinocarvone  | 0,42  |
| 17,449 | Endo-Borneol   | 1,61  |
| 17,899 | Terpinen-4-ol  | 0,91  |
| 18,264 | Tricyclo [4.3.0.0(3,8)] nonan-2-ol,2-(aminomethyl) stereoisomer  | 0,06  |
| 18,544 | $\alpha$ -Terpineol  | 0,41  |
| 19,344 | 2-Cyclohexen-1-ol, 3-methyl-6-(1-methylethyl)-, cis-             | 0,55  |
| 19,84  | Ethanol, 2-(3,3-dimethylbicyclo [2.2.1] hept-2-ylidene) -        | 0,77  |
| 23,081 | Thymol   | 0,19  |
| 25,612 | 3-Cyclohexene-1-methanol, $\alpha,\alpha,4$ -trimethyl-, acetate | 1,51  |
| 27,728 | 3,5-Heptadienal, 2-ethylidene-6-methyl-                          | 1,00  |
| 28,138 | 3-Methyl-2-pent-2-enyl-cyclopent-2-enone                         | 0,80  |
| 35,621 | (-) -Spathulenol   | 0,82  |
| 36,086 | Davanone   | 48,84 |

## DISCUSSION

More than 2,000 plant species with insecticidal activity have already been identified (JACOBSON, 1989), some plants have evolved a wide range of physical conditions and chemical defenses against a variety of insects through substances such as (phenols and polyphenols, terpenoids, alkaloids) that can be isolated using various extraction methods (DUBEY, 2011).

The experiences of PRANATI et al. (2018) have shown the larvicidal and pupicidal effect of extracts of *Clerodendrum philippinum* leaves against *Aedes aegypti* and *Anopheles stephensi* with considerable mortality rates. A study by KAURA et al. (2019) reveals the larvicidal and pupicidal effect of the essential oil of *Eucalyptus globulus* which acts quickly on the larvae and pupae of *Aedes aegypti* and *Aedes albopictus*.

The results obtained reveal a considerable and variable sensitivity translated by rates of low to very high mortality which correlates with the extension of time from one concentration to the other. This activity can be expressed by the diversification of the bioactive molecules which compose this essential oil being able to carry out a singular action of one of the major components, of which it is dominated by Davanone, or a synergistic effect between several compounds towards the larvae and the nymphs of mosquitos which are exposed to it.

JUN-HYUNG & MURRAY (2015) note that insecticidal activity is the result of a series of complex actions and contractions between a toxic tissue and an insect tissue. This mechanism of toxicity can be expressed in three steps: penetration, activation (target site interaction) and detoxification. Plant extracts act in two possible ways; a larvicidal action that can cause an appreciable mortality of larvae in 1 to 12 days, or a juvenile hormone mimetic action, with an extension of the larval life span that can inhibit pupation (RAGEAU & DELAVEAU, 1980).

## CONCLUSION

This study indicates that essential oil of *Artemisia herba alba* having toxic properties on larvae and pupae of *Culex pipiens*. These results are encouraging and open up interesting and promising horizons for its application in the production of bioinsecticides, these are readily available and the cost constraint can be overcome by the low value of the LC50. However, another deep chemical study would be necessary in order to precise and to isolate the molecule responsible for this toxic effect, in addition a histological study is desirable in order to know the mode of action of this oil on the tissues of larvae and pupae.

**REFERENCES**

- Abe E, Stanilas G, Claude A (2010). Extraction Liquide-Liquide: Théorie, Applications, Difficultés. *Ann Toxicol. Anal.* 22 (2): 51-59.
- Acheuk F, Abdellaoui K, Lakhdari W, Dehliz A, Ramdani M, Barika F, Et Allouane F (2017). Potentiel Bio-Insecticide De L'extrait Brut De La Plante Saharienne *Artemisia Judaica* En Lutte Anti-Vectorielle: Cas Du Moustique Commun *Culiseta longiareolata*. *Journal Algérien des Régions Arides (Jara)* 14: 109-116.
- Air Paris (2016). Les Pesticides Dans L'air Francilien Partie 1: État Des Connaissances. Observatoire De L'air En Îles De France, 29 P.
- Belhattab R, Loubna Amor L, Barroso J, Pedro L, Figueiredo C (2014). Essential Oil from *Artemisia Herba Alba* Asso. Grown Wild in Algeria: Variability Assessment and Comparison with An Updated Literature Survey. *Arabian Journal of Chemistry* 7: 243-251.
- Benhissen S, Rebbas K, Habbachi W, Masna F (2018). Bioactivity of *Nicotianaglauca* (Solanaceae) And Its Toxic Effects on *Culiseta longiareolata* (Diptera; Culicidae). *Int. J. Res. Ayurveda Pharm* 9 (1): 123-126.
- Brunhes J, Rhaim A, Geoffroy B, Angel G, Hervy Jp (1999). Les Moustiques De L'Afrique Méditerranéenne, Logiciel D'identification Et D'enseignement, I.R.D. Edition.
- Clevenger Jf (1928). Apparatus for Volatile Oil Determination: Description of New Type Clevenger. *Am. Perf. Ess. Oil. Review* 467-503.
- Couzin F (2006). Bone Disease Gene Finally Found. *Science* 312, Issue 5773, 514-515.
- Delaunay P, Fauran P, Marty P (2001). Les Moustiques D'intérêt Médical. *Revue Française des Laboratoires* 338: 27-36.
- Delazar A, Reid G, Sarker D. (2004). Gc-MS Analysis of The Essential Oil from The Oleoresin of *Pistacia Atlantica* Var. *Mutica*. *Chemistry of Natural Compounds* 40 (1): 24-27.
- Dubey N (2011). Natural Products in Pest Management. London, Cab International.
- Falleh H, Ksouri R, Chaieb K, Karray-Bouraoui N, Trabelsi N, Boulaaba M, Abdely C (2008). Phenolic Composition of *Cynara Cardunculus* L. Organs, And Their Biological Activities. *C.R. Biologie* 331: 372-379.
- Feknous S, Saidi F, Mohamed Said R (2014). Extraction, Caractérisation Et Identification De Quelques Métabolites Secondaires Actifs De La Mélisse (*Melissa Officinalis* L.). *Revue « Nature & Technologie ». A- Sciences Fondamentales Et Engineering*, 11: 07-13.
- Habbachi W, Benhissen S, Ouakid Ml, Farine Jp (2013). Effets Biologiques D'extraits Aqueux De *Peganum Harmala* (L.) (Zygophyllaceae) Sur La Mortalité Et Le Développement Larvaire De *Drosophila Melanogaster* (Diptera, Drosophilidae). *Algerian Journal of Arid Environment* 3(1): 1-14.
- Hellenic Pharmacopoeia, 5th Ed., National Organization for Medicines of Greece, Chapter 28.12, Athens (2002).
- Imbahale S, Mweresa K, Takken W, Mukabana R (2011). Development of Environmental Tools for Anopheline Larval Control. *Parasites & Vectors* 4: 1-10.
- Jacobson M (1989). Botanical Pesticides, Past Present and Future In: Arnason Jt Et Al. (Ed.). *Insecticides of Plantorigin*. Washington, D.C: American Chemical Society Symposium, Series 387, Pp. 1-10.
- Jiafeng W, Nick Ho, Huaiping Z (2011). The Impact of Weather Conditions on *Culex Pipiens* And *Culex Restuans* (Diptera: Culicidae) Abundance: A Case Study in Peel Region. *J. Med. Entomol.* 48(2): 468-475.
- Jun-Hyung T., Murray B. (2015). Enhanced Cuticular Penetration as The Mechanism for Synergy of Insecticidal Constituents of Rosemary Essential Oil in *Trichoplusia Ni*. *Sci. Rep.* 5: 1-10.
- Kaura T, Mewara A, Zaman K, Sharma A, Agrawal Sk, Thakur V, Garg A, Sehgal R. (2019). Utilizing Larvicidal And Pupicidal Efficacy of *Eucalyptus* and Neem Oil Against *Aedes* Mosquito: An Approach for Mosquito Control. *Trop Parasitol.* 9: 12-17.
- Manickam J, Arivoli S, Rajasingh R, Tennyson S (2016). Larvicidal And Pupicidal Efficacy of Plant Oils Against *Culex Quinquefasciatus* Say 1823 (Diptera, Culicidae). *Journal of Entomology and Zoology Studies* 4 (5): 449-456.
- Matoug H, Merabti B, Tadjer W, El Bah D, Ouakid L (2017). Biological Control Test of Ethanol Extracts of *Peganum Harmala* (L.) On the Mortality and Development of *Culex Pipiens* (Diptera). *World J. Environ. Biosci.* 6 (4): 15-19.
- Merabti B, Lebouz I, Adamou A, Ouakid L (2015). Effet Toxique De L'extrait Aqueux Des Fruits De *Citrullus Colocynthis* (L.) Sur Les Larves Des Culicidae. *Revue Des Bio Ressources.* 5 (2): 120-130.
- Merabti B, Lebouz I, Ouakid L (2017). Larvicidal Activity and Influence of Azadirachtin (Neem Tree Extract) On the Longevity and Fecundity of Mosquito Species. *Acta Zool. Bulg.* 69 (3): 429-435.
- Michaelakis A, Mihou Ap, Couladouros Ea, Zounos Ask, Koliopoulos G (2005). Oviposition Responses of *Culex Pipiens* To A Synthetic Racemic *Culex Quinquefasciatus* Oviposition Aggregation Pheromone. *Journal of Agricultural and Food Chemistry* 4: 1-5.

- Mohammad Abu Darwish, Célia Cabral, Maria José Gonçalves, Carlos Cavaleiro (2015) *Artemisia Herba-Alba* Essential Oil from Buseirah (South Jordan): Chemical Characterization and Assessment of Safe Antifungal and Anti-Inflammatory Doses. *Journal of Ethnopharmacology* 174: Jepd1501495:24pp
- Pranati D, Jyoti Ranjan R, Preeti K, Sagorika P, Pallabi P, Chandi C, Chinmay P, Santi Lata S (2018). Larvicidal And Pupicidal Activity of *Clerodendrum Philippinum* Schauer Leaf Extracts Against *Anopheles Stephensi* And *Aedes Aegypti*. *Pharmacognosy Journal* 10 (6): 1137-1142.
- Rageau J, Delaveau P (1980). Effets Toxiques D'extraits De Végétaux Sur Les Larves De Moustiques. *Bulletin De La Société De Pathologie Exotique* 72: 168-171.
- Rehimi N, Soltani N (1999). Laboratory Evolution of Alsystine. A Chitin Synthesis Inhibitor Agonist *Culex Pipiens* L. (Diptera, Culicidae). Effects on Development and Cuticule Secretion. *J. Appl. Ent.* 123: 437-441.
- Resseguier P (2011). Contribution À L'étude Du Repas Sanguin De *Culex Pipiens Pipiens*. Thèse De Doctorat, Université Paul – Sabatier. Toulouse, 80 P.
- Tilaoui M, Ait Mouse H, Jaafari A, Zyad A (2015). Comparative Phytochemical Analysis of Essential Oils from Different Biological Parts of *Artemisiaherbaalba* And Their Cytotoxic Effect on Cancer Cells. *Plos One* 21: 1-15.
- Who. (2005). Guidelines for Laboratory and Field Testing of Mosquito Larvicides. World Health Organization, 41 P.

Oral Presentation  
Thursday  
Population Ecology

## Bruchinae Latreille 1802 Species Detected on Edible Grain Legumes and Forage Crops in Southeastern Anatolia Region

Melek Güdek Güçlü<sup>1\*</sup>, Celalettin Gözüaık<sup>2</sup>, Neslihan Gültekin<sup>2</sup>, Klaus-Werner Anton<sup>3</sup>

<sup>1</sup>Atatürk University, Faculty of Agriculture, Department of Plant Protection, Erzurum, Turkey.

<sup>2</sup>Iğdır University, Faculty of Agriculture, Department of Plant Protection, Iğdır, Turkey.

<sup>3</sup>Grünwaldstr.13, D-79312 Emmendingen, Germany

\*Corresponding author e-mail: mlk.gdk9@gmail.com

### Abstract

Southeastern Anatolia Region is a very important region for the cultivation of legumes and forage crops. In this study, the species diversity of the subfamily Bruchinae Latreille, 1802, which includes species that feed especially on forage crops and edible legumes, and which can cause significant damage was investigated. Although Bruchinae species are small body size and a small number of species diversity, they are a very important group in terms of damage to leguminous plants. There are approximately 1700 species in the world, and 113 species is known from Turkey based on recent years finding. In this study, 26 species belonging to five genera were identified from Southeast Anatolia. These are *Bruchus* Linnaeus 1767 (8 species), *Bruchidius* Schilsky 1905 (13 species), *Acanthobruchidius* Borowiec, 1980 (1 species), *Spermophagus* Schoenherr, 1833 (3 species) and *Paleoacanthoscelides* Borowiec 1985 (1 species). Among of these, *Bruchus ervi* J.A Frölich in lentils which are grown at an important level, *Bruchidius trifolii* (Motschulsky, 1874) and *B. foveolatus* (Gyllenhal, 1833) in alfalfa, *P. gilvus* (Gyllenhal, 1839) in sainfoin are the most abundant species. In addition, *Bruchus dentipes* Baudi di Selve 1886 has been detected at a significant level in vetch.

**Keywords:** Bruchinae, biodiversity, Southeastern Anatolia Region, legumes.

**Acknowledgement:** The second and third authors have been partly supported by a project TUBITAK (Project Number: 120O352).

Oral Presentation  
Thursday  
Population Ecology

## Changes in Carbon Concentration of Tree Components for Calabrian Pine Forests in the Western Black Sea Region of Turkey

Şükrü Teoman Güner<sup>1\*</sup>

<sup>1</sup>Bartın University, Ulus Vocational School, Department of Forestry, Ulus, Bartın, Turkey.

\*Corresponding author e-mail: stguner@gmail.com

### Abstract

In the framework of the Kyoto Protocol, the countries, which signed the protocol, need to prepare their national inventory reports in every year and submit to the secretariat of Convention on Climate Change of United Nations. Countries prepare their reports according to AFOLU (Agriculture, Forestry and Land Use) guidelines. However, countries need to produce tree species-specific coefficients in order to prepare more precise reports. This study was carried out in order to determine the carbon ratios of the tree components (needle, wood, bark, root) and the weighted carbon ratios of the above-ground and total tree mass in natural Calabrian pine (*Pinus brutia* Ten.) forests in the Western Black Sea Region. The samplings were made in 10 stands in the wooded age stage (dbh = 20.0-51.9 cm) that are different in terms of habitat characteristics. First, habitat characteristics of the sampling areas were determined. Later, samples of needle, wood, bark and root were taken from dominant three trees in each sampling area. Carbon analysis were performed in the laboratory in samples (10 sample areas  $\times$  3 repetitions  $\times$  4 components = 120 samples) of wood components taken from sampling areas. The data obtained from laboratory analyses were evaluated by analysis of variance and Duncan multiple comparison test. Statistically significant differences were determined between carbon ratios of tree components ( $p < 0.001$ ). The lowest carbon density (50.25%) was found in root and the highest (54.90%) in the bark, among the tree components. The weighted carbon ratio was calculated as 52.07% for the above-ground tree mass and 51.77% for the total tree mass in natural Calabrian pine forests. The results obtained in this study can be used for calculation of the carbon stocks stored in both whole and in different components of trees in Calabrian pine forests.

**Keywords:** *Pinus brutia*, weighted carbon concentration, site properties

Oral Presentation  
Thursday  
Population Ecology

### The Usage of Sage (*Salvia* sp.) Taxa as Traditional Folk Medicine

Ahmet Efe<sup>1\*</sup>, Derya Karakoyun<sup>1</sup>, Çağla Güvenç Biçer<sup>1</sup>, Dudu Özlem Mavi İdman<sup>1</sup>

<sup>1</sup>National Botanical Garden of Turkey, Republic of Turkey Ministry of Agriculture and Forestry,  
Ankara, Turkey

\*Corresponding author e-mail: ahmet.efe@tarimorman.gov.tr

#### Abstract

The methods of treatment with herbs, which are as old as the history of humanity, have been passed down from generation to generation and have reached the present day. Throughout history, people have used different organs of plants in the treatment of diseases in various ways. Even today, many synthetic drugs are obtained directly or indirectly from plant-derived materials. Although herbal treatment methods have remained in the background with the appearance of synthetic drugs, there has been an increase in the use of plants used as traditional folk medicine in recent years, since the use of drugs obtained by synthetic means is both expensive and causes side effects in humans. Turkey is very rich in plant diversity as it is located in three different plant geographies. In addition, approximately one third of the plants in our country are endemic. In addition to this, our country's geography is home to many different civilizations, so it keeps different cultures together. Traditional folk medicine is perhaps the most important one of these cultures.

This study is about the sage (*Salvia* sp.) taxa, which belong to the Lamiaceae (Ballıbabagiller) family and are used as a traditional folk medicine in Turkey and different parts of the world. Different organs of sage taxa in different parts of the world are used in the treatment of many health problems such as respiratory diseases, eye diseases, abdominal pain, muscle diseases, urological diseases. In the study, information about the use of sage species as a traditional folk medicine in various countries is given. In addition, information about the use of sage taxa grown in Turkey by our people is also extensively mentioned.

**Keywords:** *Salvia*, traditional folk medicine, synthetic drugs, treatment methods



Poster Presentation

Friday

Diversity of Animal Species, Systematics and  
Phylogeny; Population Ecology; Biodiversity,  
Landscape, Tourism**Biodiversity of Fresh Water Macro Invertebrates *From of The Aurès Region, North-Est  
Algeria***Meriem Taferghoust<sup>1,3\*</sup>, Wissem Hezil<sup>1,3</sup>, Boudjéma Samraoui<sup>2,3</sup>, Farrah Samraoui<sup>1,3</sup><sup>1</sup>Department of Ecology and Environmental Engineering, Faculty of Sciences of Nature and Life, Earth and Universe Sciences, University May 08, 1945, Guelma, Algeria.<sup>2</sup>Biology Department, Faculty of Sciences, University Badji Mokhtar, Annaba, Algeria.<sup>3</sup>Laboratoire de Conservation des Zones Humides, Université 8 Mai 1945, Guelma, Algeria.

\*Corresponding author e-mail: taferghoustmeriem@gmail.com

**Abstract**

Water is the source of life, but due to the environmental and anthropogenic impacts to which it is exposed, its quality and properties may change. Therefore, it has become necessary to analyze it before allocating it for any use. There are a number of ways in which the scientific community and environmental agencies test water quality, such as physico-chemical analyzes, although these tests are limited and expensive. The purpose of this study was to determine the biodiversity of fresh water macroinvertebrates that are known as good indicators of the health of aquatic ecosystems due to their varying tolerance to pollution and habitat degradation. In order to know the state of health in the rivers of the Eastern Aurès massif with an efficient and less expensive way. The sampling has been initiated in June 2019 to June 2020. We have carried out monthly sampling at 16 localities using a dipnet. In addition, a number of environmental factors have also been measured. The collected macroinvertebrates samples have been preserved in 100% Ethanol pending identification according to relevant keys and literature. A total of 10548 individuals representing 11 groups were collected. The Ephemeroptera is the most abundant with a total of 4373 individuals, and a frequency of 42%, followed by the Amphipods, with 2262 individuals and a frequency of 22%, followed by Trichoptera with 1365 individuals and a frequency of 13%, and Diptera, with 1323 individuals and a frequency of 12%. The other orders are poorly represented with values below 11%. The predominance of groups of Ephemeroptera, Amphipods, Trichoptera which are pollution-sensitive organisms, suggest that the rivers of the Eastern Aurès massif are of a good quality.

**Keywords:** Fresh water, macro invertebrates, biodiversity, Eastern Aurès Massif, Algeria

Poster Presentation

Friday

Diversity of Animal Species, Systematics and  
Phylogeny; Population Ecology; Biodiversity,  
Landscape, Tourism

### Extensive Road Mortality of *Bufo bufo* (Linnaeus 1758) in İkizdere, Rize

Cantekin Dursun<sup>1\*</sup>, Serkan Gül<sup>1</sup>, Nurhayat Özdemir<sup>1</sup>

<sup>1</sup>Recep Tayyip Erdogan University, Faculty of Arts and Sciences, Department of Biology, Rize, Turkey.

\*Corresponding author e-mail: cantekin.dursun@erdogan.edu.tr

#### Abstract

In the last decades, amphibians have been seriously threatened by anthropogenic factors. The road mortality represents one of the serious concerns for amphibians and it is causing more risk for poor disperser species, especially during rainy nights. *Bufo bufo* is known as a nocturnal pool-breeding species, and it has annually overland migrations to arrive waterbodies during breeding season. In this study, we have reported a high mortality of *B. bufo* during three subsequent fieldworks along the different routes on main roads in İkizdere, Rize between April and June 2021. At the end of these trips, we have recorded 173 death individuals. Most of the recorded locations were placed around waterbodies and next to creeks. The species is slow-moving organism migrating between aquatic and terrestrial habitats and vulnerable to defence itself due to remaining immobile at the approach of a vehicle. Since the town is located at the main road of Rize-Erzurum transition and has transhumance activities via the village and upland roads, the vehicle traffic was the main reason among the potential factors in spring period. Besides, the forestry activities, tourism, roadworks and hydroelectric power plant and quarry construction sides were thought as the other factors. Although the intensity of *B. bufo* population was appeared high in this town, the number of losses observed in these trips indicated that the protection measures should be taken by local authority.

**Keywords:** Amphibia, common toad, conservation, traffic

**Acknowledgement:** The study was supported by RTEU BAP with the grant number FBA-2019-1047.

Poster Presentation

Friday

Diversity of Animal Species, Systematics and  
Phylogeny; Population Ecology; Biodiversity,  
Landscape, Tourism

### New Record of Biting Midge (Diptera: Ceratopogonidae) for Sinop (Turkey):

#### *Leptoconops bidentatus* Gutsevich, 1960

Fethi Turgut<sup>1\*</sup>

<sup>1</sup>Sinop University, School of Health Services, Environmental Health, Sinop, Turkey.

\*Corresponding author e-mail: fturgut@sinop.edu.tr

#### Abstract

Three genera of the family Ceratopogonidae include species that feed on blood-sucking. The genus *Leptoconops* is one of them. There are few studies on the species composition of other genera except *Culicoides* in Turkey. In the study carried out to determine the Ceratopogonidae fauna of the Sarikum Nature Reserve Area (Sinop-Turkey) in 2013 and 2017, it was determined that the *Leptoconops bidentatus* Gutsevich, 1960 species was distributed in this area. The samples were collected with light trap in the evening hours, and were stored in bottles containing 70% ethyl alcohol. They were mounted on microscope slides in phenol-Canada balsam and were examined under a microscope. At the end of the study, one female on 04.06.2013 and six males on 26.06.2013 belonging to this species were identified. In the other months of 2013 and in 2017, no *Leptoconops* samples were caught. With this result, *L. bidentatus* species was detected for the first time in Sinop. This species was previously recorded only from Tokat in Turkey. Thus, *L. bidentatus* was reported for the second time from Sinop in Turkey. The morphological features with photographs of this species and its distribution are given.

**Keywords:** Biting midges, *Leptoconops*, Ceratopogonidae, Sinop, Turkey

Poster Presentation

Friday

Diversity of Animal Species, Systematics and  
Phylogeny; Population Ecology; Biodiversity,  
Landscape, Tourism**The First Record of *Atrichopogon infuscus* Goetghebuer, 1929****(Diptera: Ceratopogonidae) in Sinop (Turkey)**Fethi Turgut<sup>1\*</sup><sup>1</sup>Sinop University, School of Health Services, Environmental Health, Sinop, Turkey.

\*Corresponding author e-mail: fturgut@sinop.edu.tr

**Abstract**

About 6500 species belonging to Ceratopogonidae (Diptera), known as biting midges, have been reported. *Atrichopogon* is one of the important genus of this family with its more than 500 species. There are few studies on the species composition of other genera except *Culicoides* in Turkey. In this study, it is aimed to contribute to the Ceratopogonidae fauna of Turkey. The study was carried out in Aklıman district of Sinop (Turkey) in 2014 and 2015. Biting midges were collected with light traps in the evening hours, and were stored in bottles containing 70% ethyl alcohol. They were mounted on microscope slides in phenol-Canada balsam and were examined under a microscope. At the end of the study *Atrichopogon infuscus* Goetghebuer, 1929 was determined in this area. With this result, *A. infuscus* species was detected for the first time in Sinop. The distribution of this species has been reported in all the provinces of Amasya, Çorum, Ordu, Samsun and Tokat, which are the provinces of the Central Black Sea Region. Detection of the existence of this species in Sinop shows that it has a very wide distribution in this region. The morphological features with photographs of this species and its distribution in the World and in Turkey are given.

**Keywords:** Biting midges, *Atrichopogon*, Ceratopogonidae, Sinop, Turkey

Poster Presentation

Friday

Diversity of Animal Species, Systematics and  
Phylogeny; Population Ecology; Biodiversity,  
Landscape, Tourism

**Morphological Investigation of Some Populations of *Podarcis muralis* (Laurenti, 1768) (Squamata: Lacertidae) in The Anatolian and Thrace Regions**

Melis Karakoç<sup>1\*</sup>, Murat Tosunoğlu<sup>2</sup>

<sup>\*1</sup> Çanakkale Onsekiz Mart University, School of Graduate Studies,

Department of Biology, Çanakkale, Turkey

<sup>2</sup> Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, 17100,  
Çanakkale, Turkey.

\*Corresponding author e-mail: meliskarakoc1996@gmail.com

**Abstract**

In this study, two populations of *Podarcis muralis* that belonging to the regions of Anatolia and Thrace, from Lacertidae family which has a worldwide distribution, were studied morphologically. The pholidosis, body measurement, ratio and index values of a total of 24 *Podarcis muralis* individuals in both populations were examined, similarities and dissimilarities between populations were revealed. As a result, 27 pholidosis and 24 morphometric parameters were examined, it was determined that there was no sexual dimorphism and no statistically significant difference between the two *Podarcis muralis* populations.

**Keywords:** Lacertidae, *Podarcis muralis*, morphology, Tekirdağ, Çanakkale

**Acknowledgement:** This study is a part of the master's thesis titled “Morphological and Osteological Investigation of Some Populations of *Podarcis muralis* (Laurenti, 1768) (Squamata: Lacertidae) in Anatolia and Thrace Region” at Çanakkale Onsekiz Mart University, School of Graduate Studies.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Leaf Geometric Morphometrics Among A Natural Population of Norway Maple (*Acer  
Platanoides* L.) in Northern Algeria**Rida Mohammed Mediouni<sup>1\*</sup>, Sarra Said<sup>1</sup>, Faiza Ilias<sup>2</sup>, Gaouar Semir Bechir Suheil<sup>1</sup><sup>1</sup>Laboratory of pathophysiology and Biochemistry of nutrition (PPABIONUT), Department of Biology,  
Faculty of Natural and Life sciences (SNV-STU), Abou-Bekr Belkaid Tlemcen University<sup>2</sup>University of Ain Temouchent, Department of Natural and Life Sciences (SNV)\*Corresponding author: Rida Mohammed Mediouni, Abou Bekr Belkaid University, Tlemcen, Algeria,  
e-mail: Redamediouni191@gmail.com, Tel.: +213(0)553454209, Orcid Id: <https://orcid.org/0000-0002-5514-8286>**Abstract**

Maple (*Acer* L.) is a diverse tree genera that includes more than a hundred of deciduous and evergreen species in Northern hemisphere, *Acer platanoides* is a species from the maple's genus with an invasive aptitude in Europe and North America, this species had never been recorded in North Africa and the main aim of this work is to investigate the shape and size variability within a natural population in Northern Algeria. The study was carried out using a collection of multivariate, bivariate and univariate statistics, 303 *A. platanoides* leaves were included in the analysis counting 2 taxa from 8 countries. The analyzed data shows some very close results between Algerian and European *A. platanoides*, One Way ANOVA of size provided a significant p.value <0.001 between the three studied populations, the Bonferroni correction doesn't show any significant p. values between Algerian and European *A. platanoides* but confirmed the difference of *A. platanoides* ssp *turkestanicum* from the others, linear regression of shape and size shows a significant p.value of <0.001 but a low negative coefficient of correlation  $r = -0.18$  and a low coefficient of determination  $r^2 = 0.033$ , Principal component analysis (PCA) shows an inertia of 53.48% between the first two components and revealed three different forms, MANOVA based on shape data revealed a significant p.value <0.001 between groups of taxa, a Pillai trace of 1.108, and a Wilks lambda coefficient of 0.192, the closest squared Mahalanobis distance ( $d=8.01$ ) was

reported between Algerian and European *A. platanoides* populations while the largest ( $d=16.74$ ) was scored between Algerian and Iranian populations, clustering using Kmeans was depending on both Elbow and Silhouette methods, the typical number of clusters according to the two methods was  $k=2$ , however, clustering doesn't reveal any specific shape or group of leaves, the statistical analysis proved a small phenotypic plasticity between Algerian and European *A. platanoides* leaves in terms of shape while size remain conserved between both populations, the provided statistical tools confirms the ability of *A. platanoides* to show an environmental adaptation additionally also approves *A. platanoides* ssp *turkestanicum* as distinguished subspecies.

**Keywords:** north african maples, *Acer platanoides* l., pca, Algeria, geometric morphometric

## INTRODUCTION

Maple (*Acer* L.) is a large and diverse tree genera that includes more than 130 of deciduous and evergreen species (Parsa 2014) it is a part of Sapindales order's and the family of Aceraceae (Siahkoliae et al. 2017), maples are known with their dispersion along the temperate regions of the northern hemisphere from North America, to East Asia, passing by Europe, North Africa and Middle East (Gibbs & Chen 2009).

The European continent is considered the home of many native maples and a number of infraspecific taxa (Blondel 2018), until now, It is not clear how many subspecies and varieties should be included into the genus *Acer* as the south-east limits with Asia are not appropriately inspected, however some species are totally well known all over the continent, this includes, *Acer Pseudoplatanus*, *Acer lobelii* and *Acer platanoides* (Turok et al. 1996), in the other hand the rest and the majority of European maples lies across the Euro-Mediterranean area expending toward the West of Asia, actually, there are 7 native maple species reported by (FAO 2013) across the Mediterranean area with North Africa, these maples are reported as spontaneous species, with a very heterogeneous geographic distribution, (Mediouni & Azira 1992) creating some kind of mixed forest formations with Oak, Numidian fir, Yew and Atlas Cedar (Trabut & Battandier 1890), According to (Quézel & Santa 1963), 4 taxa of maples occurs mainly in Northern Algeria which are *Acer monspessulanum*, *Acer campestre*, *Acer opalus* and *Acer obtusatum*.

Maples taxonomy was always questionable, in fact a lot of investigations are established in order to determine an accurate classification for species within this genus, Early revisions were those of Pax 1902 and Pojarkova 1933 followed by Fang 1966 until the late of the late 20<sup>th</sup> century where (De Jong 2002) subdivided the *Acer* genus into sixteen sections that are further subdivided into

nineteen series, however and with all these efforts North African maples seems to be unbenefited at all and a remarkable lack of data and scientific works regarding the mentioned genus in the stated area is clearly exposed.

*Acer platanoides* is a shade intolerant, deciduous maple that could achieve 10 meters high, it is native to Europe but also has a large distribution and found in many parts of the world (Gelderen Van et al. 1994) it belongs to the section *platanoides* with *Acer campestre* a well-known species around the Mediterranean rim (Nagy & Ducci 2004), *Acer platanoides* is recognized with two taxa, *Acer platanoides ssp platanoides* and *Acer platanoides ssp turkestanicum* a subspecies with smaller leaves that is native to west Asia including Afghanistan, Iran and Turkestan (Murray 1969) *Acer platanoides* has a unique capability of high seed survivability, even under difficult conditions, this could achieve several years in freezing temperature (Hong & Ellis 1990).

Recent studies reveals the importance of morphological characters for identifying maple species and resolving difficulties that occurs into its taxonomical identification (Chikhaoui 2016), for many reasons, the use of both classical and geometric morphometrics with different statistical models became a powerful tool to illustrate variations among groups of taxa (Savriama 2018), weather linear measurement appeared to be quite dealing with quantitative traits, this latter remains very limited in topics, experiments and studies that aims to characterize and distinguish between taxa, a reason could be that the complex shape of an organism cannot be easily summarized by using only linear measurements (Zelditch et al. 2012), conversely, the morpho-geometrics allows to better explore the deformations and graphically display any changes across the anatomical parts of the plants, and this could be a beneficial advantage for species classification and identification (Liu et al. 2018).

Until this moment, we don't have any bibliographic source that confirms the presence of *Acer platanoides* nor its origins in North Africa and this would rise some interesting hypothesis regarding its presence in Chr ea forest and its environmental adaptation in Algeria, this work should answer the following questions: could a geometric morphometric method reveal any variations regarding shape and size among the studied *A. platanoides* populations?

Are there any forms of environmental adaptation that influenced leaves shape and size of Algerian *A. platanoides*?

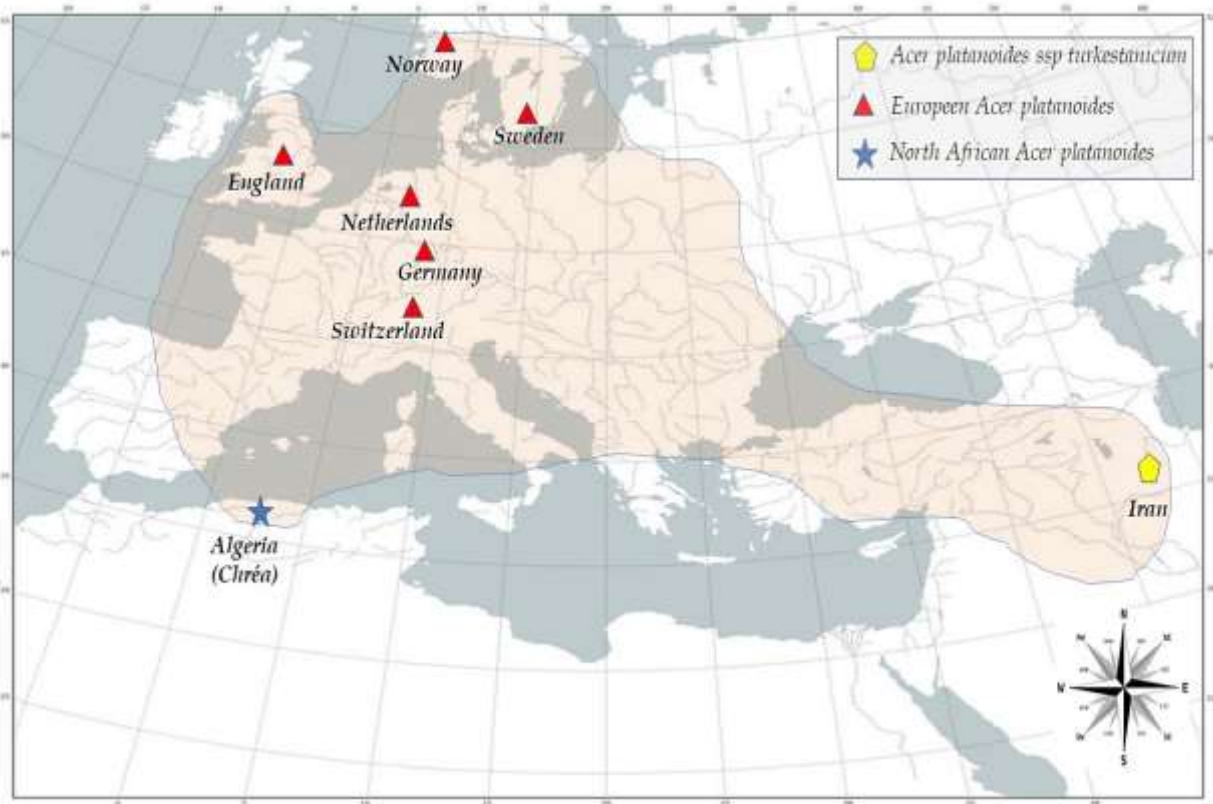
The exposed work has the objective to highlight some essential informations regarding this species in Algeria and the main aim of this study is to compare collections of Algerian and worldwide *A. platanoides* leaves that are very different in terms of ecological and geographical conditions, the



presented work also highlight the variability of shape and size of an isolated and naturally grown population of Norway maple using a morpho-geometric method and a collection of statistical tools.

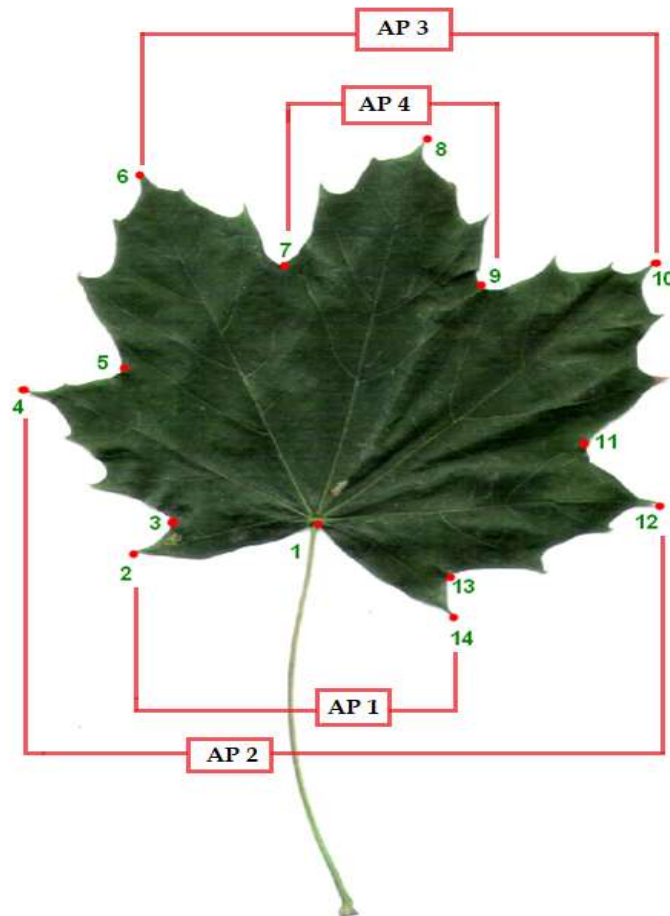
## MATERIALS AND METHODS

This study took in consideration three populations of Norway maple, the first population is represented by Algerian *A. platanoides*, the second population is represented by European *A. platanoides* and finally the third population is corresponding to Iranian *A. platanoides* ssp *turkestanicum*, 303 landmarked leaves participates in the analysis matching to 8 localities (Figure 1), including Algeria, Germany, England, Switzerland, Netherlands, Sweden, Norway and Iran, Algerian leaf samples were collected from the forest of Chr ea an area of the national park in northern Algeria at an elevation of 1250m to 1350m, 14 mature and healthy Norway maple trees were selected, naturally grown and dispersed along the forest, mainly with species of *Cedrus*, *Juniperus* and *Quercus*, leaves were scanned on their fresh form using a combined printer (HP all in one 123) to 300 DPI, European *A. platanoides* and *A. platanoides* ssp *turkestanicum* samples were downloaded from virtual herbariums registered at the Global Biodiversity Information Facility data base (GBIF 2021), specimens were chosen from different countries in order to cover a wide geographical area (Table 1), damaged leaves were initially excluded from digitizing, a scale factor was adjusted in order to remove the effect of using pictures in various resolutions.



**Figure 1.** Theoretical distribution map of *Acer platanoides* species in Eurasia, Algerian *Acer platanoides* appeared in blue, European *Acer platanoides* appeared in red while *Acer platanoides ssp turkestanicum* appeared in yellow.

A configuration of 14 landmarks (LM's) was used in leaf digitizing (Figure 2), this procedure is done through TPSdig32 ver2.31, a software from Rohlf's geometric morphometrics packages, during the analysis LM 1 represents leaf base, 2 and 14 are apex of the lower teeth's , 3 and 13 are the inner sinus between the lower teeth's and the lower lateral lobes, 4 and 12 apex of lower lateral lobes, 5 and 11 are the inner sinus between upper lateral lobes and lower lateral lobes, 6 and 10 are the apex of upper lateral lobes, 7 and 9 are the inner sinus between the upper lateral lobes and central lobe, 8 is the apex of central lobe, this configuration is very similar to this of (Wahlsteen 2021), petioles had been excluded from the analysis, in general, petioles are highly unstable while scanning leaves and it is really difficult to estimate their correct length due to its curvature and deviation from a straight line hence this could indicate errors while digitizing.



**Figure 2.** The 14 landmarks (LM's) configuration applied on Algerian *Acer platanoides* leaf with the analyzed anatomical parts (AP's) here AP1: shows the left and right lower teeth's, AP2 shows left and right lower lateral lobes, AP3: shows the upper lateral lobes and finally AP4: reveals the central lobe.

The used statistical methods varied between multivariate, bivariate and univariate statistics including principal component analysis (PCA) a very common method in geometric morphometrics used for exploring the shape trends and variability among specimens, leaves Centroid Size (CZ) was calculated according to Procrustes shape distances, the scale factor plays an important role in this operation (Hammer & Harper 2005), size was tested using descriptive statistics and One way analysis of variance (ANOVA) in addition to a supplementary post-hoc test using Bonferroni coefficients for identifying similar groups, according to (Ghasemi & Zahediasl 2012) normality should be ignored due to the large number of observations 303, the relationship between shape and size was tested using linear regression, shape data were represented by the scores of "PC1" the leading component in PCA function with the highest inertia and variations, discrimination between groups of taxa was tested using multivariate analysis of variance (MANOVA) at a confidence level equal to 95%.

Finally the clustering was done using Kmeans method, the typical number of clusters for this method was estimated using both Silhouette and Elbow methods, both are depending on the visual selection (Kodinariya & Makwana 2013)

**Table 1.** Number of digitized *Acer platanoides* leaves from each country and continent according to their taxa, here Algeria is represented by 206 leaves in total, European *Acer platanoides* by 68 including England, Germany, Netherlands, Sweden, Norway and Switzerland, finally *Acer platanoides ssp turkestanicum* is represented by 29 from Iran.

| Country                     | Number of leaves           | Continent                    | Taxon   |
|-----------------------------|----------------------------|------------------------------|---|
| Algeria                     | 206                        | North Africa                 | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| England                     | 11                         | Europe                       | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| Germany                     | 11                         | Europe                       | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| Norway                      | 30                         | Europe                       | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| Sweden                      | 3                          | Europe                       | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| Switzerland                 | 6                          | Europe                       | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| Netherlands                 | 7                          | Europe                       | <i>A. platanoides</i> subsp. <i>platanoides</i> L.                  |
| Iran                        | 29                         | Asia                         | <i>A. platanoides</i> subsp. <i>turkestanicum</i> (pax) P.C.de Jong |
| <b>8 countries in Total</b> | <b>303 leaves in Total</b> | <b>3 continents in Total</b> | <b>2 taxa in Total</b>  |

The statistical analysis was purified from some outliers and carried out using a collection of softwares and packages, Initial Shape data were stored in files of TPS format created by TPSutil ver1.78, leaf landmarking (digitizing) was done using TPSdig32 ver2.31 (Rohlf 2015), before running the statistical analysis all the shape data were transferred to a two dimensional Procrustes fit by Past software. (R Core Team 2020) version 3.6.3 was used to calculate ANOVA, MANOVA, Descriptive statistics and also to estimate the optimal number of clusters, the package factoextra version 1.0.7 in R (Kassambara & Mundt 2020) was an R extension used to plot the Kmeans function, the PCA was released using MorphoJ version 1.06d (Klingenberg 2011), Past software version 4.03 (Hammer et al. 2001) was used for testing linear regression

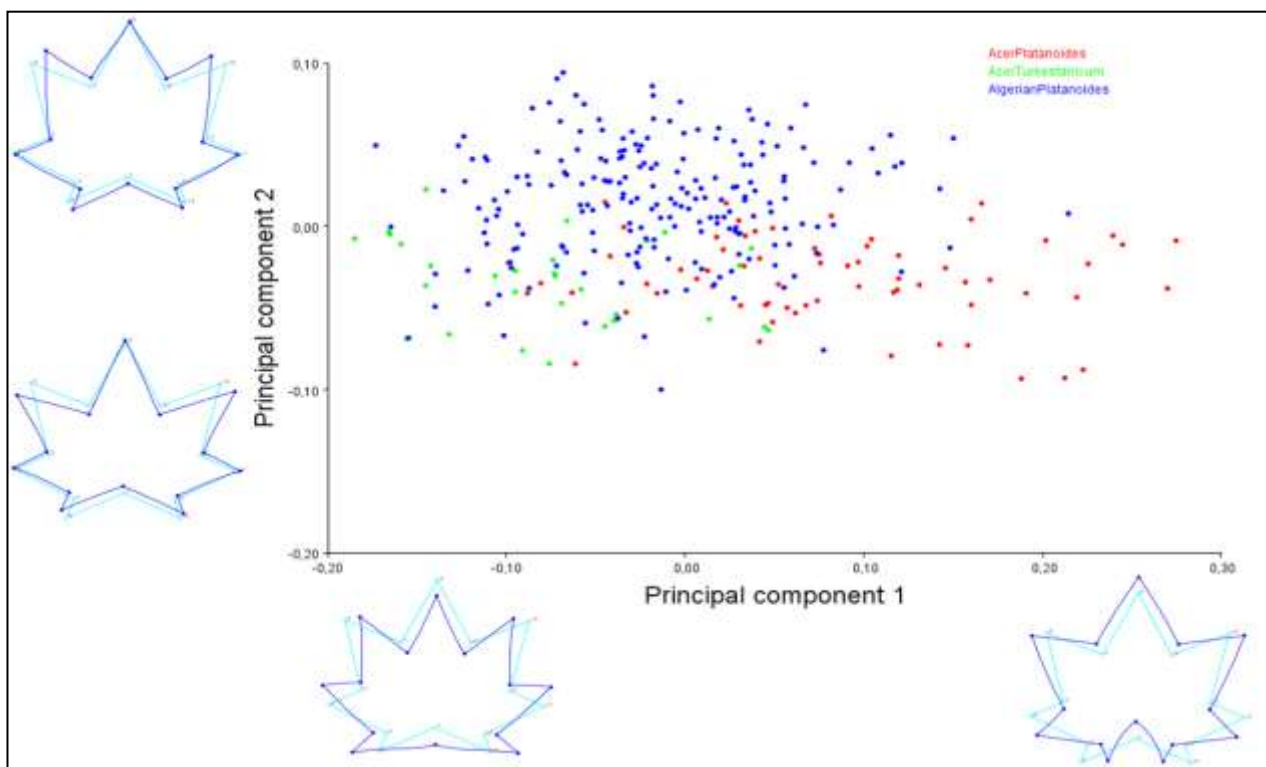


**Figure 3.** Algerian *Acer Platanoides* located in the forest of Chr ea, Original picture from the Author Mediouni Mohammed Rida

## RESULTS

### Principal Component Analysis and Global Shape Trends

Most of the variance in the PCA is explained by the first two components, the function is dealing with *A. platanoides* leaves that are originally recorded from different regions (Figure 4), PC1 leads the highest value of inertia by 44.56% followed by PC2 explaining an inertia of 8.92% (53.48% in total).



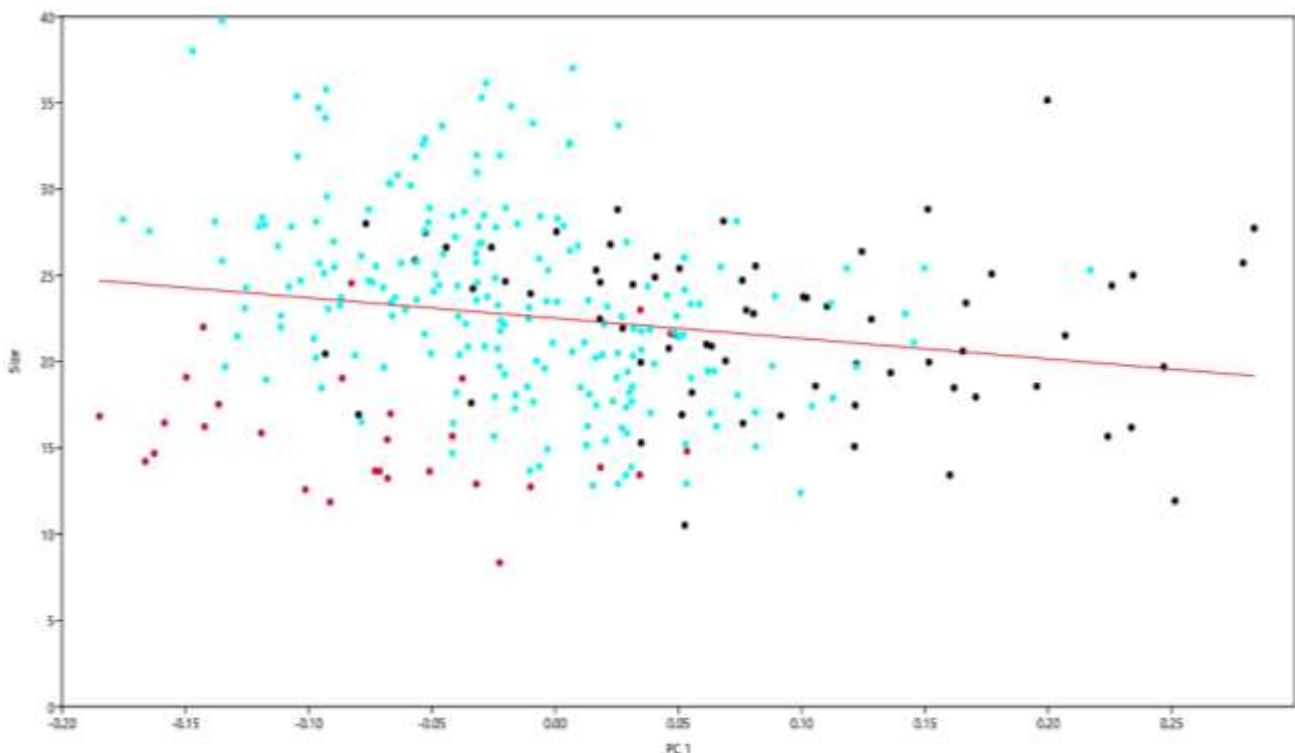
**Figure 4.** Principal Component Analysis of 303 *Acer platanoides* leaves, here Algerian *Acer platanoides* appears in (Blue), European *Acer platanoides* appears in (Red) and Iranian *Acer platanoides* ssp *turkestanicum* appears in (Green)

The scatter plot revealed three distinguished populations in terms of shape, European *A. platanoides* appeared on the positive score of the first component while *A. platanoides* ssp *turkestanicum* dominated the negative score of the same component, Algerian *A. platanoides* appeared mainly in the positive side of the second principal component, leaf shapes could be also clearly distinguished, European *A. platanoides* appeared with five palmately lobes, a wide middle lobe, lateral lobes were radially positioned with an open angle however and conversely, a very narrow angle of approximately 60° at the level of lower teeth's while Algerian *A. platanoides*

appeared with contracted lateral lobes leveled at the same direction of middle lobe also with an open angle at the level of lower teeth's, in the other hand, *A. platanoides* ssp *turkestanicum* appeared different to the previous taxa with smaller and narrow middle and lateral lobes compared to those of European *A. platanoides* however the main difference occurs in the open angle at the level of lower teeth's that almost reached 180°.

### Regression Analysis of Shape Versus Size

The relationship between shape and size was tested by linear regression (Figure 5), this latter shows a significant <0.001 but negative correlation  $r = (-0.18)$  between the two studied parameters, also shows a low determination coefficient  $r^2 = (0.033)$ , the reported results indicates some slight changes in shape over the diminution of size therefore the correlation coefficients remains low and doesn't allow us to confirm a strong relationship between the shape and size.



**Figure 5.** linear regression of shape and size data based on three *Acer platanoides* leaf collections, here Algerian *Acer platanoides* appeared in (Blue), European *Acer platanoides* in (Black) and Asian *Acer platanoides* ssp *turkestanicum* in (Red)

According to the regression, *Acer platanoides* ssp *turketanicum* appeared with a considerably smaller size compared with Algerian *Acer platanoides* and European *Acer platanoides*, the two later appeared with a very heterogenous forms and shapes however it is noticeable that Algerian

*Acer platanoides* gain a slight amount of size against European *Acer platanoides*.

### Size Analysis Using ANOVA and Descriptive Statistics

Descriptive statistics shows that Algerian *A. platanoides* and European *A. platanoides* are very close in terms of size (Table 2) since the two populations achieved a mean of 23.57 and 22.09, a median of 23.33 and 22.63 and a standard deviation of 5.53 and 4.57 respectively, while *A. platanoides ssp turkestanicum* appeared very different from these populations with considerably lower values compared to continental *A. Platanoides*, the Analysis of variance showed no significant difference between Algerian *A. platanoides* and European *A. platanoides* but a very significant difference *A. platanoides ssp. turkestanicum* and continental *A. platanoides*.

**Table 2.** Descriptive statistics and One-way ANOVA p. values based on leaves centroide size (cz)

| Population | N*  | Min   | Max   | Mean  | Median | Variance | Std<br>Deviation | Std<br>Error | Overall<br>P.value | Post-Hoc<br>Significance** |
|------------|-----|-------|-------|-------|--------|----------|------------------|--------------|--------------------|----------------------------|
| Algeria    | 206 | 12.40 | 39.78 | 23.57 | 23.33  | 30.66    | 5.53             | 0.38         |                    | Non-<br>Significant        |
| Europe***  | 68  | 10.51 | 35.16 | 22.09 | 22.63  | 20.96    | 4.57             | 0.55         | <<br>0.0001        | Non-<br>Significant        |
| Asia       | 29  | 8.35  | 24.54 | 15.97 | 15.48  | 13.26    | 3.64             | 0.67         |                    | Significant                |

\*: Number of leaves according to each population.

\*\*: Post Hoc Significance was calculated according to Bonferroni coefficients.

\*\*\*: Participating countries from Europe were England, Sweden, Germany, Netherlands, Norway & Switzerland

#### *Shape discrimination using MANOVA:*

The multivariate analysis of variance based on shape data (Table 3) provided a high significant overall probability  $p < 0.001$ , a Pillai trace of 1.108, which indicates the good contribution of the applied landmark data to the test (Pillai 1955), additionally, a Wilks lambda value of 0.19, which indicates some important statistical variations among the groups of taxa (Shi 2019), the Bonferroni correction also provided high significant probabilities between the tested groups and indicates a clear discrimination between the studied populations.

MANOVA also scored a matrix of squared Mahalanobis distances, the highest values were reported between Algerian *A. platanoides* and *A. platanoides ssp turkestanicum* giving a value of 16.74 followed by 13.92 between European *A. platanoides* and *A. platanoides ssp turkestanicum* while the lowest distance was of 8.01 between Algerian *A. platanoides* and European *A. platanoides*, the results shows the clear separation of *A. platanoides ssp turkestanicum* from the other taxa, while Algerian and European *A. platanoides* remains very close in terms of shape.

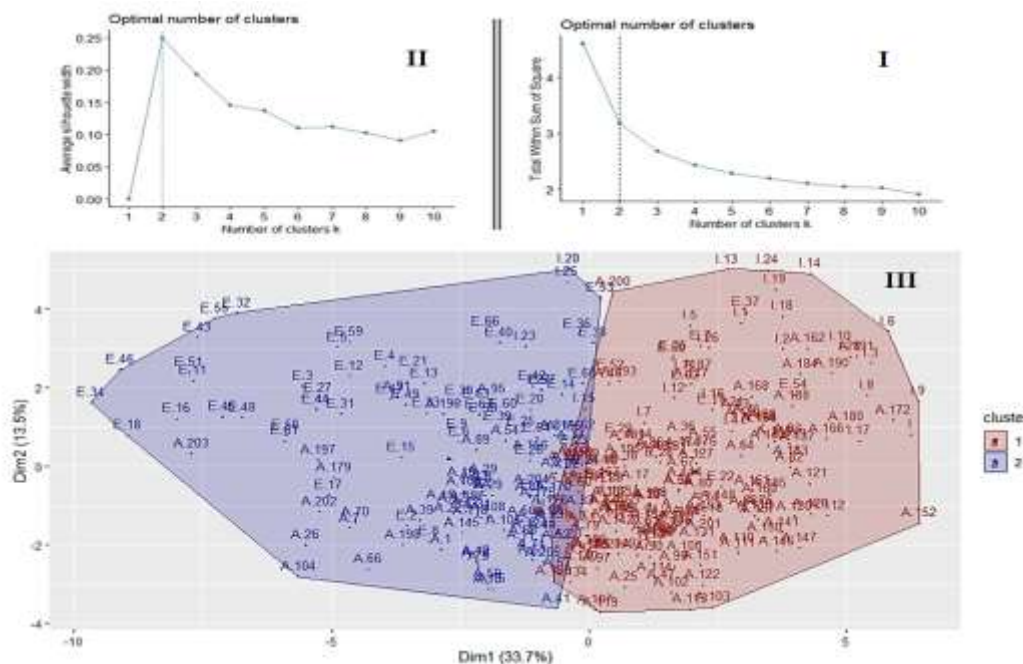


**Table 3.** Results of MANOVA according to the three studied populations

| Compared Taxa  | Squared Mahalanobis distances | Bonferroni Correction | Wilk's lambda |       | Overall P value |
|--|-------------------------------|-----------------------|---------------|-------|-----------------|
|  |                               |                       | Pillai trace  | trace |                 |
| <i>A. platanoides ssp turkestanicum</i> – Algerian <i>A. platanoides</i> | 16.74                         | <0.0001               |               |       |                 |
| <i>A. platanoides ssp turkestanicum</i> – European <i>A. platanoides</i> | 13.92                         | <0.0001               | 0.192         | 1.108 | <0.0001         |
| Algerian <i>A. platanoides</i> – European <i>A. platanoides</i>          | 8.01                          | <0.0001               |               |       |                 |

*Kmeans clustering:*

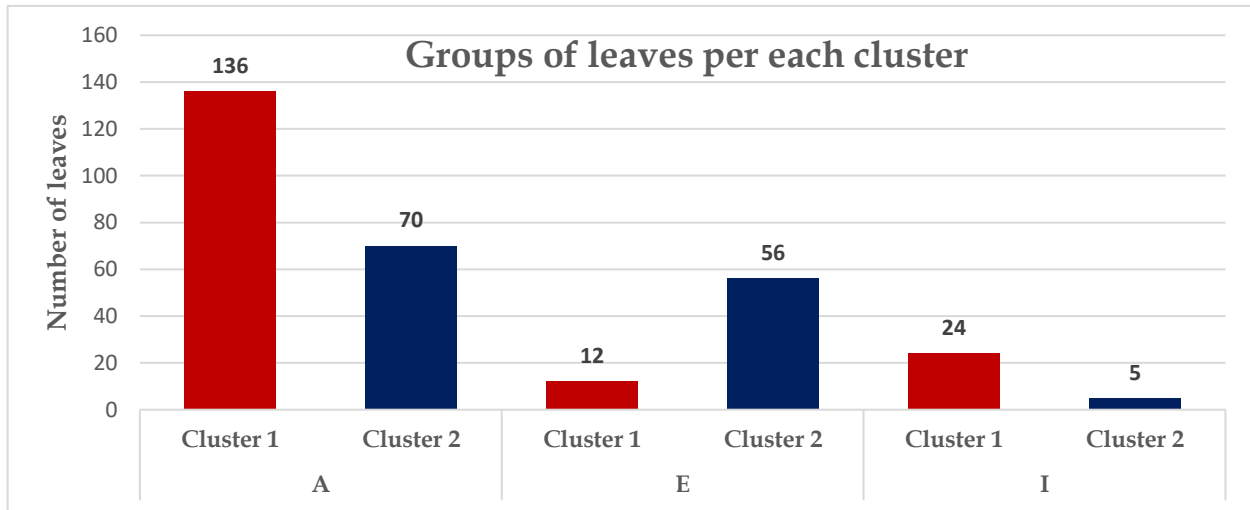
During the analysis both Elbow and Silhouette methods proposed an optimal number of clusters equal to k=2 (Figure 6), the scatter plot of the Kmeans revealed two main groups with a very heterogenous leaf composition, the first dimension provided and inertia of 33.7% While the second dimension provided and inertia of 13.5% (47.2% in total)



**Figure 6.** Kmeans clustering revealing 2 clusters according to both Elbow “(I)” and silhouette “(II)” methods, in the scatterplot “(III)”, Algerian *Acer platanoides* appears with abbreviation “A”, European *Acer platanoides* appears with abbreviation “E” while “I” means Iranian *A. platanoides ssp turkestanicum*.

Kmeans was depending on 1000 permutations, additionally, 2 initial random centroids were set at the beginning of the analysis, the function does not reveal any specific or distinct group of leaves (Figure 7) however the first Cluster appears dominated with 136 Algerian *Acer platanoides* leaves

followed by 24 Iranian *Acer platanoides* ssp *turkestanicum* and finally come European *Acer platanoides* with only 12 leaves, in the other hand the second cluster appears to be balanced with Algerian *Acer platanoides* and European *Acer platanoides* with 70 to 56 recorded leaves respectively and only 5 leaves of *Acer platanoides* ssp *turkestanicum*.



**Figure 7.** leaves distribution according to each cluster, Algerian *Acer platanoides* appears with abbreviation “A”, European *Acer platanoides* appears with abbreviation “E” while “I” means Iranian *A. platanoides* ssp *turkestanicum*, Cluster1 (Red) is represented by 172 leaves while Cluster2 (Blue) is represented by 131 leaves.

## DISCUSSION

The value of this work is not occurring only on its content as a research paper that deals with North African maples however as one of the rare articles that deals with *Acer platanoides* geometric morphometrics, in the mean time we can find many manuscripts dealing with maple’s diversity, classification, and taxonomy, using different methods from geometric morphometrics counting (Jensen et al. 2002) on *Acer rubrum* and *Acer saccharinum* (Kostic et al. 2017) on *Acer pseudoplatanus*, (Wahlsteen 2020) on *Acer campestre*, to molecular analysis including (Khademi et al. 2016) on *Acer monspessulanum*, (Pandey 2005) and (Grimm et al. 2007) on *Acer pseudoplatanus*. Actually, the data concerning *A. platanoides* morphogeometrics are so few, and the reason why could depend on the technical difficulties that appears while studying a rather complicated leaf shape (Gavrikova & Ignatyuk 2014), the morphometrical method applied in this manuscript could give us an idea on how Algerian *A. platanoides* appears in terms of shape and size rather than provide us with a simple leaf configuration based on 14 landmarks for further geometric morphometrics studies. The statistical analysis in this manuscript tested leaves size

using descriptive statistics, ANOVA and linear regression, the mentioned tools confirms the smaller size of *A. platanoides* ssp *turkestanicum* on the other hand it does not allow us to separate between the Algerian and the European Norway maple, since this latter remains independent from shape variations according to the regression results but conserved between both populations. In the next step we managed to use Principal component analysis, a multivariate tool that mainly deal's with leaves shapes and trends in geometric morphometrics, the PCA distinguished 3 different types of shape conformation where *A. platanoides* ssp *turkestanicum* was clearly noticed to be different and this is totally expected since it is from a very different ecology that extends from East of Iran to Mid Afghanistan and other neighboring regions, therefore, the main shape differences were reported between Algerian and European *A. platanoides* principally at the level of lateral lobes and lower teeth. The discrimination based on shape data using MANOVA showed a significant difference between all the studied groups of taxa and provided a matrix of squared Mahalanobis distances where Asian and Algerian populations of *A. platanoides* appears very different, and this would confirm the ability of discrimination tools in geometric morphometrics to distinguish groups of taxa based on their shape data. The clustering using Kmeans method provided a set of 2 optimal clusters however it failed to identify a specific group of shapes nor a group of taxa, all what we can justify is that cluster 1 was almost dominated with Algerian *A. platanoides* while cluster 2 was a hybrid of the groups of taxa. According to the provided results, the problematic of differentiation between North African and continental *A. platanoides* appears to be a matter of shape not size since this latter remains conserved between the two studied populations of Europe and Algeria, this would rise some interesting hypothesis regarding leaf phenotypic plasticity of this species and its adaptation capacities to different environments and climates, it should be noted that this is not the first report regarding North African maple's behaviors since a most recent study done by (Mediouni et al. 2021) reveals that both shape and size of three separated populations of Algerian *A. monspessulanum* were influenced by environmental conditions compared to Eurasian groups of *A. monspessulanum*. An expression of isolation by distance phenomena is not excluded also from the list of hypothesis meanwhile we don't have the accurate information concerning the presence of the studied species in Algeria and for this reason, studies concerning the genetic diversity using molecular markers like SSR's or SNP's are highly important at this stage since SSR's are relatively reliable and does not require much efforts nor costs (Kvesić et al. 2020), Further studies should also include the statistical analysis of samaras taking in consideration that the anatomy of this compartment plays an

important and discriminant role in the identification and evaluation of worldwide maples.

According to our field prospections, the species does not show any tendency or behaviors of invasiveness contrarily to its influence in Europe and North America (Straigyte & Baliuckas 2015) hence Norway maple in Chr ea forest now is considered as a richness to the Algeria and North African flora.

### Funding

This research received no external funding.

### ACKNOWLEDGEMENTS

The authors are grateful to Dr Ramdan Dahel, Dr Faiza Takarli, and Dr Boutkhil Morseli, also thankful to the administration of Chr ea National Parc for their field support.

### Conflicts of Interest

The authors declare no conflict of interest.

### Plant Identification

Algerian *Acer platanoides* collections were examined and identified by Professor Medjahdi Boumediene from the university of Tlemcen, Departement of Forestry.

### REFERENCES

- Blondel J. 2018. Conna tre le pass  pour comprendre le pr sent : histoires d'arbres et d'oiseaux dans l'espace m diterran en. *Ecol Mediterr.* 44(2):88–93.
- Chikhaoui Z. 2016. Analyse inter-stationnelle et interindividuelle de la morphologie de la morphologie des feuilles d'Erables ( *Acer monspessulanum* L. et *Acer obtusatum* W. et K.) au Djurdjura [Internet]. Tizi-Ouzou: Mouloud Mammeri University. <https://dl.ummtto.dz/handle/ummtto/1649>
- FAO. 2013. State of Mediterranean Forests 2013 [Internet]. :49–50. <http://www.fao.org/3/i3226e/i3226e.pdf>
- Gavrikova VS, Ignatyuk OA. 2014. The dynamics of fluctuating asymmetry of *Acer platanoides* L. leaves in urbanized environment. *Ecol Noospherology.* 25(3–4):34–44.
- GBIF. 2021. Global Biodiversity Information Facility Occurrence Download. DK-2100 Copenhagen - Denmark. <https://api.gbif.org/v1/occurrence/download/request/0220683-200613084148143.zip>
- Gelderen Van D., De Jong P., Oterdoom H. 1994. Maples of the world. Oregon USA: Timber Press Portland.
- Ghasemi A, Zahediasl S. 2012. Normality tests for statistical analysis: a guide for non-statisticians. *Int J Endocrinol Metab* [Internet]. 10(2):486–489. <https://pubmed.ncbi.nlm.nih.gov/23843808>
- Gibbs D, Chen Y. 2009. The Red List of Maples. In: Richmond, UK: BOTANIC GARDENS CONSERVATION INTERNATIONAL (BGCI); p. 5.
- Grimm GW, Denk T, Hemleben V. 2007. and Evolution Evolutionary history and systematics of *Acer* section *Acer* – a case study of low-level phylogenetics. *Plant Syst Evol.* 267:215–253.
- Hammer  , Harper D. 2005. Paleontological Data Analysis. Wiley-Blac. [place unknown]. <https://doi.org/10.1002/9780470750711.ch4>
- Hammer O, Harper D, Ryan P. 2001. PAST: Paleontological Statistics Software Package for Education and Data Analysis. *Palaeontol Electron.* 4:1–9.
- Hong TD, Ellis RH. 1990. A comparison of maturation drying, germination, and desiccation tolerance between developing seeds of *Acer pseudoplatanus* L. and *Acer platanoides* L. *New Phytol* [Internet]. 116(4):589–596. <https://doi.org/10.1111/j.1469-8137.1990.tb00543.x>

- Jensen RJ, Ciofani KM, Miramontes LC. 2002. Lines, outlines, and landmarks: Morphometric analyses of leaves of *Acer rubrum*, *Acer saccharinum* (Aceraceae) and their hybrid. *Taxon*. 51(3):475–492.
- De Jong PC. 2002. Worldwide maple diversity. *Int Maple Symp* 02.:2–11.
- Kassambara A, Mundt F. 2020. factoextra: Extract and Visualize the Results of Multivariate Data Analyses [Internet]. <https://cran.r-project.org/package=factoextra>
- Khademi H, Mehregan I, Assadi M, Nejadstari T, Zarre S. 2016. Molecular phylogeny of *Acer monspessulanum* L. subspecies from Iran inferred using the ITS region of nuclear ribosomal DNA. *BIODIVERSITAS*. 17(1):1623.
- Klingenberg P. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour*. 11:353–357.
- Kodinariya T, Makwana P. 2013. Review on determining number of Cluster in K-Means Clustering. *Int J Adv Res Comput Sci Manag Stud* [Internet]. 1(6):90–95. <http://www.ijarcsms.com/>
- Kostic S, Cukanovic J, Ljubojevic M, Mladenovic E, Mrdjan S, Svilokos N. 2017. Morphometric characteristics of sycamore maple (*Acer pseudoplatanus* L.) fruits in Novi Sad urban populations. *Glas Sumar Fak Fac For*.(116):69–98.
- Kvesić S, Hodžić MM, Ballian D, Gömöry D, Fussi B. 2020. Genetic variation of a widespread subdominant tree species (*Acer campestre* L.) in Bosnia and Herzegovina. *Tree Genet Genomes*. 16(6):1–12.
- Liu Y, Li Y, Song J, Zhang R, Yan Y, Wang Y, Du FK. 2018. Geometric morphometric analyses of leaf shapes in two sympatric Chinese oaks: *Quercus dentata* Thunberg and *Quercus aliena* Blume (Fagaceae). *Ann For Sci*. 75(4).
- Mediouni K, Azira F. 1992. Contribution à l'étude de la dynamique des formations à Erables (*Acer*) d'Ait Ouabane (Djurdjura). *Forêt méditerranéenne* [Internet]. 13(2):109–114. <http://www.foret-mediterraneenne.org/fr/catalogue/id-379-contribution-a-l-etude-de-la-dynamique-des-formations-a-erables-acer-d-ait-ouabane-djurdjura->
- Mediouni RM, Wahlsteen E, Gaouar SBS. 2021. Leaf shape variability among North African and Eurasian populations of Montpellier maple (*Acer monspessulanum* L.). Tlemcen: Abou Bekr Belkaid University.
- Murray E. 1969. *Flora Iranica: Aceraceae*. 61:1–11.
- Nagy L, Ducci F. 2004. EUFORGEN Technical Guidelines for genetic conservation and use for field maple (*Acer campestre*). Rome, Italy.
- Pandey M. 2005. Development of microsatellites in sycamore maple (*Acer pseudoplatanus* L.) and their application in population genetics. [place unknown]: Georg-August University of Göttingen.
- Parsa A. 2014. AFRĀ. *Encycl Iran* [Internet]. 1(6):569–570. <http://www.iranicaonline.org/articles/afra-persian-term-for-the-maple-tree-genus-acer-so-embracing-a-few-shrubs-of-the-family-aceraceae>
- Pillai KCS. 1955. Some New Test Criteria in Multivariate Analysis. *Ann Math Stat* [Internet]. 26(1):117–121. <https://doi.org/10.1214/aoms/1177728599>
- Quézel P, Santa S. 1963. Nouvelle Flore de l'Algérie et des régions désertiques méridionales. In: Paris; p. 615.
- R Core Team. 2020. R: A Language and Environment for Statistical Computing [Internet]. <https://www.r-project.org>
- Rohlf FJ. 2015. The tps series of software. *Hystrix, Ital J Mammal* [Internet]. 26(1):9–12. <http://dx.doi.org/10.4404/hystrix-26.1-11264>
- Savriama Y. 2018. A Step-by-step guide for geometric morphometrics of floral symmetry. *Front Plant Sci*. 9(October):1–23.
- Shi F. 2019. Learn About Wilks' Lambda in SPSS With Data From the Global Health Observatory (2016). *SAGE Res methods* [Internet].:1–8. <https://methods.sagepub.com/dataset/wilks-in-gho-2016>
- Siahkolaei SN, Sheidai M, Assadi M, Noormohammadi Z. 2017. Pollen morphological diversity in the genus *Acer* L. (Sapindaceae) in Iran. *Acta Biol Szeged*. 61(1):95–104.
- Straigyte L, Baliuckas V. 2015. Spread intensity and invasiveness of sycamore maple (*Acer pseudoplatanus* L.) in Lithuanian forests. *iForest - Biogeosciences For*. 8(5):693–699.
- Trabut LC, Battandier J. 1890. Flore de l'Algérie : Description de toutes les plantes signalées jusqu'à ce jour comme spontanée en Algérie – Les dicotyledones –. In: Alger; p. 855.
- Turok J, Eriksson G, Kleinschmit J, Canger S, Campilers. 1996. Noble Hardwoods Network. Escherode, Germany: International Plant Genetic Resources Institute, Rome, Italy.
- Wahlsteen E. 2020. Morphometrical methods as tools for identifying field maple (*Acer campestre* L.) trees. *Feddes Rept*. 131(1):72–81.
- Wahlsteen E. 2021. Morphometrical characteristics of cryptic invasive and indigenous gene pools of field maple *Acer campestre* L. in southern Sweden. *Nord J Bot* [Internet]. 39(2). <https://doi.org/10.1111/njb.02901>
- Zelditch ML, Swiderski DL, Sheets HD. 2012. Geometric Morphometrics for Biologists [Internet]. In: 2nd ed. San Diego: Academic Press; p. 1–20. <https://www.sciencedirect.com/science/article/pii/B9780123869036000010>

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Plant Diversity of Belezma Cedar -Batna-**Neffar Fahima<sup>\*1</sup><sup>\*1</sup>Mostefa Ben Boulaid University -Batna2-

neffar.fa@gmail.com

**Abstract**

Cedar is degraded by human action and overgrazing which transforms its favorable environment into a drier biotope, particularly in warmer and less snowy exposure, negatively influencing tree growth and natural regeneration, so dieback is the problem major of Belezma National Park. With the decline of the forest, significant genetic potential, a factor of biodiversity, can be irreversibly lost. The hypotheses put forward on the causes of this problem and, which seems to be generated by the combination of several factors such as the climate, the soil, and the attacks of the processionary caterpillar and the xylophagous, but also of the irrational overgrazing, the result is a " crazy " decline which sometimes affects a whole slope. The floristic bypass carried out at the stations of "Tuggurt" and "Boumerzoug" reveals a richness and a great floristic diversity of the PNB, in particular with regard to the botanical family of Asteraceae.

**Keywords:** *Asteraceae*, diversity, pnb, tuggurt, boumerzoug

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Bio-Ecological & Demo-Ecological Approach of Avifauna at Sector Level 'Hamla (Djebel Tuggurt) & Fesdis (Kasrou)' of the National Park of Belezma -Batna-**Neffar Fahima<sup>\*1</sup><sup>\*1</sup>Mostefa Ben Boulaid University -Batna2-

neffar.fa@gmail.com

**Abstract**

In Algeria, there are 406 species distributed over several orders and families. This proves the richness and diversity of the Algerian avifauna which is in fact closely linked to the diversity of cultivated and spontaneous flora. As in the world, the most important order is that of the Passeriformes, encompassing the *Hirundinidae*, *Motacillidae*, *Turdidae*, *Muscicapidae*, *Sylvidae*, *Laniidae*, *Paridae*, etc. Birds fall into several categories, namely granivores, insectivores, carnivores and frugivores. Birds have a very rich instinctive life and exceptional vocal abilities, their usefulness comes mainly from the role they play in nature, by destroying harmful insects and rodents, by propagating the seeds of plants and, by participating in the various cycles. subjects. The birds are part of the branch of the Chordés; The order richest in species is that of Passeriformes which brings together the passerines representing more than half of living species, and more than a third of families. 41 avian species recorded are distributed among 8 orders, 21 families. The most important order in family and in species is that of Passeriformes with 13 families or 61.90% of all families and 30 species or 73.17% of all species. The two stations, namely Hamla and Fesdis have revealed an incredible wealth of avian species.

**Keywords:** Avifauna, kasrou, tuggurt, pnb, Batna

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Study of Edaphic Biodiversity Under *Olea europea. L* Arbori-cultural Ecosystem in The  
Semi-Arid Region of Batna in Algeria**Ghanem Nadra<sup>1\*</sup>, Zekri Djihane<sup>2</sup>, Kherbache Amel<sup>3</sup>, Medjoudj Amina<sup>4</sup><sup>\*1,2,3,4</sup> Batna 2 University, Faculty of Sciences and Life, Department of Ecology and Environment, Fesdis  
05110, Algeria.

\*Corresponding author e-mail: n.ghanem@univ-batna2.dz

**Abstract**

In the Mediterranean basin, olive tree (*Olea europea. L*) constitutes a main fruit species, both in terms of number of varieties cultivated as well as the social and economic importance of its cultivation and its environmental role. However, soil organisms are living things that perform at least part of their life cycle in the soil, on the soil surface in decaying organic matter. A large number of organisms live in soil and perform various ecological functions there. This soil fauna is particularly studied in case of agro-systems because it has an impact on primary production. It intervenes in recycling of nutrients, in structure of the soil, in control of pests and can modify the interactions between plant species. Our work therefore aimed to assess the biodiversity of edaphic invertebrates under a garlic crop. Random soil sampling at six sites was carried out in the spring period in a plot cultivated with Garlic in the Chemora region of the wilaya of Batna, which is characterized by a semi-arid climate with cold winters. It was followed by an extraction and identification of invertebrates carried out with the naked eye, and by means of the Berlése trap, from a soil volume of 30/30/30 cm<sup>3</sup>, ie a weight of about 8 kg. The results made it possible to identify ten varieties of invertebrates: Lombricidae of the Genus Aporetodea; Larvae Coleoptera; Tipulid larvae; Dermaptres; Diptera larvae; Beetle larvae; Trichoptera larvae; Mites (Pseudoscopion); larva Hemiptera Aphidoedae and finally Carabeadae. The correlation matrix revealed a positive correlation between the biomass of earthworms and the larvae of different species of invertebrates extracted from the soil. The principal component analysis made it possible to record a group of variables explained by the observation of earthworm biomass. This group of variables includes larvae of Coleoptera, Diptera and Hemiptera.

**Keywords:** Biodiversity, invertebrates, soil, ecosystem, olive tree.



Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Study of Edaphic Biodiversity Under *Allium sativum L* Culture Ecosystem in The Semi-Arid Region of Batna in Algeria**Ghanem Nadra<sup>1\*</sup>, Zekri Djihane<sup>2</sup>, Mokhtari bouthaina<sup>3</sup><sup>\*1,2,3</sup> Batna 2 University, Faculty of Sciences and Life, Department of Ecology and Environment, Fesdis 05110, Algeria.

\*Corresponding author e-mail: n.ghanem@univ-batna2.dz

**Abstract**

The garlic (*Allium sativum L*) cultivation is one of market gardening crops widely used in semi-arid regions in Algeria especially in later years, given its economic and social interest. This vegetable constitutes the most important element of a balanced diet thanks to their valuable nutritional component values and micronutrients essential for human health. The organic matter that is deposited in soil via aerial or root litter specific to garlic crop, therefore constitutes special energy and carbon sources for underground biodiversity, especially in rather distinct climatic conditions. A large number of organisms live in soil and perform various ecological functions there. This soil fauna is particularly studied in the case of agrosystems because it has an impact on primary production. The objective of this study was therefore to study the biodiversity of edaphic invertebrates under a garlic culture. Random soil sampling at six sites was carried out in the spring period in a plot at Batna region, characterized by a semi-arid climate with cold winter. It was followed by an extraction and identification of invertebrates carried out with the naked eye, and by means of a Berlése trap with a soil volume of 30/30/30 cm<sup>3</sup> of about 8 kg of soil. The results allowed us to identify eight varieties of invertebrates: Lombricidae of the Genus Aporetodea, Allolobophora and Proctodrilus; Larvae Coleoptera; Tipulid larvae; Dermaptres; Diptera larvae; Beetle larvae; mites; and Carabidae. The correlation matrix revealed a positive correlation between the biomass of earthworms as well as their number of genus and the larvae of different species of invertebrates extracted from the soil. The principal component analysis made it possible to record a group of variables explained by the observation of earthworm biomass and earthworm genus. This group of variables brings together the larvae of beetles and Diptera.

**Keywords:** Biodiversity, invertebrates, soil, ecosystem, garlic

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Some New Alien Plant Species and Their Invasive Potential in the Flora of Adjara  
(Georgia)**Irakli Mikeladze<sup>1\*</sup>, Gia Bolkvadze<sup>1</sup>, Murman Davitadze<sup>2</sup><sup>1</sup>Institute of Phytopathology and Biodiversity, Batumi Shota Rustaveli State University (BSU), Kobuleti,  
Georgia;<sup>2</sup>Department of Biology, Faculty of Natural Sciences and Health Care, Batumi Shota Rustaveli State  
University (BSU), Batumi, Georgia

\*Corresponding author e-mail: irakli.mikeladze@bsu.edu.ge

**Abstract**

In the modern world, including Georgia one of the most threats to the biodiversity are alien species, among them are alien plant species. Unlike other parts of Georgia, the spread of foreign plants continues more intensely in the Adjara florist region. There are over 2000 species, among them about 500 species are of foreign origin. As a result of the research after 2010 year many new alien species were described by us, they are: North American origins - *Verbena brasiliensis* Vell., *Solidago canadensis* L., *Sicyos angulatus* L., East Asian origin - *Youngia japonica* (L.) DC., *Mazus pumilus* (Burm.f.) Steenes., and European- *Lobelia urens* L. *Verbena brasiliensis* is widespread in seaside Adjara - roadways, ruderal sites, abandoned pastures, forest margins and abandoned lawns. In spreading areas, it takes the dominant position and completely changes plant environment. *Solidago canadensis* massive spread on roadsides, canals, ruderal areas, tea plantation, cultivated fields, forest margins and semi natural phytocoenoses. The plant is characterized with vegetative and generative propagation, which provides its fast spread. Its invasive potential is high. *Sicyos angulatus* is spread on the river banks and nearby territories, mainly in the swampy and moist soils. It is widely spread on the agricultural grounds, particularly maize field and represents as a serious weed for farmers. *Youngia japonica* starts vegetation in early spring, grows as an agricultural and environmental weed. It is found in disturbed areas, roadsides, abandoned pastures, lawns, cultivated fields and forest margins. *Mazus pumilus* is a fairly common plant at seaside Adjara. It is mainly described sidewalk cracks, trail sides, in paving stones and waste ground. At this stage *Lobelia urens* is not widespread. found on wet soils, water canal edges and abandoned fields.

**Keywords:** Georgia, Adjara, invasive, alien, flora**Acknowledgement:** The research was funded by Batumi Shota Rustaveli State University.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

**Mapping of *Testudo graeca* Linnaeus, 1758 (Reptilia: Testudinidae) Living in Bozcaada  
According to Habitat Preferences**

Ceren Nur Özgül<sup>1\*</sup>, Çiğdem Gül<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies,  
Department of Biology, Çanakkale, Turkey

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, 17100,  
Çanakkale, Turkey.

\*Corresponding author e-mail: cerennurozgul@gmail.com

**Abstract**

For scientists, knowing the geographical distribution of species and the factors affecting their distribution is an important, current and successful way for the species to survive in the future. In addition, knowing the habitat preferences is very important for biodiversity. In this study, a distribution map was created using ArcGIS 10.8 package program according to habitat preferences of *Testudo graeca* (Tortoise) species in Bozcaada, which has a closed ecosystem and different habitat diversity. As a result, among 6 different habitat types selected in Bozcaada, it was determined that *T. graeca* species is preferred stony-hilly habitat type the most with 57,69% and 17,30% woodland habitat, 9,61% agricultural habitat, 7,69% dune habitat, 3,86% shrubby habitat and 3,84% around wetlands respectively. At the same time, 6 different breeding points have been identified on the island and shown on the map. Breeding points were determined in stony-hilly area, dune area and shrubby area respectively according to observation of nest and juvenile individuals.

**Keywords:** Bozcaada, *Testudo graeca*, distribution, mapping

**Acknowledgement:** This study is a part of Master's Thesis titled "Bozcaada'da Yaşayan Amfibi ve Reptil Türlerinin Habitat ve Çevresel Değişimlere Göre Dağılım Haritalarının Oluşturulması", Çanakkale Onsekiz Mart Üniversitesi, School of Graduate Studies.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

**Color- Pattern Analysis of *Hemidactylus turcicus* (Linnaeus 1758) (Sauria: Lacertilia:  
Gekkonidae) Populations Distributed in Çanakkale**

Didem Kurtul<sup>1\*</sup>, Çiğdem Gül<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies,

Department of Biology, Çanakkale, Turkey

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology,  
Çanakkale, Turkey

\*Corresponding author e-mail: didemkurtul17@gmail.com

**Abstract**

Color-pattern, pholidosis and morphometric measurements are used in the classical classification of reptiles. As well as other reptile species, the color and pattern characteristics of *Hemidactylus turcicus* (Linnaeus 1758) (Mediterranean House Gecko) species are important parameters for identifying. In this study, 15 different color pattern characters were selected qualitatively and similarities or dissimilarities were determined from a total of 46 individuals belonging to *Hemidactylus turcicus* populations that distributed in Çanakkale and Bozcaada. As a result, it was determined that the Ayvacık population was larger than the Bozcaada population in terms of head+body length. Differences in dorsal ground color, tubercule color on the tail and dorsal patterning were found between Ayvacık and Bozcaada populations of *Hemidactylus turcicus*, and it was determined that there was no differences between two populations in other parameters examined.

**Keywords:** *Hemidactylus turcicus*, color- pattern, morphology, Çanakkale, Bozcaada

**Acknowledgement:** This study is a part of Master's Thesis titled "Ayvacık ve Bozcaada (Çanakkale)'da Dağılışı Gösteren *Hemidactylus turcicus* (Linnaeus, 1758) (Sauria: Lacertilia: Gekkonidae) Popülasyonlarının Morfolojik ve Osteolojik Karşılaştırılması", Çanakkale Onsekiz Mart Üniversitesi, School of Graduate Studies.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

### Distribution of Breeding Anatidae Family in Çanakkale Province

Ibrahim Uysal<sup>1\*</sup>, Ibrahim Uysal<sup>2</sup><sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Biology. e-mail:

ibrahimuysal@hotmail.com, Orcid ID: 0000-0001-7180-5488

<sup>2</sup>Çanakkale Onsekiz Mart University, Vocational School of Health Services, e-

mail:uysalibrahim@comu.edu.tr, Orcid ID: 0000-0002-7507-3322

#### Abstract

The Anatidae (ducks, geese, and swans) family are a group of waterbird that are ecologically dependent on wetlands for at least some parts of their annual cycle. Anatidae species use a wide variety of wetlands such as tundras, swamps, rivers, estuaries, freshwater or brackish lakes, coastal lagoons and mud flats. Çanakkale, located on one of the important migration routes in the Western Palearctic Region, and there are important wetland areas for breeding waterbird populations. Although there are regular censuses and researches on waterbird wintering and breeding in Çanakkale, there is no scientific study on breeding species. Within the scope of the research, the breeding duck species and distribution in the wetlands of Çanakkale province, including the early and late breeding periods, were investigated using atlas methodology between the years 2020-2021. As a result of field studies, breeding codes were given to 7 species included in the anatidae family. Probable breeding code for *Cygnus olor* (1 pair), *Aythya ferina* (1 pair), *Aythya nyroca* (2 pairs) and exact breeding code for *Spatula querquedula* (6 pairs), *Anas platyrhynchos* (18 pairs), *Tadorna tadorna* (6 pairs), *Tadorna ferruginea* (12 pairs) species have been given. The number of habitat types and size of wetlands were proportioned according to breeding species and number of pairs. The significance of the wetlands was tested with the "single sample chi-square" test using the ratios obtained. Although wetlands are critical importance for the *Anatidae* family, biodiversity and therefore sustainability are damaged by increasing anthropogenic activities in wetlands, which are one of the ecosystems where human influence is intense. Detection and monitoring of breeding waterbird will provide critical information for the conservation of wetlands and breeding

populations in the region. The number of breeding duck pairs in the wetlands where the research was conducted is quite low. It is great importance to maintain regular monitoring in order to better understand the dynamics of breeding duck populations in Çanakkale province.

**Keywords:** Anatidae, breeding, wetland, population, Çanakkale

**Acknowledgements:** This study was prepared with data collected within the scope of the thesis study to cover part of the master's thesis entitled “Evaluation of Midwinter waterbirds counts and research of breeding waterbirds”, which is being carried out at Çanakkale Onsekiz March University, Graduate Education Institute, Department of Biology.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

### Researches on Bio-Ecology of Small Dove (*Spilopelia senegalensis* L.)

#### Population in Çanakkale City Center

Sinan Marangoz<sup>1\*</sup>, Murat Tosunoğlu<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Biology, Çanakkale, Turkey.

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Science and Literature, Department of Biology.

\*Corresponding author e-mail: snnmrngz@gmail.com

#### Abstract

The first records of the *Spilopelia senegalensis* (Small Dove) which is not in its natural habitat and whose homeland is the African continent and Arabian Peninsula, belong to the southeastern Anatolia region and Istanbul. Çanakkale province, which is the research area, constitutes one of the most distant distribution areas of the species in the northwest. In this study, the Bio-Ecology of the population of the *S. senegalensis* species in Çanakkale prefecture was investigated. The spread areas of the species in the region, factors affecting the spread, population size in the area of spread, intrasite and interpersonal behaviors, and incubation biology were investigated. Within the scope of the research, the working area (City center) is divided into 32 grids of 1x1 km<sup>2</sup> in order to determine the field distribution of the species and to perform comparison analyses with different variables. In 2021, data was collected by linear transect method to cover the reproductive period (January – September). Descriptive statistical analyses were used in the analysis of the data. Since the first registration of the species in Çanakkale province, 248 individuals have been recorded in 16 grids to date. Incubation activities continued during the observed period (January – September). April and August were determined as the period of the most intense reproductive activity in the research area. In sampling areas, offspring output was observed in 18 of the 22 nests detected throughout the year. Incubation success was calculated as 80.55% according to the total number of eggs and 87.87% according to the number of eggs opened. The relationship of the species with the natural species in which it competes, the growth rate of the population, and the rate of its spread should be monitored.

**Keywords:** Çanakkale, *Spilopelia senegalensis*, population

**Acknowledgement:** This study is a part of Master's Thesis titled "Researches on Bio-Ecology of Small Dove (*Spilopelia senegalensis* L.) Population in Çanakkale City Center", Çanakkale Onsekiz Mart University, School of Graduate Studies.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

### Effects of Tourism Activities on Rock Nuthatch (*Sitta neumayer*) Population in Nevşehir

Bilge Yeni<sup>1\*</sup>, Ahmet Karataş<sup>2</sup>

<sup>1</sup>Department Of Biology, Faculty Of Sciences And Arts, Hacı Bektaş Veli University, Nevşehir, Turkey

<sup>2</sup>Department of Biology, Faculty of Sciences And Arts, Niğde Ömer Halisdemir University, Niğde,  
Turkey.

\*Corresponding author e-mail: bilge.yeni@nevsehir.edu.tr

#### Abstract

It is very important to determine the impact of human activities on wildlife for the conservation of species. Therefore, one of the studies conducted during bio-ecology study of the Rock nuthatch (*Sitta neumayer*) population at Nevşehir was how the species responded to anthropogenic activities. Observations of bio-ecology study was made between 2014 and 2018. Binoculars and camera were used for records. The reason to choose Nevşehir as study area is Cappadocia is a popular tourism region and the population increases four times in a short time due to art and sports activities and people coming for vacation, especially in the breeding season. Balkandere valley is preferred for both trekking and activities with an average of 10k participants such as Cappadox and Salomon Ultra Trail due to its location and structural features. Especially since events such as festivals or sports activities are short notice that they do not allow the species to adapt, examining the effects on wildlife and informing regulatory organizations about this issue is important for conservation. Unfortunately, it has been observed that these activities have a negative impact on the Rock nuthatch individuals, such as leaving the nest, which ecological tolerance is low due to being in the breeding season, and this effect continues for a long time afterward. The results of the observations were shared with the organizers of the events and public institutions, and ideas were exchanged on how to follow a path for conservation of future events.

**Keywords:** *Sitta neumayer*, cappadox, salomon ultra trail, Cappadocia, Balkandere

#### INTRODUCTION

Nevşehir is one of the cities in the Cappadocia region. The city is at UNESCO's world heritage list. Due to these features, it is visited by more than 2 million people annually especially in the



breeding season. Cultural tourism is more dominant than ecological tourism in the region. This situation has a positive economic impact on the region. But the negative effect on wildlife to be ignored (Bateman and Fleming, 2017). Therefore, one of the studies conducted during bio-ecology study of the Rock nuthatch (*Sitta neumayer*) population at Nevşehir was how the species responded to anthropogenic activities. Balkandere valley is one of valleys in Nevşehir, preferred for both trekking and activities due to its location and structural features. It is important to determine the effect of these activities on the Rock Nuthatch population.

### **MATERIALS AND METHODS**

Observations of bio-ecology study was made between 2014 and 2018. Binoculars and camera were used for records. Salomon Ultra Trail was held in October 2017 and Cappadox Festival was held in June 2018. During the events, observations were made and photographs were taken in the area. Hidden Worlds, Night Landscape installation by RAAAF (Rietveld Architecture-Art-Affordances) was the focus of observations. Because fire was lit inside the caves for this exhibition.

### **RESULTS AND DISCUSSION**

Since the Balkandere valley is one of the tracks used for the Salomon ultra trail in 2017, approximately 700 racers passed through the valley during the event. After this event, a couple of *Sitta neumayer* who had a nest there moved their nest to another. In the place where the nest was moved, the following year, an exhibition called Hidden Worlds was held as part of the Cappadox festival. Within the scope of this exhibition, fires were lit in the caves under the nest for 4 days. As a result, the couple abandoned that nest. As seen these activities have a negative impact on the Rock nuthatch individuals which ecological tolerance is low due to being in the breeding season, and this effect continues for a long time afterward. Especially since events such as festivals or sports activities are short notice that they do not allow the species to adapt, examining the effects on wildlife and informing regulatory organizations about this issue is important for conservation. The results of the observations were shared with the organizers of the events and public institutions, and ideas were exchanged on how to follow a path for conservation of future events.

### **REFERENCES**

Bateman, P. W., & Fleming, P. A. (2017). Are negative effects of tourist activities on wildlife over-reported? A review of assessment methods and empirical results. *Biological Conservation*, 211, 10-19.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**The Influence of Sluices on Zooplankton Diversity in Canal – Case Study**Nikola Kolarova<sup>1\*</sup>, Paweł Napiórkowski<sup>1</sup>

<sup>1</sup>Kazimierz Wielki University, Faculty of Biological Sciences, Department of Hydrobiology, Bydgoszcz, Poland.

\*Corresponding author e-mail: nikol77@student.ukw.edu.pl

**Abstract**

The poster presents the results of studies on changes in the structure of the zooplankton community caused by environmental conditions in the canal before and after sluices. Water samples were collected in June and July 2021 in the Bydgoszcz Canal and the Noteć Canal at sites before and after sluices. We analyzed how water parameters and selected parameters changed after passing the sluice and affected zooplankton diversity (T) and density (N). In total 55 species were determined with an average density 294 ind/L. The zooplankton was the most diverse at sites before sluices in Bydgoszcz Canal. Zooplankton density and biomass was higher at sites after sluices in the Bydgoszcz Canal. The most dominant species among rotifers was *Lecane closterocerca* and *Synchaeta oblonga*. Among crustaceans the most dominant was *Bosmina longirostris*, *Ceriodaphnia pulchella* and nauplii (larval forms of copepods). At all sites before sluices the zooplankton community was qualitatively and quantitatively dominated by rotifers compared to crustaceans. At sites after sluices zooplankton community differed in comparison to zooplankton before sluices. In the Bydgoszcz Canal crustaceans dominated quantitatively, but rotifers qualitatively. At site of Noteć Canal rotifers were dominated group of all zooplankton. The water in the canals in front of the sluices slowed down very much, which could create good conditions for the development of zooplankton (shaped in diversity). On the other hand, below the sluice, the water began to flow quite quickly, bypassing the sluice with relief channels, and this could have contributed to the deterioration of the living conditions for the zooplankton.

**Keywords:** microinvertebrates, water parameters, artificial waterways, sluices

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

**Study on Micropropagation of *Paeonia mascula* subsp. *bodurii***

Ebru CAMBAZ<sup>1\*</sup>, Nurşen ÇÖRDÜK<sup>2</sup>, Bahar KÖKÇÜ<sup>1</sup>, Ersin KARABACAK<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Biology, Çanakkale, Turkey.

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Arts and Sciences, Department of Biology, Çanakkale, Turkey.

\*Corresponding author e-mail: ebrucmbzz@gmail.com

**Abstract**

*Paeonia* species are known peony and used as a folk medicine in the treatment of many diseases. These species, which have herbaceous and woody forms, are rooted plants with a tuberous base and fleshy roots. *Paeonia mascula* subsp. *bodurii* subspecies is an endemic plant and distributed in Çanakkale, Turkey. The conservation status of this species has been declared as Endangered (EN) according to the red data book of Turkish Plants. It takes 2 years for the seeds to germinate in nature, as their seeds have dormancy one after the other. For this reason, plant tissue culture techniques are used for their production. The aim of this study, establish to *in vitro* micropropagation of *Paeonia mascula* subsp. *bodurii*. Shoot tip explants were cultured on ½ MS medium supplemented with 1 mg/L BAP, 1 mg/L GA<sub>3</sub>, 1 g/L PVP, 3% (w/v) sucrose and 0.7% (w/v) agar for shoot induction. All cultures were maintained at 25±2°C under the 16/8 h photoperiod with a light intensity of 72 µmol m<sup>-2</sup>s<sup>-1</sup>. The shoots were cultured on ½ MS medium containing 1 mg/L IAA and 0.3 g/L AC in darkness at +4°C for different period (10, 15, and 20 days) for shoot development and tuberous fleshy storage root induction. Each stage of micropropagation for *P. mascula* had been optimized under optimum conditions, shoot and tuberous fleshy storage root induction treatments were compared. Shoot induction were achieved with SI medium. Our result showed that culturing the shoots on ½ MS medium containing 1 mg/L IAA and 0.3 g/L AC in darkness at +4°C for 15 days was effective for shoot development but these conditions were insufficient for tuberous fleshy storage root induction.

**Keywords:** endemic, peony, propagation, tissue culture

## INTRODUCTION

Turkey has a very rich biodiversity because of many features such as its geographical location, geological structure, climatic conditions, having three different biogeographic regions such as Europe-Siberia, Mediterranean and Iran-Turan, and their transition zones. This genetic diversity is especially important with the species diversity of endemic, rare, medicinal, and cultivated plants. The flora of Turkey has an endemism rate of 31.82% with 3649 endemic plant species (Demirayak, 2002; Avcı 2005; Güner et al., 2012). Endemic plant species may be endangered due to their distribution specific to a certain region and the decrease in the number of individuals in the population. Endemic plant species should be taken under protection in *ex situ* as well as *in situ* for the biodiversity of our country to be transferred to future generations, for the gene resources not to be destroyed, especially for the wild forms of many cultivated species not to be lost, and for the sustainability of plant production. Improved biotechnological methods used in culturing plant cells and tissues provide new tools for rapid propagation and protection of valuable, rare, and endemic medicinal plants (Mikulík, 1999; Rout et al., 2000).

The genus *Paeonia* (Peony), which is a geophyte, is the only genus of the Paeoniaceae family and includes 52 taxa under 36 species in the world (The Plant List, 2021). *Paeonia* is divided into three sections: sect. *Moutan* DC. (in China), sect. *Onaepia* Lindl. (in North America) and sect. *Paeonia* (Europe, North Africa and Asia) (Hong, 2010). All Turkish species belong to the sect. *Paeonia*. 6 species and 8 under species taxa were reported in Turkey (Körüklü, 2012). Although, it is generally known as peony in our country, its local name is Tombak. These peony species, which have herbaceous and woody forms, are rooted plants with a tuberous base and fleshy roots. Asynchronous development of different embryo parts and prolonged seed germination are characteristic feature of this genus. Seed germination takes 2 years in nature due to consecutive dormancy of seeds, (Griess and Meyer 1976; Tian et al., 2010). Although, seed production and propagation methods are used in peony plants, grafting and tuber methods are the most frequently used methods. *Paeonia* species, are used as folk medicine in various countries. Known for its analgesic, sedative, anti-inflammatory, antimicrobial, antiepileptic properties, *Paeonia* plants are also used for the treatment of cardiovascular and genital diseases (Miyazawa et al., 1984; Zhu 1998; Lin et al., 1999; Müller et al., 1999). In addition to its medicinal importance, *Paeonia* species are cultivated as a garden, potted ornamental plant, or cut flower in many countries.

*P. mascula* subsp. *bodurii* (Beyaz Tombak) was described as a new subspecies from Çanakkale in 1995 by Özhatay. The morphological features of this species are as follows; stem glabrous,

purplish, striate 50-80 cm. Lower leaves biternate with (7-)9(-11) leaflets; leaflets obovate, broadly elliptic or nearly orbicular, shortly acuminate, 9-11 cm long, 5-11 cm broad, terminal leaflets attenuate into a shortly decurrent petiole. Leaves glabrous, greyish green above, glaucous beneath. Upper cauline leaves ternate, leaflets 6-13 cm long, 6-9 cm broad, shortly acuminate. Flowers 11-12 cm across. Petals 5-7, obovate, white, purplish at the base. Filaments dark purplish, 10-13 mm; anthers pink or yellow. Carpels 3-4 short very dense white tomentose and fertile seeds dark purplish (Özhatay and Özhatay, 1995). This local endemic peony is under high pressure from deforestation, road constructions, legal mining activities and illegal collecting tubers, and individual numbers are negatively affected. It is accepted that the risk of extinction in nature is high (EN) according to the red data book of Turkish Plants (Ekim et al., 2000).

In recent years, many studies have been devoted to *in vitro* cultivation, propagation, and protection of genetic resources of rare, endemic, and economically valuable plants (Rout et al., 2000; Nishitha et al., 2006; Sarasan et al., 2006; Bunn et al., 2011; Çördük and Akı 2010; 2011; Çördük and Esen, 2014). *In vitro* culture of some *Paeonia* species such as regeneration of *P. mlokosewitschii*, *P. tenuifolia* (Orlikowska et al., 1998) and *P. lactiflora* (Tian vd., 2010), micropropagation of *P. lactiflora* (Hosoki et al., 1989), *P. arborea* (Černá et al., 2001) and *P. suffruticosa* (Beruto et al., 2004), somatic embryo production of *P. lactiflora* (Lee et al., 1992; Kim and Lee 1996; Jana et al., 2013) and *P. anomala* (Brukhin and Batygina, 1995), somatic embryo cryopreservation of *P. lactiflora* (Kim et al., 2006), and secondary metabolite production with callus culture of *P. lactiflora* (Hu et al., 2015).

The aim of this study, establish to *in vitro* micropropagation of *Paeonia mascula* subsp. *bodurii* and transfer to the external environment by rooting.

## MATERIALS AND METHODS

### Plant Material

*Paeonia mascula* subsp. *bodurii* (Figure 1A) plant samples collected from the Lapseki-Ağı Mountain, Çanakkale, Turkey in December 2020. Plant samples identified by Assoc. Prof. Dr. Ersin Karabacak and were kept humid until study was started. Plant samples were prepared as herbarium materials with voucher specimen number (505) and deposited in the Çanakkale Botanic Garden Herbarium (CBB, Çanakkale, Turkey).

### Surface Sterilization

The underground buds (Figure 1B) were kept under running tap water for 1 hour to remove contaminants such as dust-soil. Buds were surface-disinfected by 75% ethanol (EtOH) for 30 seconds. Then, buds were disinfected by 10% and 20% (v/v) sodium hypochlorite (NaOCl) for 20 and 30 minutes under sterile conditions, followed by 5 rinses in sterile water.



**Figure 1.** A) *Paeonia mascula* subsp. *bodurii* plant from the Lapseki-Ağı Mountain, B) underground buds.

### Shoots Induction

Shoot induction cultures (SI) of *P. mascula* were established using shoot tip explants excised aseptically from the bud scales of the underground buds. The explants were cultured on  $\frac{1}{2}$  MS medium supplemented with 1 mg/L benzylaminopurine (BAP), 1 mg/L gibberellic acid ( $GA_3$ ), 1 g/L polyvinylpyrrolidone (PVP) and 3% (w/v) sucrose. All medium were gelled with 0.7% (w/v) agar and the pH was adjusted to 5.75 before autoclaving. All cultures were maintained at  $25 \pm 2^\circ C$  under the 16/8 h photoperiod with a light intensity of  $72 \mu mol m^{-2} s^{-1}$ . Ten explants were cultured per magenta for shoot tips explant, and five replicates were used each treatment. The mean number of regenerated shoots per explant was recorded in each culture after 6 weeks.

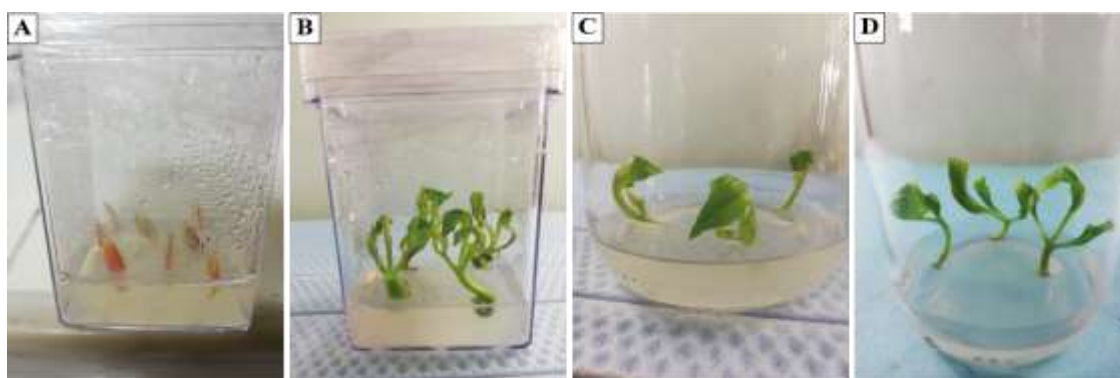
### Shoot Multiplication, Rooting

In order to multiply shoots grown for 6 weeks on shoot induction medium were excised and transferred to fresh  $\frac{1}{2}$  MS medium containing the same PGRs as the shoot induction medium. The well-developed shoots were cultured on  $\frac{1}{2}$  MS medium containing 1 mg/L indole-3-acetic acid (IAA) and 0.3 g/L activated carbon (AC) in darkness at  $+4^\circ C$  for different period (10, 15, and 20 days) for shoot development and storage root induction (RI). Two shoots were cultured per magenta and at least two replicates were used for each treatment. Three weeks later, shoots were transferred to fresh  $\frac{1}{2}$  MS medium containing the same plant growth regulators (PGRs) as the tuberous fleshy storage root induction medium.

## RESULTS

### Surface Sterilization

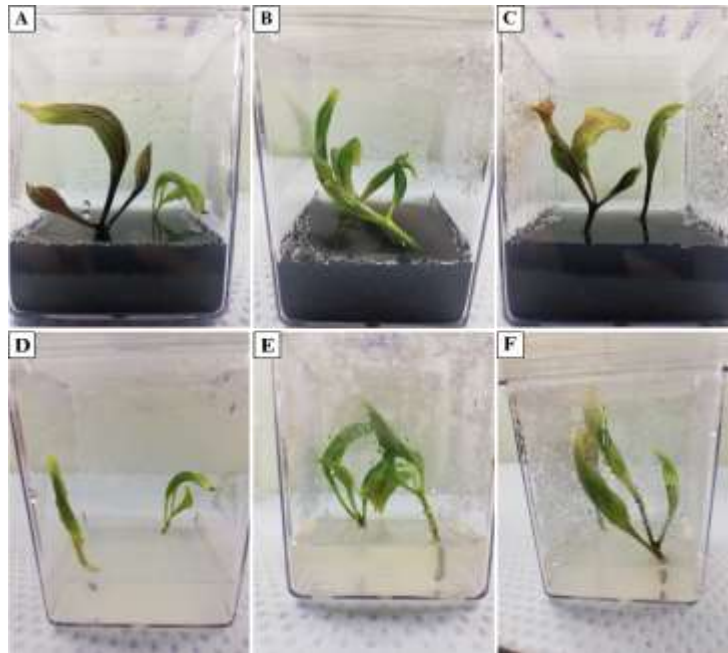
In the first stage, surface sterilization of underground buds was carried out. After surface sterilization, shoot tip explants were obtained and cultured. In the surface sterilization of the underground bud, it was observed that keeping it in 75% EtOH for 30 seconds and 20% NaOCl for 30 minutes have been found more effective for sterilization of underground buds in comparison to treatment with 10% NaOCl for 20 minutes.



**Figure 2.** A) Shoot tip explants cultured on SI medium, B) Shoot induction, C) Shoot multiplication, D) Development shoots from explants.

### *In Vitro* Culture

In this study, shoot tips explants of *P. mascula* were cultured on ½ MS medium containing concentrations of 1 mg/L BAP, 1 mg/L GA<sub>3</sub> and 1 g/L PVP (SI medium) for shoot induction (Figure 2A). The effect of GA<sub>3</sub> in combination with BAP on shoot regeneration from all cultured explants of *P. mascula* was evaluated. PVP was added to prevent shoot browning. The shoots were induced from all of shoot tip explants grown in SI medium (Figure 2B). Three weeks after culture, uncontaminated shoot tip explants were subcultured and grown in fresh ½ MS medium containing the same PGRs as the SI medium. After the second subculture, to multiplied the shoots, shoots grown in SI medium were excised and transferred to fresh ½ MS medium containing the same PGRs as the SI medium (Figure 2C). The well-developed shoots were transferred to the RI medium (Figure 2D). It was observed that leaf yellowing was occurred at shoots that cultured in darkness and cold treatment 10, 15, and 20 days (Figure 3A-3C).



**Figure 3.** Shoots in  $\frac{1}{2}$  MS medium containing 1 mg/L IAA and 0.3 g/L AC and were kept for A) 10 days, B) 15days, C) 20 days in darkness and cold (+4°C) treatment. Shoots transferred from RI medium to SI medium D) 10 days, E) 15 days, F) 20 days.

Shoots in RI medium were cultured in SI medium 3 weeks after darkness and cold treatment (Figure 3D-3E). It was observed that shoots did not tuberous fleshy storage root. Shoots did not survive due to lack of roots. Our result showed that culturing the shoots on  $\frac{1}{2}$  MS medium containing 1 mg/L IAA and 0.3 g/L AC in darkness at +4°C for 15 days was effective for shoot development but these conditions were insufficient for tuberous fleshy storage root induction (Figure 3B and 3E).

## DISCUSSION

In this study, the underground buds were chosen as a explants source because of the difficulties of breaking seed dormancy. Seed dormancy is usually found in *Paeonia* species. It is known that the germination of seeds takes 2-3 years and their germination depends on the season (Barton and Chandler, 1958). Plants bloom 4-5 years after planting (Qin, 2004). Clonal propagation can be done with *in vitro* cultures to shorten the reproductive cycle. Underground buds contain many primordia and are one of the most used explants in *in vitro* cultures of *Paeonia* species. Guo (2001) used the underground buds of *P. lactiflora* 'Qi Hua Lu Shuang' and 'Zhong Sheng Fen' variate as explant source.

In our study, underground buds of *Paeonia mascula* subsp. *bodurii* were collected during dormancy in December, considering the phenological study of Kökçü and Karabacak (2021). It was observed that 70% of the buds sprouted in the SI medium. Geophyte plants spend their



dormancy period underground and sampling period of underground buds affects the success of *in vitro* culture experiments. It has been reported that the most suitable sampling period is between November and March when the buds are dormant at low temperature (He et al., 2009). During these months, the underground buds differentiate, accumulate nutrients, and have a lower contamination rate. Thus, it has been reported that the survival rate and sprouting rate are higher (Zhang, 2006). *P. lactiflora* 'Zhong Sheng Fen' buds were determined the sprouting and differentiation rates of the buds collected in November-December (100% sprouting and 90-92.7% differentiation) and the buds collected in September-October (47.2% sprouting and 44.4% differentiation) and showed the importance of bud sampling period for *in vitro* cultures (Guo, 2001).

In the surface sterilization of the underground bud, it was observed that treatment underground buds with 75% EtOH for 30 seconds and 20% NaOCl for 30 minutes have been found more effective for surface sterilization in comparison to treatment with 10% NaOCl for 20 minutes. One of the most important elements of the initial stage of *in vitro* cultures is the sterilization of explants. There are many methods for the bud sterilization procedure of *Paeonia* species (Zhao and Yu 2008; Wu et al., 2011b; Yu et al., 2012).

In our study,  $\frac{1}{2}$  MS medium containing 1 mg/L BAP, 1 mg/L GA<sub>3</sub> and 1 g/L PVP was used for shoot induction. It was observed that 100% of the explants were stimulated for induction. It was observed that the addition of BAP and GA<sub>3</sub> to the medium was also effective for *Paeonia mascula* subsp. *bodurii* species. It has been reported that different ratios and concentrations of auxin and cytokinin have been used for shoot induction from underground buds of herbaceous and tree peonies (Li et al., 1984; Harris and Mantell, 1991; Černa et al., 2001; Wang et al., 2018). As a result of the studies, it has been reported that GA<sub>3</sub> alone is not effective for shoot induction, but combination GA<sub>3</sub> with BAP significantly increases shoot induction and breaks dormancy buds (Bouza et al., 1994; Kong and Zhang, 1998; Pan, 2010; Wen et al., 2016; Wang et al., 2016). In the propagation studies of *Paeonia*, it was reported that supplementation BAP alone or with calcium chloride (CaCl<sub>2</sub>) or other PGRs to the medium is effective for shoot propagation (Guo, 2001; Yu et al., 2011a). Li (2004) reported that MS medium containing 0.5 and 1.0 mg/L BAP are the most suitable for shoot propagation. In our study, we used  $\frac{1}{2}$  MS medium containing 1 mg/L BAP, 1 mg/L GA<sub>3</sub> and 1 g/L PVP for shoot induction, this medium is 100% effective in shoot multiplication as well as shoot induction.

It was stated that the two-stage rooting protocol is generally more efficient for *Paeonia* species in root induction studies. Beruto et al. (2004) achieved root induction in 20 peony trees, first by cold treatment (2°C) and then by keeping them in the dark for 7 days. In many studies, for root induction, dark and cold treatment (10 - 4°C) for 10-30 days, followed by AC (%0.03-0.3 (w/v)) and used ½ MS medium with IBA (Indole-3-butyric acid) or without PGRs (Gua, 2001; Li, 2004; Zhang, 2006; He, 2009). We tried to carry out the shoot development and tuberous fleshy storage root induction of *P. mascula* subsp. *bodurii* via different conditions. Our result showed that culturing the shoots on ½ MS medium containing 1 mg/L IAA and 0.3 g/L AC in darkness at +4°C for 15 days was effective for shoot development. Culturing the shoots on ½ MS medium containing 1 mg/L IAA and 0.3 g/L AC in darkness at +4°C for different period (10, 15, and 20 days) were insufficient for tuberous fleshy storage root induction. Leaf yellowing was occurred during culturing the shoots on ½ MS medium containing 1 mg/L IAA and 0.3 g/L AC in darkness at +4°C for 10 days and 20 days. Shoots were transferred to the SI medium, but they did not survive due to not rooting.

This is the first report on micropropagation study of *P. mascula* subsp. *bodurii*. We tried to carry out shoot induction, shoot development and storage root induction of *P. mascula* via different conditions. For achieving micropropagation of this species, storage root induction of *P. mascula* subsp. *bodurii* has to be optimized by different conditions.

## ACKNOWLEDGEMENTS

This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

## REFERENCES

- Avcı M (2005). *Çeşitlilik ve Endemizm Açısından Türkiye'nin Bitki Örtüsü*. İstanbul Üniversitesi Edebiyat Fakültesi, *Coğrafya Dergisi* 13, 27-55.
- Barton LV & Chandler C (1958). Physiological and morphological effects of gibberellic acid on epicotyl dormancy of tree peony. *Contributions from Boyce Thompson Institute* 19:201-214.
- Beruto M, Lanteri & Portogallo C (2004). Micropropagation of tree peony (*Paeonia suffruticosa*). *Plant Cell, Tissue and Organ Culture* 79: 249-255, 2004.
- Bouza L, Jacques M & Miginiac E (1994). Requirements for *in vitro* rooting of *Paeonia suffruticosa* Andr. cv. 'Mme de Vatry'. *Scientia Horticulturae* 4: 223-233.
- Brukhin VB, Batygina TB, (1994). Embryo culture and somatic embryogenesis in culture of *Paeonia anomala*. *Phytomorphology* 44: 151-157.
- Bunn E, Turner SR & Dixon KW (2011). Biotechnology for saving rare and threatened flora in a biodiversity hotspot. *In Vitro Cellular & Developmental Biology - Plant* 47,188-200.
- Černá K, Dedičová B & Borbélyová D (2001). Micropropagation of *Paeonia arborea* Donn, Syn. *P. suffruticosa* Andr. *Acta Fytotechnica et Zootechnica* 4: 51-54.
- Çördük N & Akı C (2020). *Endangered Plants: In Vitro Propagation of Digitalis trojana Ivanina., an Endemic Medicinal Plant of Turkey*. Sanjeet Kumar (ed.), Intech Open, London, 1-10.

- Çördük N & Akı C (2011). Inhibition of Browning Problem During Micropropagation of *Sideritis trojana* Bornm., an Endemic Medicinal Herb of Turkey. *Romanian Biotechnological Letters* 16(6):6760-6765.
- Çördük N & Esen O (2014). The effect of plant growth regulators on adventitious shoot regeneration of *Silene bolanthoides*. The 3rd International Congress of the Molecular Biology Association of Turkey, İzmir, Turkey. 85.
- Demirayak F (2002). *Biyolojik Çeşitlilik-Doğa Koruma ve Sürdürülebilir Kalkınma*. TÜBİTAK, Vizyon 2023, Biyolojik Çeşitliliğin Korunması ve Sürdürülebilir Kalkınma, [http://www.tubitak.gov.tr/tubitak\\_content\\_files/vizyon2023/csk/EK-14.pdf](http://www.tubitak.gov.tr/tubitak_content_files/vizyon2023/csk/EK-14.pdf). Son Erişim tarihi: 26.04.2015.
- Ekim T, Koyuncu M, Vural M, Duman H, Aytaç Z & Adıgüzel N (2000). *Türkiye Bitkileri Kırmızı Kitabı (Eğrelti ve Tohumlu Bitkiler)*. 100. Yıl Üniversitesi ve Türkiye Tabiatını Koruma Derneği, Ankara.
- Griess JL & Meyer MM (1976). Dormancy and survival of perennial plants. *American Peony Society Bulletin* 231:39–42.
- Guo FY (2001). Study on tissue culture of *Paeonia lactiflora*. Master's Thesis. Beijing Forestry University, Chinese.
- Güner A, Aslan S, Ekim T, Vural M & Babaç MT (ed/r.) (2012). *Türkiye Bitkileri Listesi (Damarlı Bitkiler)*. Nezahat Gökyiğit Botanik Bahçesi ve Flora Araştırma Derneği Yayını, İstanbul, 1290 s.
- Harris AR & Mantell SH (1991). Effects of stage: subculture durations on the multiplication rate and rooting capacity of micropropagated shoots of tree peony (*Paeonia suffruticosa* Andr.). *Journal of Horticultural Sciences* 66: 95-102.
- He D (2009). The control on rooting culture of *Paeonia suffruticosa in vitro*. Master's Thesis. Henan Agricultural University, Chinese.
- Hong DY (2010). *Paeonies of the World: Taxonomy and Phytogeography*. Royal Botanic Gardens, Kew, London, 302.
- Hosoki T, Ando M, Kubara T, Hamada M & Itami M (1989). *In vitro* propagation of herbaceous peony (*Paeonia lactiflora* Pall.) by a longitudinal shoot-split method. *Plant Cell Reports* 8: 243–246.
- Hu S, Ma Y, Jiang H, Feng D, Yu W, Dai D & Mei L (2015). Production of paeoniflorin and albiflorin by callus tissue culture of *Paeonia lactiflora* Pall. *Chinese Journal of Chemical Engineering* 23: 451–455.
- Jana S, Sivanesan I, Lim MY & Jeong BR (2013). *In vitro* zygotic embryo germination and somatic embryogenesis through cotyledonary explants of *Paeonia lactiflora* Pall. *Flower Research Journal* 21(1):17-22.
- Kim YS & Lee BK (1996). Somatic embryogenesis and plant regeneration in cotyledon culture of *Paeonia albiflora*. *Journal of the Korean Society for Horticultural Science* 37:827-830.
- Kim HM, Shin JH & Sohn JK (2006). Cryopreservation of somatic embryos of the herbaceous peony (*Paeonia lactiflora* Pall.) by air drying. *Cryobiology* 53:69-74.
- Kong XS & Zhang MX (1998). The research of peony propagation technology *in vitro*. *Northwest Horticulture* 3:87-89.
- Kökçü B & Karabacak E (2021). Phenological behaviours of the local endemic *Paeonia mascula* (L.) Mill. subsp. *bodurii* Özhatay in Çanakkale, Turkey. *Trakya University Journal of Natural Sciences* 22(2): 207-213.
- Körüklü ST (2012). Paeoniaceae, 659-660. In: Güner, A., Aslan, S., Ekim, T., Vural, M. & Babaç, M.T. (eds). *Türkiye Bitkileri Listesi-Damarlı Bitkiler*. Nezahat Gökyiğit Botanik Bahçesi Yayınları Flora Dizisi 1, İstanbul, xxi + 1290.
- Lee BK, Ko JA & Kim YS (1992). Studies on thidiazuron treatment on anther culture of *Paeonia albiflora*. *Journal of Korean Society of Horticultural Sciences* 33(5): 384-395.
- Li YL, Wu DY & Pan SL (1984). The research of peony tube seedlings reproduction technology. *Science Bulletin* 8: 500-502.
- Li YM (2004). Studies on the tissue culture of three cultivars of *Paeonia suffruticosa* Andr. Master's Thesis. Beijing Forestry University, Chinese.
- Lin HC, Ding HY, Ko FN, Teng CM & Wu YC (1999). Aggregation inhibitory activity of minor acetophenones from *Paeonia* species. *Planta Medica* 65:595-599.
- Mikulík J (1999). Propagation of endangered plant species by tissue cultures. *Acta Universitatis Palackianae Olomucensis Facultas Rerum Naturalium Biologica* 37: 27-33.
- Miyazawa M, Maruyama H & Kameoka H (1984). Essential oil constituents of "Paeoniae Radix" *Paeonia lactiflora* Pall. (*Paeonia albiflora* Pall.). *Agricultural and Biological Chemistry* 48: 2847-2849.
- Müller AA, Reiter SA, Heider KG & Wagner H (1999). Plant derived acetophenones with antiasthmatic and anti-inflammatory properties: inhibitory effects on chemotaxis, right angle light scatter and actin polymerization of polymorphonuclear granulocytes. *Planta Medica* 65, 590-594.
- Murashige T & Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiologia Plantarum* 15, 473-497.

- Nishitha IK, Martin KP, Ligimol, Beegum AS & Madhusoodanan PV (2006). Micropropagation and encapsulation of medicinally important *Chonemorpha grandiflora*. *In Vitro Cellular & Developmental Biology - Plant* 42, 385-8.
- Orlikowska T, Marasek A & Kucharska D (1998). Regeneration of *Paeonia mlokosewitschii* Lom. and *P. tenuifolia* L. *in vitro* from different explants. *Acta Societatis Botanicorum Poloniae* 67(3/4): 223-227.
- Özhatay N & Özhatay E (1995). A new white *Paeonia* L. from NW Turkey: *P. mascula* Miller subsp. *bodurii* N. Özhatay. *The Karaca Arboretum Magazine* 3: 17-26.
- Pan T (2010). Studies on the tissue culture of multiple shoots and callus of *Paeonia lactiflora*. Master's Thesis. Beijing Forestry University, Chinese.
- Rout GR, Samantaray S & Das P (2000). *In vitro* manipulation and propagation of medicinal plants. *Biotechnology Advances* 18:91-120.
- Qin KJ (2004). Herbaceous Peony. China's Forestry Press, Beijing, Chinese.
- Sarasan V, Cripps R, Ramsay MM, Atherton C, McMichen M, Prendergast G & Rowntree JK (2006). Conservation *in vitro* of threatened plants – progress in the past decade. *In Vitro Cellular & Developmental Biology - Plant* 42:206–214.
- Tian D, Tilt KM, Dane F, Woods FM & Sibley JL (2010). Comparison of shoot induction ability of different explants in herbaceous peony (*Paeonia lactiflora* Pall.). *Scientia Horticulturae* 123:385-389.
- The Plant List (2021). <http://theplantlist.org/1.1/browse/A/Paeoniaceae/> (Date accessed: 22.06.2021).
- Wang MM, Pu XP & Zhang Q (2018). Study on *in vitro* rapid propagation technology on *Paeonia ostii* var. *Lishizheni*. *Molecular Plant Breeding* 16(2):526–534
- Wang X, Cheng FY, Zhong Y, Wen SS, Li LZ & Huang NZ (2016). Establishment of *in vitro* rapid propagation system for tree peony (*Paeonia ostii*). *Scientia Silvae Sinicae* 05:101–110.
- Wen SS (2016). Studies on the micropropagation of tree peony (*Paeonia* × *lemoinei* 'High Noon'). *Propagation of Ornamental Plants* 19-27.
- Wu HJ, Yu XN, Teixeira da Silva JA & Lu GP (2011b). Direct shoot induction of *Paeonia lactiflora* 'Zhong Sheng Fen' and rejuvenation of hyperhydric shoots. *New Zealand Journal of Crop and Horticultural Science* 39 (4), 271–278.
- Yu XN, Wu HJ, Cheng FY, Teixeira da Silva JA & Shen MM (2011a). Studies on multiple shoot induction and proliferation of *Paeonia lactiflora* Pall. 'Zhong Sheng Fen'. *Propagation of Ornamental Plants* 11 (3), 144–148.
- Yu XN, Wu HJ, Teixeira da Silva JA & Shen MM (2012). Multiple shoot induction and rooting of *Paeonia lactiflora* 'Da Fu Gui'. *African Journal of Biotechnology* 11 (41), 9776–9781.
- Zhang QR (2006). The preliminary research on the tissue culture of herbaceous peony. Masters's Thesis. Henan Agricultural University, Chinese.
- Zhao R & Yu XN (2008). Shoot and callus induction of *Paeonia lactiflora* 'Tao Hua Fei Xue'. *China Ornament. Hortic. Res. Prog* 275–278.
- Zhu YP (1998). *Chinese Materia Medica*. Overseas Publishers Association, Amsterdam.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Screening for Indole Acetic Acid Production in Halophilic and Halotolerant****Gram-Positive Bacteria**Sabrina Behairi<sup>1\*</sup>, Nassima Baha<sup>1</sup>, Wafa Achouak<sup>2</sup>, Thierry Heulin<sup>2</sup>, Yahia Kaci<sup>1</sup><sup>1</sup>University of Science and Technology Houari Boumediene, Faculty of Biological Sciences, Laboratory of Biology and Physiology of Organisms, Team of Soil Biology, Algiers, Algeria.<sup>2</sup>Aix-Marseille Univ, CEA, CNRS, UMR7265, LEMiRE, Laboratory of Microbial Ecology of the Rhizosphere, ECCOREV FR 3098, F-13108 Saint Paul Lez Durance, France.

\*behairi-sabrina@hotmail.com

**Abstract**

Microbial synthesis of the phytohormone auxin, particularly indole acetic acid (IAA) is widespread among bacteria including those that are salt-adapted. IAA plays many different roles in plant growth and development, especially under saline conditions. Therefore, it seems interesting to evaluate the production of this secondary metabolite and to test its presence in halophilic and halotolerant bacteria. In the present study, we tested the IAA production ability of 157 Gram-positive bacteria associated with the halophyte plant *Halocnemum strobilaceum* and the bulk soil. These bacteria phylogenetically belong to Firmicutes (110) and Actinobacteria (47). The screening was performed on TSB 1/10 medium containing 0.8 M NaCl and added 0.5 mg/ml tryptophan. After incubation, the presence of the phytohormone was revealed by adding Salkowski's solution to the medium, which resulted in the color change to pink. The results showed that few bacteria responded positively to this test. Out of a total of 157 bacteria, only 28 (18%) had the ability to synthesize and release IAA, contrary to what has been reported in many studies. This could be explained by the low availability of the natural precursor tryptophan in the studied environment which caused the non-adaptation of these bacteria to produce this phytohormone. Moreover, among Firmicutes, only 13% were positive while the number of IAA-producing bacteria was considerably higher in Actinobacteria (30%). Halophilic and halotolerant Actinobacteria appear to be the most IAA-producing and best adapted to hostile environmental conditions.

**Keywords:** Halophilic and halotolerant bacteria, Firmicutes, Actinobacteria, IAA.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

**Investigation of the Interaction of Smoke Tree (*Cotinus coggygia* Scop.) Leaf Extracts with  
Plasmid DNA by Agarose Gel Electrophoresis Method**

Büşra Dalgıç<sup>1\*</sup>, Neslihan Demir<sup>2</sup>

<sup>1</sup>Çanakkale Onsekiz Mart University, School of Graduate Studies, Department of Biology, Çanakkale, Turkey.

<sup>2</sup>Çanakkale Onsekiz Mart University, Faculty of Arts and Science, Department of Biology, Çanakkale, Turkey.

\*Corresponding author e-mail: busrdlgc@gmail.com

**Abstract**

*Cotinus coggygia* Scop. is a commercial ornamental plant belonging to the Anacardiaceae family, also known as "smoke tree" among the people with medicinal use. In this study, the interactions of different extracts (ethyl acetate, methanol and aqueous) of *C. coggygia* leaves with pBR322 plasmid DNA were investigated hydrolytically and oxidatively. Excessive production of reactive oxygen species (ROS) leads to oxidative stress. Oxidative stress can cause damage to DNA, protein, carbohydrates and lipids. *C. coggygia* has strong antioxidant activity with the phenols and flavonoids it contains. The secondary metabolites in its content have the prevention activity of oxidative stress. In this study, *C. coggygia* extracts were prepared at different concentrations (25, 50, 100, 200, 400 µg/mL), and their interactions with DNA were determined by agarose gel electrophoresis method using supercoiled (SC) pBR322 plasmid DNA in TAE buffer. This test examines whether the SC plasmid DNA, transforms into open circular (OC) form and/or linear form (LN). The results show that ethyl acetate, methanol and aqueous extracts hydrolytically cleaved DNA. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was added to the DNA as an oxidizing agent. The result of methanol and aqueous extracts show that they prevent H<sub>2</sub>O<sub>2</sub>-induced oxidative DNA damage, OC structure of DNA is completely denaturated depending on the increasing concentration. Although ethyl acetate decreases the percentage of OC structure of DNA at low concentrations, increases it at 400 µg/mL.

**Keywords:** *Cotinus coggygia*, dna cleavage activity, agarose gel electrophoresis

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

**Determination of The Acute Effects of Olive Mill Wastewater on *Gammarus komareki*  
Schäferna, 1923 (Amphipoda: Gammaridae)**

Deniz Anıl Odabaşı<sup>1\*</sup>, Aytuğ Zilifli<sup>2</sup>, Sevdan Yılmaz<sup>3</sup>

<sup>1\*</sup>Department of Marine and Inland Water Sciences, Faculty of Marine Science and Technology,  
Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

<sup>2</sup>School of Graduate Studies, Çanakkale Onsekiz Mart University, Çanakkale, Turkey.

<sup>3</sup>Department of Aquaculture, Faculty of Marine Sciences and Technology, Çanakkale Onsekiz Mart  
University, Çanakkale, Turkey.

**Abstract**

In this study, acute effects of olive mill wastewater (OMW) that causing water pollution in our country and the Mediterranean circle countries were investigated on the freshwater crustacea, *Gammarus komareki* Schäferna, 1923, in a laboratory setting. *Gammarus komareki* individuals were transported from their habitat to the tanks in the laboratory as a model organism. Totally thirty individuals (males and females) of *G. komareki* were firstly placed and adapted to the laboratory conditions which mimicking the natural habitat conditions of the season. OMW was obtained from an olive oil facility around the Çanakkale Province. After the adaptation stage of the organisms, OMW was introduced into experiment tanks with different ratios such as 2.3%, 2.67%, 3%, 3.33%, and 4%. According to the results of the present study, the LC50 value of the OMW was determined as 3.65% for 72 hours respectively.

**Keywords:** Olive mill wastewater, toxicity, acute effects, Crustacea, freshwater

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

### Greater Inter-Individual than Inter-Population Variability of *Calendula*

#### *suffruticosa* subsp. *algarbiensis* Hexane Extract

Silvana Ohse<sup>1</sup>, Mariza B. Marques<sup>2</sup>, Joaquim J. F. Neto<sup>3</sup>, Paulo C. Silveira<sup>4\*</sup>, Diana C.G.A.  
Pinto<sup>5</sup>

<sup>1</sup>Department of Phytotechnics and Fitossanitary, State University of Ponta Grossa, Campus Uvaranas,  
General Carlos Cavalcanti Avenue-4748, 84030-900, Ponta Grossa-Paraná, Brazil.

<sup>2</sup>Department of Chemistry, State University of Ponta Grossa, 84030-900, Ponta Grossa-Paraná, Brazil.

<sup>3</sup>Northeastern Center of Environmental Research, Montevideu Avenue, 172, Rooms 1105 and 1106,  
Recife Pernambuco, Brazil.

<sup>4</sup>Cesam-Centre for Environmental and Marine Studies, Department of Biology, University of Aveiro,  
3810-193 Aveiro, Portugal.

<sup>5</sup>Laqv-Requimte & Department of Chemistry, University of Aveiro, Campus de Santiago, 3810-193  
Aveiro, Portugal.

\*Corresponding author e-mail: [psilveira@ua.pt](mailto:psilveira@ua.pt)

#### Abstract

The genus *Calendula* L. includes 10 to 27 species depending on the taxonomic concept since it is an extraordinarily complex and poorly understood genus due to its wide morphological and karyological variability. Morphological characteristics have been considered insufficient for correct taxonomic identification. Thus, phytochemical characterization can become an additional tool for their botanical classification, both interspecific and intraspecific. Given this, GC-MS profiles of hexane extract from five specimens of *Calendula suffruticosa* subsp. *algarbiensis* collected in the same geographic region were compared with samples mixing fragments of several individuals (populations) from different local environments. Overall, hexane extracts analysis by GC-MS allowed the identification of 42 compounds, eight fatty acids, 24 terpenoids, three alcohols, five alkanes, and two pollutants. Plants of *C. suffruticosa* subsp. *algarbiensis* collected near urban areas absorbed two compounds considered pollutants, indicating the necessity to pay



attention to the place of cultivation when used in traditional medicine, cosmetics, or as food. Some of the compounds found in significant quantities are known for their medicinal and nutritional properties. Twenty-five secondary metabolites were detected for the first time in the *C. suffruticosa* subsp. *algarbiensis* providing detailed information about the intraspecific variation. The individual samples' variability was even higher than that of mixed samples from different and distant populations. Therefore, sampling of significant numbers of individuals should be considered in future chemotaxonomic studies in *Calendula*, although it should be assumed that each individual plant must have enough mass to obtain sufficient extract for the chromatographic analysis.

**Keywords:** *Calendula*, gc/ms, *Calendula suffruticosa* subsp. *algarbiensis*, chemical variability

**Acknowledgement:** Thanks are due to the University of Aveiro and FCT/ MCT for the financial support for the QOPNA research Unit (UID/QUI/00062/2019) and the LAQV-REQUIMTE (UIDB/50006/2020) through national funds and, where applicable, co-financed by the FEDER, within the PT2020 Partnership Agreement. Thanks, are, also, due to FCT/MCTES for the financial support to CESAM (UIDM/50017/2020+UIDB/50017/2020), through national funds.

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity

### Uses of PGPR for Decrease Salt Stress Effects

Bekkaye Massakib<sup>1\*</sup>, Behairi Sabrina<sup>1</sup>, Karaali Karima<sup>1</sup>, Issad Samia<sup>1</sup>, Baha Nassima<sup>1</sup>

& Kaci Yahia.

<sup>1</sup>Soil Biology Team - Laboratory of Biology and Physiology of Organisms Faculty of Biological Sciences, USTHB BP 32 El Alia - Bab Ezzouar, 16111 Algiers.

\*Corresponding author e-mail: massakb.bekkaye@hotmail.com

#### Abstract

The growth and development of plants is affected by climate changes which are becoming more and more restrictive especially in arid and semi-arid areas. Nearly 80% of Algerian soils are affected by salinity, which causes enormous reduction in the crop production. This problem has forced us to think about the strategies to be undertaken to understand, on the one hand, the mechanisms put in place by plants in order to face new environmental conditions and maintain their growth and development, and on the other apart from limiting the effects of soil salinization by using biological agents (microorganisms). The usefulness of bacteria with "PGPR" effects is one of the methods used to minimize the negative effect of salt stress by synthesizing plant growth regulating hormones, osmoprotectors, exopolysaccharides... etc and it can considerably limit the use of inputs (chemical fertilizers, herbicides, pesticides, fungicides, etc.). In this work, we are interested in the study of the effect of the interaction between rhizobacteria and plants under saline stress, a large number of bacteria isolated from the rhizosphere of halophytic plant from arid areas in Algeria are found as highly salt-tolerant, growing in TSA medium supplemented with different concentration of NaCl. For test the effects of these bacteria on wheat plants under salt stress conditions, wheat seeds were inoculated by bacterial suspension, then sown in pots, each one was watered with solutions containing different concentration of NaCl, for 30 days of growth. Results revealed that some of bacterial strains could promote growth of the seedlings significantly, which showed an increase in the length and biomass of the leaves as well as the roots. Inoculation has in fact removed the deleterious effects of saline stress from durum wheat seedlings, via maintenance of water status and protection of photosynthetic pigments and membrane integrity.

**Keywords:** Durum wheat, rhizosphere, inoculation, pgpr, salinity

Poster Presentation

Friday

Diversity of Plant Species, Systematics and  
Phylogeny; Environmental Toxicology &  
Microbial Biodiversity**Palmyraculture: The Role of Palmyra as Potential Life Support for Plant Species Diversity**Christine Thevamirtha<sup>1\*</sup>, Sherin Monichan<sup>2</sup>, M. Jefwin Paul<sup>2</sup>, Paulraj Mosae Selvakumar<sup>1,2</sup><sup>1</sup>Science and Math program, Asian University for Women, Chittagong, Bangladesh-4000.<sup>2</sup>Panaiyaanmai (Palmyraculture), The Centre for self-reliance and sustainable development, Kadayam,  
Tenkasi, Tamil Nadu, India, 627415

\*Corresponding author e-mail: alexis.thevamirtha@auw.edu.bd &amp; p.selvakumar@auw.edu.bd

**Abstract**

Palmyra palm trees are the gift of nature, help in maintaining plant diversity by acting as life support for many plants. The roots of the Palmyra are found by storing a huge amount of water and helping in transforming arid land into fertile land. It also acts as a host plant for epiphytes and support climber plants. The findings of this study show that the growth status and the survival rate of the plants growing near young Palmyra are relatively high.

**Keywords:** *Borassus flabellifer*, palmyra palm, life support, plant diversity, biodiversity**INTRODUCTION**

Asian Palmyra palm, botanically known as *Borassus flabellifer*, is a tall fan-shaped tree that belongs to group Palmae. It is about 25-30m tall and the trunk is black having a diameter of nearly 1m. Palmyra can be mainly in South Asian countries including Tamilnadu, the Northern regions of Srilanka, Bangladesh, etc., is an important tree that contributes a lot to biodiversity, it acts as life support for other plants, and a bio-fence. (Morton, 1988). Palmyra palm has a fibrous root system. The leaves of the palm grow only one leaf per month and it is then divided into 60-80 segments. The diameter of the segmented leaves is nearly 25feet. (Mariselvam, *et al.*, 2020). The leaf stalks have thorny edges. (Gummadi, *et al.*, 2016). The sex of the tree is determined 12-20yrs after it starts flowering. The male Palmyra has 2m long spadix, is stout and branched, whereas the

female Palmyra gives rise to 4-10 flower-bearing spikes and is branched (Davis & Johnson, 1987). The Palmyra fruits are 4-7 inches in diameter, are fibrous and usually contain 3 seeds. (Gummadi, *et al.*, 2016). The lifespan of Palmyra is 120 years. (Uluwaduge & Thillainathan, 2018). Palmyra tree has been used for many uses from the past and the Tamil literature *Tala vilasam* says that Palmyra has been providing 801 uses. (Franco, *et al.*, 2020) “Palmyraculture” is the cultivation and utilization of the Palmyra tree to live a self-reliant lifestyle towards sustainable development (Selvakumar *et al.*, 2020) (Varadaraju, *et al.*, 2020) (Mariselvam, *et al.*, 2020).

Palmyra trees are found to be having the potential to store a huge volume of water in their tubular roots and this can help in increasing the underground water level of the land. This could be the reason why the farmers have planted Palmyra trees near the water resources like rivers, tanks, and wells. The tree also has the ability to turn arid land into fertile land. (Sridevi Krishnaveni, *et al.*, 2020). Palmyra trees are planted in the fields to help harvest and conserve the underground water. (Veilmuthu, n.d.). In this background, we have studied the survival rate and the growth status of the plants growing in between young Palmyra trees.



**Male Palmyra Palm**



**Female Palmyra Palm**

**Figure 1: Male and Female Palmyra palm trees**

## MATERIALS AND METHODS

The experiment was conducted in Panaiyaanmai (Palmyraculture), The Centre for self-reliance and sustainable development, Kadayam, Tenkasi, Tamilnadu, India. In the experimental group the plants Tamarind, Portia, Cashew, Neem and Pineapple each of six in numbers were planted in between young Palmyra trees. The distance between two young Palmyra trees was 7 feet. In the control group the above mentioned plants of the same amount were grown without any young Palmyra trees. The timeframe for the experiment was one year and three months. It was started on 1st July 2020 and we concluded it on 1st October 2021. The land where we experimented was dry land. During the study period, the plants have been watered only twice and there was rainfall once a month. However, from June to August there was no rain and the plants were grown without any source of water.

## RESULTS AND DISCUSSION

The results of this experiment were significant. In the experimental group, all the plants survived, except one portia tree and the survival rate and the growth status of the plants were significantly high compared to the control group. The results of the experiment are shown in the table below.

**Table 1:** The Survival Rate and the Growth Status of the Plants

|   | Common Name (Sapling) | Botanical Name                | In Presence of Palmyra            |               |                 | In Absence of Palmyra |               |              |
|---|-----------------------|-------------------------------|-----------------------------------|---------------|-----------------|-----------------------|---------------|--------------|
|   |                       |                               | No of plants (In between Palmyra) | Survival Rate | Growth Status   | No of plants          | Survival Rate | No of plants |
| 1 | Tamarind tree         | <i>Tamarind indica</i>        | 6                                 | 6             | Good            | 6                     | 3             | Thin and dry |
| 2 | Portia tree           | <i>Thespesia populnea</i>     | 6                                 | 5             | Good, One dried | 6                     | 0             | All dried    |
| 3 | Cashew tree           | <i>Anacardium occidentale</i> | 6                                 | 6             | Good            | 6                     | 4             | Thin and dry |
| 4 | Neem tree             | <i>Azadirachta indica</i>     | 6                                 | 6             | Good            | 6                     | 4             | Thin and dry |
| 5 | Pineapple tree        | <i>Ananas comosus</i>         | 6                                 | 6             | Good            | 6                     | 1             | Dry          |



**Figure 2:** The experimental group plants

Mixed cropping is one of the oldest forms of agriculture where two or more plants are grown simultaneously and the plants will mutually benefit. Palmyra trees support mixed cropping, as they can help to enhance soil fertility and increase the water level of the land. The experimental results demonstrate that the Palmyra palm is life support for the plants that grow in the dry zones and it helps in mixed cropping. Also, Palmyra palm can be a potential water reservoir that can store water for years in its roots and the plants that grow nearby can utilize that stored water for their growth during the dry seasons. It creates a microclimate that is needed for plant growth. The fan-shaped leaves of the Palmyra tree provide shade for the saplings/plants to avoid getting direct sunlight and protect them from animals and insects.



**Figure 3:** Using Palmyra leaves to protect the saplings

Apart from the experiment, we observed some other phenomena of Palmyra acting as life support for plants. Palmyra tree helps in maintaining plant diversity, by acting as a host for the epiphytes, such as

banyan, peepal, neem, Nuna, athi, and orchids. The birds eat the fruit of the banyan tree and when they leave their excretion on the sheath of the young Palmyra tree the banyan tree can germinate. The Palmyra tree provides a suitable growth place for the banyan tree. The trunk, leaves, and leaf stalks of the Palmyra act as a support for climbers such as bottle gourd, and ivy gourd. Also, this can be used as a support for the black pepper plant cultivation. Also, some shrubs and trees grow very close to the root system of the Palmyra tree, so that they can get nutrition and water from the soil near the Palmyra tree. An array of palmyra trees around the particular land/place is acting as bio-fence and an ecosystem that enhances biodiversity.



**Figure 4:** Climbers growing on Palmyra palm tree



**Figure 5:** Epiphytes growing on Palmyra palm tree



**Figure 6:** Plants growing close to the root system of Palmyra palm tree

## CONCLUSION

Palmyra palm is useful in many ways to both plants and animals. It helps the plants by enhancing the fertility of the soil, increasing the water level of the land, acting as a host/ support, providing shadow, and a place for some plants to germinate. Thus, the Palmyra palm plays an important role in keeping the plant diversity around it. This further contributes to the conservation of biodiversity by acting as a host to plants, animals, and micro-organisms. Hence, a single Palmyra tree can be seen as an ecosystem. Palmyra tree-based bio fences also act as eco-system and help enrich biodiversity. Further research can be conducted to study the feasibility of using Palmyra palm in mixed cropping to improve the agricultural economy of the country.

## ACKNOWLEDGEMENTS

We thank all the Palmyra warriors (also known as palmyra climbers/toddy tappers) of Tamil Nadu, India, Sri Lanka, Bangladesh, and other countries for their self-sufficient lifestyle and eco-friendly community living (PALMYRACULTURE) in pursuit of sustainable development, as well as their dedication to the use and protection of Asian palmyra trees. PMSK thanks the government of Tamilnadu for the initiatives to develop palmyraculture in Tamilnadu and also he requests the govt. of Tamilnadu to allow the usage of palmyra toddy in Tamilnadu.



**REFERENCES**

- Davis, T.A., & Johnson, D. (1987). Current Utilization And Further Development Of The Palmyra Palm (Borassus Flabellifer L., Arecaceae) In Tamil Nadu State, India. *Economic Botany*, 41(2):247-266. Doi: 10.1007/BF02858972
- Franco, F.M., Samuel, G., & Francis, T. (2020). Mutualism Between Humans And Palms: The Curious Case Of The Palmyra Palm (Borassus Flabellifer L.), And Its Tapper. *Institute Of Asian Studies Working Paper*, 59. Retrieved From [https://www.researchgate.net/publication/344347248\\_Mutualism\\_Between\\_Humans\\_And\\_Palms\\_The\\_Curious\\_Case\\_Of\\_The\\_Palmyra\\_Palm\\_Borassus\\_Flabellifer\\_L\\_And\\_Its\\_Tapper](https://www.researchgate.net/publication/344347248_Mutualism_Between_Humans_And_Palms_The_Curious_Case_Of_The_Palmyra_Palm_Borassus_Flabellifer_L_And_Its_Tapper)
- Selvakumar, P. M., & Thanapaul, R. J. R. S. (2021). An Insight Into The Polymeric Structures In Asian Palmyra Palm (Borassus Flabellifer Linn). *Organic Polymer Material Research*, 2(2). <https://doi.org/10.30564/OPMR.V2I2.2639>
- Gummadi, V. P., G. R. Battu, K. D. M. S., And K. Manda. (2016). "A REVIEW ON PALMYRA PALM (BORASSUS FLABELLIFER)". *International Journal Of Current Pharmaceutical Research*, 8(2): 17-20. Doi:<https://innovareacademics.in/journals/index.php/ijcpr/article/view/12102>
- Veilmuthu, P. (N.D.). *Palmyra- Nature's Perennial Gift In The Face Of Climate Crisis- Climate South Asia Network*. Retrieved From <http://climatesouthasia.org/palmyra-natures-perennial-gift-in-the-face-of-climate-crisis/>
- Sridevi Krishnaveni, T.R., Arunachalam, R., Chandrakumar, M., Parthasarathi, G., & Nisha, R. (2020). Potential Review On Palmyra (Borassus Flabellifer L.). *Advances In Research*, 21(9): 29-40. <https://journalair.com/index.php/AIR/article/view/30229/56724>
- Uluwaduge, I., & Thillainathan, K. (2018). Palmyra Research In Srilanka: A Way Forward. Doi:10.13140/RG.2.2.16244.60805
- Morton, J.F. (1988). Notes On Distribution, Propagation, And Products Of *Borassus* Palms (Arecaceae). *Economic Botany* 42(3): 420-441
- Mariselvam, R., Ignacimuthu, S., Ranjitsingh, A.J.A., & Selvakumar, P. M. (2021). An Insight Into Leaf Secretions Of Asian Palmyra Palm: A Wound-Healing Material From Nature. *Materials Today: Proceedings* 47(3), 733-738, <https://doi.org/10.1016/J.Matpr.2020.05.393>
- Mariselvam, R., Ighnachimuthu, S.J., & Selvakumar, P.M. (2020) Review On The Nutraceutical Values Of Borassus Flabelifer Linn. *J Pharm Drug Res*, 3(1): 268-275.
- Varadaraju, C., Selvakumar Paulraj, M., Tamil Selvan, G., Et Al., (2021). An Insight Into Asian Palmyra Palm Fruit Pulp: A Fluorescent Sensor For Fe<sup>2+</sup> And Cd<sup>2+</sup> Ions, *Materials Today: Proceedings*, 47(3), 747-750, <https://doi.org/10.1016/J.Matpr.2020.06.532>

Oral Presentation  
Thursday  
Effects of Biodiversity to Human Health-1

*In Vitro* Assessment of Hemostatic Performances of *Salvia verticillata*, *Achillea biebersteinii*,  
*Tragopogon aureus*, and *Cephalaria procera*

Songül Karakaya<sup>1\*</sup>, Özlem Özdemir Tozlu<sup>2</sup>, Ümit İncekara<sup>2,4</sup>, Hasan Türkez<sup>3</sup>, Özkan Aksakal<sup>4</sup>

<sup>1</sup>Atatürk University, Faculty of Pharmacy, Department of Pharmaceutical Botany, Erzurum, Turkey.

<sup>2</sup>Erzurum Technical University, Faculty of Science, Erzurum, Turkey.

<sup>3</sup>Atatürk University, Faculty of Medicine, Department of Medical Biology, Erzurum, Turkey.

<sup>4</sup>Atatürk University, Faculty of Science, Department of Biology, Erzurum, Turkey.

\*Corresponding author e-mail: ecz-songul@hotmail.com

**Abstract**

Hemostasis is an inherent function and natural processes to prevent or stop bleeding. Nowadays great efforts are being made to develop novel, economic and high-performance products to control bleeding. In this study, we aimed to assess the *in vitro* hemostatic effects by four several plant species used in folk medicine for different purposes including *Salvia verticillata*, *Achillea biebersteinii*, *Tragopogon aureus*, and *Cephalaria procera*. The extracts with different solvent nature were prepared and their hemostatic efficacy were determined using optical aggregometry. The present results clearly revealed that the extracts of *S. verticillata* showed the highest efficacy on platelet aggregation in presence of adenosine-diphosphate (ADP) (80.77%), collagen (80.78%), and arachidonic acid (AA) (73.71%) when compared to other plant extracts. Again, the most effective platelet aggregation (47.27%) was determined after application *C. procera* with in the presence of epinephrine (EPI). Moreover, we firstly executed that *n*-butanol and ethyl acetate extracts led to the highest percentages of platelet aggregation in the presence of APD, collagen, AA and EPI. In a conclusion, our findings suggested that the tested medicinal plants in particular *S. verticillata* and *C. procera* could be novel and natural sources of effective hemostatic agents.

**Keywords:** *Achillea*, *Cephalaria*, hemostasis, *Salvia*, *Tragopogon*.

**Acknowledgement:** This work was supported by Research Fund of the Atatürk University (FBA-2018-6832).

## INTRODUCTION

Bleeding is the blood flowing out of the vein due to injuries and some other reasons. This is vital for all humans and animals and it causes serious risks including death. Hence, extensive efforts using actual techniques and novel drugs/compounds are being made to prevent or stop bleeding (Yılmaz, 2013). In the 1960s, the revolutionary platelet aggregation experiment in platelet-rich plasma (PRP), light transmission aggregometry was the key technique for diagnosing platelet function (Born, 1962). This approach, enable to determine the *in vitro* ability of platelets to agglomerate each other in response to external aggregating agents or agonists, such as adenosine-diphosphate (ADP), arachidonic acid (AA), collagen, and epinephrine (EPI). Nowadays great efforts are being made to develop novel agents that alter blood coagulation. Indications for drugs that alter coagulation is closely associated with platelet inhibitors. Most platelet inhibitors block receptors on platelets to prevent adhesion. Bleeding (including bleeding caused by toothbrushing and excessive bleeding after injury) is the most common adverse effect (Zhou, 2005). There are variegated number of products on the market dealing with this issue. However, the best products are still unexplored and the increasing number of researches are being performed to obtain novel candidates exhibiting more performance and more economical features than available ones. The drug named Ankaferd Blood Stopper (ABS) is the newest product in this field, which is considered to be the greatest invention of Turkish experts after the smallpox vaccine. This drug, which has no side effects, stops bleeding much shorter time than its peers. It comprises of a standardized mix of five plants: *Glycyrrhiza glabra* L., *Thymus vulgaris* L., *Urtica dioica* L., *Alpinia officinarum* Hance, and *Vitis vinifera* L (Yarali et al., 2010; Okten et al., 2011).

In this investigation we aimed to assess the *in vitro* hemostatic potential by the extracts of *S. verticillata*, *A. biebersteinii*, *T. aureus*, and *C. procera* with various nature solvents. Our research was performed within the scope of the project titled “Determination of Traditional Knowledge Based on Biodiversity in Erzurum Province” conducted under the rights of General Directorate of Nature Conservation and National Parks of Ministry of Forestry and Water Affairs of Turkey. Plants were investigated for the first time within the proposed project with the method of maximum aggregation using optical aggregometry of platelet.

## MATERIAL AND METHODS

### Plant Examples

*Salvia verticillata*, *Achillea biebersteinii*, *Tragopogon aureus*, and *Cephalaria procera* were

collected within the scope of the current project titled “Determination of Traditional Knowledge Based on Biodiversity in Erzurum Province”. They were diagnosed by Dr. Ozkan Aksakal. Plant samples were recorded in Atatürk University, Faculty of Pharmacy Herbarium. The localities and voucher specimens of collected plant samples were shown in Table 1.

### **Extraction and Fractionation**

The aerial parts of *S. verticillata*, *A. biebersteinii*, and *C. procera* (50 g) and roots of *T. aureus*, (50 g) were powderized and subjected to mobile maceration with methanol (3x200 ml) for three days at room temperature. The filtrate was evaporated to dryness and dispersed in a mixture of methanol: water (1: 9), followed by n-hexane (3x150 ml), dichloromethane (3x150 ml), ethyl acetate (3x150 ml), and n-butanol (3x150 ml), and each fraction was evaporated to dryness and weighed. The sums of obtained extracts and sub-extracts of *S. verticillata*, *A. biebersteinii*, *T. aureus*, and *C. procera* were presented in Table 2. In addition, taking into consideration the use of plants among the public, while the plants were fresh, the aerial parts were pounded in a mortar and plant juices were obtained. Only the latex of *T. aureus* root was obtained via incision.

### **Chemicals**

ADP, Collagen, AA and EPI for platelet aggregation were purchased from Hart Biologicals (Hartlepool, UK).

### **Blood Collection and Preparation of Prps**

Blood was collected from three healthy and non-smoking female volunteers between the ages of 20 and 25 ( $22.5 \pm 1.3$ ) into a polypropylene tube containing 0.105 M buffered sodium citrate. None of the donors had received any medication known to affect platelet function for at least 10 days before blood collection. PRP was collected by the centrifugation of whole blood at 150 g for 15 min at room temperature (RT). Subsequently, platelet-poor plasma (PPP) was collected by the centrifugation at 2000 g for 20 min at RT. The platelet count was adjusted to  $2 \times 10^8$  cells /ml with PPP.

### **Incubation of PRP with Extracts**

PRP was incubated with extracts (1/5 and 1/10) for 15 min at 37 °C. PRP without any compound was used as a control group.

### **Measurement of Maximum Aggregation by Optical Aggregometry**

The measurement of platelet aggregation in PRP was performed using four-channel aggregometer (APACT 4004; LABiTec, Ahrensburg, Germany). After the incubation of PRP with extracts, platelet aggregation was stimulated by ADP (5 $\mu$ M), Collagen (10 $\mu$ g/ml), AA (5mM) and EPI (10 $\mu$ M) and percentage of aggregation was monitored for 10 min. Assay was performed immediately after blood collection and finished within 2 h. Platelet aggregation experiments were performed in triplicates.

### Statistical Analysis

SPSS 20.0 package program (SPSS Inc., Chicago, USA) was used in the statistical analysis of the findings. Variance analysis was used to determine whether platelet aggregation varies between control and different samples treated groups. Oneway Anova and Fisher's Least Significant Difference (LSD) tests were used for analysis of variance. A p-value less than 0.05 was considered as statistically significant.

### RESULTS

The methanolic extracts and *n*-hexane, CH<sub>2</sub>Cl<sub>2</sub>, EtOAc, BuOH, aqueous sub-extracts of aerial parts of *S. verticillata*, *A. biebersteinii*, *T. aureus*, and *C. procera* were tested for bleeding stopping performance in the presence of ADP, collagen, AA, and EPI. In addition, juices of *S. verticillata*, *A. biebersteinii*, and *C. procera* aerial parts and latex of *T. aureus* root was tested. The results of bleeding stopping performance of the samples in the presence of ADP, collagen, AA, and EPI were presented in Figures 1-4.

Among the *S. verticillata* samples, the most effective sample on platelet aggregation was found to be in the presence of ADP (80.77%). Among the *A. biebersteinii* samples, the most effective sample on platelet aggregation was found to be in the presence of ADP (45.62%). Likewise, among the *T. aureus* samples, the most effective sample on platelet aggregation was found to be in the presence of ADP (37.94%). And, among the *C. procera* samples, the most effective sample on platelet aggregation was observed in the presence of ADP (61.61%). Notably, among all samples, *S. verticillata* was determined as the most effective specimen on platelet aggregation in the presence of ADP (Figure 1).

Among the *S. verticillata* samples, the most effective sample on platelet aggregation in the presence of collagen was determined as 80.78%. Similarly, the most effective sample on platelet aggregation in the presence of collagen for *A. biebersteinii*, *T. aureus* and *C. procera* was determined as 80.27%, 36.74% and 38.55%, respectively. *S. verticillata* was the most effective

specimen among all samples on platelet aggregation in the presence of collagen (Figure 2).

The most effective sample among *S. verticillata* samples on platelet aggregation in the presence of AA was found to be the ethyl acetate sub-extract (73.71%). The most effective sample of *A. biebersteinii* on platelet aggregation in the presence of AA was found to be the hexane sub-extract (22.92%). Likewise, among the *T. aureus* samples, the most effective sample on platelet aggregation in the presence of AA was found to be the aqueous residue sub-extract (36.39%). The most effective *C. procera* sample on platelet aggregation in the presence of AA was determined to be the methanol sub-extract (26.99%). As compared to other specimens *S. verticillata* was found to be the most effective one on platelet aggregation in the presence of AA (Figure 3).

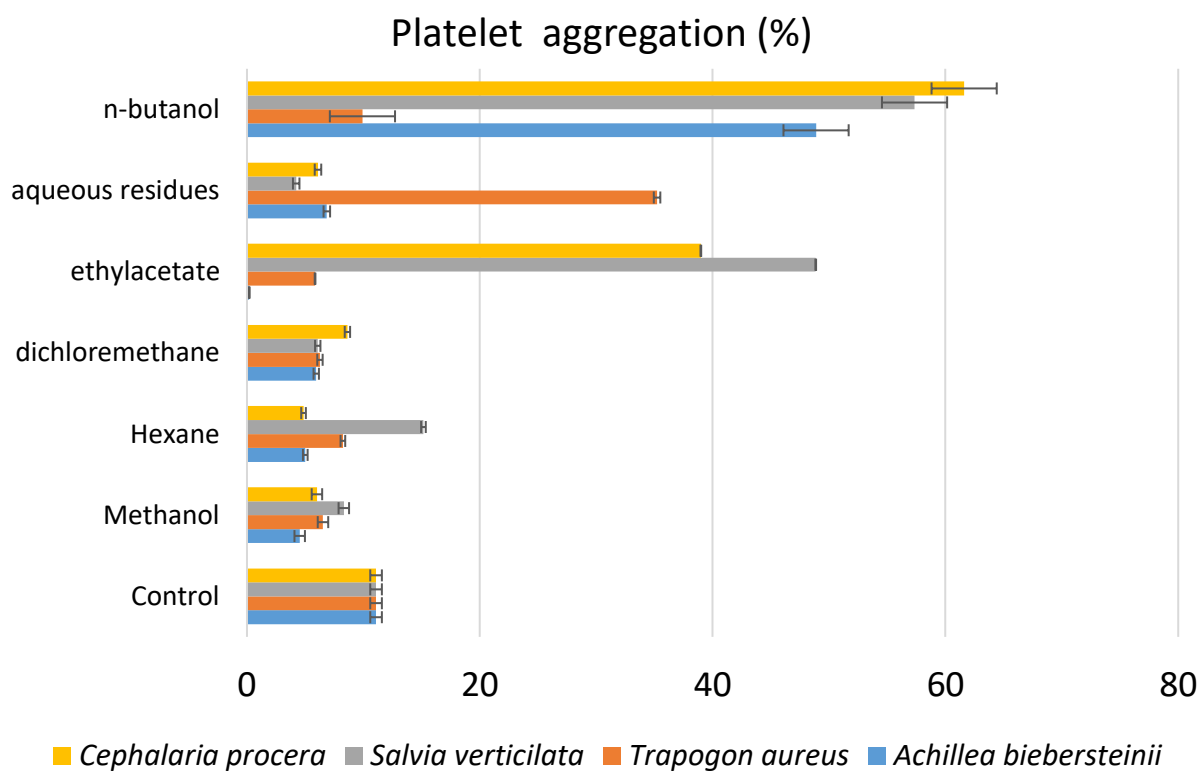
Among all tested samples, the most effective one on platelet aggregation in the presence of EPI was found to be the ethyl acetate sub-extract of *S. verticillata* (30.14%). Among the *A. biebersteinii* samples, the most effective one on platelet aggregation in the presence of EPI was found to be the methanol sub-extract (13.45%). And, the most effective *T. aureus* extract on platelet aggregation in the presence of EPI was found to be the methanol sub-extract (47.02%). Moreover, *C. procera* was determined as the most effective specimen on platelet aggregation in the presence of EPI (47.27%) (Figure 4).

**Table 1.** The localities and voucher specimens of collected plant samples.

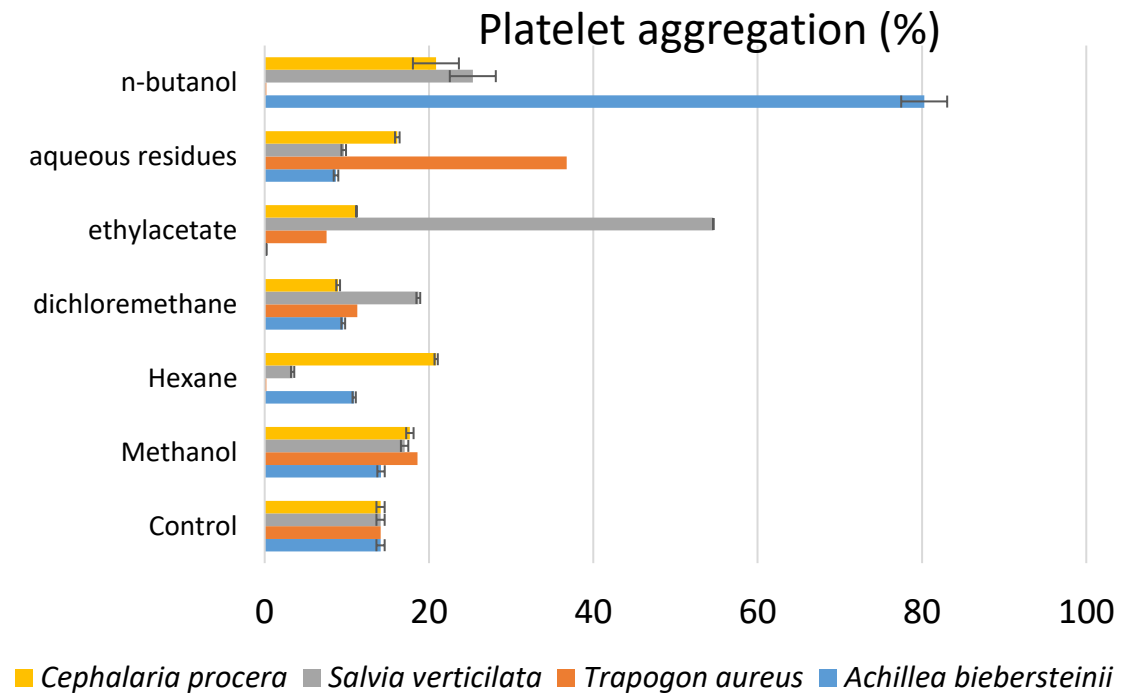
| <b>Species</b>                               | <b>Localities</b>  | <b>Voucher Specimens</b> |
|--|--|--------------------------|
| <i>Salvia verticillata</i> L.                | B8 Erzurum: Narman, Göllü village,<br>03.06.2018, 1900 m   | AUEF 1264                |
| <i>Achillea biebersteinii</i> Hub.-Mor.      | B8 Erzurum: Ataturk University<br>campus, the garden of Faculty of<br>Pharmacy, 05.06.2018, 1890 m | AUEF 1359                |
| <i>Tragopogon aureus</i> Boiss.              | B8 Erzurum: Erzurum Kent forest,<br>07.06.2018, 1910 m   | AUEF 1360                |
| <i>Cephalaria procera</i> Fisch. & Avé-Lall. | B8 Erzurum: Erzurum Kent forest,<br>07.06.2018, 1910 m   | AUEF 1361                |

**Table 2.** The sums of obtained extracts and sub-extracts of *salvia verticillata*, *achillea biebersteinii*, *tragopogon aureus*, and *cephalaria procera*.

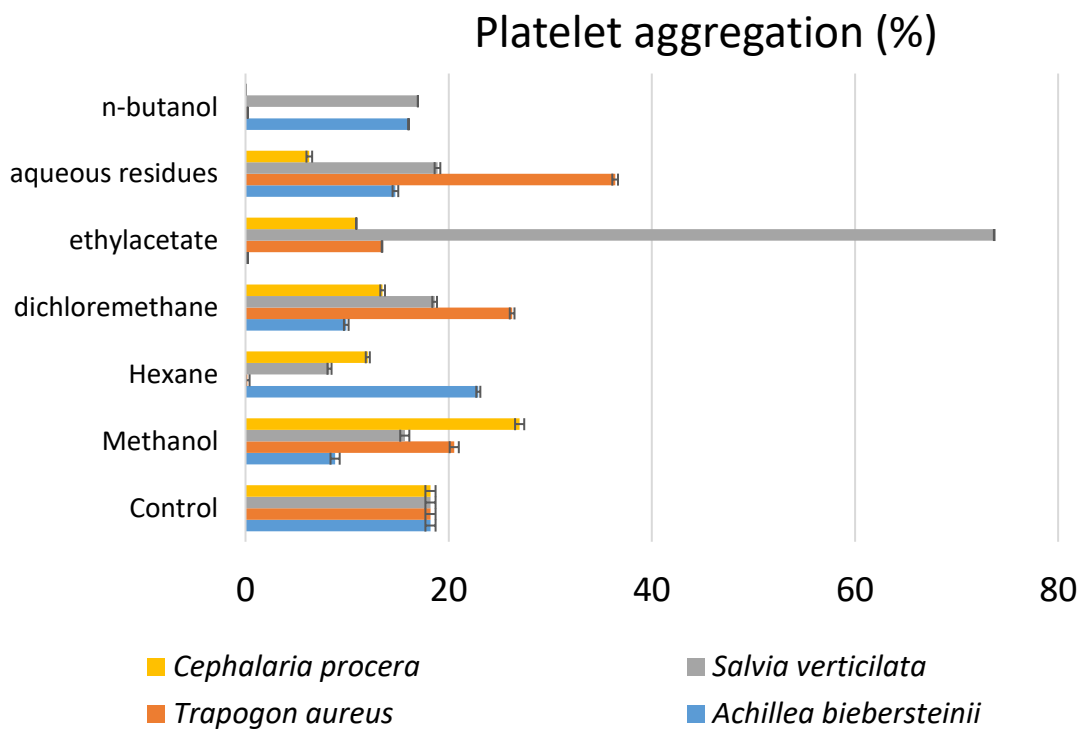
| Extracts and Sub-Extracts           | <i>Salvia verticillata</i> | <i>Achillea biebersteinii</i> | <i>Tragopogon aureus</i> | <i>Cephalaria procera</i> |
|-------------------------------------|----------------------------|-------------------------------|--------------------------|---------------------------|
| MeOH (g)                            | 10.09                      | 10.25                         | 11.24                    | 9.87                      |
| Hexane (g)                          | 1.20                       | 1.22                          | 1.51                     | 1.26                      |
| CH <sub>2</sub> Cl <sub>2</sub> (g) | 2.89                       | 2.92                          | 3.02                     | 2.04                      |
| EtOAc (g)                           | 0.76                       | 0.69                          | 1.03                     | 0.93                      |
| BuOH (g)                            | 2.77                       | 2.91                          | 3.01                     | 2.28                      |
| Aqueous residue                     | 2.23                       | 2.04                          | 2.41                     | 2.22                      |



**Figure 1.** The effect of obtained plant extracts with different solvents on platelet aggregation in the presence of ADP. Values are shown as mean $\pm$ SD.

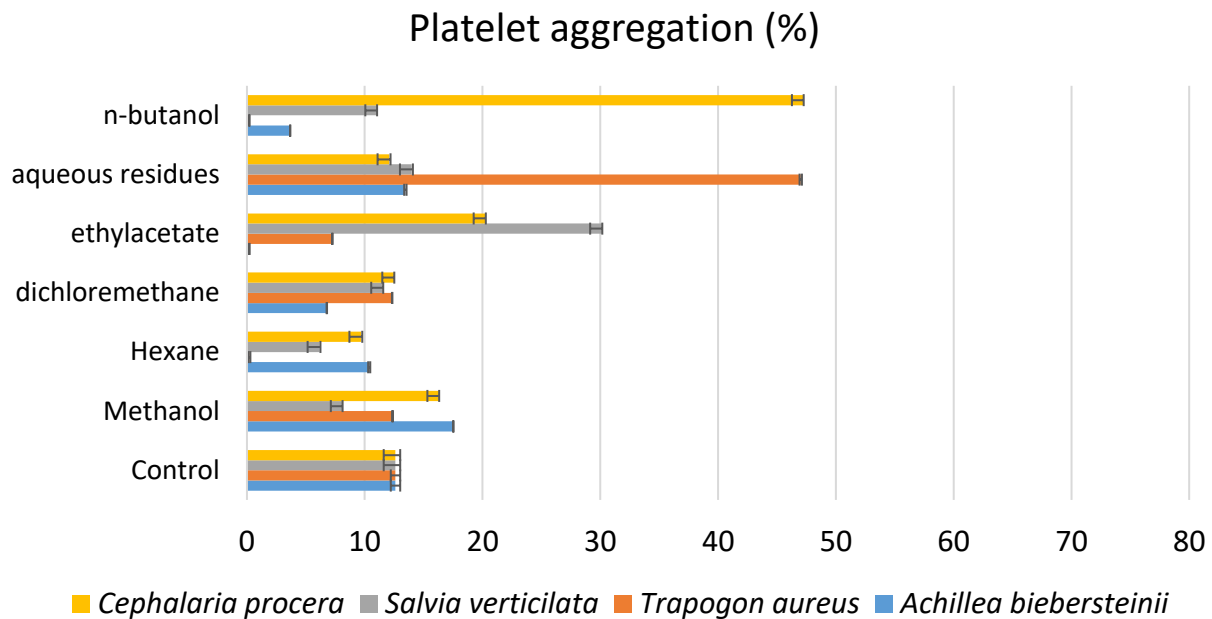


**Figure 2.** The effect of obtained plant extracts with different solvents on platelet aggregation in the presence of collagen. Values are shown as mean $\pm$ SD.



**Figure 3.** The effect of obtained plant extracts with different solvents on platelet aggregation in the presence of arachidonic acid. Values are shown as mean $\pm$ SD.





**Figure 4.** The effect of obtained plant extracts with different solvents on platelet aggregation in the presence of ephinephrine. Values are shown as mean±SD.

## DISCUSSION

Bleeding could be the outcome of injury, blood diseases, or medication effect. And stopping haemorrhages are frequently requested medical actions. There are available effective several hemostatic medications like desmopressin, aprotinin, and antifibrinolytic amino acids (as tranexamic acid and aminocaproic acid). They are utilized in congenital bleeding, diseases, internal bleedings, or cardiac surgery, however, these drugs are not intended for local usage. Topical hemostatic agents like fibrin tissue adhesives, prothrombin, collagen, and thrombin are pricy and not easily accessible. Thus, efficient topical agents are urgently needed not only for critical bleeding cases but also for widespread wounds or mucosa bleeding. Several natural compounds have been traditionally used efficiently to stop bleeding (Páez and Hernández, 2003). Bleeding as an outcome of surgical processes is an unusual reason for death in plastic surgery practices other than those including burns. The conventional medicinal plant extracts is an alternate therapy modality utilized for the administration of dermal, external traumatic and post-surgical, and dental bleeding (Kose et al., 2012). The phytochemical finding demonstrated that all the studied herbs included polyphenolic substituents. Actually, it was reported that flavonoids and tannins were existing in *Arbutus unedo*, *Equisetum arvense*, *Petroselinum crispum*, *Cistus ladaniferus*, and *Urtica dioica*. At this point the observed hemostatic properties by tested plant

species could be attributed to their biologically active ingredients. And previous investigations have indicated that flavonoids considerably inhibited platelet aggregation, secretion, and adhesion. In fact, it was demonstrated that a polyphenolic compound (3,5,4'-trihydroxy-trans-stilbene or *t*-resveratrol) included in the red wine, inhibited platelet aggregation in the hypercholesterolemic rabbits (Mekhfi et al., 2004).

## CONCLUSION

Further investigations are necessary for elucidating the underlying mechanisms of hemostasis by these species as well as revealing the associated active compound or compounds that simplify coagulation or inhibit fibrinolysis. As a matter of fact, the present findings firstly suggested that the tested four plant species in particular *S. verticillata* might be cheap, natural, safe, and easy available sources of topical hemostatic agent for both skin or mucosal damages.

## FUNDING

This work was supported by Research Fund of the Atatürk University (FBA-2018-6832).

## REFERENCES

- Born, G.V., 1962. Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature*, 194:927-929.
- Kose, R., Sogut, O., Erdemir, T., Koruk, I., 2012. Hemostatic efficacy of folkloric medicinal plant extract in a rat skin bleeding model. *Dermatol Surg.*, 38:760-766.
- Mekhfi, H., El Haouari, M., Legssyer, A., Bnouham, M., Aziz, M., Atmani, F., Remmal, A., Ziyat, A., 2004. Platelet anti-aggregant property of some Moroccan medicinal plants. *Journal of Ethnopharmacology*, 94:317-322.
- Okten, S., Kurt, M., Onal, I.K., Haznedaroglu, I.C., 2011. Use of Ankaferd Blood Stopper for controlling actively bleeding fundal varices. *Singapore Med J.*, 52(1): e10.
- Páez, X., Hernández, L., 2003. Topical Hemostatic Effect of a Common Ornamental Plant, the Geraniaceae *Pelargonium zonale*. *J Clin Pharmacol.*, 43:291-295.
- Yarali, N., Oruc, M., Bay, A., Dalgic, B., Bozkaya, I.O., Arıkoğlu, T., Kara, A., Tunc, B., 2010. A New hemostatic agent—Ankaferd Blood Stopper: Management of gastrointestinal bleeding in an infant and other experiences in children. *Pediatric Hematology and Oncology*, 27:592-596.
- Yılmaz, Y.K., 2013. Vascular injuries: Introduction, history, the diagnosis and treatment methods. *Turkiye Klinikleri J Cardiovasc Surg-Special Topics*, 5(2):18-25.
- Zhou, L., Schmaier, A.H., 2005. Platelet aggregation testing in platelet-rich plasma: description of procedures with the aim to develop standards in the field. *Am J Clin Pathol.*, 123:172-183.