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Dear colleagues;

As Elegans Biotechnology Consultancy and Organization Joint Stock Company, we are excited about the first international conference we will organize. 1st International Conference on Life Science will be an online event open to researchers working on life science from all over the world. Abstracts of all papers will be carefully examined by our scientific committees. Our main goal is to serve as a bridge for quality original studies to reach other scientists around the world.

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Kind regards

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23.11.2024	1. DAY	
11:00-11:15	OPENING CEROMONY	
11:15-11:45	**Keynote Speaker: Prof. Dr. İsmail ÖÇSOY**	**"APPLICATIONS OF NANOTECHNOLOGY IN BIOANALYTICAL AND MEDICINE"**
11:45-12:15	**Keynote Speaker: Dr. Bülent GÖZCELİOĞLU**	**"MARINE BIODIVERSITY OF TURKISH SEAS"**
12:15-13:00	BREAK	

13:00-14:00	Room 1	
	Moderator	**Prof. Dr. Mehmet Gökhan HALICI**
13:00-13:15	Yavuz Aslan & Ayşe Sena Yumbul Kardas	THE IMPACT OF THE COVID-19 PANDEMIC ON DIVING ROUTINES AND DIVING HYGIENE
13:15-13:30	H. Abdullah Uçan, Bülent Gözcelioğlu & Alper Evcen	PRELIMINARY INVESTIGATION OF THE BIODIVERSITY OF THE KAYNAR SEA CAVE
13:30-13:45	Djihane Bouzid, Kaouter Haridi & Mohamed Mihoub Zerroug	*Spirulina platensis* PRODUCTION USING BIOREACTORS AND A NEW CULTIVATION PROCESS TO ENHANCE YIELD
13:45-14:00	Ayşe Sena Yumbul Kardas & Yavuz Aslan	PROBLEMS THAT MAY BE EXPERIENCED IN KELP DIVING AND INDEPENDENT SCUBA DIVING METHOD

13:00-14:00	Room 2	
	Moderator	**Prof. Dr. Osman SEYYAR**
13:00-13:15	Mariam Wasif Dar & Nazish Mazhar Ali	RIBOTYING AND ANTIBIOTIC RESISTANCE OF PATHOGENIC STRAINS OF *Streptococcus pneumonia* ISOLATED FROM DIFFERENT OPHTHALMIC INFECTIONS
13:15-13:30	Cihan Dusgun & Elif Yurumez Canpola	KOMBUCHA MICROBIOTA: DYNAMICS OF MICROBIAL SPECIES AND HEALTH BENEFITS
13:30-13:45	Mahnoor Naveed, Nazish Mazhar Ali, Memuna Ghafoor Shahid & Bushra Mazhar	SCREENING AND CHARACTERIZATION OF PATHOGENIC BACTERIA FROM SOIL AND THEIR RESISTANCE AGAINST DIFFERENT ANTIBACTERIAL AGENTS
13:45-14:00	Cihan Dusgun & Elif Yurumez Canpola	SUBSTRATE SELECTION AND ITS EFFECT ON KOMBUCHA FERMENTATION

14:30-15:30	Room 1	
	Moderator	**Prof. Dr. Mehmet Gökhan HALICI**
14:30-14:45	Ivan Frolov, Ilya Prokopiev & Polina Tumanina	PRELIMINARY ANALYSIS OF CHANGES IN THE SPECIES COMPOSITION OF LICHENS OF THE FAMILY TELOSCHISTACEAE ALONG THE GRADIENT OF CONTINENTALITY IN THE RUSSIAN FAR EAST
14:45-15:00	Aleksandr Yatsyna	COLLECTION OF LICHENS (MSK-L) OF THE INSTITUTE EXPERIMENTAL BOTANY OF THE NATIONAL ACADEMY OF SCIENCE (MINSK, BELARUS)
15:00-15:15	Areesha Sohail & Memuna Ghafoor Shahid	EXTRACTION AND DETERMINATION OF SECONDARY METABOLITES OF LICHENS COLLECTED FROM JHIKA GALI, MURREE HILLS, KP, PAKISTAN
15:15-15:30	Veronica Karmanova	MYCOBIOTA OF THE BELARUSIAN STATION "MOUNT VECHERNYAYA" (EAST ANTARCTICA)

14:30-15:30	Room 2	
	Moderator	**Dr. Cihan DÜŞGÜN**
14:30-14:45	Ceyda Berceste Karabulut, Teoman Kankılıç & İlkay Civelek	METAGENOMIC CHARACTERIZATION OF THE BACTERIAL MICROBIOTA IN DOMESTIC CAT (*Felis catus*) SALİVA
14:45-15:00	Ayser I. AbdulAziz, Hind A. Hanoon & Suhair Dakhil Neamah	DETECTION OF *Toxoplasma gondii* BY RAPID TEST ,ELISA,PCR ANALYSIS IN IRAQI WOMEN WITH RECURRENT ABORTION
15:00-15:15	Yunus Emre Atay	NANOTECHNOLOGY BASED STRATEGIES FOR NON-SURGICAL TREATMENT OF CANINE MAMMARY TUMORS
15:15-15:30	Osman Seyyar & Merve Karakaya	NOTES ON *Argyroneta aquatica* (WATER SPIDER) (ARANEAE, DICTYNIDAE) IN ANATOLIA

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	Moderator	**Assoc. Prof. Dr. Fatih Doğan KOCA**
16:00-16:15	Fatima Arif, Memuna Ghafoor Shahid, Amina Tanveer, Mehak Iftikhar & Sumayyah Zia	PRODUCTION AND PURIFICATION OF ERGOT ALKALOIDS FROM *Pleurotus ostreatus*
16:15-16:30	Iqra Gillani, Saira Atta & Memuna Ghafoor Shahid	PRODUCTION OF ACETYL-XYLAN ESTERASE FROM *Penicillium digitatum*
16:30-16:45	Djellel Nadjiha & Larous Larbi	MYCOFLORA OF POULTRY FEEDS AND MYCOTOXINS PRODUCING POTENTIEL OF *Aspergillus flavus*
16:45-17:00	Zohaib Anjum, Memuna G. Shahid, Ikram-ul-Haq, Mehak Iftikhar, Nazish Mazhar, Areesha Sohail & Iqra Gillani	EXTRACTION AND PRODUCTION OF ERGOT ALKALOIDS PRODUCED BY FUNGAL CONSORTIUM

16:00-17:00	Room 2	
	Moderator	**Assoc. Prof. Dr. Burcu Yılmaz ÇITAK**
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16:15-16:30	Bouaouina Sarah & Aouf Abdelhakim	ANTIBACTERIAL ACTION OF OREGANO ESSENTIAL OIL FREE AND ENCAPSULATED AGAINST UROPATHOGENIC BACTERIA
16:30-16:45	Recep KARA & Hatice TAŞPINAR	BRYOPHYTE VEGETATION OF CAPPADOCIA REGION (NEVSEHIR)
16:45-17:00	Ayşe Nur Deniz Aydın & Burcu Yılmaz Çıtak	ALLERGIC TREES SPREADING IN SELÇUK UNIVERSITY ALAEDDIN KEYKUBAT CAMPUS

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	Moderator	**Dr. Nazish Mazhar ALİ**
17:30-17:45	Muhammad Fatiq, Memuna Ghafoor Shahid1, Gul Zareen Ghafoor, Nazish Mazhar Ali & Ikram-ul-Haq	EXTRACTION AND DETERMINATION OF SECONDARY METABOLITES OF LICHENS COLLECTED FROM AYUBIA NATİONALPARK, MURREE HILLS, KP, PAKISTAN
17:45-18:00	Memuna Ghafoor Shahid, Sameera Zikria, Gul Zareen Ghafoor, Nazish Mazhar Ali & Ikram-ul-Haq	DETERMİNATION AND EVALUATION OF MICROCRYSTALLİNE SUBSTANCES IN SOME LİCHENS OF MURREE HILLS, PAKISTAN
18:00-18:15	Zahara Nassoro MKWAYU, Mehmet Gökhan HALICI & Merve YİĞİT	ANATOMICAL AND MORPHOLOGICAL INVESTIGATION OF SOME LICHENIZED FUNGI FROM USAMBARA MOUNTAINS -TANGA, TANZANIA
18:15-18:30	Zeycan HELVACI	COMPARATIVE ANALYSIS OF NICHE BREADTH AND ENVIRONMENTAL ADAPTABILITY IN *Apodemus mystacinus* AND *Apodemus epimelas*

17:30-18:30	Room 2	
	Moderator	**Dr. Memuna Ghafoor SHAHİD**
17:30-17:45	Elif Erdemirci Kılınç, Şafak Bulut, Zeynel Arslan Gündoğdu, Süreyya İsfendiyaroğlu	DIETARY ANALYSİS OF BARN OWL (*Tyto alba*) POPULATION IN SOUTHEASTERN ANATOLIA REGION (CEYLANPINAR - SANLIURFA)
17:45-18:00	Sumayyah Zia &Memuna Ghafoor Shahid	PRODUCTION AND EXTRACTION OF COMMERCIALLY IMPORTANT GLUCOAMYLASE FROM WILD TYPE AND MUTANT STRAINS OF *Trichoderma viride*
18:00-18:15	Saira Naeem & Memuna Ghafoor Shahid	PURIFICATION AND CHARACTERİZATION OF COMMERCIALLY IMPORTANT KERATINASE BY WILD TYPE AND MUTANT STRAINS OF *Mucor mucedo*
18:15-18:30	Mehak Iftikhar, Dr. Memuna G. Shahid, Zohaib Anjum & Sumayyah Zia	PURIFICATION AND CHARACTERIZATION OF A-AMYLASE PRODUCED FROM *Penicillium citrinum*

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	Moderator	**Prof. Dr. Mehmet Gökhan HALICI**
11:00-11:15	Djama Goumaneh AWALEH, Abdillahi Houssein ABDALLAH, Ibrahim Souleiman ABDALLAH, Tahir ATICI & Bülent GOZCELIOGLU	FIRST SIGN CORAL BLEACHING IN SOUTHERN DJIBOUTI RED SEA
11:15-11:30	Ahmet Yesari SELÇUK & Batuhan Yaman YAKIN	A COLORATION ANOMALY IN *Microtus obscurus* FROM TURKIYE
11:30-11:45	Ecem ÇANTALI, Elif BAKIŞ, Evrim Öykü ONAY, Ulvi Kerem GÜNAY, Batuhan Yaman YAKIN, Kerim ÇİÇEK & Cemal Varol TOK	AGE ESTIMATION OF THE TRABZON POPULATION of *Mertensiella djanaschvilii* TARTARASHVILI AND BAKRADZE 1989
11:45-12:00	Ulvi Kerem GÜNAY, Batuhan Yaman YAKIN, Cemal Varol TOK	IS SKELETOCHRONOLOGY A PRACTICABLE METHOD FOR LONGEVITY OF THE TAWNY OWL, *Strix aluco* (Linnaeus, 1758) (STRIGIFORMES: STRIGIDAE)
11:00-12:00	Room 2	
	Moderator	**Assoc. Prof. Dr. Fatih Doğan KOCA**
11:00-11:15	Maham Chaudhry, Nazish Mazhar Ali, Memuna Ghafoor Shahid & Bushra Mazhar	EVALUATION OF BACTERICIDAL POTENTIAL OF DIFFERENT ANTIBIOTICS AND GREEN SYNTHESIZED NANOPARTICLES AGAINST *Pseudomonas aeruginosa* ASSOCIATED WITH PULMONARY INFECTIONS IN INFANTS
11:15-11:30	Merve KARAKAYA & Osman SEYYAR	THE PRODUCTION OF RECOMBINANT SPIDER SILK THROUGH INSECT SYSTEMS
11:30-11:45	Sidra Munir, Nazish Mazhar Ali &Memuna Ghafoor Shahid	DETERMINATION OF ANTIBACTERIAL ACTIVITY OF DIFFERENT PLANT EXTRACTS AND GREEN SYNTHESIZED NANOPARTICLES AGAINST BETA HEMOLYTIC STRAINS OF *Staphylococcus aureus* FROM EYE INFECTIONS
11:45-12:00	Dilara BİŞGİN, Mehmet Gökhan HALICI & Merve YİĞİT	DNA BARCODING OF SOME LICHENIZED FUNGI FROM DISMAL ISLAND

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	Moderator	**Dr. Yunus Emre ATAY**
12:30-12:45	Tülin Günver, Tahir Atıcı	HIGH SCHOOL STUDENTS' VIEWS ON VIRTUAL AND REAL FIELD TRIPS AFTER THE FOREST FIRE CREATED WITH 3600 VIDEOS ON ENVIRONMENT AND ECOLOGY
12:45-13:00	Hind Amira, Fatima Benchikh , Walid Mamache, Hassiba Benabdallah, Islam Amira, Asma Ouare, Mohamed Abdallah Torki, Aya Amira & Amira Smain	PHYTOCHEMICAL PROFILING AND ANTIOXIDANT POTENTIAL OF ESSENTIAL OILS FROM *Achillea odorata* L.
13:00-13:15	Sellanı Halima & Megherbı Aicha	VALORIZATION OF *Pistacia lentiscus* (*Pistacia lentiscus* L.) SEED OIL: "STUDY OF PHYSICOCHEMICAL AND BIOLOGICAL CHARACTERISTICS (ANTIBACTERIAL AND ANTIFUNGAL) FROM THREE REGIONS (EL-TARF, SKİKDA, GUELMA)
13:15-13:30	Mohamed Abdallah Torkı, Lakhdar Gasmı, Walid Mamache, Hind Amıra, Fatima Benchıkh, Hassiba Benabdallah & Islem	ANTIDIABETIC AND ANTIOXIDANT PROPERTIES OF *Agrimonia eupatoria*: A NATURAL THERAPEUTIC AGEN

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	Moderator	**Dr. Merve YİĞİT**
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12:45-13:00	Esham Tahir & Memuna Ghafoor Shahid	PRODUCTION OF ETHANOL FROM NATURAL BIO-RESOURCES USING *Mucor mucedo*
13:00-13:15	Hind Amira, Fatima Benchikh, Walid Mamache, Hassiba Benabdallah, Islem, Amira, Mohamed Abdallah Torki, Asma Ouaret, Aya Amira & Smain Amira	PHYTOCHEMICAL PROFILE AND THERAPEUTIC POTENTIAL OF *Solidago virgaurea* L.: ANTIOXIDANT AND ANALGESIC EFFECTS
13:15-13:30	Mamen Nassima, Mayouf Rabah & Benabdallaha Amina	ETUDE ETHNOBOTANIQUE DU ROMARIN OF *Rosmarinus officinalis* FROM THE REGION OF KHENCHELA (ALEGRIA)

14:00-15:00	Room 1	
	Moderator	**Assoc. Prof. Dr. İzzet Burçin SATICIOĞLU**
14:00-14:15	Shanza Asim, Memuna Ghafoor Shahid, Esham Tahir, Areesha Sohail, Saira Naeem, Nazish Mazhar Ali, Iqram-ul-Haq, & Gul-Zareen Ghafoor	PRODUCTION OF ETHANOL FROM NATURAL BIO-RESOURCES USİNG *Saccharomyces cerevisiae*
14:15-14:30	Ali Erdem Öztürk, Mustafa Bodu, Yunus Emre Atay, Serpil Sarıözkan, Zeliha Kılınç & İsmail Öçsoy	EFFECTS OF BSA NANOPARTICLES ON THE FREEZABILITY OF ANKARA GOAT SEMEN
14:30-14:45	Aysel Kekillioğlu & Ömer Eren Bostan	BIOECOLOGICAL AND FAUNISTIC INVESTIGATİON OF INSECT TAXA IN BEAN AGRICULTURAL AREAS AND SURROUNDINGS OF DERINKUYU DISTRICT
14:45-15:00	Tülay Ezer, Ahmet Uygur, Ali Keskin, Züleyha Aslan Ergenekon, Mevlüt Alataş & Nevzat Batan	NEW LOCALITY DATA OF *Cyrtomnium hymenophylloides* (BRYOPHYTA: MNIACEAE) FROM BOLKAR MOUNTAINS IN TURKIYE

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	Moderator	**Assoc. Prof. Dr. Fatih Doğan KOCA**
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14:30-14:45	Negar Kashefı & Fatih Doğan Koca	SYNTHESIS AND CHARACTERIZATION OF ORGANIC@INORGANIC Cu HYBRID NANOFLOWER WİTH *Salix alba* EXTRACT
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15:00-15:15	Rachid Mouedden, Abbassia Ayache	NEW DATA ABOUT OVERLOOKED URBAN LİCHEN-FORMİNG OF SİDİ BEL ABBES WESTERN ALGERİA

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ABSTRACTS

Ribotying and Antibiotic Resistance of Pathogenic Strains of *Streptococcus pneumonia* Isolated From Different Ophthalmic Infections

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Abstract

Streptococcus pneumoniae (S. pneumoniae) is an important pathogen responsible for a range of infections, including ocular infections such as conjunctivitis, keratitis, blepharitis, and trachoma. The present study aims to explore and review the biochemical tests commonly used for the identification, differentiation of *S. pneumoniae*, antibiotic susceptibility testing and the molecular characterization of pathogenic strains of *S. pneumoniae* that cause eye infections. Eye infection samples were obtained from Rashid Latif Medical Complex (RLMC) in Lahore. Following collection, these samples were spread onto chocolate agar to obtain a pure culture of *S. pneumoniae*. Once pure bacterial strains were isolated, their pathogenicity was assessed through a blood agar test. The strains demonstrating alpha hemolysis were selected for further testing, and their susceptibility to different antibiotics and green synthesized silver Nano-particles of various plant extract was analyzed. The pathogenic strains that showed the sensitivity against antibiotics, plant extracts and green-synthesized silver nanoparticles were sent for molecular characterization. The findings indicated that all antibiotics tested were significantly effective against the pathogenic bacteria isolated. Five strains exhibited alpha hemolysis, indicating the presence of pathogenic strains of *S. pneumoniae*, which are known to be linked to conjunctivitis and keratitis. Most isolates were optochin-sensitive and bile-soluble. Different antibiotics and green synthesized silver Nano-particles of various plant extract resulted in observable zones of inhibition (ZOI) but others showed resistance. The accession numbers of the pathogenic strain of *S. pneumoniae* were received that showed sensitivity against antibiotics Biochemical tests and molecular characterization remain a cornerstone in the identification and treatment of *S. pneumoniae* in ophthalmic infections. Combining these tests with antibiotic susceptibility testing ensures accurate diagnosis and optimal treatment, reducing the risk of complications associated with untreated or poorly treated infections.

Keywords: Ophthalmic infections, *S. pneumonia*, Molecular characterization, Antibiotics susceptibility

Extraction and Determination of Secondary Metabolites of Lichens Collected From Jhika Gali, Murree Hills, Kp, Pakistan

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Abstract

Lichens are symbiotic entities that are made up of algae, fungi, or cyanobacteria and are well known for their capability to synthesize distinctive secondary metabolites that have crucial pharmacological and ecological importance. The main purpose of this study was to identify, and analyze two lichen species, in order to investigate their ability as a source of usnic acid and other bioactive compounds. The lichens were collected from Jhika Gali which is situated in Murree hills of Khyber Pakhtunkhwa KPK, Pakistan, at an elevation of around 2300 meters (7545 feet) above sea level. The species of lichens that were collected are *Dermatocarpon miniatum* from *Cedrus deodara* tree, *Parmelia aurulenta* from *Pinus* tree, *Xanthoria sorediata* from *Quercus* tree and *Physcia orbicularis* from *Acer saccharum* tree. The methodology includes culturing fungal member on Potato Dextrose Agar (PDA) along with Malt Extract Agar Media (MEA), and the inoculation as well as incubation of the samples of lichen under aseptic environment. Mycobiont was successfully cultured on prepared media. The growth was observed and properly grown cultures were used to prepare a suspension medium of fungal partner (mycobiont) for further experiments. Mycobiont in the lichen was identified using various identification keys. After that lichen samples were used to extract the secondary metabolites with the help of ethanol and n-hexane, which led to the identification of usnic acid which was observed as a yellowish-colored crystal from the lichens of *Xanthoria* and *Parmelia*. Thin Layer Chromatography (TLC) was utilized to observe the presence as well as purity of usnic acid was measured with the performance of two different mobile phase systems. The results of chromatographic analysis showed clear separations which confirmed the presence of usnic acid in both lichen species, i.e., *Parmelia* and *Xanthoria*, with minor differences in their metabolites.

Keywords: Secondary Metabolites, *S. pneumonia*, Lichens, Antibiotics susceptibility

Allergic Trees Spreading in Selçuk University Alaeddin Keykubat Campus

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Abstract

Pollen grains are one of the most common airborne living particles deposited in the atmosphere. Pollen grains are generally small and can spread over very long distances, so it can easily settle in the human respiratory system and cause allergic reactions in sensitive individuals. The amount or density of pollen is effective in the onset of allergic reactions. Knowing the concentration of pollen grains is important in preventing allergic diseases. Wind-pollinated plants produce large numbers of pollen grains to ensure pollination. Excessive amounts of pollen stored in the atmosphere cause allergic reactions in sensitive people. In this study, to detect the trees with allergenic effects spread in Selçuk University Alaeddin Keykubat Campus. Samples of trees for example stems with flowers were collected from the area where the plants distribute during their flowering periods and their herbarium examples were made using standard herbarium methods. Descriptions of trees made according to Flora of Turkey and Akkemik (2018). Twenty-five allergenic tree were identified in campus area. *Betula alba, Juniperus excelsa, Pinus nigra, Viburnum lantana, Catalpa bignonioides, and Ailanthus altissima* etc. are some of collected allergic trees. As a result, allergenic trees spread in the campus area were identified and listed, and were given their botanical characteristics.

Keywords: Alder tree, Allergenicity, Betula, Birch tree, Konya, Wolfeat tree.

DNA Barcoding of Some Lichenized Fungi From Dismal Island

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Abstract

Lichenized fungi are the most dominant macroorganisms in Antarctic terrestrial ecosystems. Studies on lichens in Antarctica have a history of approximately two centuries. Especially with the recent use of DNA-based techniques in taxonomy studies, lichen biodiversity studies in Antarctica has accelerated. Dismal Island is the largest of the Faure Islands, 1.9 kilometers long and 60 meters high, mostly covered with ice in Marguerite Bay on the west coast of Graham Land. In the literature, there is no study directly addressing or examining lichen biodiversity on Dismal Island. In this context, the aim of this study is to examine anatomically-morphologically and to perform DNA barcoding of some lichenized fungi species from Dismal Island. In this context, lichen samples collected from Dismal Island during the 6th Turkish National Antarctic Scientific Expedition by the second author. The collected samples were identified by anatomical and morphological examinations and DNA barcoding was performed with ITS gene region primers. As a result of the study, DNA barcoding of 12 species was performed: *Austroplaca hookerii* (C.W. Dodge) Søchting, Frödén &Arup, *Buellia russa* (Hue) Darb., *Candelariella flava* (C.W. Dodge & Baker) Castello & Nimis, *Lecanora fuscobrunnea* C.W.Dodge & G.E.Baker, *Mastodia tessellata* (Hook & Harv.) Hook & Harv., *Polycauliona candelaria* (L.) Frödén, Arup & Søchting, *Rhizoplaca aspidophora* (Vain.) Follmann, *Rhizocarpon geographicum* (L.) DC., *Tephromela atra* (Huds.) Hafellner ex Kalb., *Tetramelas anisomerus* (Vain.) Elix., *Umbilicaria antarctica* Frey & I.M. Lamb., *Usnea antarctica* Du Rietz.

Keywords: Lichenized fungi, biodiversity, Antarctica, Dismal Island, DNA barcoding.

Biochemical and Molecular Characterization of Bacterial Pathogens from *Bombyx mori*

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Abstract

Bombyx mori, commonly known as the domestic silkworm, is a fascinating insect of great economic significance. It belongs to the family Bombycidae within the order Lepidoptera. This insect species has been domesticated for thousands of years, primarily for its ability to produce silk, a valuable natural fiber used in textile production. Despite its critical role in silk production, the silkworm, *Bombyx mori*, is susceptible to various bacterial diseases that can have detrimental effects on sericulture. Common bacterial diseases in silkworms include *bacterial flacherie, grasserie, bacterial wilt, enterococcal infections,* and *staphylococcal infections,* which can result in substantial economic losses in the sericulture industry. Several bacterial pathogens pose significant threats to silkworm population. *Bacillus thuringiensis, Bacillus cereus, Clostridium perfringens, Staphylococcus aureus, Bacillus bombyseptieus, Enterococcus faecalis, Pseudomonas aeruginosa, Serratia marcescens* and *Staphylococcus aureus* are just few examples of bacterial strains that can infect silkworm. Previously use of antibiotics was found useful against these antibiotics hence effectiveness is decreased with time. In current study, pathogenic bacteria were isolated from diseased silkworm larvae. Isolates were characterized through traditional microbiological techniques, including microscopy, colony morphology, and biochemical assays. Pathogenicity was checked by blood agar test. Molecular techniques, such as DNA extraction through phenol-chloroform method was used and 16S rRNA gene sequencing was employed for precise taxonomic classification. The resistance developed in these pathogenic strains against various antibiotics specifically against Ciprofloxacin, Azithromycin, Metronidazole and Levofloxacin was checked by following disc diffusion method. Overall, this research contributes to a better understanding of the biochemistry and molecular biology of pathogenic bacteria in silkworms. The findings are essential not only for the management of bacterial diseases in sericulture but also for advancing our knowledge of insect-bacteria interactions. This thesis work offers valuable insights into the molecular basis of silkworm-pathogen interactions, which can potentially inform strategies to enhance the health and productivity of silkworm populations in the sericulture industry.

Keywords: Silkworm, Molecular characterizations, 16srRNA gene, Bacterial infections

Determination of the Effects of Sodium Copper Chlorophyllin on *Artemia salina* nauplii

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Abstract

Sodium Copper Chlorophyllin has many uses in food, such as health, cosmetics and additives. It is usefully stated that no harmful effects were observed in the studies reviewed. Determining the effects of Sodium Copper Chlorophyllin (SCC) will contribute to fisheries as it is of great importance both in determining the effects on the aquatic ecosystem and in terms of aquatic products to be used as live bait. In this study, the toxic effects, positive and/or negative effects of SCC on *Artemia salina* nauplii were examined. The aim was to investigate the toxicity of SCC on *A. salina* nauplii, by evaluating mortality. *A. salina* nauplii were exposed to SCC for 24, 48, 72 and 96 h in seawater, from 10^{-6} to 10^{-3} Molar. The test organisms were not fed during the toxicity tests. Bioassays were run in six replicates. High concentrations of SCC had a negative effect on egg hatching. Swimming speed significantly decreased in *A. salina* nauplii exposed to SCC. It was found that SCC had a hormetic effect on *A. salina* nauplii at a concentration of $1x10^{-4}$ M and a negative effect at high concentrations. Due to the increase in concentration, deaths were observed in larvae that passed from the nauplius larva stage to a more advanced stage. In conclusion, swimming alteration, deaths and SCC toxicity represent valid endpoints for exposure. More studies are needed to examine that SCC can contribute to live feed cultivation and hormetic and toxic effects in aquatic ecosystems.

Keywords: Sodium Copper Chlorophyll, aquatic ecosystem, *Artemia*, nauplii

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Production of Acetyl-xylan Rsterase from *Penicillium digitatum*

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Abstract

Current research focuses on the Production of Acetyl-Xylan esterase by *Penicillium digitatum* under submerged culture fermentation. The synthesis of Acetyl-Xylan esterase was optimized using bio-industrial waste at a 5g level as the basal substrate. The study most likely found certain circumstances, such as substrate type, moisture content, pH, temperature, and incubation duration that enhance enzyme synthesis. These parameters are critical for increasing the production of acetyl-Xylan esterase. The optimal parameters of the fermentation medium for the maximum synthesis of Acetyl-Xylan esterase enzyme by Penicillium digitatum were found to be pH 4-5, incubation temperature of 25°C-27 0°C, incubation length of 15days. A 5g glucose, 5g bio-industrial waste, 8g yeast extract, 1g KH_2PO_4, 1g NaCl, and 0.5g $(NH_4)2SO4$, $FeSO_4$, $ZnSO_4$, and $MnSO_4$ mixture was added to 100 ml of mineral salt solution (MSS). This resulted in an increase in enzyme activity. Yeast shows Maximum growth of Acetyl-Xylan esterase by penicillium digitatum among beef Extract, peptone, Ammonium chloride and Malt extract.

Keywords: Acetyl-xylan, *Penicillium digitatum*

Purification and Characterization of A-Amylase Produced From *Penicillium citrinum*

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Abstract

This research work was conducted on the Purification and Characterization of α-Amylase enzyme by *Penicillium citrinum* using surface culture fermentation technique. The optimization study was done for α-Amylase enzyme production using fermentation parameters using OFAT (one factor at a time) technique. Various parameters such as effect of incubation temperature, incubation period, pH, various carbon and nitrogen sources. Various concentration levels of sucrose, yeast extract, potato peel, KH_2PO_4, $MgSO_4$, $MnSO_4$, $FeSO_4$, $ZnSO_4$ and NaCl were observed on the production of α-Amylase enzyme. Results indicated that 2.5g sucrose, 5g yeast extract, 3.5g potato peel, 2g KH_2PO_4, 1g $MgSO_4$, 1g $MnSO_4$, 0.25g $FeSO_4$, 0.5g $ZnSO_4$ and 1.25g NaCl gave the highest yield of α-Amylase enzyme. The maximum α-Amylase production was observed as 1.518±0.1 U/ml for extracellular extract and 2.547±0.1 U/ml for intracellular extract while keeping the pH of fermentation medium at 5.5 kept at 27℃ with all the optimized culture medium ingredients for 5 days. The α-Amylase enzyme was partially purified by addition of Sodium Sulphate to the crude enzyme obtained after solid state fermentation, with constant stirring at room temperature. And after its purification characterization of enzyme was also done. The α-Amylase was checked by varying the temperature, pH and substrate concentration. It was observed that α-Amylase was stable till the 37℃ of temperature and gave the maximum activity at pH 5.5. The yield of α-Amylase from *Penicillium citrinum* appeared as the potential source for up-scaling the cost effective and sustainable process through which α-Amylase can be used food processing, bioremediation and in various biotechnological applications.

Keywords: α-Amylase, *Penicillium citrinum*, Potato peel, OFAT, Parameters, Purification, Characterization.

Production and Purification of Ergot Alkaloids From *Pleurotus ostreatus*

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Abstract

The fungal strain *Pleurotus ostreatus* was used in the present study which has the ability to produce ergot alkaloids. The production of ergot alkaloids was achieved from the extracellular extract and intracellular extract of *P. ostreatus* using surface culture fermentation technique. Various culture conditions were optimized such as effect of different substrates i.e. nitrogen and carbon sources, other culture medium ingredients i.e. effect of KH_2PO_4, $CaCl_2$, $ZnSO_4$, $MgSO_4.7H_2$ O and $FeSO_4.7H_2O$, and various process parameters i.e. effect of pH level, incubation time period and inoculum sizes by using one factor at a time method (OFAT). Maximum yield of ergot alkaloids was obtained from the extracellular extracts at optimum level of sucrose (20%), tryptone (20%), KH_2PO_4 (2.5%), $CaCl_2$ (2%), $ZnSO_4$ (1%), $MgSO_4.7H_2O$ (0.01%), $FeSO_4.7H_2O$ (0.05%), at pH 5.5 and 25℃ after 5days of incubation. The highest yield of ergot alkaloids was also achieved in the extracellular extract at incubation period (9days) and inoculum size (10ml). Partial purification of ergot alkaloids was also performed using chloroform extraction method and purify extract contain maximum quantity of ergot alkaloids (1.117 mg/ml). TLC was performed for the further analytical analysis of ergot alkaloids and it was found that *Rf* value (0.84) for the extracellular extract was attained in the mobile phase F which indicating the presence of ergocryptine alkaloids.

Keywords: Ergot Alkaloids, *Pleurotus ostreatus*

Extraction and Determination of Secondary Metabolites of Lichens Collected From Ayubia Nationalpark, Murree Hills, Kp, Pakistan

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Abstract

Lichens are the entities consisting of mycobiont and phycobiont forming a symbiotic relation between them. They contain large amount of secondary metabolites holding an important part in pharmacological industry and importance in economic development. The main objective of this study was to identify and visualize the two important species of lichens *Parmelia* and *Xanthoria* to determine their ability as a source of usnic acid and other secondary metabolites. Different species of lichens were collected from Ayubia National Park in the month of June, 2023. The lichens collected were foliose and crustose such as *Dermatocarpon reticulum*, *Physcia sp*, *Xanthoria sp*, *Parmelia sp*, *Ramalina sp* from the host *Quercus, Pinus excelsa, Aesculus indica*, *Abies pindrow*, *Euphorbia* respectively. The process encompasses the culturing of mycobiont on PDA and MEA containing media, along with incubation and inoculation by suspension medium of lichen under aseptic environment. The spores of fungal partner were successfully isolated from the growth media and were identified under microscope with the help of the identification keys. Then the isolated lichens, *Xanthoria* and *Parmelia* were converted into powder form and were used in extraction of usnic acid using ethanol and n-hexane resulting in formation of yellowish coloured crystals considered as usnic acid. TLC was used to determine andprovide clarity about the yellow crystals as usnic acid. The mobile phases in this process were allowed to move along with the stationary phase and result shows the presence of usnic acid in both the lichen species with minor differences in its presence

Keywords: Secondary metabolites, Lichens, Ayubia Nationalpark

Evaluation of Bactericidal Potential of Different Antibiotics and Green Synthesized Nanoparticles Against *Pseudomonas aeruginosa* Associated With Pulmonary Infections in Infants

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Abstract

Bacteria are known to spread diseases and infections. *Pseudomonas aeruginosa* is an opportunistic pathogenic bacterial strain which causes serious issues among immunocompromised persons. *P. aeruginosa* is a Gram-negative bacterium which causes nosocomial infections among infants such as respiratory distress syndrome associated pneumonia and other pulmonary infections. The intention to conduct the current study was to the isolate the different bacterial strains from sputum of infants suffering from pulmonary infections from intensive care unit of hospitals and isolate the *P. aeruginosa* from samples and screening of pathogenic bacterial strains and check their sensitivity against different antibacterial agents along with antibiotics. Sputum samples of infants were collected and bacterial strains were isolated on nutrient gar media then different biochemical and virulence tests were performed to isolate and to ensure the presence of *P. aeruginosa.* Antibacterial activity of antibiotics, plant extracts and green synthesized silver nanoparticles were checked against pathogenic strains of isolated *P. aeruginosa* by using well diffusion method. Molecular characterization of pathogenic strains which showed sensitivity against different antibacterial agents was done and sent for sequencing. Pathogenic isolates of *P. aeruginosa* showed sensitivity against antibiotics but some strains showed resistance against antibiotics, but it wonder that all strains showed maximum, intermediate and minimum sensitivity against plant extracts and green synthesized silver nanoparticles and no strain showed resistance against them. Accession number of pathogenic strains which showed sensitivity against plant extracts and green synthesized silver nanoparticles were obtained. Plant extracts and green synthesized silver nanoparticles can be used to inhibit the growth of *P. aeruginosa,* as bacteria become resistant by continuously usage of antibiotics and dangerous side effects also occur due to excessive use of antibiotics.

Keywords: Bacteria, *Pseudomonas aeruginosa*, Infants, Pulmonary infections, Biochemical test, Virulence, Antibiotics, Plant extracts, Green synthesized silver nanoparticles

Screening and Characterization of Pathogenic Bacteria From Soil and Their Resistance Against Different Antibacterial Agents

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Abstract

The present study was conducted to characterize and screen the pathogenic bacteria from the soil of Shahdara Lahore. Two pathogenic strains were isolated. Blood agar test was performed to check the pathogenicity of the isolated strains. Both the strains showed the beta hemolysis that confirmed their pathogenicity. Evaluation for the susceptibility of pathogenic bacteria against nanoparticles and various plant extracts was done. There was no significant difference occurred in the antibacterial activity of nanoparticles ($p > 0.05$) and they showed good antibacterial activity as demonstrated by their zone of inhibition. Plant extract of *Eucalyptus radiata* and *Azadirachta indica* demonstrated good antibacterial activity against the isolated pathogens. Assessment for the resistance of pathogenic bacteria against variety of antibiotics was done. There was a significant difference in the values of various antibiotics ($p < 0.05$) as indicated by the results of one-way ANOVA. Further post hoc analysis by tukey and bonferroni test indicated that the value of chloramphenicol was quite different while all the other antibiotics showed similar antibacterial activity. All the antibiotics showed good antibacterial activity except chloramphenicol that showed less antibacterial activity. Ribotyping results revealed that the isolated strains were *Bacillus spp.* and *Pseudomonas spp.* The study concluded that soil of Shahdara Lahore contain different pathogenic bacteria that cause various infections in humans and animals. These pathogenic bacteria present in the soil are also harmful for the plants. Antibiotic resistance has been established in bacteria as a result of overuse and misuse of these drugs to treat infections. It is advised that various biological tools, such as plant extracts and nanoparticles, be examined more as antibacterial techniques so that pathogens may not have evolved resistance.

Keywords: Pathogenic Bacteria, Nanoparticles, Plant Extracts, Antibiotic Resistance, Molecular characterization

Antibacterial Action of Oregano Essential Oil Free and Encapsulated Against Uropathogenic Bacteria

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Abstract

Urinary tract infections (UTI) are an extremely common health problem, they constitute 50 % of the nosocomial infections, the present study aimed to determine the prevalence and the susceptibility pattern of bacteria isolated from urinary tract infections and to evaluate the antibacterial activity of an encapsulated and free essential oil against pathogenic isolates . During a three months , 59 isolates from 431 samples were identified ; *E.coli* (46%) and *K .pneumoniae* (29%) were the most predominant spices , Analysis of results showed that women were more prone to UTI with a sex ratio of 1.54 (W/M) . The susceptibilities of the gram-negative bacilli to antibiotics revealed a high resistance frequency to penicillin , cephalosporin and ciprofloxacin , while the gram-positive mainly represented by *S.aureus* were weakly resistant to the antibiotics tested . The nanocapsules containing oregano were developed by high speed homogenization (HSP) . The antibacterial activity using a disk diffusion method and a minimum inhibitory concentration (MIC) demonstrated a microbiocid effect against the pathogenic bacteria , the inhibition zone were ranging between 11 and 40 mm , the chemical analysis revealed the presence of the thymol and carvacrol in encapsulated and free oil , the results obtained show the effectiveness of oregano against uropathogenic strains

Keywords: Essential oil, Uropathogenic bacteria, Nanocapsules, Antimicrobial resistance

Determination of Antibacterial Activity of Different Plant Extracts and Green Synthesized Nanoparticles Against Beta Hemolytic Strains of *Staphylococcus aureus* From Eye Infections

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Abstract

In recent years, eye infections have become a widespread medical issue, which may be partially attributed to the rising number of contact lens users. The aim of this study was to identify and characterize the pathogenic strains of *Staphylococcus aureus* that involved in corneal infections. There is a major public health problem regarding eye infections, especially in contaminated environments. Eye swab samples were collected from Fatima Memorial Hospital (FMH), Lahore. The samples were inoculated on Mannitol salt agar (MSA) isolate pure colonies of *S.aureus.* After isolation of pure strains of bacteria, their pathogenicity was assessed using a Blood Agar test. Strains exhibiting beta hemolysis were selected and evaluated for their susceptibility to various antibiotics, green-synthesized silver nanoparticles (AgNPs) derived from different plant extracts, and also again different plant extracts. The pathogenic strains that showed the sensitivity against antibiotics, plant extracts and green-synthesized silver nanoparticles were sent for molecular characterization. Four isolated strains demonstrated beta hemolysis that is the indication of pathogenic *Staphylococcus aureus* strains, which are associated with eye infections. The Zones of inhibition were then noted against various, green-synthesized silver nanoparticles (AgNPs) derived from different plant extracts, antibiotics and certain plant extracts by some bacterial strains, while others displayed resistance. The advancement of an effective and available treatment could enhance global public health consequences. Improved accessibility to treatments may result in well health results, especially in areas where eye infections are common but progressive medical interventions are restricted.

Keywords: Eye infections, *S. aureus,* Silver nanoparticles, Antibacterial activity, Antibiotic resistance

Production and Extraction of Commercially Important Glucoamylase From Wild Type and Mutant Strains of *Trichoderma viride*

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Abstract

This present research deals with the Production and Extraction of commercially important Glucoamylase from wild-type and mutant strains of *Trichoderma viride*. Industrially important glucoamylase production was done by *Trichoderma viride* under optimized conditions using a submerged fermentation technique. The enzyme was produced at 25°C temperature, 5.5p H, and 5ml inoculation for about 5 days. The glucoamylase showed maximum production as glucose and nitrogen in sucrose and yeast extract source. The mineral salts used 2g KH_2PO_4, 2g $MgSO_4$, 0.5g $MnSO_4$, 2g NaCl, 2g $FeSO_4$, and 2g $ZnSO_4$ also showed the highest growth of glucoamylase. The highest enzyme yield was observed in Sucrose (3.00±0.005mg/ml), ammonium solution (3.00±0.065mg/ml). *Trichoderma viride* were subjected to mutagenesis with chemical (Ethane Methane Sulfonate) and physical (UV) mutagen to enhance the production of enzyme. The yield of wild and mutant *Trichoderma viride* was also compared. The highest yield was observed from UV irradiation (2.097±0.005mg/ml). It was also observed that *Trichoderma viride* and the process of surface fermentation using OFAT was cost-effective and sustainable.

Keywords: *Trichoderma viride*, Glucoamylase

Extractıon And Production Of Ergot Alkaloids Produced By Fungal Consortıum

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Abstract

The current research focused on the Extraction and Production of Ergot alkaloids produced by a fungal consortium utilizing the surface culture fermentation approach. The Ergot alkaloids production was optimized utilizing fermentation parameters and the OFAT (one factor at a time) technique. Several characteristics including the effect of incubation temperature, pH, inoculum size and various carbon and nitrogen sources. Sucrose, peptone, KH_2PO_4, $MgSO_4$, $FeSO_4$, $ZnSO_4$, and $CaCl_2$ were used at different concentrations to produce ergot alkaloids. After 5 days of incubation, the maximum output of Ergot alkaloids was obtained with 25g sucrose, 20g peptone, 0.5g KH_2PO_4, 1.25g $MgSO_4$, 0.75g $CaCl_2$, 1g $FeSO_4$, and 0.75g $ZnSO_4$ at pH 5.5 and 25℃. The extracellular extract yielded the most Ergot alkaloids at the 9-day incubation period and 30mL inoculum size. Ergot alkaloids were partially purified using the chloroform extraction method, and the purified extract contained the highest concentration of ergot alkaloids (1.22 mg/ml). TLC was used for further examination of Ergot alkaloids, and it was discovered that the Rf value (0.81) for the extracellular extract was obtained in the mobile phase F, indicating the presence of Ergocriptine alkaloids.

Keywords: Fungal consortium, Ergot Alkaloids, OFAT, Extraction, Production, Parameter.

Collection of Lichens (MSK-L) of the Institute Experimental Botany of the National Academy of Science (Minsk, Belarus)

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Abstract

Herbarium of the Institute of Experimental Botany (IEB) NAS of Belarus is the oldest and largest herbarium in the Belarus. The founder of the lichen collection is considered to be the famous lichenologist Mikhail Petrovich Tomin. The number of species of lichens, lichenicolous and non-lichenized fungi in the collection includes about 1,730 and more than 65 thousand specimens, from them 1670 species of lichens, 35 lichenicolous and 30 non-lichenized fungi. The collection of lichens consists of two parts: a geographical sector, all continents, except Belarus and a sector with specimens only from Belarus. Species of lichens and closely related fungi in the collection (MSK-L) are represented from 7 continents: Europe – 1185 species, Asia – 628, North America – 375, Antarctica – 86, Australia – 85, Africa – 25 and South America – 7 species. Lichens and closely related fungi in the collection are noted from 60 countrie: Belarus – 646 species, Russia – 617, Finland – 436, USA – 334, Poland – 178, Lithuania – 163, Slovakia – 155, Uzbekistan – 154, Latvia – 153, Ukraine – 139. The collection (MSK-L) contains about 230 type specimens of lichens described by M.P. Tomin: *Anaptychia isidiata* Tomin, *Blastenia gordejevii* Tomin, *Caloplaca desertorum* Tomin, *Physcia mereschkowskii* Tomin, *Pyxine sibirica* Tomin and some others. The collection contains isotypes transferred from Kherson State University (KHER) and Royal Botanic Gardens Victoria (MEL): *Caloplaca albopustulata* Khodos. & S.Y.Kondr. (KHER), *Caloplaca conranii* S.Y. Kondr. & Kärnefelt (MEL), Xanthoria coomae S.Y. Kondr. & Kärnefelt (MEL).

Keywords: Lichens, lichenicolous and non-lichenized fungi, collection, species, Belarus.

Anatomical and Morphological Investigation of Some Lichenized Fungi from Usambara Mountains -Tanga, Tanzania

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Abstract

This study used morphological and anatomical ivestigations to determine the biodiversity of lichenized fungi in the Usambara Mountains located in Tanga region of Tanzania. Lichens are symbiotic associations formed by the combination of a fungal partner and an algal and/or cyanobacterial partner. Usambara mountains have a significant range in elevation with peaks ranging up to 2400 metres. Knowledge of lichenized fungi is retatively limited regardless Usambara Mountains being a biodiversity hotspot with a variety of endemic species. In this context, the aim of this study is to examine anatomically-morphologically selected lichenized fungi species from Usambara Mountains. The collected lichen samples were identified by anatomical and morphological examinations which resulted into identification of 5 species of lichenized fungi: *Chaenothecopsis debilis* (Sm.) Tibell, *Calicium hyperelloides* Nyl., *Hypogymnia tubulosa* (Schaer.) Hav., *Lobaria pulmonaria* (L.) Hoffm. and *Sticta dichotoma* Bory ex Delise. The findings contribute to the understanding of lichen diversity and adaptation in the East African highlands, with implications for biodiversity conservation and ecosystem monitoring.

Keywords: Lichenized fungi, Biodiversity, Tanzania, Usambara Mountains.

Bryophyte Vegetation of Cappadocia Region (Nevsehir)

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Abstract

In this study, bryophyte vegetation in the Cappadocia region (Nevşehir) in the Central Anatolia Region of Turkey was investigated. In 30 field studies, 208 relevé (30 x 30 cm) were selected from the research area between 2020-2021. 132 of these randomly selected relevé were found suitable for the study and analyzed using the PAST (PAleontological STatistics) program with ordination methods. Cluster analysis (NJA) was used to distictive communities, and detrended correspondence analysis (DCA) was used to determining ecological factors. With this method, which is new for bryophyte vegetation studies in Turkey, 6 communities (*Encalypta vulgaris - Syntrichia ruralis; Syntrichia ruraliformis - Pterygoneurum ovatum; Syntrichia ruralis - Didymodon acutus, Grimmia pulvinata - Grimmia anodon, Lewinskya rupestris- Grimmia pulvinata* and *Grimmia crinita- Grimmia pulvinata*) were determined from the research area according to substrate type and humidity.

Keywords: Moss, Vegetation, PAST, DCA, NJA, Cappadocia, Nevşehir.

Acknowledgements: This work was supported by the Scientific and Technological Research Council of Türkiye, project number 119Z205.

Spirulina platensis Production Using Bioreactors and a New Cultivation Process to Enhance Yield

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Abstract

The optimization of *Spirulina platensis*, a cyanobacterium of significant nutritional interest, is a key challenge in agricultural and industrial biotechnology. Our research focuses on the application of continuous-flow bioreactors and the implementation of a novel cultivation process aimed at maximizing the yield of this microalga under Algerian conditions. The use of bioreactors enables optimal control of physico-chemical parameters such as temperature, pH, dissolved oxygen concentration, and light intensity, all of which are essential for promoting homogeneous and accelerated growth of *Spirulina platensis*. The newly developed process involves the optimization of culture media, nutrient adjustments, and the implementation of more efficient harvesting strategies, leading to a significant increase in biomass production. This innovative approach not only improves cellular productivity but also enhances the nutritional properties of *Spirulina*. The results demonstrate the potential impact on the profitability of local production and the quality of the final product, thereby meeting the growing demand in the food and pharmaceutical markets. By participating in this online seminar, we aim to share the outcomes of this research and encourage scientific and industrial collaborations to implement sustainable and effective *Spirulina platensis* production solutions in the region.

Keywords: *Spirulina platensis*, Bioreactor, Nutrient optimization, Biomass yield, Sustainable production.

Mycoflora of Poultry Feeds And Mycotoxins Producing Potentiel Of *Aspergillus flavus*

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Abstract

This study dealt with the isolation and diagnosis of contaminants fungi from six samples of Poultry feed brought from the Eastern Poultry Group (ENAP) El-Eulma Sétif. The most important contaminated fungal are identified is *Penicillium* which was found with a rate of 74.58% (44 isolates), followed by *Aspergillus* with 20.34% (12 isolates), *Trichoderma* with 3.39% (two isolates) and finally the lowest rate (1.7%) was obtained with *Rhizopus* (one isolate). *Aspergillus flavus* has been identified as one of the most important fungal fungi. The results showed its ability to produce aflatoxin B1 and B2 as well as beta cyclopiazonique.

Keywords: Poultry feeds, *Aspergillus flavus*, Aflatoxin B1, *Penicillium.*

Metagenomic Characterization of the Bacterial Microbiota in Domestic Cat (*Felis catus*) Saliva

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Abstract

Felis catus is a domestic animal that interacts with people frequently, both directly and indirectly. Human-animal interactions have resulted in zoonotic and parasitic diseases. These interactions ultimately result in changes to each organism's microbiome. Studying the microbiota of species in contact with humans enables data collection on potential health risks and provides insights into microbiota changes. This study used metagenomic analysis of *Felis catus* saliva to determine its bacterial composition at various taxonomic levels. Saliva sample from a domestic cat was sequenced using next-generation technology, targeting the V3-V4 region of bacterial 16S rRNA gene. This approach allowed us to obtain a detailed profile of the oral microbiota, contributing to a more comprehensive understanding of animal microbiomes and their potential impact on human health, particularly in the context of zoonoses and bacterial infections. At the phylum level, Bacterioidota (38%), Pseudomonadota (21%), Fusobacteriota (19%), Bacillota (11%), Spirochaetota (5%), and Mycoplasmatota (3%) were dominant. At the genus level, key genera included Porphyromonas (30%), Fusobacterium (14%), Treponema (5%), Peptostreptococcus (5%), Campylobacter (5%), and Oceanivirga (4%). These results provide valuable insights into the microbial composition of the cat's oral cavity and shed light on the diversity and role of microorganisms in their microbiome. This study expands our understanding of the cat oral microbiome and provides a foundation for future research on microbial transmission between animals and humans. By using advanced sequencing techniques, we can better study the relationships between bacterial communities in domestic animals and their potential impact on human health, particularly through zoonotic pathways.

Keywords: *Felis catus*, metagenomics, saliva, microbiota

Detection of *Toxoplasma gondii* by Rapid Test, ELISA,PCR Analysis in Iraqi Women with Recurrent Abortion

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Abstract

Toxoplasma gondii represents one of the reasons for miscarriage and congenital deviation in infected females, which causes critical alterations in pregnancy. The current study aimed to detect the prevalence toxoplasmosis in recurrent abortion women by applying several tests and comparison between them. Four hundred fifty blood specimens were collected from Al- Kadhimiya Teaching Hospital and Ibn Al-Balady Hospital in Baghdad(December 2023- April 2024).TORCH rapid test and ELISA were used to detect IgG and IgM antibody in recurrent abortion women .Besides, PCR test was applied to detect B1 gene for Toxoplasma gondii . The outcomes for rapid test showed 30% for IgG and IgM in recurrent abortion with toxoplasmosis. While the ELISA results for recurrent abortion women with toxoplasmosis (IgG and IgM) recorded 60%.There are significant differences (P<0.05) when comparing between rapid test and ELISA. On the other hand PCR analysis diagnosed 78 samples with B1 gene for Toxoplasma gondii out of 140 samples which were diagnosed by ELISA. Concerning the results of diagnostic parameters of rapid test versus ELISA, it was observed that a rapid test for IgG had a sensitivity of 88.68%, a specificity of 100%, and an accuracy of 98.3%. Meanwhile, the results of diagnostic parameters of a rapid test for IgM were 42.9%, the specificity of 100%, and the accuracy of 77.4%.Moreover, the results of the diagnostic accuracy for ELISA versus PCR test showed 55.7%, the specificity of 100%, and the accuracy of 75.2% . Furthermore, odd ratio (OR) values were 255 ,0.14and 0.2 in the comparison between control and patients for (IgM+IgG), IgG respectively.Although ELISA test is considered the most common test for diagnosing toxoplasmosis, it is preferable to use supporting tests such as PCR analysis for accurate diagnosis, especially with the emergence of congenital malformations at present in Iraq .

Keywords: ELISA ,Rapid test,PCR, Toxoplasmosis

The Seroepidemiology of Toxoplasmosis among Premarital Females Students in Baghdad City University

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Abstract

This study aims to show some seroprevalence of toxoplasmosis in some premarital females students in some Baghdad collages. Using enzyme linked immunosorbent assay (ELISA) IgG and IgM, to determine the prevalence of toxoplasmosis in (90) blood samples. Premarital females infected with Toxoplasma. gondii were appeared with 37 (41.1%) seropositive anti-Toxoplasma IgG and 25 (22.22%) seropositive anti- Toxoplasma IgM. Domestic cat breeder had high percentage 12 (60%) of anti- Toxoplasma (IgG -- &IgM +) antibodies in comparison with patients who do not have cats. Females students who eat in restaurants show 5(100%) for (IgM+,IgG+) and 15(75%), 22(68.75%) for(IgM+,IgG-) and (IgM-,IgG+) respectively . ELISA IgG avidity test which used as distinguish mean between acute and latent infection, showed highly significant (p<0.01) percentage between samples with low avidity 7 (18.91%) or acute phase and with high avidity 30 (81.08%) or chronic phase of toxoplasmosis.

Keywords: Toxoplasmosis, Premarital females

The Impact of the COVID-19 Pandemic on Diving Routines and Diving Hygiene

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Abstract

The COVID-19 pandemic led to a significant reduction in diving activities in Turkey. While knowledge about the virus increased and specific safety measures were adopted to encourage a return to diving, it seems that recovery of the pre-pandemic levels will take time. The purpose of this study was to identify changes in divers' routines and perceptions of hygiene compared to the pre-pandemic period. A 20-question survey, designed to assess demographic data, diving routines, and hygiene perceptions, was electronically sent to four diving schools, inviting participation from their members. A total of 48 divers participated in the survey. The majority of participants were experienced divers in the middle-aged group who had been diving regularly for a long period. A significant increase in hygiene awareness was observed post-pandemic. However, many participants reported shortcomings in the implementation of safety measures and expressed a lack of sufficient motivation to return to diving. This study, though based on a limited sample, revealed the impact of the pandemic on the diving population in Turkey. It highlighted the need for efforts to restore diving operations to pre-pandemic levels of activity. These results also confirmed that diving hygiene is a crucial component of diving safety.

Keywords: COVID-19, Diving hygiene, Diving safety

Problems that may be Experienced in Kelp Diving and Independent Scuba Diving Method

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Abstract

The purpose of this review is to be cautious against the problems that may occur in independent scuba diving with a cap and to explain the solutions to the problems that may occur in diving. Kelp is a type of seaweed and comes together to form an underwater forest. Kelp forest creates habitat for many living species. Kelp grows in waters with temperatures between 10-20°C, at depths of 15-30 meters, depending on water clarity. It can grow up to 61 cm per day and faces threats from storms and overfeeding by sea urchins. Dried kelp is used as a thickening agent in food products and as a source of vitamins, minerals, and antioxidants in medicine. Scuba diving in a kelp forest requires special training and equipment. This guide focuses on preparing for and addressing potential issues during a kelp forest dive. Proper equipment preparation is crucial, with wetsuit or drysuit selection based on water temperature to prevent hypothermia. Kelp entanglement is a common risk, so divers should avoid dangling gear and ensure all equipment is secured. Dive planning must be thorough, including routes, depth, and emergency strategies. In kelp forests, divers should descend vertically, avoid large fin strokes, and swim horizontally once in the canopy. If entangled, stay calm and carefully untangle or use a dive knife if needed. Navigating in kelp requires good compass and natural navigation skills, and controlled ascents are essential to avoid decompression sickness. Regular air checks are vital, and sufficient air must be reserved for a safe return. Upon finishing the dive, exit carefully, ensuring no entanglement as you surface. Before diving in a kelp forest, divers must master essential skills like buoyancy control, air management, and navigation. A diver with no kelp forest experience should dive cautiously, avoiding combining it with other first-time experiences. Safety should always come first, followed by exploration.

Keywords: Kelp diving, SCUBA diving, Recreation management, Kelp's canopy

High School Students' Views on Virtual and Real Field Trips After the Forest Fire Created with 3600 Videos on Environment and Ecology

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Abstract

Today, environmental pollution, which has reached global dimensions, brings the importance of environmental education to the agenda. One of the current environmental problems, especially caused by lack of awareness and education, is forest fires. The deficiencies in environmental education lie at the root of fires that cause irreversible damage to biodiversity and nature. In this context, effective and efficient environmental education becomes important. One of the important components of an effective environmental education that includes much more than transferring information is field trips. Field trips that allow observing events and facts in their natural environment and collecting information first hand cannot be carried out due to the burden of procedures such as permission, time and financial inadequacies. This situation encourages the use of virtual field trips, which have become widespread with the effect of developing technology in recent years, in education. Virtual field trips carried out in the comfort of the classroom increase interest, motivation and focus, and positively affect student success, attitudes and behaviors. In this study, it was aimed to determine the student views after a real field trip and a virtual field trip created by the researcher with 360° videos in order to raise awareness by observing the destruction in the area after a forest fire, which is one of the current environmental problems. The study group of the qualitatively designed study consists of 30 students studying in the tenth grade of a state school. The virtual field trip to the area after the forest fire in the Marmaris İçmeler Region was applied to the students who formed the study group with VR glasses. A real field trip was carried out with the same group after the forest fire in the Bolu Göynük district. The students' opinions about the virtual field trip and the real field trip were obtained with semi-structured interview forms and the opinions were explained with a finite analysis.

Keywords: Environment, Ecology, Forest Fire

Synthesis and Characterization of Organic@Inorganic Cu Hybrid Nanoflower With *Salix alba* Extract

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Abstract

In this study, organic@inorganic Cu hybrid nanoflowers (hNFs) were synthesized by the coordination of *Salix alba* extract and Cu^{+2}. The optimum synthesis condition was determined by characterization tests with FE-SEM, EDX and EDX analysis of hNFs synthesized with 0.5, 1 and 5 mg/L plant extract in PBS buffer prepared at different pHs. hNFs were not formed in conditions where the PBS buffer was acidic (pH 5). The optimum synthesis of hNFs was achieved in the presence of 1 ml of extract and the pH of the PBS buffer at 7.4. It was recorded that hNFs synthesized under optimum conditions had a diameter range of 24-41 μm and a petal thickness of 21-31 nm, similar to the ideal flower morphology. In addition, it was observed that the petals forming the hNFs obtained under these synthesis conditions came together irregularly and had a morphology far from an ideal flower form. The presence of C, O, P, N and Cu elements in the structural component of hNFs formed by the coordination of *S. alba* extract and Cu element in PBS buffer (pH 7.4) was demonstrated by EDX mapping. The presence of O-H (alcohol), N-H (amine) and primary phosphate crystals was revealed by FT-IR analysis.

Keywords: *Salix alba*, Nanoflower, Copper

Preliminary Analysis of Changes in the Species Composition of Lichens of the Family Teloschistaceae Along the Gradient of Continentality in the Russian Far East

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Abstract

As part of our study of the biodiversity of lichens of the order Teloschistales in the Russian Far East, we have preliminary analyzed the distribution of its family Teloschistaceae along the longitudinal transect Yakutsk–Magadan–northern Kamchatka–Commander Islands (arranged from the west to the east). This transect runs in the northern part of the boreal macrobioclimate from the continental sector of continentality in the west to the oceanic sector in the east through the maritime and suboceanic sectors in the intermediate part of transect. The material was collected by the authors as well as by D. Himelbrant, I. Stepanchikova and A. Zueva. We preliminarily compared the species composition of the Teloschistaceae in the localities along this transect using hierarchical cluster analysis (Ward's method). The localities are grouped into two main clusters: (1) all localities of the continental sector and localities of the maritime sector not associated with the seacoast, (2) all localities of the oceanic and suboceanic sectors and localities of the maritime sector located directly on the seacoast. Within these two clusters, localities are grouped mainly according to their type of plant community (coniferous forest, floodplain forest, tundra etc.).

Keywords: Boreal macrobioclimate, *Caloplaca* s. lat., sectors of continentality, *Xanthoria* s. lat.

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Preliminary Investigation of the Biodiversity of the Kaynar Sea Cave

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Abstract

Sea caves are extraordinary but also very sensitive and vulnerable habitats containing rich biodiversity. Sea caves are recognised as priority habitats by the EU Habitats Directive and the Barcelona Convention, as they represent a range of habitats for specific species communities. Sea caves are widely distributed throughout the Mediterranean, but the lack of quantitative data on their structure and function hinders their conservation status assessment. Species diversity of sea caves in the Eastern Mediterranean, including Turkish coasts, has been addressed in a limited number of studies compared to the Western Mediterranean. The aim of this research is to reveal the biodiversity characteristics of Kaynar sea cave located in Aydıncık district of Mersin and to form the basis of a scientific framework for the protection of sensitive species and important habitats. In this context, the mega-flora and fauna of the cave were tried to be revealed by experienced divers with direct observations and, as much as possible, by photographing during scuba diving. When the photographs taken were examined, 119 species belonging to 12 taxonomic groups (Phaeophyceae, Rhodophyta, Chlorophyta, Porifera, Cnidaria, Polychaeta, Crustacea, Mollusca, Bryozoa, Echinodermata, Tunicata, Pisces) were identified. However, it is estimated that the number of species living in the cave is much higher. Comprehensive research is needed to determine the spatial-temporal variations and functional characteristics of benthic species communities in sea caves. In future studies, the cryptic and soft bottom habitats of Kaynar Cave should be addressed and examined within this broad scope.

Keywords: Biodiversity, Sea Cave, Aegean Sea, Eastern Mediterranean

Notes on *Argyroneta aquatica* (Water Spider) (Araneae, Dictynidae) in Anatolia

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Abstract

Argyroneta aquatica, commonly known as the water spider, is the only spider that lives its life entirely underwater. This species has been recorded from several localities in Anatolia. Some biological and ecological observations, habitat photographs, habitat preferences and localities of this species are also given. The data show that *Argyroneta aquatica* inhabits eutrophic lakes and ponds, as well as marshes, swamps and slow-flowing streams in waters with relatively low pH and dissolved oxygen concentration.

Keywords: *Argyroneta aquatica*, Araneae, Ecological datas, Anatolia

The Production of Recombinant Spider Silk Through Insect Systems

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Abstract

The unique mechanical and biophysical properties of spider silks have made them a prominent area of research for several years. The farming of most spiders is not viable as their cannibalistic behavior poses significant challenges. The only practical method for achieving large-scale spider silk production is through the biotechnological generation of spidroins. Advances in genetic engineering have recently allowed for the synthesis of recombinant spider silks. The large-scale production of spider silk proteins, known as spidroins, is being attempted through recombinant techniques in various expression systems, including plants, bacteria, yeasts, insects, silkworms, mammalian cells, and animals. The natural spinning abilities of silkworms make them a highly suitable candidate for the generation of recombinant spider silk. A crucial consideration in the investigation of spider silk protein production through insect cells is the minimal evolutionary divergence observed between spiders and insects. This study focusing on the production of recombinant spider silk through insect systems.

Keywords: Spider silk, Silkworm silk, Recombinant production, Spidroin

New locality data of *Cyrtomnium hymenophylloides* (Bryophyta: Mniaceae) from Bolkar Mountains in Türkiye

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Abstract

During bryological field study to the Bolkar Mountains, the arctic-alpine moss *Cyrtomnium hymenophylloides* (Hübener) T.J.Kop., previously known only from Ordu province, was rediscovered from Niğde province in Türkiye. Firstly, the species was collected on wet soil (about 195-540 m a.s.l.) in Kalıcak and Kadıncık Villages (Ulubey-Ordu) where located in the central and eastern part of Black Sea Region in 2013. Ten years later, it was collected on wet limestone bedrock around Çiniligöl (Ulukışla-Niğde), a glacial lake in the Central Anatolian part of the Bolkar Mountains. This site, located approximately 2680 m a.s.l., situated the highest altitude shelter for this species which makes it interesting and remarkable. *C. hymenophylloides* which is characterised erect tuft life form, broadly-ovate leaves, acute to apiculate leaf apices, is distributing mainly in the northern boreal–arctic region. While it is including in the IUCN Red List of Threatened Species in the LC category in Europe, it is including in the NT category in EU-28.

Keywords: Bolkar Mountains, Bryophyte, *Cyrtomnium,* Türkiye.

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Purification and Characterization of Commercially Important Keratinase by Wild Type and Mutant Strains of *Mucor mucedo*

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Abstract

The abundance and recalcitrance of chicken feather waste, which is almost 80% composed of keratin, produced as a byproduct of poultry industries can be significantly degraded by ecofriendly and monetary processes involving microbial keratinases. This study focused on the keratinolytic activity of wild and mutant strains of *Mucor mucedo* in vitro as a feather keratin-degrading agent. The production of keratinase in the modified recipe of 1% (w/v) feather substrate fermentation media was optimized using a one-factorat-a-time (OFAT) technique for purifying and characterizing the stability of keratinase for various pH, temperatures, and incubation periods. The best carbon and nitrogen sources for keratinase production were maltose (0.779 ± 0.007 U/ml) and yeast extract (0.507 ± 0.007 U/ml), respectively. The optimized fermentation conditions were 3% substrate, 5g carbon, 4g nitrogen, 1.5g peptone, $CaCl_2$, & $MnSO_4$, 1g $FeSO_4$, 0.75g $MgSO_4$, 0.5g KH_2PO_4, and 0.25g $ZnSO_4$. After optimization of fermentation media components, a 1-liter fermenter inoculated with wild-type *Mucor mucedo* strain was run and maximum yield was obtained (1.228 ± 0.005 U/ml). Then the wild-type strain was subjected to physical (UV) and chemical (Ethidium bromide) mutagenesis. The survival rate of *Mucor mucedo* colonies for physical mutation was up to 107% and for chemical mutation up to 80-90%. The best keratinase activity was observed by physically mutated fungal keratinase (2.987 ± 0.004 U/ml). The maximum stability of keratinase extracted from the fermenter with wild-type strain was observed at pH 8.0, 50°C for 100 minutes (0.814 ± 0.006 U/ml). The most stable conditions for the physical mutant were pH 7.0, 30°C for 40 minutes (2.427 ± 0.008 U/ml), and for the chemical mutant, it was pH 7.0, 70°C for 20 minutes (2.348 ± 0.009 U/ml). The study showed that Mucor mucedo is an efficient producer of keratinase with high keratinolytic activity and it improved its capabilities with physical and chemical mutations. This leads to the possible potential of mutated keratinase for industrial purposes making the environment safe, healthy, and pollution-free.

Keywords: Surface culture fermentation, Keratinase, Keratin, Poultry feather degradation, *Mucor mucedo*, Mutation, OFAT, Stability, Purification, Characterization.

Synthesis and Characterization of Organic@Inorganic Cu Hybrid Nanoflower with *Mentha piperita* Extract

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Abstract

In this study where hNFs were synthesized with Mentha piperita extract, the organic component (plant extract) increasing concentration from 0.5 mg/l to 1 mg/l and inorganic component (Cu^{+2}) were coordinated in acidic, neutral and basic PBS buffer. The optimum synthesis condition was determined by characterization tests with FE-SEM, EDX and EDX. The morphologies of hNFs with organic components of mint extract were determined by FE-SEM analysis. hNFs was not formed in the tubes under pH 5 conditions of PBS buffer. In addition, hNFs was not synthesized in the presence of 0.5 mg/L plant extract under all pH conditions. hNFs were synthesized under optimum conditions by adjusting the pH of PBS buffer to 7.4 and using 1 ml of plant extract. According to FE-SEM images of hNFs with ideal flower morphology, it was recorded that their diameters were 45 μm and their petal thicknesses were distributed in the range of 31-47 nm. EDX analysis of hNFs synthesized under optimum conditions revealed the presence of C, O, N, P and Cu, which constitute their structural elements. FT-IR analysis confirmed the presence of alcohol (O-H), alkene (C=C) and primary phosphate crystals.

Keywords: *Mentha piperita*, Nanoflower, Copper

Nanotechnology-Based Strategies for Non-Surgical Treatment of Canine Mammary Tumors

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Abstract

Canine mammary tumors (CMTs) are among the most prevalent malignancies in female dogs. Surgery has historically been the gold treatment approach; however, certain tumor types are not suitable for surgical intervention, and various complications may arise from surgical procedures. Whether used independently or as part of multimodal treatments, researchers are increasingly focusing on strategies for effective non-surgical interventions. Chemotherapy, as a non-surgical intervention, is limited by three main problems: the inability to specifically target cancer cells, the development of resistance to therapeutic drugs, and the negative effects that these agents can have on healthy tissues and organs. The integration of chemotherapy agents with nanomaterials offers substantial potential to mitigate the adverse effects of cancer treatment and improve the efficacy of chemotherapy. Nanotechnology provides unique methods for precision therapy, enabling targeted drug delivery, improving bioavailability, and reducing systemic toxicity. Nanoparticles are also used as photothermal agents to increase the effectiveness of photothermal therapy for superficially localised solid CMTs. It provides combined therapies like photothermal therapy with chemotherapy. This presentation reviews and discusses the current studies using nanotechnology-based therapeutic approaches in the management of CMTs. Research on nanomedicine for CMT treatment protocols both improves patient outcomes and offers translational insights for human breast cancer management. Further clinical studies are essential to validate these innovative approaches and establish their long-term efficacy and safety.

Keywords: Canine, Mammary Tumor, Nanotechnology, Treatment

Effects of BSA Nanoparticles on the Freezability of Angora Goat Semen

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Abstract

Cryopreservation of spermatozoa is essential for transferring genetic material to future generations. However, sperm cells are highly sensitive to endogenous and exogenous factors during freezing, often resulting in membrane damage and motility loss due to lipid peroxidation. To mitigate these issues, protective substances such as bovine serum albumin (BSA) are added to sperm extenders. Studies suggest that BSA improves sperm motility, membrane integrity, and reduces DNA damage and reactive oxygen species (ROS) levels during freezing-thawing. This raises the question: "Could nanoform BSA enhance its efficacy?" This study investigated the effects of 20 nm and 40 nm BSA nanoparticles, at 5 mg/ml and 10 mg/ml doses, on the freezability of Ankara goat semen. Samples were collected from five 2–3-year-old bucks using electroejaculation. Sperm with high motility (≥80%) and concentration ($2.5x10^9$) were pooled to minimize individual differences and grouped into control, BSA20-5, BSA20-10, BSA40-5, and BSA40-10. These groups were equilibrated at +4°C for 2 hours, frozen in liquid nitrogen vapor, and thawed at 37°C for motility, live/dead ratio, and plasma membrane functional integrity (HOST) analyses. The results showed no statistically significant difference in motility and live/dead sperm ratios between BSA nanoparticle groups and the control ($p>0.05$). However, for HOST, the control (53.06%) and BSA20-5 (55.12%) groups yielded similar results, while other BSA groups showed significantly lower outcomes ($p<0.05$). Nano BSA did not fully dissolve in distilled water, forming a colloidal suspension that reduced sperm cells' ability to benefit from it. Furthermore, nanoform BSA absorbed water, potentially impacting membrane resilience. The decline in membrane integrity may be attributed to increased water retention due to the particle size and dosage of nano BSA.

Keywords: BSA nanoparticles, Angora goat, Sperm, Freezing

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Phytochemical Profile and Therapeutic Potential of *Solidago vırgaurea* L.: Antioxidant and Analgesic Effects

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Abstract

Solidago virgaurea L. (*S.virgaurea* L.) is a perennial plant belonging to the genus Solidago and the family Asteraceae, widely used in alternative medicine. The components of *S.virgaurea* L. were extracted using boiling water, the yield of the extraction is 34.4%. Total phenolic content was determined using the Folin Ciocalteu reagent and was estimated as 143.52 ± 0.0016 mg gallic acid equivalent /g of dry extract. Flavonoids were determined using the $AlCl_3$ reagent, their content was 39.34 ± 0.0141 mg of quercetin equivalent/g of dry extract. The amount of tannins was estimated as 139.15 ± 0.00077 mg tannic acid equivalent /g of dry extract. The results also indicate that the decocted extract of *S. virgaurea* L. at the two used doses (100 and 200 mg/kg), relieved the pain caused by intraperitoneal acetic acid injections with inhibition ratio of 23.41 and 56.52%, respectively. Antioxidant activity in vitro was estimated using the Cuprac assay, where the antioxidant activity was estimated at 20,71 ± 0.07 mg/mL compared to Butylated Hydroxy Toluene (3,42 ± 0.015 mg/mL).

Keywords: *Solidago virgaurea* L., Polyphenols, Analgesic activity, Antioxidant activity

Phytochemical Profiling and Antıoxidant Potential Of Essential Oils From *Achillea odorata L.*

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Abstract

The essential oils of *Achillea odorata*, a plant renowned for its medicinal properties, were analyzed to identify their phytochemical composition. A total of 68 compounds were characterized, representing 100% of the total oil content. The primary components identified include α-Pinene (20%), Camphor (44.2%), Borneol (146.8%), Caryophyllene oxide (14.1%), and Limonene (25%). These compounds are distributed across several classes, including monoterpene hydrocarbons (4.92%), monoterpenoids (59.75%), sesquiterpene hydrocarbons (5.41%), and sesquiterpenoids (29.92%). The essential oils were extracted using hydrodistillation from the aerial parts of the plant. The chemical composition was analyzed using gas chromatography coupled with mass spectrometry (GC-MS), with identification confirmed by retention indices and co-injection with authentic standards. The antioxidant activity of the essential oils was evaluated using two assays: the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging assay and the FRAP (Ferric Reducing Antioxidant Power) assay. The DPPH assay measured the oil's capacity to neutralize free radicals, with an IC50 value of 20.85 ± 1.90 µg/mL, while the FRAP assay quantified its reducing power, yielding a value of 33.91 ± 0.16 µg/mL. Both assays were conducted in triplicate to ensure accuracy. The presence of high concentrations of monoterpenoids and sesquiterpenoids suggests significant antioxidant and antimicrobial potential. This study provides a detailed chemical profile of *Achillea odorata*, laying the foundation for future research into its pharmacological properties, particularly its potential as a natural antioxidant

Keywords: *Achillea odorata*, Essential oils, Phytochemical composition, Antioxidant activity.

Production of Ethanol From Natural Bio-Resources Using *Saccharomyces cerevisiae*

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Abstract

The fungal strain *Saccharomyces cerevisiae* was used in the present study for the production of ethanol by using fruit waste as a substrate. The study investigated the mixture of fruit waste including mango peel, apple pomace, banana peel and citrus waste that worked as a substrate for the production of bioethanol in a surface culture fermentation technique. The effect of various parameters was checked on the production of bioethanol such as the effect of different concentration levels of fruit waste, glucose, yeast extract, KH_2PO_4, $CaCl_2$, $MgSO_4$, $ZnSO_4$ and $FeSO_4$. The findings showed the potential of *Saccharomyces cerevisiae* to produce ethanol. The best yield of bioethanol was obtained from 1.25g of yeast extract (2.275±0.002mg/ml). The results concluded that waste from fruits should be converted to beneficial products like bioethanol that can work as an alternative energy source.

Keywords: *Saccharomyces cerevisiae*, Ethanol production, Fruit waste, Substrate, Mango peel, Apple pomace, Banana peel, Citrus waste, Surface culture fermentation, Bioethanol

Valorization of *Pistacia lentiscus* (*Pistacia lentiscus L.*) Seed Oil: "Study of Physicochemical and Biological Characteristics (Antibacterial and Antifungal) from Three Regions (El-Tarf, Skikda, Guelma)"

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Abstract

This study is a contribution to the valorization of the mastic tree, *Pistacia lentiscus* L., which is widely found in the eastern region of Algeria (Annaba and El Tarf). For this study, physicochemical analyses, antibacterial and antifungal activity tests were conducted on its vegetable oils, traditionally extracted. The results obtained indicate that the mastic oils tested had no antibacterial effect on the bacterial strains used. On the other hand, they showed considerable antifungal activity. The reasons for this variability can be explained by differences in environmental conditions (climate and geographic location), the harvest period, and the extraction technique. It is also worth noting that this variability may be due to the quality of the oil used. In fact, the oil seems to have been poorly preserved and stored, which led to its degradation and possibly altered its properties and/or affected its safety, explaining the results obtained.

Keywords: *Pistacia lentiscus*, vegetable oil, physicochemical parameters, antibacterial activity, antifungal activity

Ethnobotanique du romarin of Rosmarinus officinalis from the Region of Khenchela (Alegria)

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Abstract

The study aims to assess the extent to which the residents of El-Mahmal municipality use rosemary (*Rosmarinus officinalis*) as a therapeutic remedy for illnesses. Using a questionnaire distributed and collecting 65 responses, the results revealed that the residents of the area have good knowledge in the field of herbal medicine in general, the benefits and uses of rosemary in particular. The residents prefer to use rosemary by boiling the leaves and consuming it as tea, which is the most widespread method with a rate of 49%, either alone or with other herbs. The results also show that digestive system and intestinal gland disorders are the main reasons for which rosemary is used as a therapeutic remedy, with a rate of 22%. Regular doses are taken daily until complete recovery which is the most followed therapeutic regimen with a rate of 57%. The results obtained are a very valuable source of information for the region studied and for the national medicinal flora. They could also be a database for further research in the fields of phytochemistry and pharmacology.

Keywords: Medicinal plants, Ethnobotany, Traditional phytotherapy, Rosemary.

Antidiabetic and Antioxidant Properties of *Agrimonia eupatoria*: A Natural Therapeutic Agent

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Abstract

The present study evaluates the antidiabetic, antioxidant, and enzyme inhibitory properties of *Agrimonia eupatoria* methanol extract. *Agrimonia eupatoria* is commonly known as agrimony, is a perennial herb in the Rosaceae family, widely distributed in Europe, Asia, and North America, valued for its traditional medicinal uses due to its antioxidant, anti-inflammatory, and astringent properties. Antioxidant activity was assessed using ABTS•+ and metal chelating assays, with the extract exhibiting potent IC_{50} values of 3.88 ± 0.27 µg/mL and 20.42 ± 0.50 µg/mL, respectively. For antidiabetic activity, the extract demonstrated significant inhibition of α-glucosidase (78.96 ± 0.88% at 100 µg/mL, IC_{50} = 27.25 ± 0.96 µg/mL) and α-amylase (63.40 ± 0.91% at 100 µg/mL, IC_{50} = 54.70 ± 0.72 µg/mL). In comparison, acarbose, a standard antidiabetic drug, showed higher activity against both enzymes. The polyphenol and flavonoid contents of the extract were quantified as 376.63 ± 1.10 mg gallic acid equivalents and 119.43 ± 0.30 mg quercetin equivalents per gram of dry extract, respectively, supporting its bioactive potential. These findings highlight *Agrimonia eupatoria* as a promising natural source of antioxidants and enzyme inhibitors, underscoring its therapeutic potential for managing oxidative stress and diabetes.

Keywords: *Agrimonia eupatoria*, Antidiabetic properties, α-glucosidase inhibition, α-amylase inhibition, ABTS assay, Metal chelating assay.

Cholinesterase Inhibitory and Antioxidant Activities of *Agrimonia eupatoria* Methanol Extract: A Potential Natural Remedy

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Abstract

The present study investigates the cholinesterase inhibitory and antioxidant activities of methanol extract derived from *Agrimonia eupatoria* which is widely used as natural remedy in Algeria and in the world. The extract demonstrated significant inhibitory effects on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes, with inhibition rates of 65.81 ± 0.45% and 76.83 ± 0.95% at 100 µg/mL, and IC_{50} values of 58.41 ± 0.93 µg/mL and 34.58 ± 0.63 µg/mL, respectively. Comparatively, galantamine, a standard cholinesterase inhibitor, showed superior activity with IC_{50} values of 5.50 ± 0.20 µg/mL (AChE) and 42.20 ± 0.44 µg/mL (BChE). Antioxidant capacity was assessed using DPPH• and CUPRAC assays, where the extract exhibited notable IC_{50} (7.51 ± 0.34 µg/mL) and $A_{0.5}$ (10.22 ± 0.29 µg/mL) values, indicating potent free radical scavenging and reducing abilities. α-Tocopherol was used as a reference antioxidant, showing lower activity compared to the extract in the CUPRAC assay. These findings suggest that *Agrimonia eupatoria* methanol extract holds promise as a natural source of bioactive compounds for combating oxidative stress and neurodegenerative disorders.

Keywords: *Agrimonia eupatoria*, antioxidant activity, acetylcholinesterase (AChE), butyrylcholinesterase (BChE), DPPH assay, CUPRAC assay, neurodegenerative disorders.

Mycobiota of the Belarusian station “Mount Vechernyaya” (East Antarctica)

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Abstract

“Mount Vechernyaya” is a Belarusian seasonal station, which is located on the coast of the Sea of Astronauts, territory of the Tala Hills oasis, Enderby Land in East Antarctica. This region has been poorly studied for its fungal diversity. While fungal communities in Antarctica, and in the “Mount Vechernyaya” region in particular, play a role in nutrient and carbon cycling in terrestrial and marine ecosystems, perform the function of early colonization of rock outcrops, act as symbionts, mutualists, pathogens and saprotrophs. As a part of the study of the species diversity of micromycetes in the area of the “Mount Vechernyaya” station, an analysis of samples of substrates of natural (soils, substrates of plant and animal origin) and artificial (paper, plastic, wood, textiles) origin was carried out. Samples were collected according to generally accepted methods, maintaining sterility conditions. The main methods of studying fungi in the laboratory included: isolation of monocultures from the mixed cultures of micromycetes, identification by cultural-morphological characteristics, molecular-genetic methods of identification (ITS region analysis). As a result of the study, representatives of 3 phyla were identified: Ascomycota, Basidiomycota and Mucoromycota. The leading orders in terms of the number of species were Eurotiales, Hypocreales and Pleosporales. In the soils, micromycetes from the genera Penicillium, Acremonium, Cladosporium, Pseudogymnoascus and Thelebolus dominated; on substrates of animal origin - Pseudogymnoascus, Thelebolus and Chaetomium; on substrates of plant origin - Phoma, Cadophora, Penicillium. Substrates of artificial origin were characterized by the predominance of micromycetes from the genera Alternaria, Cladosporium, Penicillium. Despite the fact that active study of Antarctic mycobiota began relatively recently (in the 1960s), the diversity of species identified so far suggests that fungi may be the most diverse part of the continent's biota. Further research in this area will allow us to expand our understanding of the role of fungi in extreme ecosystems, to study the features of the formation of adaptation mechanisms of extremophilic micromycetes, to assess the influence of the anthropogenic factor on the development of the mycobiota of the continent, and to discover psychrophilic and psychrotolerant micromycetes that produce antibiotic and biologically active substances.

Keywords: Antarctic Micromycetes, Extremophilic microorganisms, Mount Vechernyaya, Psychrotolerant micromycetes, Tala Hills oasis.

Bioecological and Faunistic Investigation of Insect Taxa in Bean Agricultural Areas and Surroundings of Derinkuyu District

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Abstract

Insects are one of the most prominent living groups in terms of biodiversity, ecological role and economic impact on Earth. Although scientists have identified more than a million insect species so far, the total number of insect diversity, including undiscovered and undescribed species, is not fully known. Turkey has a rich fauna due to being a bridge between the Asian and European continents, having different climate characteristics and vegetation structures. However; studies on insect biodiversity in Turkey are limited and the lack of information in this area needs to be addressed. Accordingly, Derinkuyu district of Nevşehir, located in the center of the Central Anatolia Region, was selected as the research area of the study. As a result; The general scope and content of this study consists of the ecological and faunistic investigation of Arthropoda/Insecta taxa in the cultivation areas and surroundings of bean, a geographically registered agricultural product in Derinkuyu district, which has a continental climate and steppe vegetation, based on field studies carried out between March and November in 2022-2023.

Keywords: Insecta, Ecology, Biodiversity, Fauna, Derinkuyu, Bean plant

Kombucha Microbiota: Dynamics of Microbial Species and Health Benefits

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Abstract

Kombucha is a fermented beverage consumed worldwide that is slightly sweet, slightly acidic, refreshing and has a low carbon dioxide content. Traditionally, kombucha is obtained by fermenting sugary tea (*Camelia sinensis*) with the addition of SCOBY, a symbiotic association of bacteria and yeast. During kombucha fermentation, sucrose is hydrolyzed by yeast cells to fructose and glucose, which are then metabolized into ethanol. Fermentation is then completed by bacteria converting ethanol into organic acids. Traditionally produced from black and green tea, kombucha is fermented using different plant substrates to soften its taste, differentiate its aroma, and enrich its functional value and nutrition. Despite the worldwide popularity of kombucha as a beverage, the distribution of microorganisms in it during kombucha fermentation and how these microorganisms interact within communities have not been well characterized. Characterization of acetic acid bacteria and yeast in Kombucha starter culture may provide a better understanding of the fermentation process. The most abundant bacterial genera in Kombucha tea belongs to *Acetobacter* and *Gluconobacter*. The dominant acetic acid bacteria of these genera are *A. xylium*, *A. pasteurianus*, *A. aceti* and *G. oxydans*. Microorganisms in the culture potentially help in the production of higher quality products as they affect the production of metabolites such as organic acids that are associated with potential health benefits and sensory properties. This study aimed to reveal the microbial diversity of bacteria and yeasts in Kombucha consortium and the biochemical properties of the resulting fermented product on health.

Keywords: Kombucha, Fermentation, SCOBY, Tea, Organic Acids

Substrate Selection and Its Effect on Kombucha Fermentation

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Abstract

Studies on kombucha fermentation highlight the critical effects of substrate selection on fermentation efficiency and product quality. In kombucha fermentation, the types of sugars used by microorganisms as energy sources (such as sucrose, glucose, fructose) and the tea varieties that support fermentation (black tea, green tea, herbal teas) come to the fore. Studies have shown that sucrose is an optimal energy source for yeasts and bacteria in kombucha culture, and that this leads to rapid acid production and balanced pH reduction, increasing fermentation efficiency. Other sugars such as glucose and fructose provide lower microbial activity and fermentation efficiency. Plant material varieties create significant differences in the fermentation process and bioavailability of the product. Green tea has been shown to increase the health benefits of kombucha and provide more biological activity thanks to its high antioxidant content. Although black tea is a traditionally preferred substrate, it does not offer as high antioxidant activity as green tea. The effect of herbal teas on fermentation has been limited and has generally been found to be less effective in supporting microbial growth. Fermentation time, temperature, pH change and chemical composition of the substrate directly affect microbial diversity and metabolite production. These factors shape the final product characteristics of kombucha, such as taste, biological activity level and health benefits. Therefore, it has been shown that substrate selection is an important step to optimize quality in kombucha production. This information provides guidance for both kombucha producers and fermentation researchers and provides a basis for making future production processes more efficient.

Keywords: Kombucha, Fermentation, Biological activity, Substrate diversity

Comparative Analysis of Niche Breadth and Environmental Adaptability in *Apodemus mystacinus* and *Apodemus epimelas*

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Abstract

Ecological niche modeling (ENM) offers valuable metrics to analyze species niches and compare their distributions in geographic and environmental space. ENMTools offers tools to measure metrics like niche breadth, incorporating methods based on Levins, 1968. This study explores the niche overlap and adaptiveness of *Apodemus mystacinus* and *Apodemus epimelas*, using three key bioclimatic variables: Bio19 (Precipitation of Coldest Quarter), Bio16 (Precipitation of Wettest Quarter), and Bio13 (Precipitation of Wettest Month). Initial analyses revealed significant overlap in their fundamental niches but highlighted that *A. mystacinus* exhibits greater adaptability to varying environmental conditions compared to the more conservative *A. epimelas*. These findings were consistent with additional niche analyses, thus validating the reliability of the initial distribution map. Overall, this study emphasizes the need for nuanced interpretation of niche metrics and the value of comparative analyses in understanding species' environmental adaptability.

Keywords: ENMTools, *Apodemus mystacinus, Apodemus epimelas,* niche breadth, fundamental niche

First Sign Coral Bleaching in Southern Djibouti Red Sea

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Abstract

Coral reefs are essential components of marine ecosystems, providing exceptional biodiversity, biological density and essential ecosystem services. However, these ecosystems are facing unprecedented degradation due to the combined effects of climate change, anthropogenic activities and ecological pressures such as overexploitation, mass tourism and ship strandings. The Red Sea, a region of high biodiversity and endemism, is no exception. Djibouti, located at the convergence of biogeographic zones, hosts unique coral reef assemblages shaped by the interaction of tropical and cold-water habitats, particularly around the 7 Brothers Islands, where cold-water upwellings promote exceptional reef resilience. The 2023 global El Niño event, characterized by elevated sea surface temperatures (SSTs), marked a period of extreme thermal stress, contributing to widespread coral bleaching. This study assessed the health of coral reefs along the southern Red Sea coast of Djibouti, focusing on key sites such as Moucha and Maskali Islands, as well as Arta, Tadjourah and Obock. Analysis of historical temperature data from the Gulf of Tadjourah revealed that 2023 was the year with the highest thermal stress since 1985, with sea surface temperatures peaking at 30-32°C in September and October. Coral bleaching occurred in the southern Red Sea of Djibouti in September-October-November 2024 extending from Moucha-Maskali Islands to Obock in the northern coastal area of Djibouti, resulting from the loss or discoloration of Symbiodinium symbionts under thermal stress. The most intense bleaching was observed on reefs near the town of Obock, Ras Bir, with a rate of 30% of corals bleached. The most sensitive species were Acropora, which are particularly vulnerable to high temperatures and have diverse distribution areas. In contrast, bleaching of coral reefs at the Arta site was low compared to other sites. Our results highlight significant impacts on coral health, requiring urgent monitoring and intervention. Recommendations include implementing efficient management strategies for marine protected areas, reducing local anthropogenic stressors, and promoting resilience through the protection of critical habitats. This research highlights the importance of continued monitoring to mitigate the impacts of climate change on Djibouti's unique and vulnerable reef ecosystems.

Keywords: Djibouti, Coral reefs

A Coloration Anomaly in *Microtus obscurus* from Türkiye

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Abstract

Hair and skin pigmentation in mammals is an important factor in their survival, providing camouflage against predators, facilitating intra- or inter-species communication, protecting from ultraviolet (UV) radiation, and playing a role in physiological functions. Due to a lack or excess of pigmentation, different types of color anomalies (Albinism, Leucism, Piebaldism) can occur. Another type of chromatic anomaly, commonly known as piebaldism, is a condition in which the absence of pigment is localized and is due to the absence of melanocytes in the affected skin, feathers or fur. The aim of this study is to present records of colour anomalously one small mammals from the Türkiye. In this study, 150 Sherman type live traps were used in the field study carried out between 15-21 October 2024 in Ardahan, Türkiye. Samples were obtained from an altitude of 2500 meters. As a result of the field studies 25 *Microtus obscurus* specimens were caught. The normal fur color of *Microtus obscurus* is generally brownish-yellow. However, only one *Microtus obscurus* specimen was found to have a color anomaly. The color anomaly is in the form of a spot on the upper side of the head. Pigmentation abnormalities in wildlife are often associated with various environmental factors, including diet, pollution, infectious diseases, and more. Additionally, inbreeding and the proliferation of specific mutations within a population are linked to pigmentation abnormalities. These aberrations may serve as indicators of population health and potential threats, such as diminished genetic diversity due to isolation and small population sizes. Notably, the fragmentation of natural habitats, resulting in the contraction of ranges, can intensify inbreeding pressure within populations. Increasing inbreeding pressure poses a significant threat to the general population by contributing to the decrease in genetic diversity. In this study, a color anomaly was detected in a specimen of *Microtus obscurus*. It is crucial for researchers to report specimens with color anomalies in order to better understand the ecological and physiological impacts of this phenomenon on the population and their survival.

Keywords: Color anomaly, small mammals, Piebald

Age Estimation of the Trabzon Population of *Mertensiella djanaschvilii* Tartarashvili and Bakradze 1989

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Abstract

The Caucasus Salamander *Mertensiella caucasica* is an endemic salamander to the Caucasus region. However, with the contribution of molecular studies in recent years, *Mertensiella djanaschvilii*, which was previously defined as a subspecies of the species, was elevated to species level and the taxon in the lesser Caucasus region was named *Mertensiella djanaschvilii*. In this study, the skeletochronology method was used to estimate the ages of the specimens from Trabzon province collected in 2017 and the growth parameters of the species were revealed. Specimens were collected from Trabzon and its vicinity in 2017 within the scope of the "Species Conservation Action Plan". The phalanx and femora of the specimens were aged using the skeletochronology method. In addition, SVL measurements of the specimens were taken using a digital caliper with a precision of 0.01mm to reveal the relationship between age and SVL. Females of Trabzon population were found to be older than females both in terms of age and head+body length (SVL). The maximum age was 9 years in a female specimen with SVL values of 70.26 mm, while the minimum age was 3 years in two female (SVL= 37.26 mm, 36.99 mm) and a male specimen (SVL= 35.58 mm). The age at sexual maturity was determined as 4 years for both sexes. Mean SVL was 60.56 ± 1.81 mm (35.58 mm – 66.30 mm) for males and 56.11 ± 3.64 mm (36.99 mm - 70.26 mm) for females. In addition, the age of males ranged from 3 to 8 years, with a mean age of 5.93 ± 0.32 years, while the age of females ranged from 3 to 9 years, with a mean age of 5.60 ± 0.61 years. The age structure of the Gümüşhane and Giresun populations were previously studied. Accordingly, the results of this study are parallel with the other results. The maximum age found for *Mertensiella djanaschvilii* specimens was determined as 8 for males and 9 for females, while in other studies it was determined as 10-11 for males and 8-9 for females. In general, these results may be due to differences in elevation, feeding and access to food, and it is possible that such a result may occur in terms of the samples evaluated. In conclusion, the age-SVL relationship of the Trabzon population of *M. djanaschvilii* species were revealed. In addition, Trabzon population was compared with Gümüşhane and Giresun populations in terms of age-SVL relationship. Age estimation and similar studies are important for species affected by these environmental conditions.

Keywords: Caucasian salamander, *Mertensiella djanaschvilii*, Longevity, Age estimation.

Is Skeletochronology a Practicable Method for Longevity of the Tawny Owl, *Strix aluco* (Linnaeus, 1758) (Strigiformes: Strigidae)

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Abstract

The age and longevity of animals have long been subjects of scientific interest. Various methods are currently used to estimate the ages of different animal groups. One such method, skeletochronology, is considered reliable for determining the age and growth parameters of ectothermic organisms like fish, amphibians, and reptiles. Recent literature, however, suggests that this method can also be used for endothermic organisms, such as mammals and birds. Studies on birds have shown that the bone types in which age-related structures such as LAGs (Lines of arrested growth) are most clearly visible may vary across species. In this study, the age of a male Tawny Owl (*Strix aluco*) specimen was determined using the skeletochronology method on the axis, cervical vertebra, tibiotarsus, and phalanges. The specimen, found deceased on the Çanakkale-Güzelyalı highway in May 2024. The bones were dissected and preserved in 70% ethyl alcohol. For decalcification, a 5% nitric acid solution was applied for a period of 3 to 15 hours, depending on the size and thickness of each bone. After decalcification, the bones were rinsed in running water for 12 hours to remove any residual acid, then embedded in paraffin. 12 μm thick cross-sections were obtained using a rotary microtome. Two different staining solutions, toluidine blue and hematoxylin, were used to see differences in staining methods. The slides were examined under a light microscope and photographed. Upon examining the various bone tissues, LAGs were observed in distal regions, consistent with previous studies in literature. Analysis of the tissues revealed that the specimen was 3 years old across all bones. Furthermore, it was noted that different bone tissues varied in terms of both staining properties and their alignment with skeletochronology. The clearest images were observed in the sections taken from the longest phalanges. In the tibiotarsus sections, three LAGs were detected in the distal part of the tissue, although the image was less distinct compared to the axis. When the vertebral sections were evaluated, the same age was determined in both Toluidine blue- and hematoxylin-stained tissues, with a clearer result obtained from the Toluidine blue-stained ones. In the longest phalanges, three LAGs were identified in the distal sections which was consistent with the findings in other tissues. In this case, slides stained with Toluidine blue provided a clearer result, making the counting of LAGs easier. In this study, the axis, cervical vertebrae, tibiotarsus, and phalanges of *Strix aluco* were utilized for skeletochronology, and the estimated age across all bones was determined to be 3 years. The phalanges was found to be the most suitable tissue for age determination using this method. Additionally, staining with Toluidine blue provided much clearer results compared to hematoxylin. This study also supports the notion that skeletochronology can be a valuable method for age estimation in birds.

Keywords: Tawny owl, *Strix aluco*, road kills, Longevity, Age estimation.

Diet Analysis of Barn Owls (*Tyto alba*) in Southeastern Anatolia (Ceylanpınar, Şanlıurfa)

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Abstract

This study examines the feeding ecology and prey selection of barn owls (*Tyto alba*) in the Southeastern Anatolia Region, with a focus on pellet analysis from specimens collected in 2010 on TİGEM lands in Ceylanpınar, Şanlıurfa province. The analysis revealed that mammals, particularly rodents, constituted the primary component of the diet, while birds and reptiles were consumed as secondary prey, reflecting local ecological variability and prey availability. Pellets were dissected and subjected to microscopic analysis to identify prey remains, such as bones, teeth, and other fragments, down to the taxonomic level. The findings underscore the role of regional habitat heterogeneity and prey abundance in shaping the dietary composition of barn owls. Moreover, this study provides insights into potential new species records from the Arabian Peninsula, contributing to the biodiversity inventory of the region. The barn owl's role as a natural predator highlights its ecological significance as a biological control agent, particularly for managing rodent populations that pose agricultural challenges. These results emphasize the broader ecological and agricultural implications of barn owl conservation. Future investigations are anticipated to delve deeper into the relationship between dietary flexibility and reproductive success in TYTO ALBA, offering critical perspectives for understanding adaptive strategies in changing environments.

Keywords: Barn owl, TYTO ALBA, pellet analysis, feeding ecology, rodents, biodiversity, biological control

New data about overlooked urban lichen-forming of Sidi bel Abbes western Algeria

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Abstract

This paper reports for the first time 30 species of lichens in the urban environment of Sidi Bel Abes, 26 of these species are new to Sidi bel abbes town: *Acarospora cervina, Aspicilia cinerea, Candelariella aurella, Candelariella reflexa, Candelariella vitellina, Circinaria calcarea, Gyalolechia flavovirescens, Heteroplacidium fusculum, Kuettlingeria teicholyta, Lecania fuscella, Lecania naegelii, Lecanora campestris, Lecanora chlarotera, Lecidella elaeochroma, Lecidella stigmatea, Myriolecis dispersa, Myriolecis hagenii, Myriolecis sambuci, Protoparmeliopsis muralis, Rinodina pyrina, Sarcogyne algerica, Sarcogyne regularis, Polycauliona polycarpa, Rufoplaca arenaria, Variospora flavescens, Xanthocarpia lactea.* One additional species is new to Algeria: *Athallia cerinelloides*. Our collections were made between 2021 and 2022. For national record, we provide diagnostic descriptions.

Keywords: Habitats, Ecology, Species Distribution, Sidi bel abes

Investigation of The Excessive Increase of Jellyfish Observed In The Sea Of Marmara After Mucilage

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Abstract

In recent years, extreme jellyfish increases in the world seas have been observed. The highest increases in our country are recorded from the Marmara Sea. Excessive increases of jellyfish (bloom), which plays an important role in the marine ecosystem, damage many socioeconomic problems as well as the functioning and structure of the marine ecosystem. With this study, after the mucilage (sea snot) in 2021, in June-November 2023 (6 months), within the carried out ‘‘Project of Investigation of Reasons and Consequences Increasing of Jellyfish in the Marmara Sea (MarmANA) (I.Etap)’’ findings and solutions developed were compiled and presented. According to the results obtained, it was found that the medusae phases reached the highest abundance value in November. It was determined that the amount of planula reached maximum values especially in October and November. Species of Aurelia Aurita was widely observed during dives. In the observations made with Dron, was determined the jellyfish population showed the increase in November.

Keywords: Marmara Sea, jellyfish, mucilage, eutrophication.

Determination and Evaluation of Microcrystalline Substances in Some Lichens of Murree Hills, Pakistan

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Abstract

The present study is based the collection of different species of lichen from selected sites of Bansara Gali, Jheeka Gali, Kashmir point, Kuldana, Murree and G.P.O. road of Murree Hills, Pakistan. The species of Lichens were identified through some physical parameters such as *Phaeophyscia orbicularis*, *Dermatocarpon reticulatum*, *Ramalina polymorpha*, *Cladonia* sp., *Xanthoria parietina* and *Parmelia sulcata.* The different morphological characters and crystals of different species were observed by using the microcrystal tests. Many types of crystal shapes such as. star, brush, quadrangular, tube-like and longitudinal were observed under the microscope and drawn with the help of a camera lucida.

Keywords: Microcrystalline, lichens, Murree Hills

Production Of Ethanol From Natural Bio-Resources Using *Mucor mucedo*

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Abstract

This research deals with the use of Mucor mucedo for the production of ethanol. For this purpose, the technique of submerged fermentation was used to observe the production of ethanol from fruit waste by using Mucor mucedo as a fermentation agent. The effect of several parameters was observed in which different concentrations of Yeast, glucose, fruit waste, KH_2PO_4, $CaCl_2$, $MgSO_4$, FeSO4, and $ZnSO_4$ were used. involved. The maximum yield of ethanol was obtained from 1.5g/25ml glucose (2.82±0.02 U/ml) while the minimum amount of yield was obtained from 0.125g/25ml $ZnSO_4$ (0.12±0.02 U/ml). The highest amount of reducing sugars was found in 0.5g/25ml glucose (2.91±0.03 U/ml) and the least amount in 0.075g/25ml $FeSO_4$ (0.66±0.03 U/ml). The observed production of ethanol and the amount of reducing sugars formed was different with the different concentrations of chemicals at pH 5.5 and 29^{o}C temperature. The results showed the potential of Mucor mucedo to produce ethanol from fruit waste as an effective method for use in various industrial processes.

Keywords: Ethanol, *Mucor mucedo*

FULL TEXT

Notes on *Argyroneta aquatica* (Water Spider) (Araneae, Dictynidae) in Anatolia

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Abstract: *Argyroneta aquatica*, commonly known as the water spider, is the only spider that lives its life entirely underwater. This species has been recorded from several localities in Anatolia. Some biological and ecological observations, habitat photographs, habitat preferences and localities of this species are also given. The data show that *A. aquatica* inhabits eutrophic lakes and ponds, as well as marshes, swamps and slow-flowing streams in waters with relatively low pH and dissolved oxygen concentration.

Keywords: *Argyroneta aquatica*, Araneae, Ecological datas, Anatolia

Introduction

Spiders are occurred in various environments and must adapt to different environmental conditions. However, most species prefer a particular climatic zone and occupy a specific strategic niche in consonance with the availability of prey [1]. Dictynidae has several members associated with aquatic habitats. This family is distributed worldwide, with 461 species in 52 genera [2]. One of the members of this family, *A. aquatica*, commonly known as the water spider, is a fascinating species of spider that lives primarily underwater. It's the only known spider species that spends its entire life submerged beneath the water's surface. Unlike aquatic insects, the water spider cannot breathe underwater due to the absence of gills. Instead, it creates an air dome beneath the water's surface, where it can feed, mate, and lay eggs. When the water spider swims, the air stays attached to the fine hairs on its abdomen, thanks to surface tension [3]. The purpose of this study is to present information about some ecological and biological features of *A. aquatica* and its locality and habitat knowledge in Anatolia.

Some Biological and Ecological Features of *Argyroneta aquatica*

Geographic Range

The diving bell spider or water spider, (*A. aquatica*) is a Palearctic species with a distribution found to extend from northern and central Europe through Siberia and Central Asia. There are also isolated populations of this species in Japan that have been denoted as the subspecies *Argyroneta aquatica japonica*. [1,4].

Habitat

The water spider is the only spider that lives its life entirely underwater. It has been found to live in eutrophic lakes and ponds as well as marshes, swamps, and slow-moving streams in water of relatively low pH and dissolved oxygen concentration. Water spiders need water plants as anchors for their "bubble nests" as well as an attachment site after diving down in the water. [4-6].

Description

Males range from 7.8 to 18.7 mm in length, while females range from 7.8 to 13.1 mm. The tendency of males to be larger than females in this species is an anomaly amongst most spiders. Under the water, *A. aquatica* displays a silvery appearance due to the presence of the air bubble surrounding its abdomen. Outside of the water, the water spider has a brown cephalothorax and a dark velvety abdomen. Like other spiders, the abdomen is covered with hairs, however the water spider uses these hairs to capture a bubble of air around its abdomen. Since the respiratory organs of spiders are located on their abdomens, the bubble serves as a supply of oxygen. [3,5 and 7].

Reproduction

The mating season starts in mid to late spring. Following copulation, the female produces a dense white egg sac holding 50-100 eggs, which completely fills the upper half of the nest. Although the number of viable offspring per egg sac decreases per laying event, water spiders are able to produce six egg sacs from one copulation event throughout a year. However, females that engage in more than one copulation event tend to be more reproductively successful by avoiding a sperm deficit. After she produces her egg sac the female also produces a thick partition separating the eggs from the lower half of the nest, where she continues to live. The female is left to guard the brood until they hatch, which in captivity was found to take three to four weeks. During this time, the female seldom leaves the bell and narrows the entrance by drawing together the edges [5,7].

Behavior

A. aquatica is the only known spider to live out its entire life underwater. Females and males build separate underwater nests or "diving bells" in which each spider stores surface air (Fig.1). This diving bell is used for digestion of prey, molting, deposition of sperm and eggs, copulation, and as a brooding chamber. The diving bell is usually attached to water plants and has been said to resemble a short, wide-based thimble in structure. In construction of the

diving bell, first a platform of silk is constructed between water plants. The spider then swims to the surface, sticks its abdomen out of the water and holds its hind legs backward around the abdomen, thus enlarging the volume of air that can be captured and transported by the air-trapping hairs. Air bubble in tow, the water spider dives as best it can to the nest it is constructing, swimming down and climbing along water plants. Upon reaching the nest, the spider releases the air bubble beneath the diving bell and strengthens and extends the sides of the structure [1,5].

Fig. 1. *Argyroneta aquatica* during building of a diving bell underwater [8].

Water spiders are typically found in temperate locations, and they hibernate during the cold of winter. They are found to descend deeper in the water column and build new, sturdier diving bells during this time. These bells are eventually sealed up completely, providing the hibernating spider with oxygen from November to February. Along with these wintering structures, males of the species have been found to line empty snail shells with silk, fill them with air, and then seal themselves up inside the shell for the winter [5].

Diets

A. aquatica is a carnivorous animal with a diet differing upon location but typically including water fleas, aquatic isopods such as *Asellus aquaticus*, insect larvae, fairy shrimp and even other water spiders. While males tend to be active hunters, females are sessile ambush predators [1,5].

Predation

Due to their superior diving and swimming ability, male water spiders tend to spend more time outside of the safety of the diving bell than females. In order to avoid predation, female and juvenile water spiders are known to spend more time in the diving bell, only leaving at night. Some predators of *A. aquatica* include adult and larval beetles, dragonfly larvae, frogs,

and fish. Because water spiders can live in water of low pH and low dissolved oxygen concentration where many predatory fish cannot survive [4,5 and 7].

Ecosystem Roles

The predatory actions of the water spider are important to the marsh, lake, and pond habitats they live in by limiting the population of water insects. These actions are especially important in the water of low pH and low dissolved oxygen where other predators of these insects, such as fish, are not able to live [4,5].

Some habitats in Anatolia

Argyroneta aquatica distributed in different parts of Anatolia and has even been seen in running water. Some habitat photos are given in below (Fig. 2).

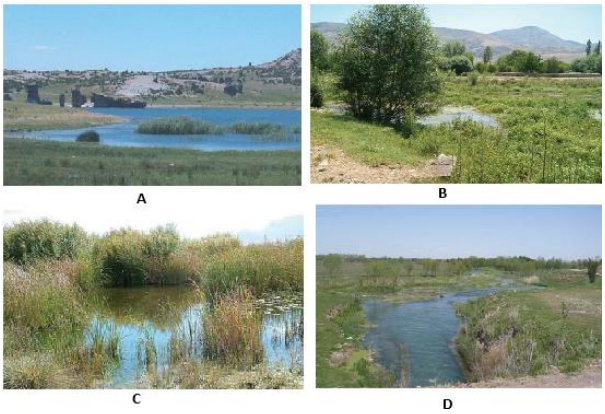

Fig. 2. Habitat photographs of *A. aquatica* in Anatolia [6].A- Emre pond (Afyon Province), B- Çayıryazı village pond (Afyon Province), C- Sultan marsh (Kayseri Province), D- Pınarbaşı spring (Afyon Province)

Conclusions

The current conservation status of this species remains unclear, but may conservatively be stated to be potentially threatened, because many similar temporary aquatic systems outside the protected areas of Anatolia have been polluted and destroyed by human activities. Following studies extended the known distribution of this species and contributed to the knowledge of ecological preferences of Turkish populations [6,9]. We expect future studies will extend the known distribution of this species towards the Black Sea and eastern Anatolian regions of Türkiye.

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The Production of Recombinant Spider Silk Through Insect Systems

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Abstract. The unique mechanical and biophysical properties of spider silks have made them a prominent area of research for several years. The farming of most spiders is not viable as their cannibalistic behavior poses significant challenges. The only practical method for achieving large-scale spider silk production is through the biotechnological generation of spidroins. Advances in genetic engineering have recently allowed for the synthesis of recombinant spider silks. The large-scale production of spider silk proteins, known as spidroins, is being attempted through recombinant techniques in various expression systems, including plants, bacteria, yeasts, insects, silkworms, mammalian cells, and animals. The natural spinning abilities of silkworms make them a highly suitable candidate for the generation of recombinant spider silk. A crucial consideration in the investigation of spider silk protein production through insect cells is the minimal evolutionary divergence observed between spiders and insects. This review focusing on the production of recombinant spider silk through insect systems.

Keywords: Spider silk, silkworm silk, recombinant production, spidroin.

Introduction

Silk, a biocompatible material widely used for a variety of purposes, is widely produced by insects and spiders [1] Spider silk has long been a subject of scientific interest, primarily owing to its unmatched visual and functional qualities, as well as the remarkable structures formed by diverse silk-producing species found in nature. It is considered one of the finest natural polymer fibers, particularly noted for its low density, exceptional tensile strength, and significant elongation before breaking [2]. Spiders produce a wide variety of high-performance silks that offer considerable potential for numerous applications; however, the cultivation of spiders is complicated by their largely solitary behavior, which frequently involves cannibalism. Recent innovations in biotechnology have led researchers to explore the transfer of spider silk genes into host organisms for recombinant protein synthesis. There are two predominant biotechnological strategies for producing silk proteins: one involves the expression of natural spidroin genes or their fragments, while the other focuses on the engineering of new genes that encode proteins that emulate the essential characteristics of spidroins [3]. A variety of hosts, both eukaryotic-such as tobacco, goats, and silkworms- and prokaryotic, have been utilized for the generation of recombinant spider silk proteins.

Silkworms, with their natural spinning capabilities, represent an ideal option for generating recombinant spider silk. A significant factor to consider in the research of spider silk protein production using insect cells is the relatively minor evolutionary divergence between spiders and insects [4].

Mechanical Properties of Silk Fiber

The synthesis of silk occurs within the epithelial cells of specialized silk glands present in diverse silkworm and spider species. The distinctions in silk obtained from these different origins are largely attributed to variations in their amino acid sequences and crystalline arrangements, leading to their specific physical attributes [5]. The structure of spidroin includes glutamine residues in its amorphous areas, which contribute to its impressive elasticity and strength. Spiders are capable of producing a diverse range of silk types, which vary in their properties and molecular weights, spanning from 70 to 700 kDa. In addition to leucine and tyrosine residues, glycine, glutamine, and alanine are found to be in significant abundance. Additionally, there exists a limited common array of amino acid motifs, and the alanine-rich domains are integral to the crystalline properties that facilitate the development of compact β-sheets [6]. Spider silk exhibits remarkable mechanical properties, characterized by a unique blend of flexibility and high ultimate tensile strength. This silk is notably durable, boasting a strength that surpasses Kevlar by a factor of two and exceeds that of steel by five times [7].

Silkworm and Spider Silk

In the natural world, numerous materials possess characteristics that are advantageous for human-made uses. One notable example of such a material, which has been utilized by humans for thousands of years, is silk, a substance generated by various arthropods. Silk derived from the larvae of the *Bombyx mori* moth, commonly known as silkworms, is extensively processed in silkworm farms for various textile applications. Spider silk has been used historically, yet it has never been harnessed for industrial purposes on a large scale. It is noteworthy that numerous spider silks exhibit superior mechanical properties compared to that of silkworm silk. Silkworms utilize their silk as a means of protection throughout their metamorphic process, whereas numerous spiders employ silk to ensnare their prey. Spiders generate a diverse array of high-performance silks that hold significant potential for various applications; however, cultivating spiders presents challenges due to their predominantly

solitary nature, which often includes cannibalistic behavior [3]. When silk is extruded from a spider's body, it solidifies, making subsequent processing challenging [8].

The Production Methods of Spider Silk

There are fundamentally two approaches to artificially produce spider silk. The first involves designing the silk by analyzing the chemistry and sequence of the constituent amino acids. The second method entails identifying the specific spider gene responsible for the synthesis of spider silk protein, followed by isolating this gene and inserting it into a different host organism [10]. An alternative method for collecting silk is known as forced silking. In this technique, the spider is immobilized and compelled to generate silk. The silk fibers are harvested by mimicking the natural behavior of spiders. The silk produced through this controlled spinning process is collected using a rotating cylinder. While this method yields pure and natural silk, the low production rate remains one of the most significant limitations of the process. Consequently, it is not suitable for industrial applications [8]. Recent advancements in biotechnology have encouraged researchers to introduce spider silk genes into host organisms for the purpose of producing recombinant proteins. Silk proteins have been produced through two primary biotechnological approaches: the first involves the expression of natural spidroin genes or their fragments, while the second entails the engineering of novel genes that encode proteins designed to replicate the fundamental components of spidroins [3]. The process commences with the acquisition of a 'blueprint' derived from the DNA sequence of natural spiders. This is followed by the design of recombinant DNA, the cloning of vectors, and the selection of an appropriate host organism for production, which may include bacteria, yeast, eukaryotic cells, or insect cells. Subsequently, protein production is achieved through culturing, culminating in the purification of the recombinant proteins [2].

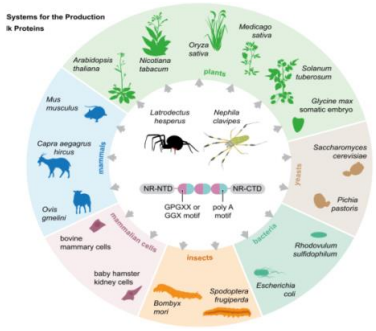

Fig.1 Recombinant Systems For The Production Silk Proteins [4]

The Production Of Recombinant Spider Silk Through Insect Systems

The production of recombinant spider silk through insect systems, particularly using transgenic silkworms, has garnered significant attention in recent years due to the unique mechanical properties and biocompatibility of spider silk. The primary focus of this research is to harness the natural spinning capabilities of silkworms (*Bombyx mori*) to produce spider silk proteins, known as spidroins, which are typically difficult to harvest from spiders due to their territorial nature and low silk yield. Various studies have demonstrated that silkworms can be genetically modified to express spider silk proteins, leading to the production of fibers with enhanced mechanical properties, which are vital for applications in biomedical engineering, textiles, and other fields [9; 4; 11].

A significant reason for taking into account insect cells in research studies of spider silk protein production is that spiders and insects have a relatively short evolutionary distance between them. Cell line Sf9, derived from *Spodoptera frugiperda*, was used to express two dragline proteins of *Araneus diadematus*, ADF3 and ADF4, targeted to the cytosol. This research showed coiled filaments forming inside the cytoplasm. Mechanical force measurement failed due to filament length limitations caused by cell size limitations [4]. Due to their natural spinning apparatus, silkworms are an excellent candidate for producing recombinant spider silk. As a result of the TALEN (transcription activator-like effector nucleases) strategy, the silkworm fibroin heavy chain gene was replaced with the MaSp1 gene (1.6 kb), and transformed cocoon shells contained up to 35.2% MaSp1 protein. CRISPR/Cas9 technology was also used to produce spider silk proteins of native size. The MaSp1 gene (6 kb) was incorporated into the genome of *Bombyx mori*. The silkworm fibroin heavy or light chain (FibH or FibL) intron (FibH) has been replaced with the spider silk gene. Spider silk fibers with FibH or FibL exhibited mechanical properties similar to natural silk. According to this study, silkworms can be used as a natural spinner for industrial purpose [4].

The mechanical properties of silk produced by transgenic silkworms have been extensively characterized. Studies indicate that the fibers exhibit high tensile strength, toughness, and elasticity, which are critical for their potential applications in various fields [9; 12; 13). For example, the tensile strength of recombinant silk fibers produced by transgenic silkworms can reach values exceeding those of conventional silks, making them suitable for use in surgical sutures, tissue engineering scaffolds, and other biomedical applications [4; 14]. Furthermore, the ability to produce silk fibers with tailored properties through genetic modification opens

up new avenues for the development of advanced materials [15]. In addition to mechanical properties, the biocompatibility of recombinant spider silk is another significant advantage. Research has shown that spider silk proteins can support cell adhesion and proliferation, making them ideal candidates for use in tissue engineering and regenerative medicine [16; 17]. The incorporation of bioactive molecules into the silk matrix can further enhance its functionality, allowing for the development of smart biomaterials that can respond to environmental stimuli [15; 18]. Moreover, the use of silkworms as a production platform offers a sustainable and scalable approach to silk production, as they can be cultivated in controlled environments, leading to consistent yields of high-quality silk fibers [4; 19].

The challenges associated with the production of recombinant spider silk, such as low expression levels and the complexity of spinning the fibers, have been addressed through innovative biotechnological approaches. For instance, researchers have explored the use of microfluidic systems for the spinning of silk fibers from aqueous solutions, which allows for better control over fiber morphology and mechanical properties [13; 20]. Additionally, the development of purification techniques for recombinant silk proteins has improved the efficiency of silk production, enabling the isolation of high-purity silk suitable for various applications [17; 21]. Huemmerich et al. conducted an analysis in 2004 on the dragline proteins ADF-3 and ADF-4 of the garden spider *Araneus diadematus*, both of which are characterized by their proline-rich composition, utilizing the baculovirus expression system for their study. This pattern is consistent with most other pairs of dragline silk proteins found in different Araneoidea species, while their proline content does not appear to play a significant role [22].

An expression system based on baculovirus was used by Zhang and colleagues to express a 70-kDa, MaSp1–EGFP fluorescent fusion protein within a *B. mori* cell line (BmN) and larvae. The authors indicated that their findings showed that the solubility is the main factor restricting the yield of spider dragline proteins. It also implied that directly generating fibrous spider silk in the silk-producing organs of transgenic silkworm larvae could be a more effective approach [23]. Wen et al., created germline-transgenic silkworms (*Bombyx mori)* that produced cocoons made of recombinant spider silk. A transformation vector based on piggyBac was created, which included cDNA for spider dragline silk (MaSp1) regulated by the sericin 1 promoter. Silkworm eggs were modified with the vector, resulting in transgenic silkworms that exhibited DsRed fluorescence in their eyes. The genotyping analysis verified that the MaSp1 gene has been incorporated into the genome of the transgenic silkworms,

while the silk protein analysis demonstrated its expression and release in the cocoon. In comparison to wild-type silk, the recombinant silk exhibited superior tensile strength and elasticity. These findings suggest the feasibility of generating recombinant spider silk through transgenic *B. mori* [24].

Teulé et al., employed piggyBac vectors to develop transgenic silkworms that express chimeric silk proteins derived from both silkworm and spider sources. The silk fibers generated by these organisms were composite materials that incorporated chimeric silkworm and spider silk proteins, which were integrated in a highly stable fashion. Additionally, these composite fibers exhibited, on average, greater toughness than the original silkworm silk fibers and demonstrated toughness comparable to that of native dragline spider silk fibers. The findings indicate that silkworms can be genetically modified to produce composite silk fibers that incorporate spider silk protein sequences in a stable manner, thereby greatly enhancing the mechanical properties of the original silkworm silk fibers. The findings indicate that it is possible to genetically modify silkworms to produce composite silk fibers that incorporate spider silk protein sequences in a stable manner, thereby enhancing the overall mechanical characteristics of the silk fibers [25]. Systems for the production of transgenic silkworm and spider silk have been established to generate robust fibers. By employing recombinant techniques, it is feasible to combine essential modules, thereby enhancing our understanding of the function of individual components and the influence of adjacent elements on their characteristics. This methodology is expected to facilitate the creation of tailored structures composed of distinct silk components [21]. Using customized zinc finger nucleases (ZFN), Ma et al., successfully edited the genome of Bmfib-H gene, which encodes silk protein, in *B. mori* with efficiency higher than any previously reported. They also showed that removing endogenous Bmfib-H protein increased exogenous protein expression [26]. Furthermore, utilizing CRISPR/Cas9 technology, spider silk proteins of native dimensions were successfully synthesized. The MaSp1 gene, measuring 6 kb, was integrated into the genome of *Bombyx mori*. The intron of the silkworm fibroin heavy or light chain was substituted with the spider silk gene. The resulting FibH or FibL-spider silk fibers exhibited mechanical properties comparable to those of natural silk, reaching 1.2 GPa. The transgenes demonstrated stability across subsequent generations. This research highlights the potential of silkworms as a natural source for industrial silk production [27].

Silkworm silk fibroin and spider silk spidroin are recognized as biocompatible and naturally biodegradable polymers, making them suitable for various biomedical applications. Due to a

decrease in molecular weight, the strength and resilience of pure recombinant silkworm fibroin and spidroin are relatively limited. Therefore, blending is recognized as the leading strategy in contemporary studies focused on optimizing the mechanical characteristics of silk fibroin and spidroin. Johari et al. provided a comprehensive summary in their article, highlighting various research studies that assess the integration of natural and synthetic polymers. The research indicated that merging natural and synthetic polymers with silk fibroin and spidroin results in changes to their conformation and structure, which effectively fine-tunes the mechanical properties of the blends [28].In a study Hu et al., constructed recombinant baculovirus *Autographa californica* multiple nucleopolyhedrovirus (AcMNPV) expressing native *Trichonephila clavipes* ampullate spidroins 2 (MaSp-G) and 1 (MaSp-C). A silkworm fibroin gene promoter is used to infect nonpermissive *Bombyx mori* larvae at the fifth instar. These chimeric silk fibers have significantly increased the mechanical properties especially strength and extensibility, due to the incorporation of MaSp-G and MaSp-C within them. By using the recombinant baculovirus AcMNPV, silkworms could be developed as nonpermissive heterologous hosts for mass production of chimeric silkworm/spider silk fibers [29].

Conclusions

Spider silk represents a remarkable class of biopolymers that have developed over the course of millions of years. The large-scale biotechnological production of recombinant spider silk represents a significant advancement in spider silk research. Recombinant production technologies enable the large-scale creation of customized silk-based biopolymers for a variety of applications in a relatively brief period. Silkworms possess a natural silk production system, rendering them ideal candidates for research into their capabilities as heterologous hosts for the synthesis of spider silk. The natural spinning capabilities of silkworms make them excellent candidates for the production of recombinant spider silk, allowing for the conversion of spider silk into fibers.

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Kombucha Microbiota: Dynamics of Microbial Species and Health Benefits

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Abstract. Kombucha is a fermented beverage that is slightly sweet, slightly acidic, refreshing, and has a low carbon dioxide content, consumed worldwide. Traditionally, kombucha is obtained by fermenting sugary tea (Camellia sinensis) with the addition of SCOBY, a symbiotic association of bacteria and yeast. During kombucha fermentation, sucrose is hydrolyzed by yeast cells to fructose and glucose, which are then metabolized into ethanol. Fermentation is then completed by the conversion of ethanol into organic acids by the bacteria. Traditionally produced from black and green tea, kombucha is fermented using different plant substrates to soften its taste, differentiate its aroma, and enrich its functional value, and nutrition. Despite the worldwide popularity of kombucha as a beverage, the distribution of microorganisms within the beverage during kombucha fermentation and how these microorganisms interact within communities have not been well characterized. Characterization of acetic acid bacteria and yeast in Kombucha starter culture may provide a better understanding of the fermentation process. The most abundant bacterial genera in Kombucha tea belongs to Acetobacter and Gluconobacter. The dominant acetic acid bacteria of these genera are A. xylium, A. pasteurianus, A. aceti, and G. oxydans. Microorganisms in the culture potentially contribute to higher quality products as they affect the production of metabolites, such as organic acids, that are associated with potential health benefits and sensory properties. This study aimed to investigate the microbial diversity of bacteria and yeasts in Kombucha consortium, and the biochemical properties of the resulting fermented product related to health.

Keywords: Kombucha, Fermentation, SCOBY, Tea, Organic Acids.

Introduction

For thousands of years, fermented drinks have been an essential part of human civilization [1]. Kefir, beer, wine, kombucha, cider, and many more are examples of these drinks. They are produced by the action of microorganisms on different substrates such as fruits, cereals, milk, or tea [2]. In addition to their sensory appeal, fermented beverages are admired for their capacity to enhance human health and wellbeing [3]. The main reason fermented drinks are beneficial to health is that they are made using microorganisms such as bacteria and yeast. The processes enhance nutritional values by enriching beverages with probiotics, antioxidants, and vitamins. Furthermore, fermented beverages enhance gut health, bolster immune system function, and may aid in the prevention of cardiovascular disorders [4].

Kombucha is a fermented beverage that originated in China over 2,000 years ago. It was first used as medicine, before being spread to Japan. 'Kombucha' is a combination of the Japanese words "Kombu," which describes a broad-leaved seaweed called Laminaria japonica; and "Cha," which means tea [5]. Kombu tea is becoming increasingly popular as a beverage with a slightly sweet and sour flavour. This fermented beverage, which is formed by the activity of microorganisms, is usually produced by fermenting black tea with kombucha starter, which has a cellulosic structure. This biofilm layer is known as 'tea fungus' or 'SCOBY'. SCOBY is the abbreviation of 'Symbiotic Culture of Bacteria and Yeast' [6]. It is a fermented product that stands out today for being vegan. Although the content of Kombu tea varies according to fermentation time and extract, it generally contains gluconic acid, lactic acid, acetic acid, probiotics, polyphenols, ethyl gluconate, ethanol; tea components such as flavonols, catechins, theoflavins; hydrolytic enzymes such as invertase, amylase; and elements such as Na, Ca, K, Cu, Mn, Fe, Zn, Ni. Kombucha is preferred for its prophylactic (disease preventive) and therapeutic properties as well as its cooling properties [7]. Studies have determined that it has antibiotic effects, supports the immune system, and possesses anticarcinogenic, antidiabetic, hypocholesterolemic, hypoglycaemic, antioxidant, and antimicrobial properties. Additionally, it has laxative properties, helps the development of intestinal flora, and supports weight loss by accelerating metabolism [7,8]. A review of the microorganisms' interactions, and composition in kombucha, as well as how they affect material changes during fermentation, is presented in this work. These developments are beneficial for extending large-scale manufacturing, improving production efficiency, and raising the yield of functional ingredients.

Species of microorganisms in Kombucha

The beverage is made by mixing a small quantity of the biofilm with 10% w/v sweetened black tea.The fermentation process is static, often lasting 7 to 12 days at ambient temperature. The microbial component of kombucha can differ depending on the fermentation matrix, local yeast and bacterial species, climate, geographic area, and culture [9]. There is no common formula because it primarily depends on the fermentation starter cultures, but it also depends on the amount of sucrose, the temperature at which the fermentation occurs, the raw materials (plants and other ingredients), and the length of time the process takes. One component of the kombucha tea microbial community is the cellulose biofilm, while the other component thrives in the soup or liquid underneath. According to previous studies yeast and acetic acid bacteria (AAB) dominate this beverage's whole microbial spectrum [10].

Previous research has looked at the bacterial and yeast species that comprise SCOBY, (symbiotic culture of bacteria and yeast), referred to as tea fungus, which is used to ferment Kombucha [11]. Kombucha has an acidic flavor. Its fragrance can be explained by its dense, jelly-like texture and vinegar-like odor. While an unpleasant or moldy smell may suggest that SCOBY is decomposing or infected, a vinegar-like smell is a favorable sign that the SCOBY is healthy [12].

A symbiotic relationship between lactic acid bacteria (LAB), acetic acid bacteria (*Acetobacter pasteurianus, Acetobacter aceti,* and *Gluconobacter oxydans*), and various yeast strains (*Saccharomyces* sp., *Pichia* sp., *Zygosaccharomyces kombuchaensis*, *Brettanomyce*s sp., *Toruposis* sp.) can prevent the growth and spread of infectious microorganisms [13]. Within 7–20 days, microorganisms can convert sugar and tea under aerobic fermentation conditions, into a refreshing beverage with a slight acidity and carbonation that contains 14 amino acids, vitamins, tea polyphenols, acetic, glucuronic, and gluconic acids, as well as some hydrolytic enzymes [9, 14]. Nonetheless, a number of microbe species, particularly AAB and yeast, are consistently found in all SCOBYs. After a nine-day fermentation cycle, the total concentration of bacteria and yeast in Kombucha is estimated to be between 10^6 and 10^8 CFU mL^{-1} [15].

According to a number of studies, when the fermentation cycle is over, there will be more yeasts than bacteria in Kombucha. *Gluconacetobacter* was the predominant genus in a study that looked at the microbial content of five distinct SCOBYs. Low quantities of Acetobacter were also discovered, whereas Lactobacillus could be detected with significant levels. The most significant AABs are *Komagataeibacter, Gluconobacter, Acetobacter, and Gluconacetobacter.* At the species level, significant roles are played by *K. intermedius, K. Hansenii, K. saccharivorans, K. rhaeticus, A. xylinum, A. pasteurians, A. aceti, A. tropicalis,* and *G. oxydans* [16]. Lactobacilli and lactococci are crucial LAB components for kombucha. It has been declared that up to 30% of the bacterial community in certain kombucha products consists of LAB. It has been demonstrated that adding some LAB strains, such as *Lactiplantibacillus plantarum* and *Lacticaseibacillus casei* enhances the antibacterial and antioxidant properties of kombucha. Additionally, it has been noted that LAB promotes the formation of glucuronic acid, 1,4 lactone (DSL), and D-saccharic acid during the fermentation process [17].

Geographical and meteorological variables, as well as interference between starter cultures, can cause the yeast species in Kombucha products to differ, particularly those from various countries. *Brettanomyces bruxellensis*, *Hanseniaspora valbyensis*, *Saccharomyces cerevisiae*,

Schizosaccharomyces pombe, Dekkera anomala, Zygosaccharomyces bailii, *Pichia, Candida*, and *Lachancea* were among the yeasts recovered in high concentrations. According to Watawana et al. [18], *Zygosaccharomyces* accounted for 84.1% of the yeast present in kombucha, followed by *Dekkera* (6%) and *Pichia* (5%).

During fermentation, yeasts—particularly osmophilic yeasts—cooperate with acetic acid bacteria to create a surface biofilm that can shield the beer from outside germs, store nutrients, and provide aerobic microorganisms inside the biofilm with access to oxygen [19].

Actinobacteria, Bacteroidetes, Deinococcus-Thermus, Firmicutes, and Proteobacteria are the five major bacterial phyla that Marsh et al. [11] discovered, when they used a metagenomic technique to analyze the microbial composition of kombucha. Ten days later, the results revealed that lactobacilli had lower recovery frequencies and that *Gluconacetobacter* was the most common genus present in all samples. The majority of yeasts (79%–99%) belonged to the genus Zygosaccharomyces. Kaashyap et al. [20] highlighted the significance and predominance of AAB, stating that *Acetobacter* sp. was the most prevalent genus, followed by *Komagataeibacter* and *Gluconobacter*. Depending on the researchers' methods and the raw material they employ, the presence of various genera varies significantly.

The fermentation of kombucha is highly intricate, involving a complex interaction of diverse process microorganisms that influence each other, to yield the distinctive flavor and efficacy. Meng et al. conducted the Spearman correlation analysis on the quantities of yeast and AAB in the microbial communities during various stages of fermentation, revealing a positive correlation and a symbiotic relationship between *Komagataeibacter, Dekkera*, and *Pichia* throughout the fermentation process, while *Komagataeibacter* demonstrating a negative correlation with other fungi [21]. The metabolic activity of yeasts in the kombucha would significantly influence the chemical composition of the product, while bacterial species have a secondary impact on the composition of organic acids. Bacteria cellulose is another major product in kombucha. Many research indicated that only specific combinations of microorganisms can mutually stimulate the formation of cellulose membranes.

Conclusions

The Kombucha starter culture has a varied and mostly unknown microbial makeup. Moreover, there is a lack of information regarding the kinetic development pattern of the predominant fermenting microorganisms during fermentation. Comprehensive information on the predominant bacteria and yeast responsible for Kombucha fermentation would be helpful

for improved control of industrial fermentation operations during the manufacturing of safe, high-quality products. Furthermore, there has been little information on the probiotic potential of Kombucha, although its live cultures have been linked to this.

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Age Estimation of the Trabzon Population of *Mertensiella djanaschvilii* Tartarashvili and Bakradze 1989

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Abstract.

•Context/Purpose With the contribution of molecular studies in recent years, *Mertensiella djanaschvilii*, which was previously defined as a subspecies of the species, was elevated to species level and named as *Mertensiella djanaschvilii*. In this study, the skeletochronology method was used to estimate the ages of the specimens from Trabzon province collected in 2017 and the growth parameters of the species were revealed

•Methods: Specimens were collected from Trabzon and its vicinity in 2017 within the scope of the "Species Conservation Action Plan". The phalanx and femora of the specimens were aged using the skeletochronology method. In addition, SVL measurements of the specimens were taken using a digital caliper with a precision of 0.01mm to reveal the relationship between age and SVL.

•Results: Females of Trabzon population were found to be older than females both in terms of age and head+body length (SVL). The maximum age was 9 years in a female specimen with SVL values of 70.26 mm, while the minimum age was 3 years in two female (SVL= 37.26 mm, 36.99 mm) and a male specimen (SVL= 35.58 mm). The age at sexual maturity was determined as 4 years for both sexes. Mean SVL was 60.56 ± 1.81 mm (35.58 mm – 66.30 mm) for males and 56.11 ± 3.64 mm (36.99 mm - 70.26 mm) for females. In addition, the age of males ranged from 3 to 8 years, with a mean age of 5.93 ± 0.32 years, while the age of females ranged from 3 to 9 years, with a mean age of 5.60 ± 0.61 years.

•Interpretation: The age structure of the Gümüşhane and Giresun populations were previously studied. Accordingly, the results of this study are parallel with the other results. The maximum age found for *Mertensiella djanaschvilii* specimens was determined as 8 for males and 9 for females, while in other studies it was determined as 10-11 for males and 8-9 for females. In general, these results may be due to differences in elevation, feeding and access to food, and it is possible that such a result may occur in terms of the samples evaluated

•Conclusion: In conclusion, the age-SVL relationship of the Trabzon population of *M. djanaschvilii* species were revealed. In addition, Trabzon population was compared with Gümüşhane and Giresun populations in terms of age-SVL relationship. Age estimation and similar studies are important for species affected by these environmental conditions.

Keywords: Caucasian salamander, *Mertensiella djanaschvilii*, Longevity, Age estimation.

Introduction

The Caucasus Salamander *Mertensiella caucasica* (Waga, 1876) s.l., is an endemic salamander to the Caucasus region (Baran et a., 2012; Tok et al., 2017; Çiçek et al., 2019). It has a distribution from distributed in northeastern Anatolia to western Georgia (IUCN, 2024). However, recent studies revealed that, *Mertensiella djanaschvilii*, which was previously defined as a subspecies of the *M. caucasica* (Tartarashvili and Bakradze, 1989; Tarkhnishvili et al., 2000; Tarkhnishvili et al., 2008), was elevated to species level and the taxon in the lesser Caucasus region was named *Mertensiella djanaschvilii* Tartarashvili and Bakradze 1989 (Raffaëlli, 2022).

Widely regarded age estimation method, skeletochronology is known to be the most suitable and safest method for determining the age of ecthotermic animals such as lizards (Castanet, 1994). With this method, species' growth patterns and demographic analyses can also be provided (Augert, 1992). Through skeletochronology, the effect of the seasonal conditions on growth rates can be seen and estimate the approximate age of individuals based on LAGs (Lines of Arrested Growths). However, body size and age do not always correlate directly in animals. For instance, the largest individuals are not necessarily the oldest. Typically, individuals with slower, more gradual growth tend to live longer (Smirina, 1994).

The aim of the study is to estimate the ages of the *M. djanaschvilii* from Trabzon province collected in 2017 by the skeletochronology method and to examine the growth parameters.

Materials and Methods

In this study a total of 26 *M. djanaschvilii* specimens (16 ♂♂, 10 ♀♀) were used collected from Trabzon and its vicinity in 2017 within the scope of the "Species Conservation Action Plan". The phalanx and femora of the specimens were used for the skeletochronology method. The bones were decalcified in 5% nitric acid for 3 hours, and cross-sections of 12 µm were stained with Ehrlich's hematoxylin. In addition, snouth-venth length (SVL) measurements of the specimens were taken using a digital caliper with a precision of 0.01mm to reveal the relationship between age and SVL.

The cross-sections were examined under a light microscope (Olympus BX51) photographed (Olympus Analysis LS). IBM SPSS Statistics 20.0 was used for statistical evaluations at a 95% confidence interval.

Results

Sexual maturity age was determined as 4 years for both sexes. The mean age of males were 5.93 ± 0.32 years (ranged from 3 to 8 years); while the mean age of females were 5.60 ± 0.61 years (ranged from 3 to 9 years) (Table1; Fig-1-2). The minimum age was estimates three years for three specimens; two female (SVL= 37.26 mm, 36.99 mm) and a male specimen (SVL= 35.58 mm) (Table 1). the maximum age was 9 years in a female specimen with SVL values of 70.26 mm (Table 1). The SVL of the males ranged from 35.58 mm to 66.30 mm (mean: 60.56 ± 1.81 mm), and the SVL of the females ranged from 36.99 mm to 70.26 mm (mean 56.11 ± 3.64 mm).

Table 1. Descriptive statistics of age and SVL of *M. djanaschvilii* (N: number of specimens Min: minimum, Max: maximum, SE: standard error, SD: standard deviation).

	N	Min.	Mean	Max.	SE	SD
Age ♂♂	16	3	5.93	8	0.32	1.28
SVL ♂♂	16	35.58	60.56	66.30	1.81	7.26
Age ♀♀	10	3	5.6	9	0.61	3.82
SVL ♀♀	10	36.99	56.11	70.26	3.64	11.54

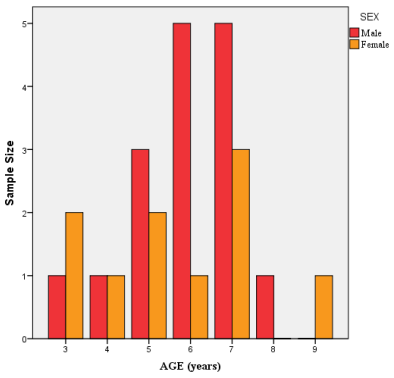

Fig. 1. Number of individuals by age class of *M. djanaschvilii* in males and females.

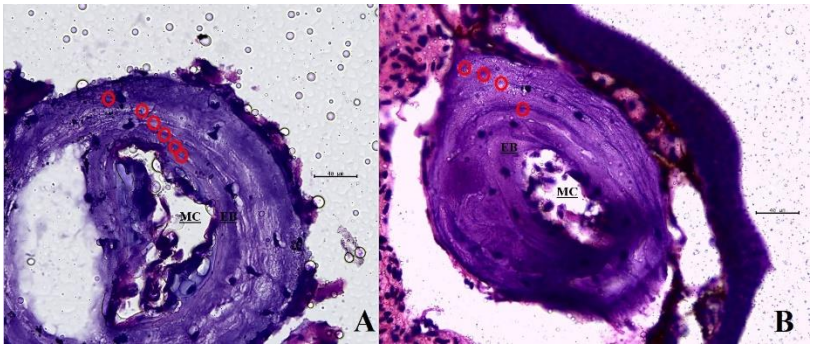

Fig. 2. A cross-section of the phalanges of **A.** a 6-year-old-male (Hematoxylen) MC: Medular cavity; EB: Endosteal Bone; O: LAG-Lines of Arrested Growth; **B.** a 4-year-old-female (Hematoxylen) MC: Medular cavity; EB: Endosteal Bone; O: LAG-Lines of Arrested Growth.

Discussion

The results of this study show similarities with the previous studies about the age estimation of *M. djanaschvilii*. Gümüşhane (Beşer et al., 2017) and Giresun (Üzüm et al., 2009) populations of *M. djanaschvilii* were studied and the ages were found as 5-10 (Üzüm et al., 2009) and 5-11 (Beşer et al., 2017) for males and 4-9 (Üzüm et al., 2009) and 4-9 (Beşer et al., 2017) for females (Table 2) while in this study age were found as 8 for males and 9 for females. Reason of the differences in results, it could be the result of feeding and access to food, and may occur in terms of the specimens evaluated.

Table 2. Comparing of age and SVL of *M. djanaschvilii* (N: number of specimens).

	Üzüm et al., 2009 Giresun			Beşer et al., 2017 Gümüşhane			This study Trabzon		
	N	Mean	Range	N	Mean	Range	N	Mean	Range
♂♂									
Age	19	7.26	5-10	28	7.42	5-11	16	5.93	3-8
SVL	19	67.53	63.10-73.36	28	64.24	57.4-74.5	16	60.56	35.58-66.30
♀♀									
Age	13	6.00	4-9	30	5.8	4-8	10	5.6	3-9
SVL	13	63.77	51.28-76.60	30	58.45	51.00-69.2	10	56.11	36.99-70.26

Conclusions

In conclusion, the age-SVL relationship of the Trabzon population of *M. djanaschvilii* species were revealed. In addition, Trabzon population was compared with Gümüşhane and Giresun populations in terms of age-SVL relationship. Age estimation and similar studies are important for species affected by these environmental conditions.

Acknowledgement

We would like to thank project entitled "The species action plan of Caucasian salamander, *Mertensiella caucasica*, in Artvin, Giresun, Gümüşhane, Rize and Trabzon provinces", funded by the Directorate of Trabzon Branch of the 12th Regional Directorate of the Department of Nature Protection and National Parks of the Republic of Turkey, Ministry of Forestry and Water Affairs, and AnaDOKU, and 1919B012339365-2209A-Tübitak project for the support to the study.

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Is Skeletochronology a Practicable Method for Longevity of the Tawny Owl, *Strix aluco* (Linnaeus, 1758) (Strigiformes: Strigidae)?

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Abstract. Context/Purpose: The age and longevity of animals have long been subjects of scientific interest. Various methods are currently used to estimate the ages of different animal groups. One such method, skeletochronology, is considered reliable for determining the age and growth parameters of ectothermic organisms like fish, amphibians, and reptiles. Recent literature, however, suggests that this method can also be used for endothermic organisms, such as mammals and birds. Studies on birds have shown that the bone types in which age-related structures such as LAGs (Lines of arrested growth) are most clearly visible may vary across species.

•Methods: In this study, the age of a male Tawny Owl (Strix aluco) specimen was determined using the skeletochronology method on the axis, cervical vertebra, tibiotarsus, and phalanges. The specimen found deceased on the Çanakkale-Güzelyalı highway in May 2024. The bones were dissected and preserved in 70% ethyl alcohol. For decalcification, a 5% nitric acid solution was applied for a period of 3 to 15 hours, depending on the size and thickness of each bone. After decalcification, the bones were rinsed in running water for 12 hours to remove any residual acid. They were then embedded in paraffin, and 12 µm thick cross-sections were obtained using a rotary microtome. Two different staining solutions, toluidine blue and hematoxylin, were used to see differences in staining methods. The slides were examined under a light microscope and photographed.

•Results: Upon examining the various bone tissues, LAGs were observed in distal regions, consistent with previous studies in literature. Analysis of the tissues revealed that the specimen was 3 years old across all bones. Furthermore, it was noted that different bone tissues varied in terms of both staining properties and their alignment with skeletochronology.

•Interpretation: The clearest images were observed in the sections taken from the longest phalanges. In the tibiotarsus sections, three LAGs were detected in the distal part of the tissue, although the image was less distinct compared to the axis. When the vertebral sections were evaluated, the same age was determined in both Toluidine blue- and hematoxylin-stained tissues, with a clearer result obtained from the Toluidine blue-stained ones. In the longest phalanges, three LAGs were identified in the distal sections which was consistent with the findings in other tissues. In this case, slides stained with Toluidine blue provided a clearer result, making the counting of LAGs easier.

•Conclusion: In this study, the axis, cervical vertebrae, tibiotarsus, and phalanges of Strix aluco were utilized for skeletochronology, and the estimated age across all bones was determined to be 3 years. The phalanges were found to be the most suitable tissue for age determination using this method.

Additionally, staining with Toluidine blue provided much clearer results compared to hematoxylin. This study also supports the notion that skeletochronology can be a valuable method for age estimation in birds.

Keywords: Tawny owl, *Strix aluco*, road kills, Longevity, Age estimation.

Introduction

Strix aluco is widely distributed across the Palearctic region. Currently, 11 subspecies of tawny owls have been identified. They inhabit open areas, deciduous or mixed forests and woodlands, agricultural landscapes with trees, parks, cemeteries, and large gardens, often favoring locations with access to water. Tawny owls are medium-sized, compact birds. They have an average body length of 38 cm and a wingspan of approximately 1 meter, features that contribute to their adaptability within diverse habitats.

The typical lifespan of *S. aluco* in the wild is approximately 4 years. However, the longest recorded lifespan of a wild tawny owl was 21 years and 5 months. In captivity, individuals can live over 27 years. Both male and female *S. aluco* typically reach reproductive maturity at an average age of 1 year. The annual survival rate for adult tawny owls is 73.8%, while the rate for juveniles is significantly lower at 30.1%. Juveniles face high mortality rates primarily due to starvation if they fail to establish a vacant territory after fledging. (Snow & Perrins, 1998; Voous, 1988).

The age and longevity of animals have long been subjects of scientific interest. Various methods are currently used to estimate the ages of different animal groups. One such method, skeletochronology, is considered reliable for determining the age and growth parameters of ectothermic organisms like fish, amphibians, and reptiles. Recent literature, however, suggests that this method can also be used for endothermic organisms, such as mammals and birds (Castanet et al., 2000; Castanet et al. 2004). Studies on birds have shown that the bone types in which age-related structures such as LAGs (Lines of arrested growth) are most clearly visible may vary across species.

Materials and Methods

In this study, the age of a male (1♂) *Strix aluco* (Tawny owl) specimen was determined using the skeletochronology method, focusing on the axis, cervical vertebra, tibiotarsus, and phalanges. The specimen was found deceased on the Çanakkale-Güzelyalı highway in May 2024. The bones were dissected and preserved in 70% ethyl alcohol. Decalcification was performed using a 5% nitric acid solution, with the duration varying between 3 and 15 hours depending on the size and thickness of the bone. Following decalcification, the bones were rinsed under running water for 12 hours to eliminate any residual acid and subsequently

embedded in paraffin. Cross-sections, 12 µm in thickness, were prepared using a rotary microtome. To assess the differences in staining methods, two different staining solutions, toluidine blue and hematoxylin, were applied. The prepared slides were examined under a light microscope and photographed for further analysis.

Results

Upon examination of the tissues, it was determined that each sample corresponded to an age of 3 years. Additionally, differences were observed among tissues in terms of staining clarity and consistency with skeletal chronology.

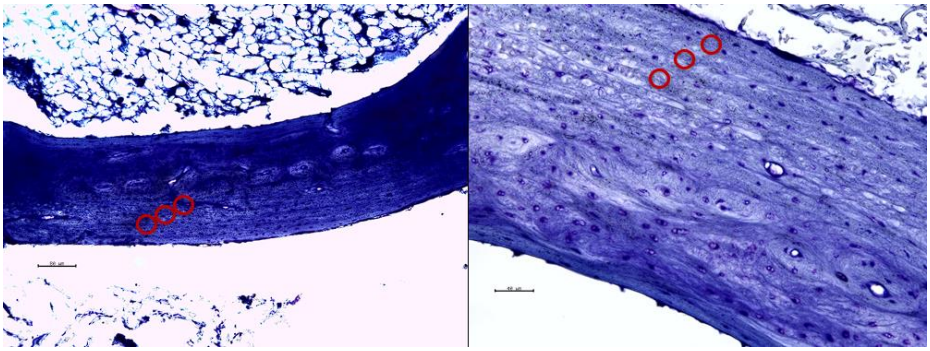

Fig. 1. Cross-sections of the phalanges stained with Toluidine Blue.

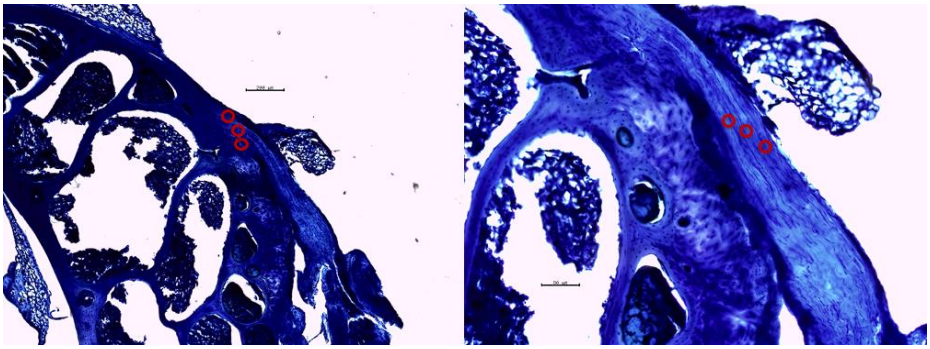

Fig. 2. Cross-sections of the tibiotarsus stained with Toluidine Blue.

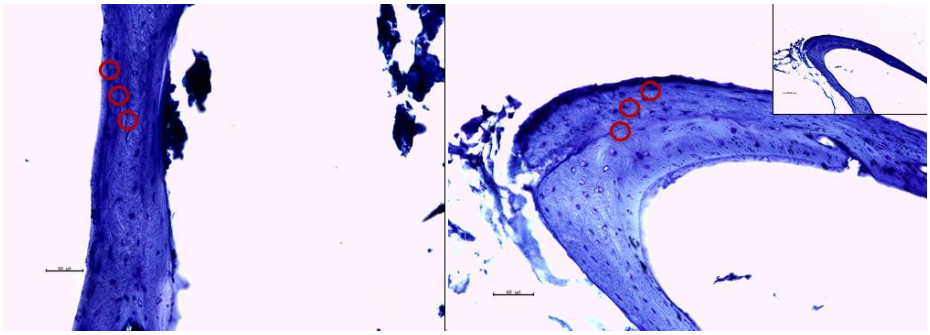

Fig. 3. Cross-sections of the axis stained with Toluidine Blue.

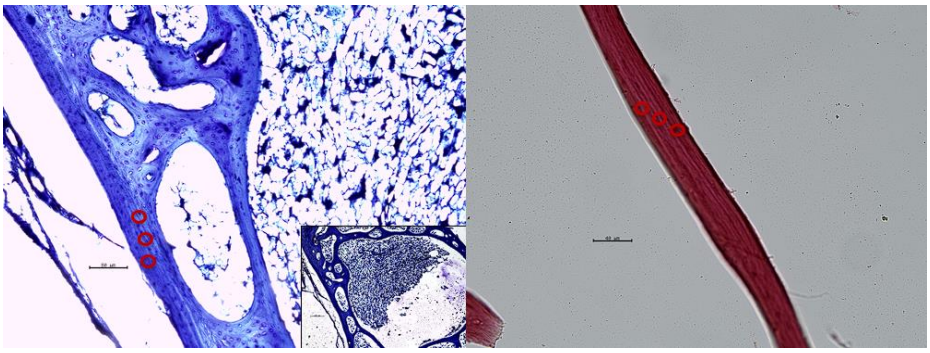

Fig. 4. Cross-sections of the vertebra stained with Toluidine Blue and Hematoxylin.

The clearest images were obtained from sections taken from phalanges (Figure 1). Conversely, in the tibiotarsus sections, 3 LAGs (lines of arrested growth) were identified at the distal end of the tissue (Figure 2); however, the clarity of these sections was lower compared to those from the axis (Figure 3).

When cross-sections of vertebral bodies were analyzed, the same age was determined in tissues stained with both Toluidine Blue and Hematoxylin. However, the tissues stained with Toluidine Blue provided clearer results (Figure 4).

Discussion

The clearest images were observed in the sections taken from the longest phalanges and axis. In the tibiotarsus sections, three LAGs were detected in the distal part of the tissue, although the image was less distinct compared to the axis. When the vertebral sections were evaluated, the same age was determined in both Toluidine blue- and hematoxylin-stained tissues, with a clearer result obtained from the Toluidine blue-stained ones. In the longest phalanges, three LAGs were identified in the distal sections which was consistent with the findings in other tissues. In this case, slides stained with Toluidine blue provided a clearer result, making the counting of LAGs easier.

Conclusions

In this study, the axis, cervical vertebrae, tibiotarsus, and phalanges of *Strix aluco* were utilized for skeletochronology, and the estimated age across all bones was determined to be 3 years. The axis and phalanges were found to be the most suitable tissue for age determination using this method. Additionally, staining with Toluidine blue provided much clearer results compared to hematoxylin. This study also supports the notion that skeletochronology can be a valuable method for age estimation in birds.

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Anatomical and Morphological Investigation of Some Lichenized Fungi from Usambara Mountains -Tanga, Tanzania

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Abstract. This study used morphological and anatomical investigations and DNA barcoding method to determine the biodiversity of lichenized fungi in the Usambara Mountains located in Tanga region of Tanzania. Lichens are symbiotic associations formed by the combination of a fungal partner and an algal and/or cyanobacterial partner. Usambara Mountains have a significant range in elevation with peaks ranging up to 2400 meters. Knowledge of lichenized fungi is relatively limited regardless of Usambara Mountains being a biodiversity hotspot with a variety of endemic species. In this context, the aim of this study is to examine anatomically-morphologically and DNA barcoding based on ITS phylogeny of selected lichenized fungi species from Usambara Mountains. The collected lichen samples were identified by anatomical, morphological examinations or/and DNA barcoding which resulted into identification of 5 species of lichenized fungi: *Bacidia schweinitzii* (Fr. ex E. Michener) A. Schneid., *Graphis sticta* (L.) Ach. *Lecanora chlarotera* Nyl, *Lecanora strobilina* Ach., *Parmelia sulcata* Taylor. The findings contribute to the understanding of lichen diversity and adaptation in the East African highlands, with implications for biodiversity conservation and ecosystem monitoring.

Keywords: Lichenized fungi, Biodiversity, Tanzania, Usambara Mountains.

Introduction

Studies on lichens in Tanzania are quite limited. In these studies, macrolichens have generally been addressed and determinations have been made using traditional methods. Molecular studies have also been quite limited. Climate change and deforestation are among the biggest threats to biological diversity in Tanzania. Lichens need to be studied both to successfully direct conservation measures in areas and habitats that are important for general biological diversity in Tanzania and to reveal lichen biodiversity. In this context, this study used morphological and anatomical investigations and DNA barcoding method to determine the biodiversity of lichenized fungi in the Usambara Mountains located in Tanga region of Tanzania.

Materials and Methods

In the study, field work was carried out in the Western Usambara Mountains in the Tanga region of Tanzania. In the field work, lichen samples were collected from natural forest reserves such as Magamba Natural Forest, Mkusu Basin and artificial forest reserves such as TAFORI Forest. Lichen samples were repackaged and brought to Kayseri (Turkey) for species identification. The transported samples were coded and kept in the Erciyes University Lichen Herbarium (ERCH). Morphological, anatomical and chemical examinations of the lichen samples were carried out in the Erciyes University Lichen Herbarium. All morphological-anatomical examinations were performed under stereomicroscope and light microscope. Chemical reagents were also used to determine the secondary components in the identification of species. DNA isolation method was performed using DNeasy Plant Mini Kit developed by Qiagen and the protocol given in the kit was taken into consideration during the isolation process. After isolation, lichen samples were loaded onto 1.2% agarose gel. After running at 80 watts for 75 min, the presence of DNA bands was determined under UV light. Primers covering the relevant region were used to amplify the ITS gene regions of DNA obtained from the studied lichen samples under appropriate PCR conditions. PCR amplification with isolated samples was performed using 2 x Taq PCR MasterMix. Polymerase chain reaction (PCR) was performed using 25 µL PCR Master Mix containing 2 µL 10 pmol bi-directional primer, 4 µL gDNA and 19 µL sterile distilled water in a final volume of 50 µL. Primers ITS1–F (CTTGGTCATTTAGAGAAGTAA) and ITS 4 (TCCTCCGCTTATTGATATGC) were used as primer pairs for the ITS gene region (Gardes and Bruns 1993; White et al. 1990). The thermal cycling conditions included an initial denaturation step of 95°C for 5min, followed by 35 cycles of 95°C for 45sec (denaturation), 54°C for 45sec (annealing), and 72°C for 60sec (extension) followed by a final extension period of 72°C for 10min.Purification of PCR samples and DNA sequence analysis were performed in two-way (forward and reverse) service acquisition. Phylogenetic analyses were performed based on the ITS gene region sequence data. Newly generated sequences were subjected to a BLAST search to evaluate their affinities and to assist in taxon sampling for phylogeny. Sequences were aligned manually using the automatic Clustal W feature in a BioEdit v.7.2 program (Hall, 1999). Uncertain regions were delimited and excluded from the alignment. Phylogenetic relationships among taxa were investigated using MEGA 11 software (Tamura et al., 2019). The dataset was analyzed using the maximum likelihood (ML) method

and Bootstrap support values were obtained using 1,000 replicates. Outgroups used in phylogenetic trees were selected to be phylogenetically related to ingroups.

Results

Bacidia schweinitzii (Fr. Ex Michener) A. Schneid.

Description: Thallus corticolous, cream-gray, matt, as continuous patches forming by small granules. Granules are small 0.05–0.1 mm diam. Prothallus present, brownish black. Apothecia present, black or sometimes brownish, sessile, round and irregular, 0.1–0.5 mm diam, sometimes slightly pruinose. Thalline margin present, very thin, prominent, concolorous with apothecial disc. Epihymenium brown, 10–20 µm. Hymenium hyaline, oil droplets present or not, 100 µm. Hypothecium brown, 60 µm. Ascus 8-spored. Ascospores hyaline, acicular, ends are pointed or not, 3–15 septate, 32–88 × 2–4 µm (Fig. 1).

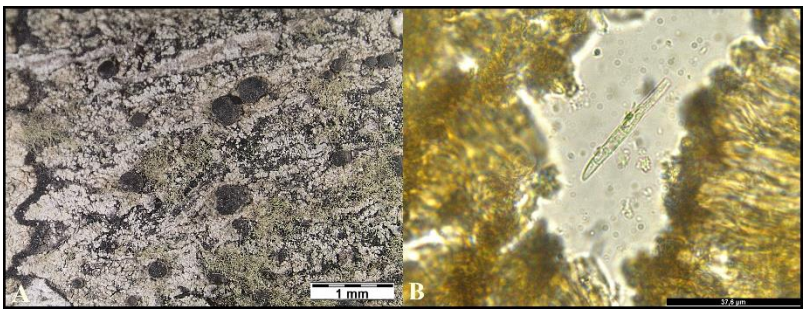

Fig. 2. *Bacidia schweinitzii.* A. Habitus, B. Ascospore.

Specimen examined: Tanzania, Tanga, West Usambara Mountains, MKUSU forest, 4.76042°S, 38.35681°E, alt. 1603m, 08.01.2021, leg. Z. N. MKWAYU. (ERCH ZAN0.076).

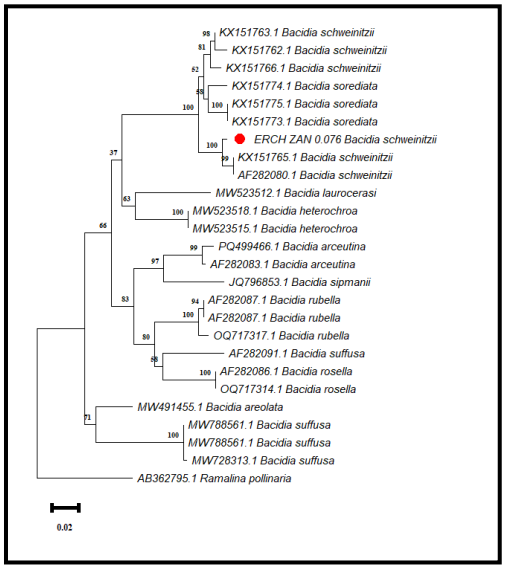

Fig. 3. Maximum Likelihood Dendrogram derived from ITS gene region sequences of *Bacidia schweinitzii* and related species retrieved from GenBank.

Ecology and Distribution: *Bacidia schweinitzii* is known from eastern North America and eastern Asia (Ekman, 2009). It has been reported from Kenya in Africa. In North America, it is widespread in temperate and subtropical regions from Florida north to Canada and the western borders of the arid steppes. It is common and abundant in moist habitats, especially in flooded marshes or mountain ridges that are frequently exposed to fog or dew. The species grows on a wide variety of hardwoods and conifers and shrubs (Lendemer, 2022).

***Graphis sticta* (L.) Ach.**

Description: Thallus on bark, almost immersed to bark, reduced. When apparent as greenish cream patches. Lirallae present, abundant, of various shapes and lengths, branched or unbranched, usually 0.7–1 mm long and 0.1–0.3 mm wide, apex usually pointed. Ascospores 5–13 septate, colorless, 20–65 × 5–10 µm. All spot tests are negative. Algae *Trentepohlia.* (Fig. 2).

Fig. 4. *Graphis sticta*

Specimen examined: Tanzania, Tanga, West Usambara Mountains, MKUSU forest, 4.74393°S, 38.28575°E, alt.1688m, 09.01.2021, leg. Z. N. MKWAYU. (ERCH ZAN 0.043).

Ecology and Distribution: It grows on the smooth bark of deciduous trees, usually in moist montane forests. It is quite widespread worldwide, including tropical regions and restricted to temperate regions (Nash et al., 2004).

***Lecanora chlarotera* Nyl.**

Description. Thallus crustose, as continuous patches with swollen-puffed granules, smooth or rough. Apothecia present, mostly aggregated, lecanorine, sessile. Apothecial disc brownish red, generally roundish, concolorous with thallus. Epihymenium light brown, 20 µm. Hymenium hyaline, 75 µm. Ascus 8-spored. Ascospores simple, hyaline, 10–15 × 6–8 µm. Thallus K+ yellow, C, KC+yellow, Pd- (Fig. 4).

Fig. 5. *Lecanora chlarotera*

Specimen examined: Tanzania, Tanga, West Usambara Mountains, MKUSU forest, 4.740705° S, 38.28091° E, alt. 1707 m, 08.01.2021, leg. Z. N. MKWAYU. (ERCH ZAN 0.001).

Ecology and Distribution: It is a cosmopolitan species occurring on a variety of tree barks in Africa, Asia, Europe and North and South America (Nash et al., 2004).

***Lecanora strobilina* (Sprengel) Kieffer**

Description: Thallus crustose, continuous, as little granules, new areoles forming in some parts of the thallus, greenish white or cream. Apothecia present, 0.1–0.9 mm, sessile, lecanorine. Apothecial disc smooth, rounded, dull brownish yellow. Apothecial margin prominent and distinct, concolorous with thallus, crenulate. Epihymenium hyaline, 25 µm. Hymenium hyaline, 80 µm. Ascus 8-spored. Ascospores simple, hyaline, 10–16 × 4–7 µm. Thallus K+ yellow, C, KC+yellow, Pd-. (Fig. 5).

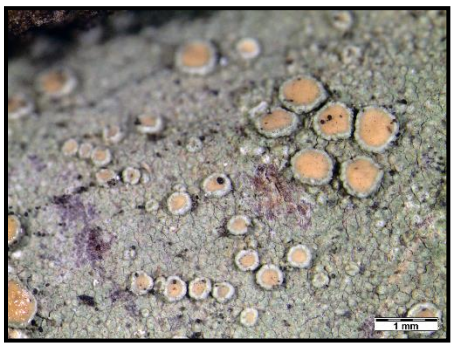

Fig. 6. *Lecanora strobilina.*

Specimen examined: Tanzania, Tanga, Tafori, West Usambara Mountains, MKUSU forest, 4.74393°S, 38.28575°E, alt.1688 m, 09.01.2021, leg. Z. N. MKWAYU (ERCH, ZAN 0.031).

Ecology and Distribution: The species, known from Europe, Macaronesia, North America, Asia and Africa, grows on various tree barks, especially conifers (Smith et al., 2009).

***Parmelia sulcata* Taylor**

Description: Thallus up to 7 cm in diameter. Branched, often forming rosettes or randomly composed of complex lobes. Lobes usually up to 5 mm in diameter, with truncated, separated or continuously smooth tips. Upper surface greyish green, sometimes with white pruinose. Pseudocyphella present. Pseudocyphella white, usually coarse. Soralia present. Soredia granular, coarse, clustered especially in the central part, whitish green. Lower part black or brown. Apothecia not observed. Thallus, medulla and soralia C-, K+ orange, Pd+ orange (Figure 6).

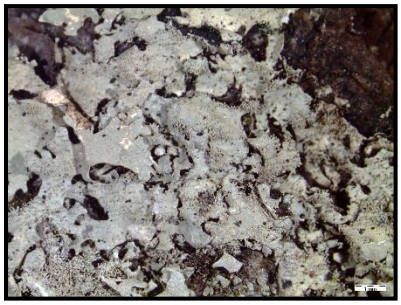

Fig. 7. *Parmelia sulcata.*

Specimen examined: Tanzania, Tanga, Shume, West Usambara Mountains, 4.73086°S, 38.2742°E, alt.1592m, 08.01.2021, leg. Z. N. MKWAYU (ERCH, ZAN 0.020).

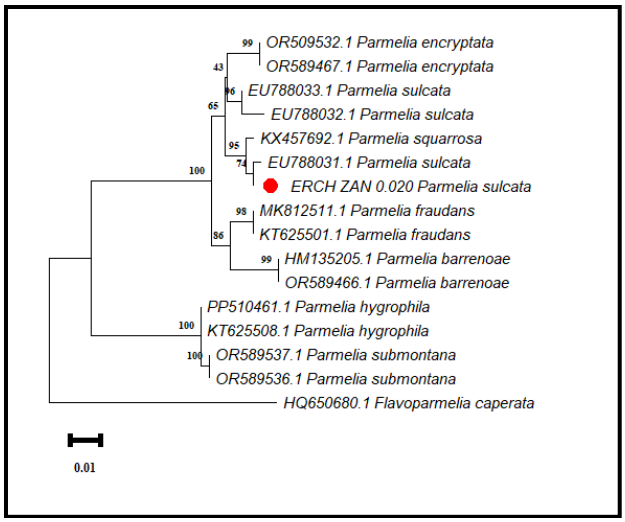

Fig. 8. Maximum Likelihood Dendrogram derived from ITS gene region sequences of *Parmelia sulcata* and related species retrieved from GenBank.

Ecology and Distribution: It is a cosmopolitan species and grows on a variety of substrates including tree barks and rocks (Smith et al., 2009).

Discussion

In this study, a species was identified as *B. schweinitzii* by examining it anatomically, morphologically, chemically, and ecologically. The species' diagnosis was also confirmed by ITS gene phylogeny. *B. schweinitzii* was determined from Tanzania for the first time in this study and is a new record for Tanzania. Anatomically and morphologically, *B. schweinitzii* is most similar to the species *Bacidia ekmaniana* R.C. Harris, Ladd & Lendemer. In both, the apothecia can be in brown, reddish-brown, and black. The most reliable distinction between the two species can be made by the presence of reddish brown pigments in the hypothecium (Lendemer, 2022). *B. schweinitzii* is also very similar to *Bacidia purpurans* Harris, Lendemer & Ladd. However, *B. purpurans* is distinguished from *B. schweinitzii* by its K+ purple epihymenium (Lendemer et al., 2016). Phylogenetically, *B. schweinitzii* is very closely related to *Bacidia sorediata* Lendemer & R.C.Harris. *B. sorediata,* differs clearly from *B. schweinitzii* by containing soredia (Lendemer et al., 2016).

In this study, a specimen was identified as *G. scripta* by examining it anatomically, morphologically, chemically and ecologically. *G. scripta* is quite similar to the species *Graphis elegans* (Borrer ex Sm.) Ach. It is quite difficult to distinguish the two species, especially in young specimens. In well-developed specimens, the thallus is almost completely immersed in the bark of both trees and the apothecia have a striated structure or remain flat. In mature forms, there are no distinct stripes on the edges of the apothecia of *G. scripta*, they are flat. However, stripes present on the edge of the apothecia of *G. elegans*. On the other hand, the thallus of *G. scripta* gives negative results with K, while K+ is red in *G. elegans* (Smith et al., 2009).

In this study, a specimen was identified as *L. chlarotera* by examining it anatomically, morphologically, chemically and ecologically. *L. chlarotera* is a highly variable species in terms of apothecial color. It can be in various colors from light yellow, cream tones to red, and from red to dark brown. Anatomically and morphologically, it is very similar to the saxicolous species *Lecanora campestris* (Schaer.) Hue. However, the two species are separated by occurring on different substrates (Smith et al., 2009).

In this study, a specimen was identified as *L. strobilina* by examining it anatomically, morphologically, chemically and ecologically. *L. strobilina* is a member of the *L. polytropa* group. C- chemotypes can be confused with *Lecanora symmicta* (Ach.) Ach. However, *L. strobilina* is distinguished from *L. symmicta* by its shorter hymenium, more yellowish thallus

and apothecial disc and corticolous thallus (Smith et al., 2009). On the other hand, this species is quite similar to *Lecanora confusoides* Bungartz & Printzen, which is seen in similar habitats. However, *L. confusoides* contains xanthones and gives C+ orange reaction. Its thallus is duller and paler, and its apothecia usually has an olive greenish or grayish black tinge (Bungartz et al., 2020).

In this study, a sample was identified as *P. sulcata* by examining it anatomically, morphologically, chemically and ecologically. The species identification was also confirmed by ITS gene phylogeny. *P. sulcata* is quite similar to *P. submontana* Hale, which is also a sorediate species. True soredia are present in *P. sulcata* and the lobes have up-rolled margins. However, soredia are very similar when comparing the isidia in *P. submontana* and the lobes characteristically have down-rolled margins (Smith et al., 2009). The species that *P. sulcata* is most confused with in the field is *Parmelia saxatilis* (L.) Ach. However, while soredia are present in *P. sulcata*, isidia are present in *P. saxatilis* (Smith et al., 2009).

Conclusions

In this study we examined anatomically-morphologically and DNA barcoding based on ITS phylogeny of selected lichenized fungi species from Usambara Mountains. This species are *Bacidia schweinitzii* (Fr. ex E. Michener) A. Schneid., *Graphis sticta* (L.) Ach. *Lecanora chlarotera* Nyl, *Lecanora strobilina* Ach., *Parmelia sulcata* Taylor. *B. schweinitzii* is a new record for Tanzania.

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Synthesis and Characterization of Organic@Inorganic Cu Hybrid Nanoflower With *Salix alba* Extract

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Abstract. In this study, organic@inorganic Cu hybrid nanoflowers (hNFs) were synthesized by the coordination of *Salix alba* extract and Cu^{+2}. The optimum synthesis condition was determined by characterization tests with FE-SEM, EDX and EDX analysis of hNFs synthesized with 0.5, 1 and 5 mg/L plant extract in PBS buffer prepared at different pHs. hNFs were not formed in conditions where the PBS buffer was acidic (pH 5). The optimum synthesis of hNFs was achieved in the presence of 1 ml of extract and the pH of the PBS buffer at 7.4. It was recorded that hNFs synthesized under optimum conditions had a diameter range of 24-41 μm and a petal thickness of 21-31 nm, similar to the ideal flower morphology. In addition, it was observed that the petals forming the hNFs obtained under these synthesis conditions came together irregularly and had a morphology far from an ideal flower form. The presence of C, O, P, N and Cu elements in the structural component of hNFs formed by the coordination of *S. alba* extract and Cu element in PBS buffer (pH 7.4) was demonstrated by EDX mapping. The presence of O-H (alcohol), N-H (amine) and primary phosphate crystals was revealed by FT-IR analysis.

Keywords: *Salix alba*, Nanoflower, Copper

Introduction

Nanoparticles (NPs) with a size range of 1-100 nm a wide range of applications in the industrial field due to their superior properties. NPs are classified into various groups considering their size, metal derivative and structural properties, and are classified as carbon-based, inorganic and organic NPs according to their structural properties [1]. Flower-shaped organic-inorganic hybrid nanostructures, which are an interesting form of nanomaterials, have attracted the attention of researchers due to their catalytic activity and enzyme immobilization. It has been stated that flower-shaped hybrid nanostructures can be used in solar cells [2], sensors [3,4], purification processes [5-7], enzyme and antimicrobial applications [8,9]. However, the use of expensive biomolecules such as enzymes, DNA and hormones as organic components in the synthesis of flower-shaped hybrid nanostructures limits their use due to both cost and limited supply [10-13], therefore, studies are being conducted on the adequacy of use of various plant extracts as an alternative to the mentioned biomolecules in the synthesis. In recent years, it is available in the literature that allicin and curcumin herbal active ingredients, lemon, orange peel extracts, *Camellia sinensis*, *Ascoseira*

mirabilis, *Persea americana* and *Kalanchoe daigremontiana* as organic components in the synthesis of flower-shaped organic-inorganic hybrid nanostructures have been synthesized and have been successful in industrial applications [14-19]. It has been observed in the literature that plant extracts are widely used in the synthesis of many metallic NPs, while there are limited studies on the synthesis of flower-shaped organic-inorganic hybrid nanostructures [19]. In this study, while there are many studies on the synthesis and potential applications of metallic NPs such as Au, Ag and cobalt ferrite [20-22], which were selected as bio-extracts, no study was found on the synthesis of hNFs with *S. alba* leaf extract. The proposed project is original and innovative in this respect.

Materyal ve Metod

After the *S. alba* leaves were sorted and washed, they were dried (1 night at 70 ^{o}C) to obtain the extract used in the synthesis of hNF. Dried leaves (10 g) were taken and brewed in 100 ml of distilled water (1 hour, 80 ^{o}C), then filtered through Whatman No 1 filter paper and the extract to be obtained was stored at +4 ^{o}C to be used in the synthesis of hNF. The effects of pH and bioagent concentration on the morphology and synthesis success of hNF were determined and the optimum synthesis conditions were determined and used in applications. For this purpose, leaf extract at different concentrations (0.5, 1 and 5 mg/L) was vortexed with $CuSO_4$ ($8x10^{-4}$ M, 0.35 ml) in PBS buffer (10 mM) at different pHs (5-9) and the reaction was ensured. After the mixture was incubated in the dark (3 days, 4 ^{o}C), the sediments formed at the bottom of the tubes were centrifuged at 10000 rpm for 10 minutes to obtain the pellet part. The pellet part containing the was washed with distilled water and dried in an oven (1 night, 70 ^{o}C) and used in characterization studies. The morphology of the hNFs was determined by FE-SEM, the elemental composition by EDX and the presence of biomolecules playing a role in the synthesis by FT-IR analysis and their characterization was evaluated [23].

Result and Discussion

The morphologies of the synthesized hNFs using *S. alba* extract as an organic component were determined by FE-SEM analysis. hNF was not formed in conditions where the PBS buffer was acidic (pH 5). The optimum synthesis of hNFs was achieved in the presence of 1 ml of extract and the pH of the PBS buffer was 7.4. It was recorded that the hNFs synthesized under optimum conditions had a diameter range of 24-41 μm and a petal thickness of 21-31 nm, similar to the ideal flower morphology (Figure 1). In addition, it was observed that the

petals forming the hNFs obtained under these synthesis conditions came together irregularly and had a morphology far from an ideal flower form.

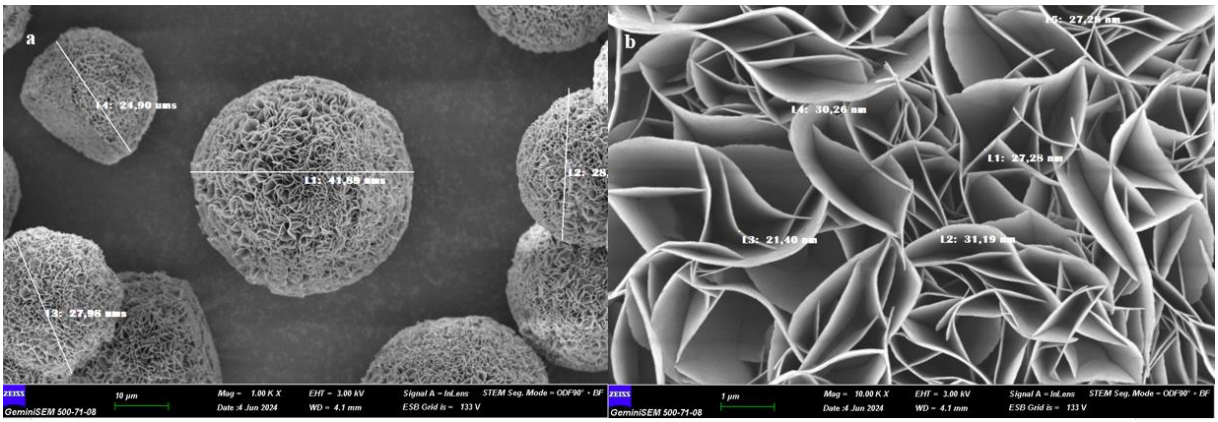

Fig. 1. FE-SEM image of hNFs. a) Diameter lengths of hNFs, b) Petal thicknesses of hNFs

The presence of elements participating in the structure of hNFs formed by the coordination of *S. alba* extract and Cu element in PBS buffer (pH 7.4) was determined by EDX analysis. The presence of C, O, P, N and Cu elements in the structural component of HNFs was shown by EDX mapping (Figure 2).

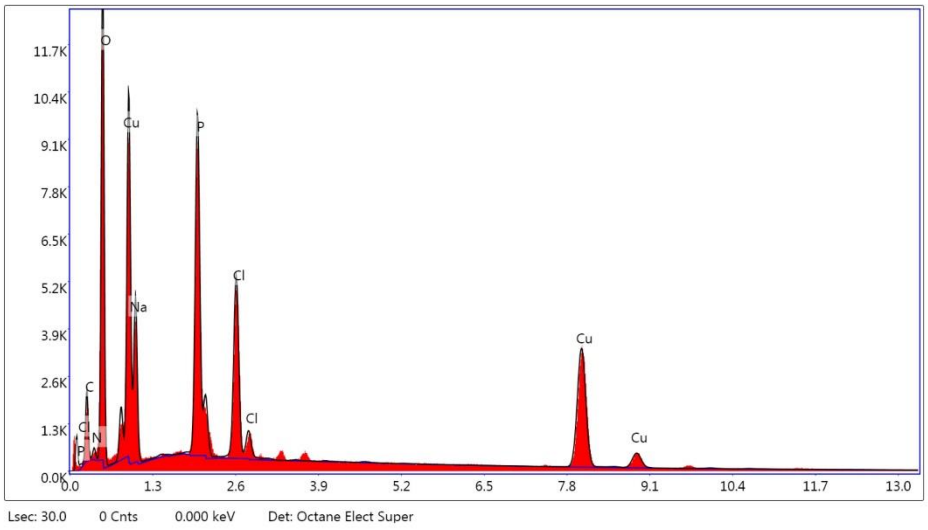

Fig. 2. EDX analyses of hNFs

FT-IR analysis was used to determine the functional groups in the structure of hNFs synthesized with *S. alba* extract (1 ml) in PBS buffer (pH 7.4) (Figure 3). The peaks observed at 2926 cm^{-1} and 1621 cm^{-1} in the FT-IR diagram correspond to O-H (alcohol) and N-H (amine) groups, respectively. The peaks observed at 1146 cm^{-1}, 1034 cm^{-1}, 986 cm^{-1}, 624 cm^{-1} and 558 cm^{-1} in the analysis indicate the formation of primary phosphate crystals formed during the nucleation stage of the synthesis of hNFs in PBS buffer [15, 23, 24].

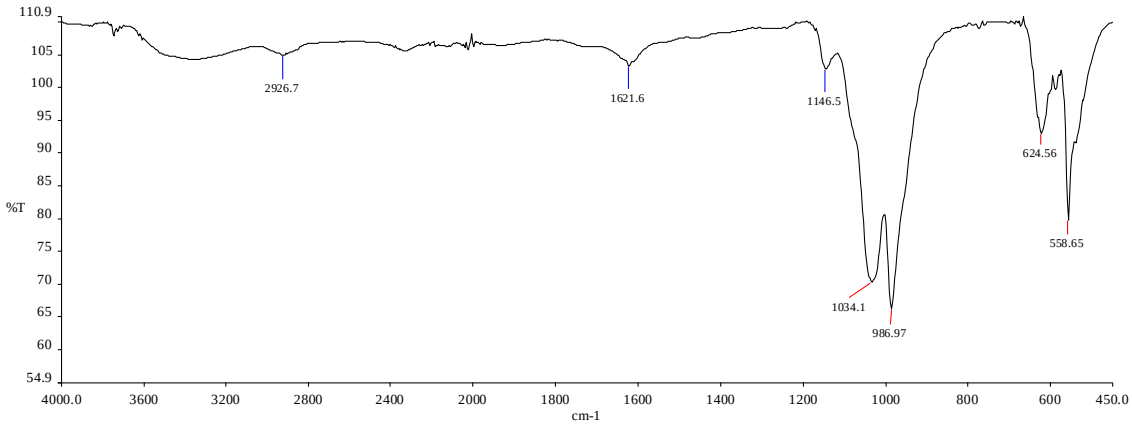

Fig. 3. FT-IR analysis of hNFs

Conclusion

As a result, the synthesis of Cu-based hNFs was carried out with *Salix alba* extract. The synthesis of hNFs depends on the organic component concentration and the pH of the PBS buffer in which the synthesis takes place. The data obtained in the study is thought to be applicable for the synthesis and different applications of hNFs.

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Synthesis and Characterization of Organic@Inorganic Cu Hybrid Nanoflower with *Mentha piperita* Extract

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Abstract. In this study where hNFs were synthesized with Mentha piperita extract, the organic component (plant extract) increasing concentration from 0.5 mg/l to 1 mg/l and inorganic component (Cu^{+2}) were coordinated in acidic, neutral and basic PBS buffer. The optimum synthesis condition was determined by characterization tests with FE-SEM, EDX and EDX. The morphologies of hNFs with organic components of mint extract were determined by FE-SEM analysis. hNFs was not formed in the tubes under pH 5 conditions of PBS buffer. In addition, hNFs was not synthesized in the presence of 0.5 mg/L plant extract under all pH conditions. hNFs were synthesized under optimum conditions by adjusting the pH of PBS buffer to 7.4 and using 1 ml of plant extract. According to FE-SEM images of hNFs with ideal flower morphology, it was recorded that their diameters were 45 μm and their petal thicknesses were distributed in the range of 31-47 nm. EDX analysis of hNFs synthesized under optimum conditions revealed the presence of C, O, N, P and Cu, which constitute their structural elements. FT-IR analysis confirmed the presence of alcohol (O-H), alkene (C=C) and primary phosphate crystals.

Keywords: *Mentha piperita*, Nanoflower, Copper

Introduction

Nanomaterials exhibit different physical, chemical and biological properties than bulk forms due to their sizes smaller than 100 nm [1]. Nanomaterials are widely used in many industrial fields such as medical, electronic and cosmetic applications due to these unique properties they exhibit [2]. Among metallic nanoparticles, Cu-based nanoparticles (Cu/CuO NP) are widely used in applications such as gas sensors, solar energy systems, solar cells, catalysts, superconductors, field emission emitters, Lithium batteries, biosensors, photodetectors, inorganic pollutant removal, magnetic storage devices, especially with photocatalytic and antimicrobial activities [3-7]. Bhagat et al. (2021) characterized the CuO NPs they synthesized with green tea extract and suggested that the green tea extract-based CuO NPs they obtained could be used in fingerprint applications in climate studies [8]. Velsankar et al. (2021) reported that CuO NPs synthesized with Capsicum frutescens leaf extract showed a distribution in the range of 20-40 nm [9]. In the same study, it was recorded that CuO NP exhibited antioxidant and antimicrobial activity against Bacillus anthracis, Listeria monocytogenes, Klebsiella pneumoniae bacterial strains. In recent years, organic@inorganic

flower-like hybrid nanostructures (hNF), a new form of nanomaterials, have attracted the attention of researchers due to their large surface area, potential to accelerate reaction kinetics, enzyme stabilization ability and many structural features [10]. With these advantages, HNF has applications such as sensors in the detection of biomolecules such as glucose, food pathogens and amyloid, enzyme purification, heavy metal and dye removal, and drug delivery [10]. The synthesis of HNFs, which are widely used in many branches of the industry, includes disadvantages such as expensive biomolecules such as enzymes and DNA used as organic components, and the fact that these molecules are expensive to obtain, and that they require complex and multi-step experimental stages [11]. It is a fact that these disadvantages of these biomolecules used as organic components in HNF synthesis will also be reflected in HNF synthesis and will limit many critical factors such as application and widespread use. However, in recent years, studies have started to use easily obtained and cheap organic components such as plant extracts instead of these biomolecules in order to eliminate these disadvantages. Güven et al. (2021) reported that Cu HNFs synthesized with cherry stalk extract have antioxidant activity [12], Koca (2021) reported that Cu HNF synthesized with thymol extract has antimicrobial activity [13], and Koca et al. (2020) reported that Cu HNF synthesized with curcumin extract has photocatalytic activity against gaykol [14]. While there are studies on the synthesis of various metallic NPs with mint leaf extract in the literature searches, no study was found on the synthesis of HNF and determination of their application potential. For the first time, Cu-based HNFs were synthesized in this study using mint leaf extract as an organic component.

Materials and Methods

Mint leaves to be used in the synthesis of HNFs were purchased from the herbalist, washed and dried in an oven overnight (70 ^{o}C). After the dried leaves were ground in a mortar, 10 g were taken and brewed in 100 ml of distilled water for 1 hour (80 oC), then filtered through Whatman No 1 filter paper and the extract to be obtained was stored at +4 oC to be used in the synthesis of HNF. In order to determine the optimized synthesis conditions in the synthesis of HNF, mint leaf extract at different concentrations (0.5, 1 and 5 mg/L) was vortexed and reacted with Cu ($8x10^{-4}$ M, 0.35 ml) in 10 mM PBS buffer prepared at different pHs (5, 7.4 and 9) in falcon tubes. After incubation of the mixture in the dark (3 days, 4 ^{o}C), the sediments formed at the bottom of the tubes were centrifuged (10000 rpm, 10 min), washed with distilled water and dried in an oven (1 night, 70 ^{o}C). The morphology of HNFs was determined by FE-SEM, the elemental composition by EDX and the presence of

biomolecules playing a role in the synthesis by FT-IR analysis and their characterization was evaluated [11].

Result and Discussion

The morphologies of HNFs with organic components of mint extract were determined by FE-SEM analysis. HNF was not formed in the tubes at pH 5 conditions of PBS buffer. In addition, HNF was not synthesized in the presence of 0.5 mg/L plant extract at all pH conditions. HNFs were synthesized under optimum conditions by adjusting the pH of PBS buffer to 7.4 and using 1 ml of plant extract. In other studies, HNFs with ideal flower morphology (synthesized under optimum conditions) were used. The morphologies of HNFs with ideal flower morphology are given in Figure 1. It was recorded that HNF was 45 µm (Figure 1a) and petal thicknesses were distributed in the range of 31-47 nm (Figure 1b). The EDX analysis of HNFs synthesized under optimum conditions (Figure 2) revealed the presence of C, O, N, P and Cu, which constitute their structural elements. FT-IR analysis (Figure 3) obtained 3349, 1621 correspond to alcohol (O-H) and alkene (C=C) groups. Other peaks at wavelengths of 1036, 988, 623 and 590 cm^{-1} observed in FT-IR analysis indicate the formation of primary phosphate crystals. These data overlap with the formation mechanism of HNFs. According to this mechanism, primary phosphate crystals are formed by the coordination of copper with phosphate from PBS buffer. The primary phosphate crystals formed are fed with the amine and diol groups of the organic component, thus providing the formation of flower-shaped hybrid nanostructures [15].

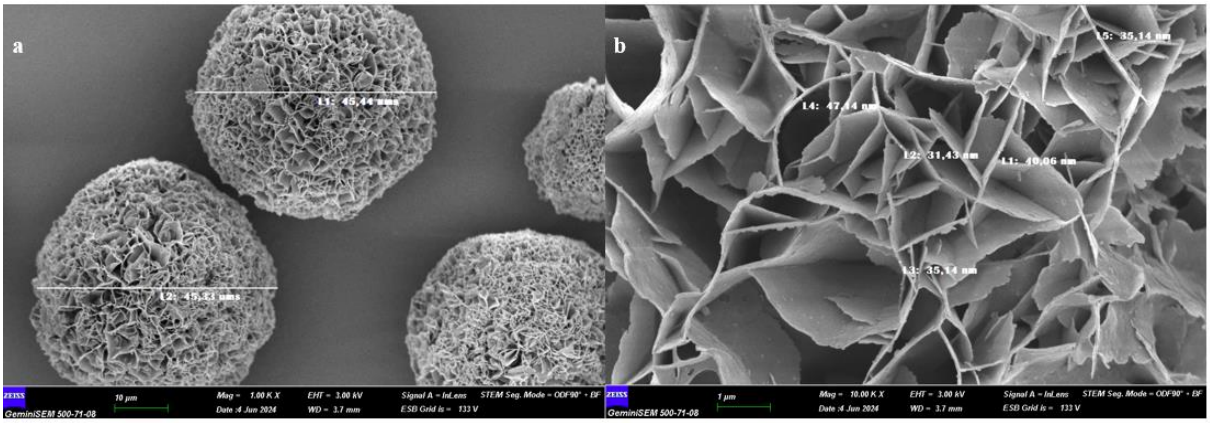

Fig. 1. FESEM image of hNFs. a) Diameter lengths of hNFs, b) Petal thicknesses of hNFs

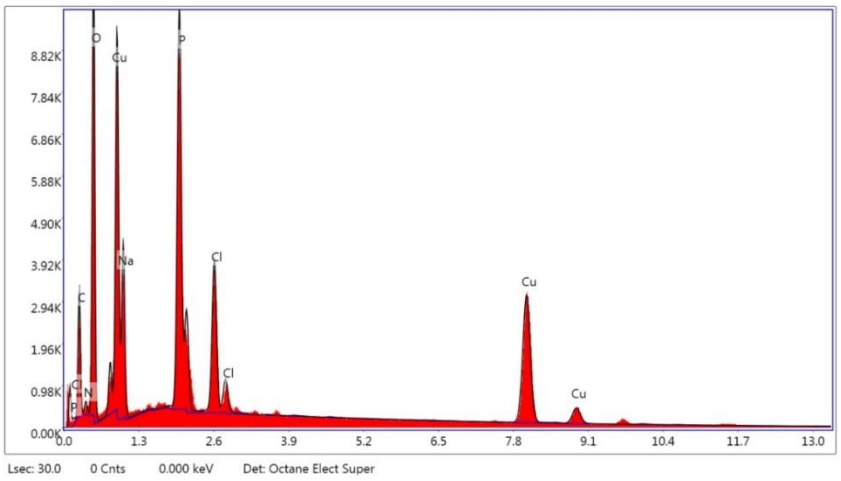

Fig. 2. EDX analyses of hNFs

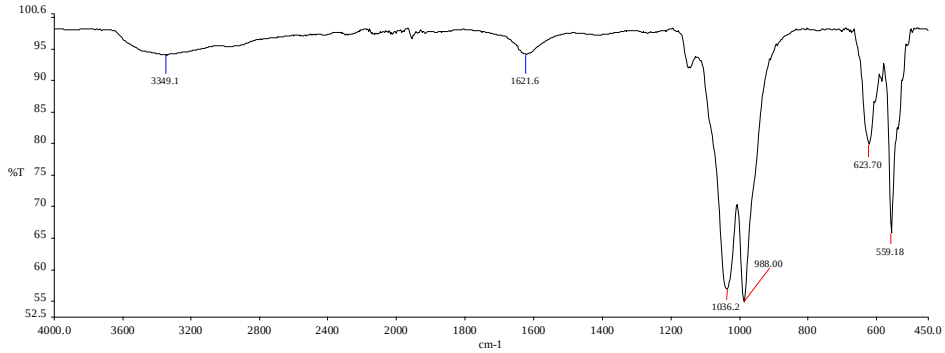

Fig. 3. FT-IR analysis of hNFs

Conclusion

In this study, it was determined that *M. piperita* extract could be used as an organic component in the synthesis of hNFs. In addition, the synthesis and morphology of hNFs depend on the plant extract concentration and PBS buffer pH. It is suggested that the obtained data can be used in biomedical, catalytic and environmental applications.

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Substrate Selection and Its Effect on Kombucha Fermentation

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Abstract. Studies on kombucha fermentation highlight the critical effects of substrate selection on fermentation efficiency and product quality. In kombucha fermentation, the types of sugars used by microorganisms as energy sources (such as sucrose, glucose, fructose) and the tea varieties that support fermentation (black tea, green tea, herbal teas) come to the fore. Studies have shown that sucrose is an optimal energy source for yeasts and bacteria in kombucha culture, and that this leads to rapid acid production and balanced pH reduction, increasing fermentation efficiency. Other sugars such as glucose and fructose provide lower microbial activity and fermentation efficiency. Plant material varieties create significant differences in the fermentation process and bioavailability of the product. Green tea has been shown to increase the health benefits of kombucha and provide more biological activity thanks to its high antioxidant content. Although black tea is a traditionally preferred substrate, it does not offer as high antioxidant activity as green tea. The effect of herbal teas on fermentation has been limited and has generally been found to be less effective in supporting microbial growth. Fermentation time, temperature, pH change and chemical composition of the substrate directly affect microbial diversity and metabolite production. These factors shape the final product characteristics of kombucha, such as taste, biological activity level and health benefits. Therefore, it has been shown that substrate selection is an important step to optimize quality in kombucha production. This information provides guidance for both kombucha producers and fermentation researchers and provides a basis for making future production processes more efficient.

Keywords: Kombucha, Fermentation, Biological activity, Substrate diversity.

Introduction

Kombucha tea is a naturally occurring, non-alcoholic beverage produced through the fermentation of sweetened tea using a kombucha culture containing yeast and bacteria. The origins of kombucha can be traced back more than 2000 years to northeast China, with its first documented use in Manchuria in 220 BC [1]. Subsequently, it is reported to have spread to Russia and Eastern Europe in the 1800s, and to North Africa and Western Europe during World War II [2]. Kombucha is produced through the fermentation of sweetened tea using a symbiotic culture of bacteria and yeast (SCOBY). The primary drivers of kombucha fermentation are acetic acid bacteria (AAB) from the genera Komagataeibacter, Acetobacter, and Gluconobacter, as well as yeasts such as Zygosaccharomyces, Saccharomyces, and

Brettanomyces [3]. The fermentation process of kombucha typically takes 7-10 days, during which the SCOBY metabolizes the sugar and tea compounds, producing a range of organic acids, vitamins, and other beneficial compounds. This fermentation not only creates the characteristic tangy flavor of kombucha but also contributes to its potential health benefits, which have been the subject of increasing scientific interest in recent years. Additionally, lactic acid bacteria (LAB) including Lactobacillus and Leuconostoc have been isolated from the beverage [4, 5]. During the fermentation process, yeasts hydrolyze sucrose to glucose and fructose as a byproduct of ethanol fermentation, while AAB subsequently convert ethanol and glucose into acetic acid and gluconic acid, respectively [6]. The complex microbial community in kombucha leads to a diverse array of metabolites, including but not limited to organic acids, enzymes, and polyphenols. These metabolites contribute not only to the unique flavor profile of kombucha but also to its potential health-promoting properties. The symbiotic relationship between the various microorganisms in the SCOBY allows for a dynamic fermentation process, where different species thrive at different stages, leading to a continuously evolving beverage composition. Further byproducts of kombucha fermentation include vitamins, minerals, phenolic compounds, lactic, citric, malic, and glucuronic acids [7, 8]. The fermentation process in kombucha production results in a complex mixture of organic acids, enzymes, and bioactive compounds that contribute to its unique flavor profile and potential health benefits. The microbial composition of the SCOBY can vary depending on factors such as geographic location, tea substrate, and fermentation conditions, leading to differences in the final product. As fermentation progresses, the pH of the kombucha decreases, creating an acidic environment that inhibits the growth of pathogenic microorganisms and contributes to the beverage's characteristic tangy taste.

Traditional kombucha fermentation substrates comprise black or green tea and sugar; however, in recent years, alternative carbon and nitrogen sources have gained prominence in an effort to enhance the functionality of the final product. The kombucha market was valued at USD 1,84 billion in 2019, with a projected growth rate of 23.2% by 2027 [9]. The microbial diversity within SCOBY can be influenced by environmental factors, leading to regional variations in kombucha flavors and properties. The production of organic acids during fermentation not only contributes to the distinct taste of kombucha but also acts as a natural preservative, extending the shelf life of the beverage. Exploring alternative substrates for kombucha fermentation opens up possibilities for creating novel flavor profiles and potentially enhancing the nutritional value of the final product. The increasing popularity of

kombucha can be attributed to its purported health benefits, which include immune system enhancement, alleviation of IBS symptoms, weight reduction assistance, and blood pressure reduction, among others. The growing appeal of kombucha as a "healthy" beverage has led to an increase in home brewing, often utilizing a SCOBY obtained from another household as a starter culture [10]. Consequently, it is imperative that individuals are informed about the potential risks associated with home-brewed kombucha and the necessary precautions to ensure safe fermentation [11]. This study aims to present a comprehensive overview of the current state of kombucha knowledge and research.

Different Substrates

While sucrose is the most widely used carbon source for kombucha fermentation, research has been conducted on the effects of other carbohydrate sources and their relative quantities on the process. Early investigations into the fermentation properties of kombucha teas containing lactose, fructose, glucose, or sucrose by Reiss [12] revealed that the type of sugar utilized had a distinct effect on the formation of lactic acid and ethanol, whereas the concentration of the sugars had no discernible impact. Malbasa et al. [13] observed higher overall acidity, higher lactic acid content, and significantly higher biomass yield in kombucha tea made with molasses as a substrate compared to sucrose-sweetened kombucha tea. This was attributed to the greater availability of nitrogen in molasses. The resulting kombucha beverage exhibited a black color and a sweet, caramelized taste; however, its sensory qualities were deemed unsatisfactory. A recent study examined molasses and coconut palm sugar as alternative substrates for sucrose. It is noteworthy that kombucha tea fermented with coconut palm sugar demonstrated the highest phenolic content and antioxidant activity, suggesting its potential application as a functional beverage [14]. A significant recent investigation explored the utilization of germinated corn as a substrate to produce a kombucha beverage without the addition of sugar. This represents the first study on kombucha fermentation using only raw seeds or grains without the supplementation of carbon or nitrogen sources. The authors posit that a viable SCOBY may be produced from grains, and soluble fiber could serve as an effective substitute for tea alkaloids [15]. Although the beneficial components of black and green tea, including flavonoids, polyphenols, and saponins, have been extensively studied, recent interest in developing a beverage high in bioactive compounds has focused on utilizing alternative teas or nitrogen sources. Recent studies have explored other teas, such as yerba mate, rooibos, and zijuan, as potential kombucha substrates. Research has demonstrated that these teas are superior to black tea kombucha in terms of their enhanced antioxidant activity,

decreased oxidative stress, and improved sensory qualities [16, 17]. An increasing number of studies are investigating the potential health benefits and functional properties of using herbal teas and medicinal plants as kombucha substrates. Initial attempts to employ herbal teas such as peppermint, lime blossom, mint, chamomile, rosemary, and sage as substitutes for kombucha tea were unsuccessful. Their volatile oils have detrimental effects, including inadequate acidification, disruption of kombucha microbe development due to antimicrobial compounds present, and unstable storage of the beverage [18]. These were not successfully utilized as alternatives in kombucha tea initially. Velićanski, et al. [19] determined that the production of kombucha with peppermint or thyme teas was suitable, resulting in fermentation times that were either similar or shorter. Tanticharakunsiri, et al. [20] and Zhang, et al. [21] successfully produced kombucha beverages using mint tea as a substrate, which were deemed sensorially acceptable. Vitas, et al. [22] reported the first use of yarrow as a kombucha substrate. They utilized the plant's therapeutic properties to create a beverage purported to have antioxidants, antibacterial, and anticancer potential. Other similar herbs and plants that have been employed as kombucha substrates include ginger, lemon balm, oak, and nettle leaf [23, 24]. Vegetables and fruits have also been identified as viable kombucha substrates; researchers are again focusing on those that are well-characterized in terms of their bioactive components and potential health benefits. These investigations have examined the use of raw materials such as spinach, grape juice, cherry juice, banana peel, and pomegranate juice [25]. These substrates are valuable not only for their functional molecules but also, under appropriate fermentation conditions, can promote the increased synthesis of organic acids such as glucuronic acid [26]. The use of alternative substrates for kombucha fermentation has gained attention in recent years, with researchers exploring various carbohydrate sources beyond traditional sucrose. These investigations have sought to understand how different sugars influence the fermentation process, including the production of organic acids, ethanol, and other metabolites. Additionally, studies have examined the potential for using non-traditional substrates, such as fruit juices or plant extracts, to create novel kombucha variants with unique flavor profiles and potential health benefits.

Conclusions

The past several decades have witnessed an increase in the popularity of kombucha due to its purported health benefits. The fermented tea known as kombucha is experiencing a global rise in popularity, as evidenced by the growth in its production, both domestically and commercially. Currently, kombucha can be found in mainstream supermarkets rather than

solely in specialty health food stores, reflecting its increasing prevalence. The fermentation conditions, microbial diversity of the kombucha consortium, and substrate utilized significantly influence the microbiological, sensory, and functional components of the beverage. The primary fermentation substrates are sucrose and either green or black tea, although alternative options have been investigated. Various substrates can impact the kombucha beverage in both positive and negative ways, potentially affecting microbial growth, metabolite production, and overall functionality. Future research should focus on how an enhanced understanding of the interactions and communication among microbial community members can modulate the sensory and functional properties of kombucha. Moreover, studies on the utilization of defined starting cultures should be prioritized, particularly to facilitate the transition to industrial-scale kombucha production. The optimization of fermentation parameters, such as temperature, pH, and duration, could lead to improved control over the final product's characteristics. Additionally, exploring the potential of novel tea varieties or herbal infusions as fermentation substrates may yield unique flavor profiles and bioactive compounds. Further investigation into the synergistic effects between different microbial species within the kombucha consortium could provide insights into enhancing probiotic properties and extending shelf life.

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Bryophyte Vegetation Of Cappadocia Region (Nevsehir)

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Abstract

In this study, bryophyte vegetation in the Cappadocia region (Nevşehir) in the Central Anatolia Region of Turkey was investigated. In 30 field studies, 208 relevé (30 x 30 cm) were selected from the research area between 2020-2021. 132 of these randomly selected relevé were found suitable for the study and analyzed using the PAST (PAleontological STatistics) program with ordination methods. Cluster analysis (NJA) was used to distictive communities, and detrended correspondence analysis (DCA) was used to determining ecological factors. With this method, which is new for bryophyte vegetation studies in Turkey, 6 communities (*Encalypta vulgaris - Syntrichia ruralis; Syntrichia ruraliformis - Pterygoneurum ovatum; Syntrichia ruralis - Didymodon acutus, Grimmia pulvinata - Grimmia anodon, Lewinskya rupestris- Grimmia pulvinata* and *Grimmia crinita- Grimmia pulvinata*) were determined from the research area according to substrate type and humidity.

Keywords: Moss, Vegetation, PAST, DCA, NJA, Cappadocia, Nevşehir.

Introduction

Bryophytes, which can show a wide distribution in many climate zones from the poles to the tropics in terms of plant geography, can generally choose rock (epilithic), soil (epigaic) and tree (epiphytic) surfaces as substrates within the vegetation they are found in (Kara and Taşpınar, 2021). The terms epilithic or saxicolous are used to describe bryophytes that grow directly on rock surfaces. The term “obligate” is used for plants that can only live on rocks and cannot grow on other substrates as substrates, while the term “facultative” is used for plants that grow on a wide variety of substrates due to their wide ecological tolerances and

can also be found epilithic (Smith. 1982). Colonization of some bryophytes on rock habitats is determined by special ecological factors such as shade, moisture content, flatness of the surface and chemistry of the substrate (Jia et al. 2014; Kara et al. 2009; Kürschner and Parolly, 1999). For epilithic bryophytes, fairy chimney surfaces are very difficult and special habitats due to high erosion and extreme drought conditions. Fairy chimneys are rock formations formed by rain, wind and flood waters eroding the structure consisting of tuffs. Fairy chimneys, which make Cappadocia a special geography, were formed by the Erciyes, Güllüdağ and Hasandağı volcanoes, which were activated by the compression of the Taurus Mountains in the south by the Anatolian fault in the north in the Tertiary period (~ 60 myo). These volcanoes erupted lava starting in the Upper Miocene and continuing until the Pliocene. The volcanic dust formed by this eruption formed a 100-150 meter thick tuff layer in the Cappadocia region. In addition to tuff, these formations also contain geological rocks such as tuffite, ignimbrite tuff, lahar, volcanic ash, clay, sandstone, marl agglomerate and basalt. This thick tuff layer has changed over time due to the effects of the floods and winds descending from the slopes of the valleys, especially the Kızılırmak River, and the hard rocks that the flood waters could not erode have formed fairy chimneys with caps and conical bodies that are unique in the world. Contrary to popular belief, the life of fairy chimneys depends on the presence of hard rock caps called caps on the conical bodies rather than the durability of the rocks in the body structure. Because the cap parts are harder and heavier than the terrain, the relatively softer body rising in the form of a cone below is protected by pressure. If the tops of fairy chimneys, called caps, fall, the fairy chimneys will disappear in a very short time due to erosion. The trunk parts of fairy chimneys are made up of tuffite, volcanic ash and tuft, also known as volcanic debris, while the cap parts are made up of hard, durable rocks such as ignimbrite and lahar. In Cappadocia, in addition to capped fairy chimneys, which are formed by erosion, mushroom-shaped, columnar and pointed fairy chimneys are also seen. The lengths of fairy chimneys vary from the height of a person to heights exceeding a 10-story building, and their diameters start from 1 meter and go up to 15 meters. Those outside these ranges are not considered fairy chimneys. Fairy chimneys, which are usually gray or white in color, are millions of years old. Apart from Cappadocia, fairy chimneys are also found in some places in the world. However, they are nowhere as dense as in Cappadocia. For this reason, the areas where fairy chimneys are located in the Cappadocia region have been included in the UNESCO World Heritage List since 1985 (URL 1). The form in which plants with similar living conditions gather together on a section of any region is called "vegetation" (Braun-Blanquet, 1964). Vegetation in a certain area can be defined through the basic

syntasonomic category of "plant association", or it can be defined as communities using orientation methods. Most of the bryo-sociological studies that have been ongoing in our country for about half a century have been carried out on epiphytic substrates with classical methods (Kara and Taşpınar, 2022). In recent years, studies have been carried out using ordination methods and defining communities to cover all substrates (epigeic, epiphytic, epilithic) (Taşpınar, 2022). This study is important because it is related to fairy chimneys, which are a world heritage with their unique geological features in the Cappadocia region, and because it includes new research methods.

Material and Method

The borders of Cappadocia were first drawn in history by the famous geographer of the Roman antiquity, Strabon (64 BC - 24 AD). According to this drawing, a wide area starting from the Taurus Mountains in the south, the Eastern Black Sea coastline in the north, Aksaray in the west, and Malatya in the east was demarcated as Cappadocia (Bulut, 2018). Today, the Cappadocia region covers the provinces of Aksaray, Nevşehir, Niğde, Kırşehir and Kayseri. The narrow area within this border covering the surroundings of Avanos, Ürgüp, Göreme, Uçhisar and Ihlara is known as the 'Rocky Cappadocia Region' and is on the UNESCO World Heritage List (Kara and Taşpınar, 2021). The study area where this study was carried out is within the Rocky Cappadocia Region (Map 1). There are thousands of fairy chimneys in different localities in the Cappadocia Region. However, they are most densely observed in the valleys within the triangle formed by Avanos-Ürgüp-Uchisar in the study area. The highest points in the area are Kermil Mountain with an altitude of 1516 m in the south of Uçhisar and Akdağ with an altitude of 1320 m in the middle part. The lowest point is the Ada Mevkii plain with an altitude of 960 m between Aktepe-Avonos. The surroundings of Akdağ, which rises in the middle of the study area and has a plateau feature, consist of steep slopes, highly indented (undulated) and deep valleys covered with numerous fairy chimneys (Vural et al. 1996). In the study area, research was conducted in Akdağ (AD), Aşıklar Valley (AV), Güvercinlik Valley (GV), Kermil Mountain (KD), Kızıl Valley (KV), Müze Valley (MV) and Zemi Valley (ZV). The continental climate generally prevails in the area; winters are cold and snowy, and the snow cover remains on the ground for a long time. However, summers are hot and dry, and precipitation generally occurs in the form of rain in the spring. The annual average precipitation varies between 272.2 mm and 523.3 mm (Kaşmer, 2011, Graph 1.). Our research material consists of 208 sample areas in 30 field studies, epilithic collected in the field study conducted in different vegetation periods in 2020-2021. The bryophyte samples

collected were placed in standard collection envelopes prepared in advance. The habitat of the plants, collection date, GPS record, altitude above sea level and other information about the locality were written on these special envelopes. The samples collected from the field were brought to the laboratory, dried and turned into herbarium samples. They were then identified using classical methods and identification keys (Zander, 1993; Smith, 2004; Kürschner and Frey, 2020). The selection and dimensions of the sample areas were made according to the Braun-Blanquet (1964) method and their ecological analyses were made according to ordination methods. Of these randomly selected sample areas, 132 were found suitable for the study, and DCA (Detrendet Correspondance Analysis) and NJA (Neighboring Join Cluster Analysis) methods in the PAST (PAleontological STatistics) program were used for community analysis with ordination methods (Kara and Taşpınar, 2021). The life forms and life strategies of the taxa forming the communities were determined according to Kurschner et al. (1998).

Results

132 of the 208 sample areas randomly selected from the fairy chimneys located in the rocky areas of Cappadocia were found suitable for the study and analyzed using the PAST (PAleontological STatistics) program with ordination methods. Cluster analysis (NJA) was used to determine the communities, and detrended correspondence analysis (DCA) was used to determine the distinguishing ecological factors. With this new method for bryophyte vegetation studies in Turkey, 6 communities (*Encalypta vulgaris - Syntrichia ruralis; Syntrichia ruraliformis - Pterygoneurum ovatum; Syntrichia ruralis - Didymodon acutus, Grimmia pulvinata - Grimmia anodon, Lewinskya rupestris- Grimmia pulvinata and Grimmia crinita- Grimmia pulvinata*) were determined from the research area according to substrate type and humidity. *Encalypta vulgaris-Syntrichia ruralis* community with 44 taxa, 7 locations, 26 sample areas, between 1070-1420 m; *Syntrichia ruraliformis-Pterygoneurum ovatum* community with 35 taxa, 8 locations, 25 sample areas, between 1110-1430 m; *Syntrichia ruralis-Didymodon acutus* community with 47 taxa, 9 locations, 25 sample areas, between 1060-1430 m; *Grimmia pulvinata-Grimmia anodon* community with 31 taxa, 7 locations, 16 sample areas, between 1060-1435 m; *Lewinskya rupestris-Grimmia pulvinata* community was defined with 44 taxa, 7 locations, 28 sample areas, between 1060-1510 m; *Grimmia crinita-Grimmia pulvinata* community was defined with 20 taxa, 5 locations, 12 sample areas, between 1110-1470 m. Euclidean similarity measure and final branching

parameter and NJA (Nearest neighbor analysis) were used as cluster analysis methods in the separation of communities.

Conclusion and Discussion

This study is also important for fairy chimneys, which are very rare geological formations in the world, where very arid microclimate conditions prevail, where there is high microerosion due to the easily soluble rock structure, and which are difficult habitats even for bryophytes. In this study, 208 sample areas were selected from the research area between 2020-2021 as a result of 30 field studies. 132 of these randomly selected sample areas were found suitable for the study and were analyzed using the PAST (PAleontological STatistics) program with ordination methods. Cluster analysis (NJA) was used to determine communities, and detrended correspondence analysis (DCA) was used to determine distinctive ecological factors. With this method, which is new for bryophyte vegetation studies in Turkey, 6 communities (*Encalypta vulgaris - Syntrichia ruralis; Syntrichia ruraliformis - Pterygoneurum ovatum; Syntrichia ruralis - Didymodon acutus, Grimmia pulvinata - Grimmia anodon, Lewinskya rupestris- Grimmia pulvinata and Grimmia crinita- Grimmia pulvinata*) were determined from the research area according to substrate type and humidity. These communities are distributed in 10 different locations between 1060-1470 meters altitude and mostly prefer Zemi Valley and Güvercinlik Valley. Liverwort is not found in the communities that generally prefer the north side of fairy chimneys. When the life form and life strategy of the taxa in the research area are examined, Tf (40%) life form in the *Encalypta vulgaris-Syntrichia ruralis* community; Ap (% 43) life strategy, Tf (% 50) life form in *Syntrichia ruraliformis-Pterygoneurum ovatum* community; Ap (% 42) life strategy, sT (% 30) life form in *Syntrichia ruralis-Didymodon acutus* community; Ap,Ag,Ba (% 26) life strategies, Tf (% 39) life form in *Grimmia pulvinata-Grimmia anodon* community; Ap (% 41) life strategy, Tf (% 29) life form in *Lewinskya rupestris-Grimmia pulvinata* community; Ap,Ba (% 29) life strategies, Tf (% 45) life form in *Grimmia crinita-Grimmia pulvinata* community; Ap (% 43) life strategy is dominant. This situation is compatible with the climate in which the communities are located.

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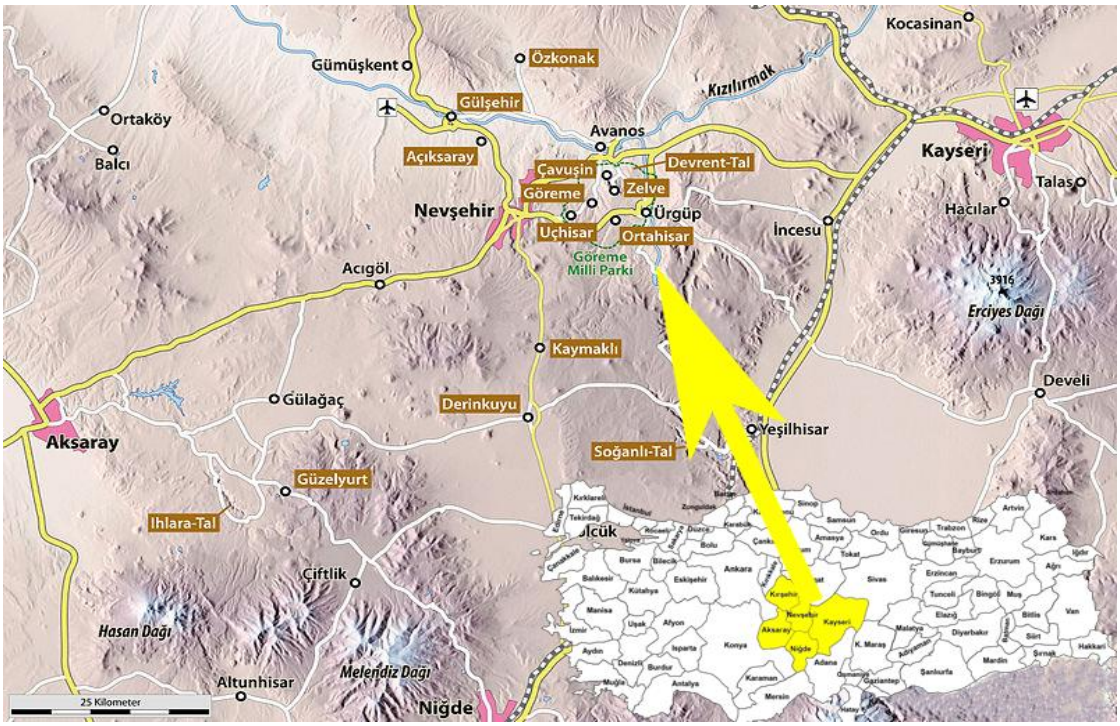
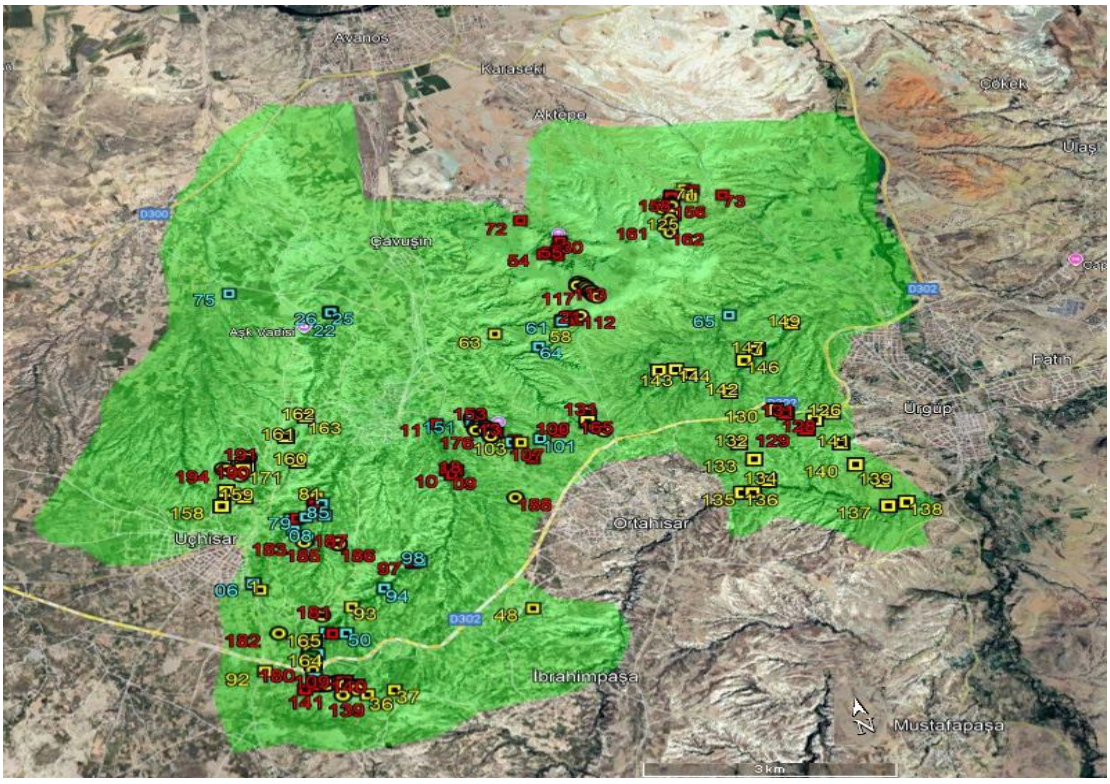
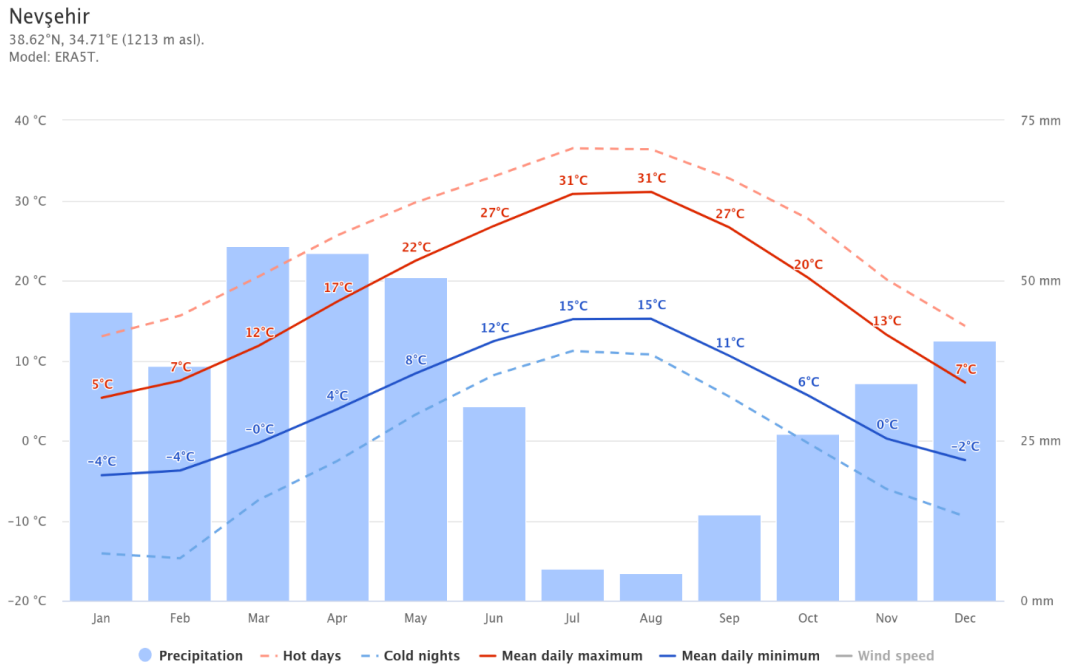


Map 1. Location of the Research Area



Map 2. Location of sample areas in the research area



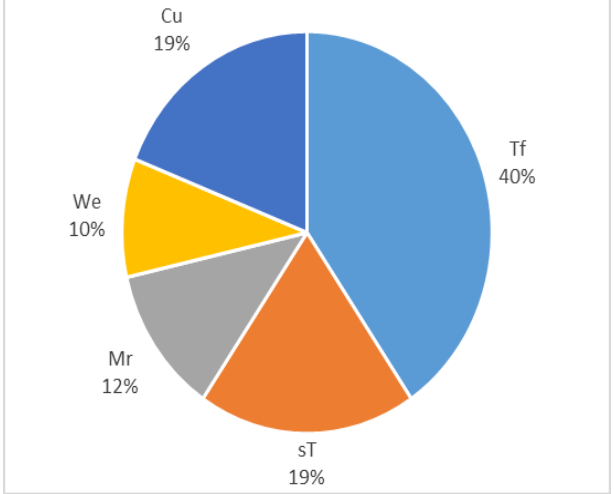
Graph 1. Average rainfall and temperature values of Nevşehir province (URL 1.)

Table 1- Life form and life strategy table

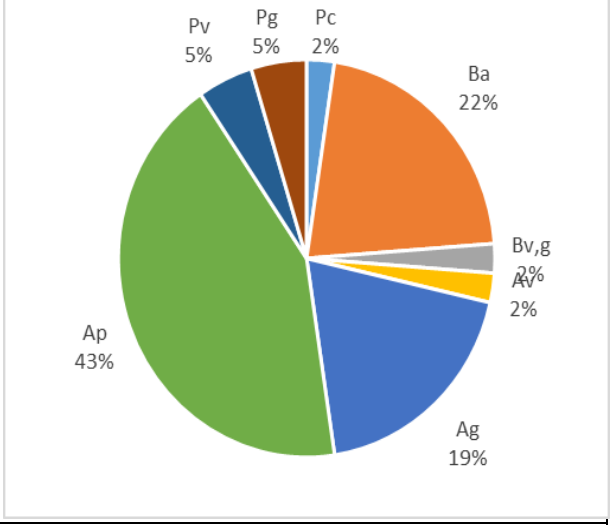
		Description	Kısaltma
Life Form	**Short turfs**	Single thalli in the form of rosettes forming smaller covers compared to thallus carpet forms forming large covers.	St
	Tall turf	Loose or tight arrangement of stems that are generally upright with limited branching.	Tf
	Cushion	Dome-shaped colonies formed by centrally originating trunks, oriented in various ways.	Cu
	Rough Mat	Arrangements in which the shoots are creeping, forming numerous upright lateral branches..	Mr
	Weft	Loosely intertwined, often abundantly branched mats.	We
Life strategy	**Annual Shuttle**	Shuttle species with a one-year lifespan	Pc
	Perennial Shuttle	Colonists capable of sexual and asexual reproduction	Bv, g
		Pauci Annual colonists	Ba
		Perennial shuttle species capable of sexual and asexual reproduction	Pv, g
	Perennial Colonist	Perennials with high sexual reproduction potential	Ag
		Perennials with high asexual reproductive potential	Av
		Perennials with moderate or low sexual and asexual reproductive capacity	Ap

Table 2- ***Encalypta vulgaris-Syntrichia ruralis*** community

Sample Area No (GMP)	70	126	58	74	85	59	125	127	139	112	154	56	65	143	145	147	137	104	141	81	82	31	98	150	151	166	Number of Repetitions	Life Forms	Life Strategy
Substrate	R	S	S	R	S	S	S	S	S	S	R	T	R	S	S	S	S	R	R	T	T	R	S	S	T	S			
Locality	GV	OV	DV	GV	ZV	DV	OV	OV	MV	OV	GV	KV	DV	MV	MV	MV	MV	OV	MV	KD	KD	KD	KV	GV	GV	ZV			
Sample Area Size (dm^2)	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9			
Altitude (m)	1220	1137	1112	1180	1290	1112	1131	1168	1110	1167	1308	1070	1124	1263	1225	1176	1131	1110	1140	1410	1410	1401	1290	1281	1320	1360			
Light (Open-Semi-Shade-Shade)	O	O	SS	S	SS	SS	O	O	S	SS	O	O	SS	O	SS	O	SS	SS	SS	S	S	O	O	O	O	S			
Humidity (Wet-Moist-Arid)	A	A	A	A	M	A	A	A	W	A	M	A	A	W	W	W	M	M	W	M	M	A	M	M	M	M			
Direction	K	K	K	K	K	K	K	K	G	K	B	K	K	K	K	K	K	G	K	K	KD	KB	G	G	GB	K			
Slope(0-45-90)	<45	<45	0	>45	>45	0	0	<45	>45	<45	>45	>45	90	0	0	0	>45	>45	0	>45	>45	<45	0	0	90	>45			
Number of species	4	4	6	4	5	4	4	4	4	4	4	5	5	5	4	5	8	6	9	4	11	5	5	5	5	5			
Encalypta vulgaris	0	0	0	0	0	1	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	0	1	1	0	0	16	Tf	Pv
Syntrichia ruralis	0	0	0	0	0	0	0	0	0	1	1	1	0	1	1	1	1	1	0	0	1	1	0	0	0	0	10	Tf	Ba
Ptychostomum imbricatulum	1	1	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	9	sT	Ap
Tortula inermis	1	1	0	1	1	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	9	Tf	Ag
Bryum dichotomum	0	0	0	0	0	0	1	1	1	0	0	1	1	1	0	0	1	0	1	0	0	0	0	0	0	1	9	Tf	Ag
Didymodon rigidulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	1	1	0	0	0	6	Tf	Ap
Syntrichia ruraliformis	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	6	Tf	Ba
Tortula muralis subsp. muralis	1	1	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	0	0	6	sT	Ba
Grimmia pulvinata	0	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	5	Cu	Ba
Didymodon acutus	1	1	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	4	Tf	Ap
Pterygoneurum ovatum	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	1	0	0	0	0	0	0	0	4	sT	Pc
Ptychostomum capillare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	3	sT	Pv,g
Syntrichia caninervis	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	3	Tf	Ba
Tortula acaulon	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	3	sT	Ba
Bryum argenteum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	2	sT	Bv,g
Didymodon luridus	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	Tf	Ap
Eurhynchiastrum pulchellum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	Mr	Ag
Funaria hygrometrica	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2	sT	Pg
Grimmia crinita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	2	Cu	Ba
Homalothecium aureum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	We	Ap
Hygroamblystegium humile	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	2	Mr	Ap
Ptychostomum pallens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	sT	Ap
Tortula subulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	2	Tf	Ag
Amblystegium serpens	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ag
Brachythecium capillaceum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ap
Bryum arachnoideum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Av
Didymodon cordatus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Tf	Ap
Didymodon fallax	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Ap
Didymodon hornschuchianum	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Ap
Didymodon imbricata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Tf	Ap
Didymodon vinealis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Ap
Encalypta pilifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Tf	Pg
Grimmia anodon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	Cu	Ba
Grimmia laevigata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	Cu	Ap
Homalothecium philippeanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	We	Ap
Homalothecium sericeum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	We	Ap
Lewinskya affinis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	Cu	Ag
Orthotrichum alpestre	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Cu	Ap
Orthotrichum diaphanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	Cu	Ag,v
Rhynchostegiella tenella	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	We	Ag
Schistidium flaccidum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	Cu	Ba
Sciuro-hypnum starkei	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ap



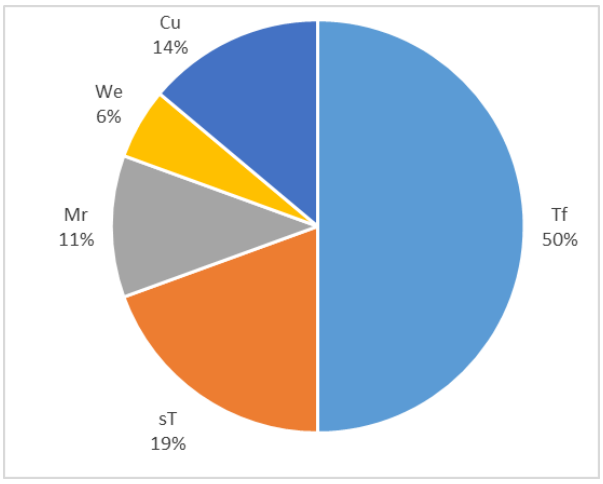
Graph 2-Life Form



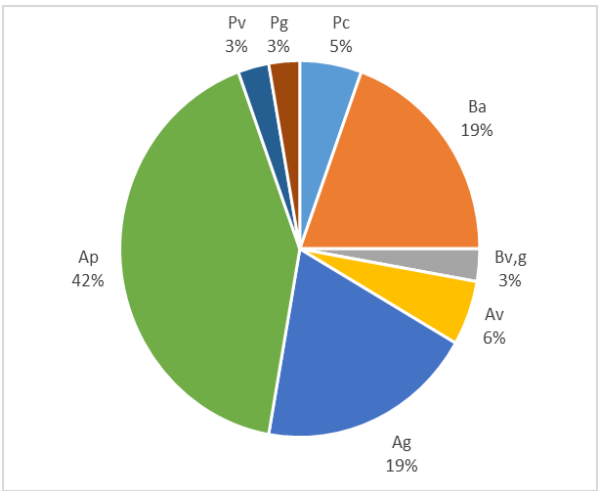
Graph 3- Life Stragedy

Table 3- *Syntrichia ruraliformis-Pterygoneurum ovatum* community

Sample Area No (GMP)	108	115	131	128	121	41	72	63	114	130	144	90	109	110	111	119	122	142	146	50	155	19	99	95	96	Number of Repetitions	Life Forms	Life Strategy
Substrate	R	S	S	S	S	S	R	R	R	S	S	R	S	S	S	S	S	S	S	S	S	R	S	S	S			
Locality	AD	OV	OV	OV	OV	ZV	GV	DV	OV	OV	MV	MV	AD	AD	OV	OV	OV	MV	MV	KV	GV	KV	KD	KV	KV			
Sample Area Size (dm^2)	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9			
Altitude (m)	1100	1172	1138	1157	1133	1360	1200	1125	1172	1173	1255	1220	1151	1230	1156	1121	1085	1253	1209	1180	1308	1220	1430	1230	1310			
Light (Open-Semi-Shade-Shade)	SS	O	O	O	O	O	O	SS	O	O	S	S	O	O	O	O	O	O	SS	O	O	S	S	O	O			
Humidity (Wet-Moist-Arid)	A	A	A	A	A	M	A	A	A	A	W	A	A	A	A	A	A	W	M	A	M	A	M	M	M			
Direction	K	K	K	K	K	D	GD	K	K	K	K	G	K	K	K	K	K	K	K	K	B	K	G	G	G			
Slope(0-45-90)	45	<45	0	0	<45	>45	>45	<45	<45	0	0	>45	0	<45	<45	<45	<45	0	0	>45	<45	>45	<45	0	0			
Number of species	4	4	4	4	5	5	5	4	4	4	4	5	5	4	6	4	4	5	5	6	3	4	4	5	5			
Syntrichia ruraliformis	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	14	Tf	Ba
Pterygoneurum ovatum	0	1	0	0	1	0	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	0	0	0	0	11	sT	Pc
Bryum dichotomum	1	1	1	1	1	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	9	Tf	Ag
Didymodon acutus	1	1	1	1	0	0	0	0	0	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	9	Tf	Ap
Syntrichia caninervis	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1	1	1	8	Tf	Ba
Tortula inermis	0	0	0	1	1	1	1	1	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	8	Tf	Ag
Bryum arachnoideum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	1	1	6	Tf	Av
Bryum argenteum	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	0	0	0	0	5	sT	Bv,g
Ptychostomum pallens	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	5	sT	Ap
Ptychostomum imbricatulum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	4	sT	Ap
Syntrichia rigescens	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	4	Tf	Ba
Brachytheciastrum velutinum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	Mr	Ag
Didymodon luridus	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	Tf	Ap
Didymodon rigidulus	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Ap
Encalypta vulgaris	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	2	Tf	Pv
Grimmia laevigata	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	Cu	Ap
Pseudocrossidium hornschuchianum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	Tf	Ap
Syntrichia ruralis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	Tf	Ba
Tortula subulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	Tf	Ag
Brachytheciastrum collinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Mr	Ag
Brachythecium salebrosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Mr	Ap
Didymodon fallax	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Ap
Didymodon hornschuchianum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Ap
Didymodon vinealis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	Tf	Ap
Distichum inclinatum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Ap
Flexitrichum flexicaule	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Tf	Ap
Grimmia anodon	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ba
Grimmia crinita	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ba
Grimmia ovalis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	Cu	Av
Homalothecium lutescens	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	We	Ap
Homalothecium philippeanum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	We	Ap
Microbryum curvicollum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	sT	Ba
Plasteurhynchium striatulum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ag
Ptychostomum capillare	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	sT	Pv,g
Tortula vlassovii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	sT	Pc



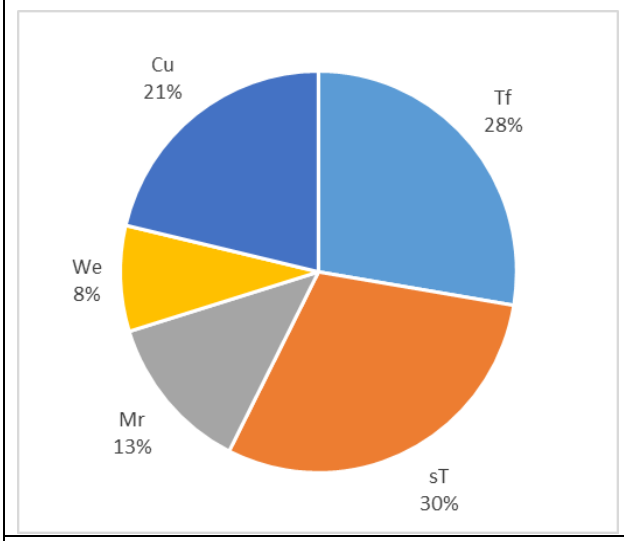
Graph 4-Life Form



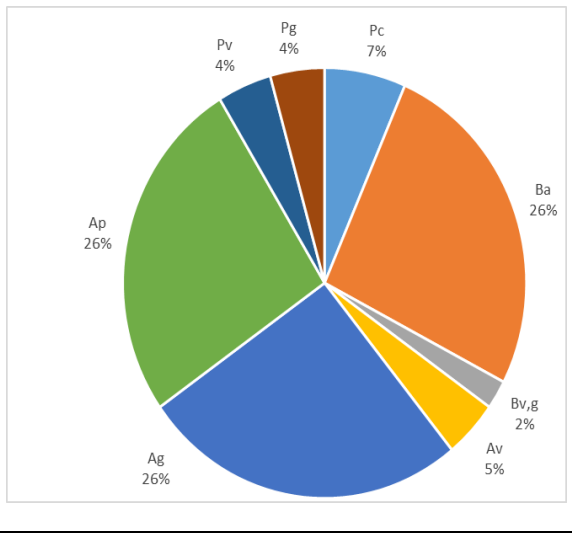
Graph 5- Life Stragedy

Table 4- *Syntrichia ruralis-Didymodon acutus* community

Sample Area No (GMP)	25	177	46	32	60	164	49	129	24	118	77	12	170	69	113	78	133	134	199	7	51	29	156	3	162	Number of Repetitions	Life Forms	Life Strategy
Substrate	R	S	R	S	S	S	S	S	T	S	R	R	S	T	R	T	S	S	R	R	S	S	S	T	R			
Locality	AV	DV	AD	KD	DV	ZV	KV	OV	AV	OV	GV	MV	KV	GV	OV	KD	OV	OV	ZV	MV	KV	KD	ZV	GV	AV			
Sample Area Size (dm^2)	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9			
Altitude (m)	1060	1125	1110	1428	1120	1428	1190	1153	1060	1156	1200	1140	1190	1220	1172	1410	1108	1071	1234	1130	1180	1421	1115	1335	1108			
Light (Open-Semi-Shade-Shade)	SS	SS	O	SS	SS	S	O	O	SS	O	S	O	SS	S	O	O	O	O	SS	O	O	SS	SS	O	O			
Humidity (Wet-Moist-Arid)	M	A	A	A	A	M	A	A	M	A	A	M	M	M	A	A	A	A	A	M	A	M	M	M	A			
Direction	K	K	G	K	K	K	G	K	K	K	KB	K	K	GB	K	K	K	K	K	G	B	KB	G	D	GB			
Slope(0-45-90)	0	<45	0	<45	0	0	0	<45	>45	0	>45	>45	0	>45	<45	>45	<45	0	>45	<45	>45	45	<45	>45	45			
Number of species	4	4	5	4	6	4	5	4	7	4	8	5	4	3	5	6	5	4	5	6	6	5	6	4	4			
Syntrichia ruralis	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	1	1	1	1	0	22	Tf	Ba
Didymodon acutus	0	1	1	0	1	0	1	1	1	1	1	1	0	0	0	0	1	0	1	0	0	0	0	1	0	12	Tf	Ap
Tortula inermis	0	0	1	1	1	1	1	0	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	10	Tf	Ag
Didymodon vinealis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	5	Tf	Ap
Ptychostomum capillare	1	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5	sT	Pv,g
Grimmia pulvinata	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	4	Cu	Ba
Homalothecium philippeanum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	1	1	0	0	4	We	Ap
Bryum argenteum	0	0	0	0	0	0	0	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	3	sT	Bv,g
Didymodon rigidulus	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	3	Tf	Ap
Syntrichia virescens	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	3	Tf	Ap
Tortula muralis subsp. muralis	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	3	sT	Ba
Tortula subulata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	1	0	0	0	0	3	Tf	Ag
Amblystegium serpens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2	Mr	Ag
Brachytheciastrum velutinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	0	0	0	2	Mr	Ag
Encalypta pilifera	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	Tf	Pg
Lewinskya affinis	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	Cu	Ag
Lewinskya rupestris	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	2	Cu	Ba
Orthotrichum diaphanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	Cu	Ag,v
Orthotrichum pumilum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	Cu	Ag
Pterygoneurum ovatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	2	sT	Pc
Ptychostomum imbricatulum	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	sT	Ap
Ptychostomum pallens	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	sT	Ap
Syntrichia ruraliformis	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	2	Tf	Ba
Brachythecia strum collinum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ag
Brachythecium albicans	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ap
Bryum dichotomum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	Tf	Ag
Encalypta vulgaris	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Pv
Grimmia crinita	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ba
Hennediella heimii	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	sT	Pg
Homalothecium lutescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	We	Ap
Homalothecium sericeum	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	We	Ap
Hygroamblystegium humile	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ap
Hygroamblystegium varium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	Mr	Ap
Microbryum curvicollum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	sT	Ba
Orthotrichum cupulatum	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ag
Orthotrichum pallens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	Cu	Ag
Orthotrichum tenellum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Cu	Av
Pterigoneurum crossidioides	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	sT	Pc
Pterygoneurum compactum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	sT	Pc
Ptychostomum inclinatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	sT	Ap
Rhynchostegium megapolitanum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	We	Ag
Schistidium trichodon	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	Cu	Ba
Syntrichia rigescens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Tf	Ba
Tortella inclinata	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	sT	Ba
Tortula acaulon	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	sT	Ba
Tortula cuneifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	sT	Ba
Tortula mucronifolia	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	Tf	Ag



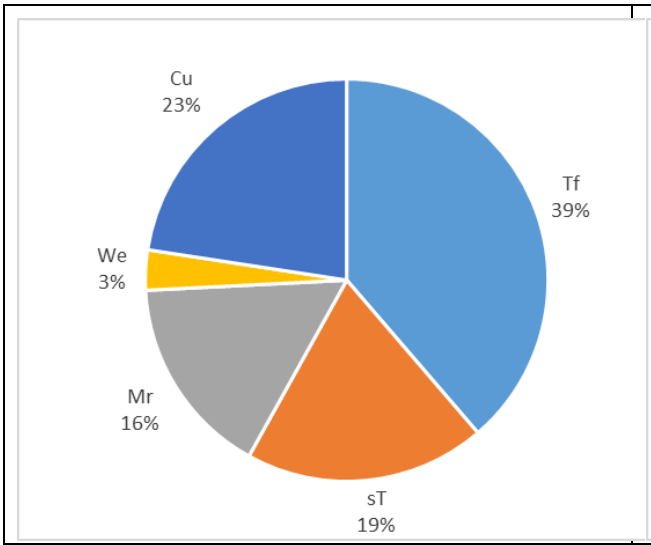
Graph 6-Life Form



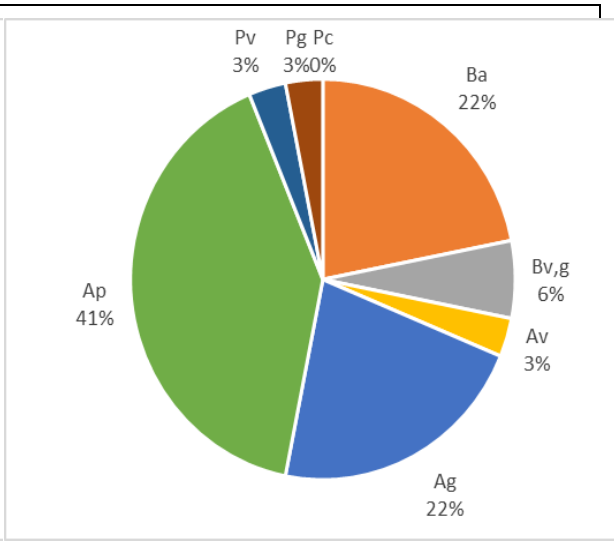
Graph 7- Life Stragedy

Table 5- *Grimmia pulvsnata-Grimmia anodon* community

Sample Area No (GMP)	97	200	194	48	188	158	196	179	169	181	182	174	55	140	13	149	Number of Repetitions	Life Forms	Life Strategy
Substrate	R	R	R	R	R	R	R	R	R	R	R	R	S	R	R	S			
Locality	KV	MV	KD	KV	AD	ZV	KD	GV	AD	GV	GV	KV	KV	MV	MV	GV			
Sample Area Size (dm^2)	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9			
Altitude (m)	1300	1259	1435	1210	1127	1117	1490	1200	1110	1170	1170	1060	1150	1130	1140	1320			
Light (Open-Semi-Shade-Shade)	O	SS	SS	O	O	O	S	O	SS	SS	S	SS	O	O	O	O			
Humidity (Wet-Moist-Arid)	M	A	A	A	A	M	A	A	A	A	M	M	A	W	M	M			
Direction	K	K	K	G	K	B	K	K	K	K	K	K	K	G	K	GB			
Slope(0-45-90)	0	>45	0	>45	<45	<45	>45	45	<45	<45	<45	45	>45	0	0	<45			
Number of species	4	4	5	4	4	5	4	4	5	4	5	6	6	4	5	4			
Grimmia pulvinata	1	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	12	Cu	Ba
Grimmia anodon	1	1	1	1	1	1	1	1	1	0	1	0	0	0	0	0	10	Cu	Ba
Syntrichia ruralis	1	1	1	1	1	0	1	1	0	0	0	0	1	0	0	0	8	Tf	Ba
Tortula inermis	0	0	0	1	1	0	0	0	0	1	1	1	1	1	0	0	7	Tf	Ag
Kindbergia praelonga	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	3	Mr	Ap
Orthotrichum pumilum	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	3	Cu	Ag
Didymodon acutus	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	2	Tf	Ap
Didymodon vinealis	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	2	Tf	Ap
Homalothecium philippeanum	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	We	Ap
Ptychostomum pallens	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	2	sT	Ap
Tortula subulata	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	2	Tf	Ag
Barbula unguiculata	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	sT	Ap
Brachytheciastrum collinum	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Mr	Ag
Brachythecium glareosum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	Mr	Ap
Bryoeryhthrophyllum rubrum	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Ag
Bryum arachnoideum	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	Tf	Av
Bryum argenteum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	sT	Bv,g
Ceratodon purpureus	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	sT	Ap
Dicranella heteromalla	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Tf	Ag
Didymodon fallax	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	Tf	Ap
Didymodon rigidulus	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	Tf	Ap
Grimmia crinita	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Cu	Ba
Grimmia orbicularis	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	Cu	Ba
Grimmia plagipodia	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ba
Hygroamblystegium tenax	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Mr	Ap
Imbribryum mildeanum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Cu	Bg
Leucodon immersus	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	Mr	Ag
Pseudocrossidium hornschuchianum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	Tf	Ap
Ptychostomum capillare	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	sT	Pv,g
Ptychostomum inclinatum	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	sT	Ap
Tortula vahliana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	Tf	Ba



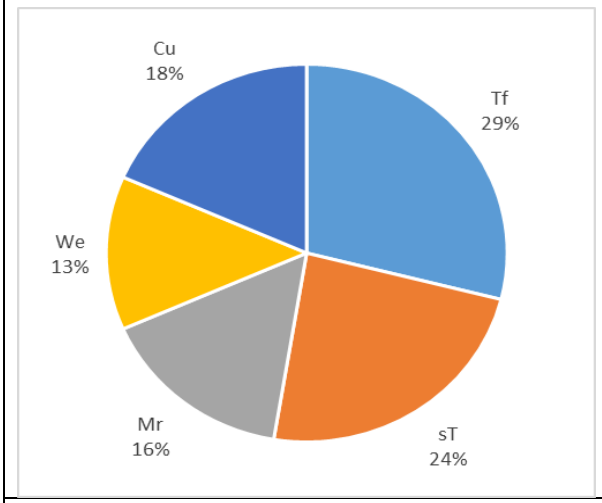
Graph 8-Life Form



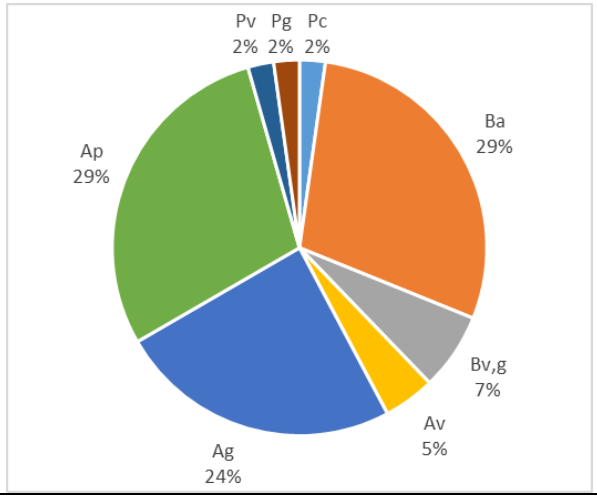
Graph 9- Life Stragedy

Table 6-*Lewinskya rupestris-Grimmia pulvinata* community

Sample Area No (GMP)	93	107	71	14	53	43	44	105	16	17	75	52	195	100	39	88	86	18	26	76	36	153	15	68	33	40	34	57	Number of Repetitions	Life Forms	Life Strategy
Substrate	T	R	T	T	T	T	T	T	R	R	R	T	R	R	T	S	T	R	R	T	T	T	T	T	T	T	T	T			
Locality	MV	AV	GV	MV	KV	ZV	ZV	OV	MV	MV	GV	KV	KD	KD	ZV	ZV	ZV	MV	AV	GV	ZV	GV	MV	GV	KD	ZV	ZV	KV			
Sample Area Size (dm^2)	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9	9			
Altitude (m)	1160	1083	1220	1130	1170	1320	1290	1110	1130	1130	1190	1170	1511	1420	1360	1220	1250	1130	1060	1190	1380	1283	1130	1240	1384	1360	1380	1120			
Light (Open-Semi-Shade-Shade)	SS	S	O	S	O	O	O	SS	S	S	SS	O	SS	SS	O	SS	SS	S	SS	SS	SS	O	S	O	SS	SS	SS	SS			
Humidity (Wet-Moist-Arid)	M	A	A	A	A	M	M	A	M	M	M	M	A	M	M	A	M	A	M	M	M	M	A	M	M	M	M	M			
Direction	G	K	B	K	K	K	KB	G	K	K	K	K	K	G	B	GB	G	K	K	K	K	G	K	D	K	B	KB	B			
Slope(0-45-90)	>45	0	0	>45	0	0	0	>45	0	>45	>45	45	>45	>45	>45	0	>45	>45	0	>45	>45	>45	>45	<45	>45	>45	>45	0			
Number of species	5	4	5	4	5	5	4	3	6	5	5	4	4	4	6	4	6	5	6	5	4	6	4	5	6	8	4	5			
Lewinskya rupestris	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	1	0	26	Cu	Ba
Grimmia pulvinata	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	0	0	1	1	0	0	0	0	0	0	0	0	0	14	Cu	Ba
Orthotrichum pumilum	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	1	9	Cu	Ag
Ptychostomum pallens	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	1	1	0	1	0	0	0	0	1	0	0	1	1	9	sT	Ap
Syntrichia ruralis	0	0	1	1	1	1	1	1	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	8	Tf	Ba
Homalothecium aureum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	1	0	0	0	0	0	0	0	0	5	We	Ap
Ptychostomum capillare	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	0	0	5	sT	Pv,g
Syntrichia ruraliformis	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	5	Tf	Ba
Brachythecium capillaceum	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	1	0	0	4	Mr	Ap
Tortula mucronifolia	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	1	0	0	4	Tf	Ag
Didymodon rigidulus	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	3	Tf	Ap
Hygroamblystegium varium	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	3	Mr	Ap
Lewinskya affinis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	1	0	0	3	Cu	Ag
Tortula muralis subsp. muralis	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	sT	Ba
Brachythecia strum mildeanum	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	Mr	Ap
Brachythecia strum velutinum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	2	Mr	Ag
Grimmia anodon	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	Cu	Ba
Grimmia crinita	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	2	Cu	Ba
Grimmia trichophylla	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	Cu	Av
Rhynchostegiella tenella	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	We	Ag
Brachythecium glareosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	Mr	Ap
Bryum radiculosum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	sT	Bg
Didymodon sinuous	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Bv,g
Didymodon vinealis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	Tf	Ap
Grimmia meridionalis	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Cu	Ba
Grimmia ovalis	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Av
Homalothecium lutescens	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	We	Ap
Homalothecium sericeum	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	We	Ap
Hypnum cupressiforme	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	We	Ag
Leucodon immersus	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Mr	Ag
Lewinskya striatum	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ag
Orthotrichum anomalum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	Cu	Ag
Orthotrichum pallens	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	Cu	Ag
Pterigoneurum crossidioides	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	sT	Pc
Ptychostomum kunzei	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	sT	Bg
Ptychostomum imbricatulum	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	sT	Ap
Ptychostomum inclinatum	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	sT	Ap
Schistidium flaccidum	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	Cu	Ba
Syntrichia calcicola	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	Tf	Ba
Syntrichia caninervis	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Ba
Syntrichia virescens	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Ap
Tortula acaulon var. pilifera	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	sT	Ba
Tortula inermis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	Tf	Ag
Tortula vahliana	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	Tf	Ba



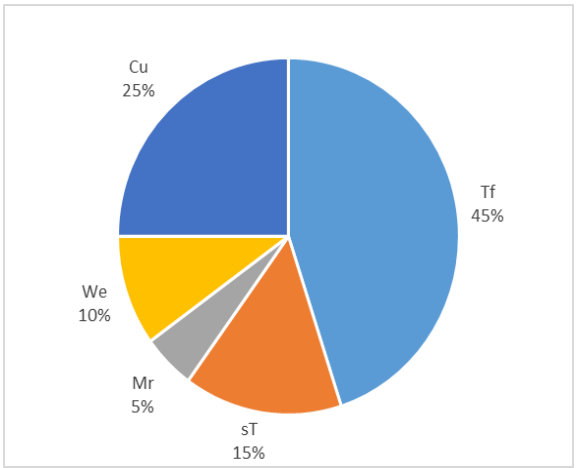
Graph 10-Life Form



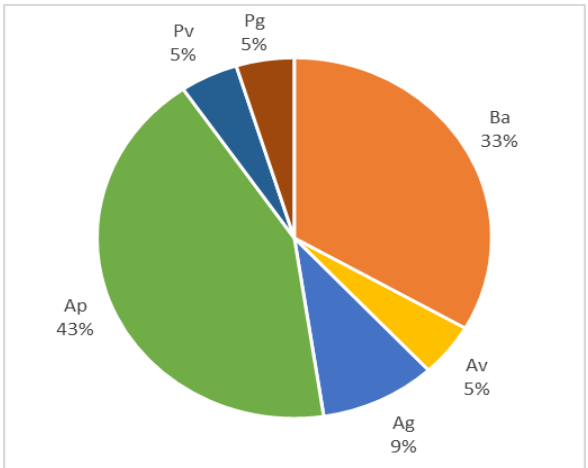
Graph 11- Life Stragedy

Table 7-*Grimmia crinita-Grimmia pulvinata* community

Sample Area No (GMP)	45	138	91	101	8	87	92	42	47	79	152	4	Number of Repetitions	Life Forms	Life Strategy
Substrate	R	R	T	R	R	R	R	R	R	R	S	R			
Locality	AD	MV	MV	KD	MV	ZV	MV	ZV	AD	KD	GV	GV			
Sample Area Size (dm^2)	9	9	9	9	9	9	9	9	9	9	9	9			
Altitude (m)	1100	1100	1200	1470	1120	1210	1180	1330	1110	1410	1283	1320			
Light (Open-Semi-Shade-Shade)	O	O	SS	SS	O	SS	S	O	O	S	O	O			
Humidity (Wet-Moist-Arid)	A	W	A	M	W	W	W	A	A	A	W	A			
Direction	G	G	G	GB	G	K	G	K	KD	K	G	G			
Slope(0-45-90)	>45	>45	0	>45	>45	>45	>45	>45	>45	>45	<45	>45			
Number of species	4	4	5	4	4	4	7	5	6	4	4	6			
Grimmia crinita	1	1	1	1	1	1	1	1	1	1	1	1	12	Cu	Ba
Grimmia pulvinata	1	1	1	1	1	1	1	0	1	1	0	0	9	Cu	Ba
Didymodon acutus	1	1	1	0	0	0	0	1	1	0	0	0	5	Tf	Ap
Tortula muralis subsp. muralis	1	1	1	1	0	0	0	0	0	0	0	1	5	sT	Ba
Homalothecium sericeum	0	0	0	0	0	1	1	0	1	0	0	0	3	We	Ap
Ptychostomum capillare	0	0	0	0	0	0	1	1	0	0	0	1	3	sT	Pv,g
Tortula subulata	0	0	0	0	0	0	0	0	1	1	1	0	3	Tf	Ag
Homalothecium philippeanum	0	0	1	0	1	0	0	0	0	0	0	0	2	We	Ap
Orthotrichum macrocephalum	0	0	0	0	0	0	0	1	1	0	0	0	2	Cu	Ap
Syntrichia ruralis	0	0	0	0	0	1	1	0	0	0	0	0	2	Tf	Ba
Tortula mucronifolia	0	0	0	0	1	0	1	0	0	0	0	0	2	Tf	Ag
Didymodon cordatus	0	0	0	0	0	0	0	0	0	0	0	1	1	Tf	Ap
Didymodon imbricata	0	0	0	0	0	0	0	1	0	0	0	0	1	Tf	Ap
Didymodon rigidulus	0	0	0	0	0	0	1	0	0	0	0	0	1	Tf	Ap
Didymodon tophaceus subsp.tophaceus	0	0	0	0	0	0	0	0	0	0	1	0	1	Tf	Ba
Grimmia ovalis	0	0	0	0	0	0	0	0	0	0	0	1	1	Cu	Av
Hygroamblystegium varium	0	0	0	0	0	0	0	0	0	0	1	0	1	Mr	Ap
Orthotrichum pellucidum	0	0	0	0	0	0	0	0	0	0	0	1	1	Cu	Ba
Ptychostomum imbricatulum	0	0	0	1	0	0	0	0	0	0	0	0	1	sT	Ap
Syntrichia ruraliformis	0	0	0	0	0	0	0	0	0	1	0	0	1	Tf	Ba



Graph 12-Life Form



Graph 13- Life Stragedy

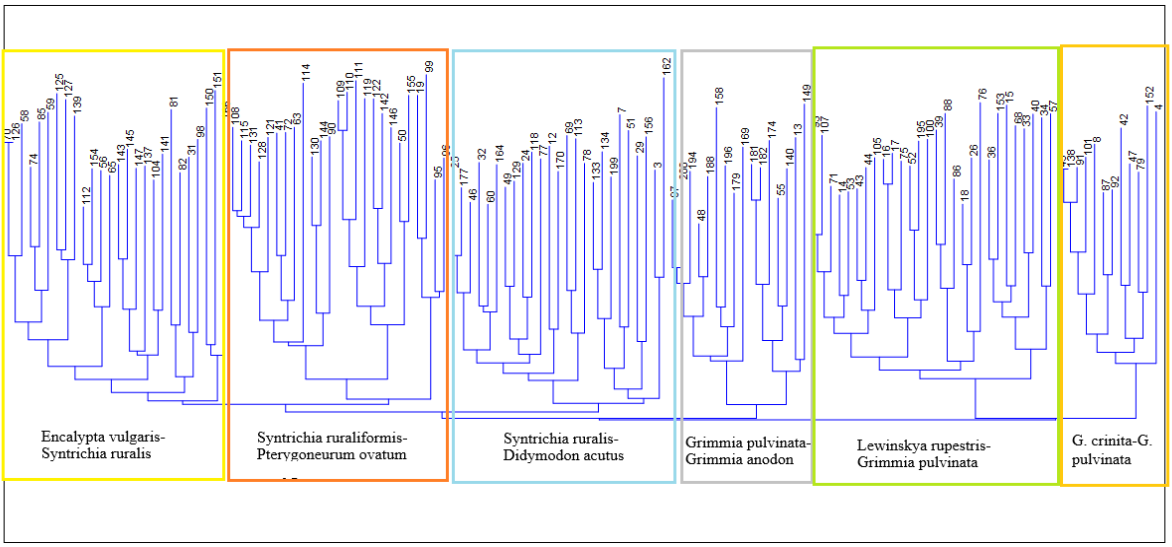


Figure 1-Position of the community in the NJA clustering graph

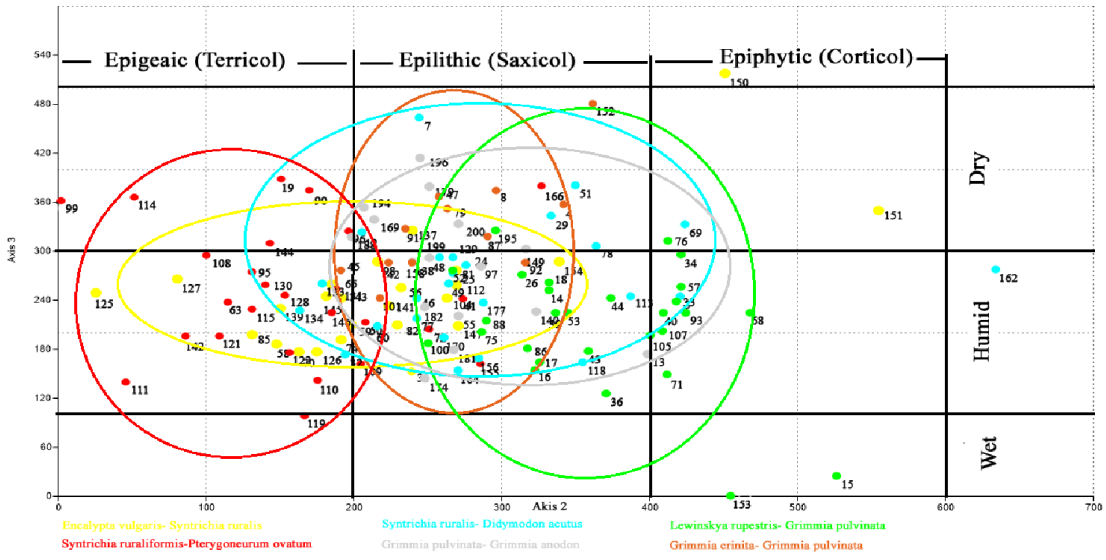


Figure 2- Distribution of sample areas according to DCA